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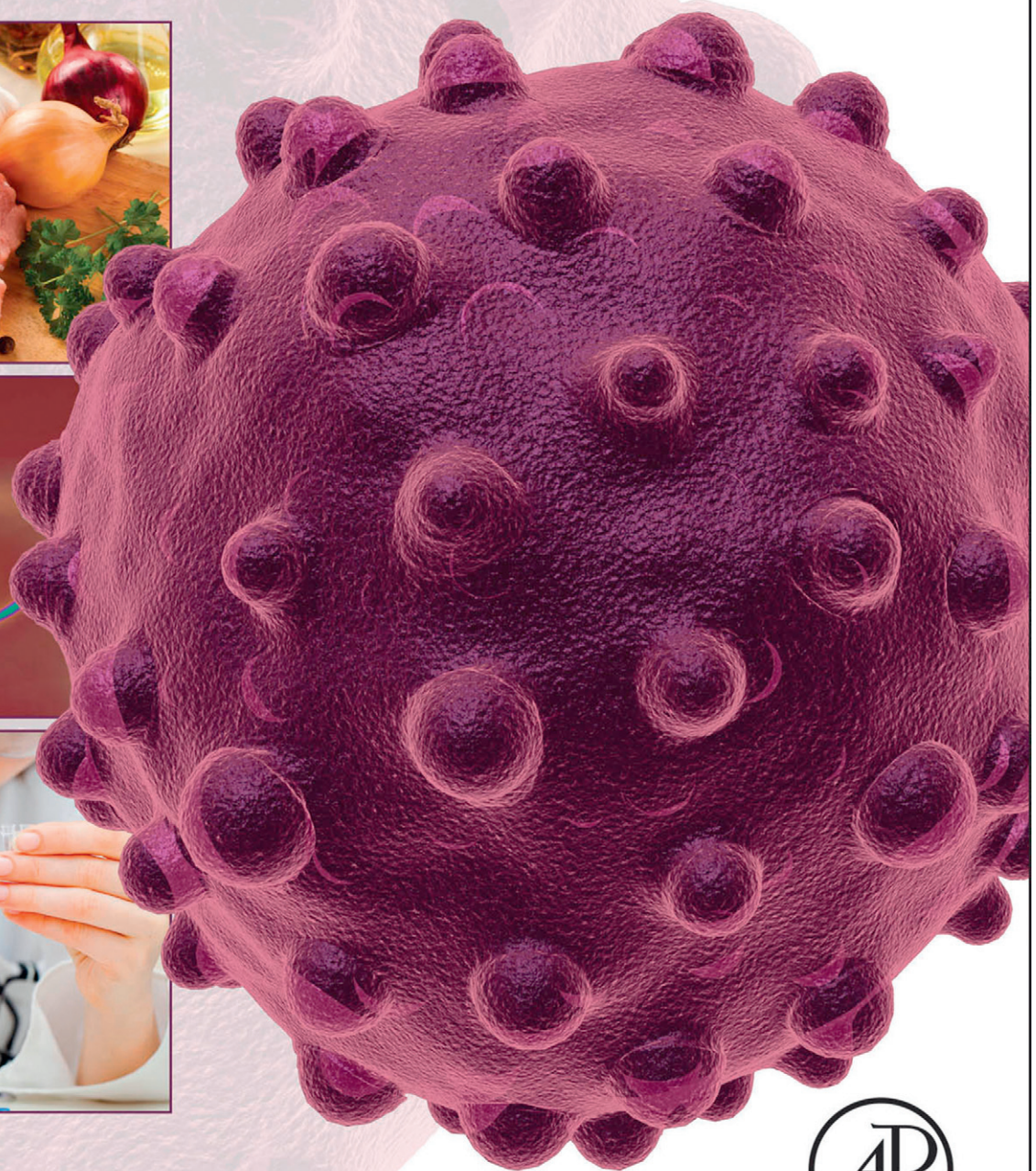
Encyclopedia of Food Safety

ENCYCLOPEDIA OF FOOD SAFETY

Edited by **Yasmine Motarjemi, Gerald Moy, Ewen Todd**



ENCYCLOPEDIA OF
FOOD SAFETY

VOLUME 1

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FOOD SAFETY

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PREFACE

Why an Encyclopedia on Food Safety?

With the world's growing population, the provision of a safe, nutritious, and wholesome food supply has become a major challenge. To achieve this, effective risk management based on sound science and unbiased information is required by all stakeholders, including the food industry, governments, and consumers themselves. In addition, the globalization of the food supply requires the harmonization of policies and standards based on a common understanding of food safety among authorities in countries around the world.

Furthermore, reports of food safety incidents and foodborne disease outbreaks in one country are disseminated almost instantaneously through the 24/7 news cycle to consumers in other countries all over the world. Consequently, food safety managers in government and industry are sometimes called on to respond to queries from politicians, the media, and the general public even before they may be aware of the problem. Taking effective intervention measures and communicating the basis of their decisions and actions are essential for maintaining confidence in the safety of the food supply.

In all the above circumstances, sound scientific information is the key to effectively and efficiently assess, manage, and communicate on food safety risks. Yet, professionals and other specialists working in this multidisciplinary field are finding it increasingly difficult to keep up with developments outside their immediate areas of expertise. The time and staff needed to provide this information are beyond the resources of most individuals and organizations. Therefore, a single source of concise, reliable, and authoritative information on food safety has, more than ever, become a necessity.

This is the role that the Encyclopedia on Food Safety sought to fulfill by gathering all of the world's knowledge and expertise covering the entire spectrum of food safety topics into one comprehensive reference work. This was done with the objective of facilitating the work of those working in the field of food safety and related fields, such as nutrition, food science and technology, and environment. The Encyclopedia also provides a platform for experts to share their state-of-the art expertise and experience with the rest of the food safety community. Furthermore, the Encyclopedia's online feature is designed for rapid search and retrieval of relevant information.

Who Will Benefit from the Food Safety Encyclopedia?

The Encyclopedia will be useful for professionals and other specialists working in, but not limited to, the following institutions:

- Regulatory and enforcement agencies.
- Food industry.
- Trade and industry organizations.
- Audit and certification bodies.
- Academic institutions.
- Private and governmental scientific and research institutions.
- International and nongovernmental organizations with an interest in food.

What Does the Encyclopedia of Food Safety Contain?

With some 280 articles, the Encyclopedia provides comprehensive coverage a broad range of food safety topics, which may be grouped under the following general categories:

- History and basic sciences that support food safety.
- Foodborne diseases, including surveillance and investigation.
- Foodborne hazards, including microbiological and chemical agents.
- Substances added to food, both directly and indirectly.
- Food technologies, including the latest developments.
- Food commodities, including their potential hazards and controls.
- Food safety management systems, including their elements and the roles of stakeholders.

In developing the Encyclopedia, the editors and members of the Editorial Advisory Board have aimed to ensure that the Encyclopedia provides:

- Contributions by the foremost authorities in their fields.
- Unbiased and concise overviews on a multitude of food safety subjects.
- References for further information
- Specialized and general definitions for food safety terminology.

While the editors have made every effort to ensure that the Encyclopedia reflects the most complete and up-to-date information available, new scientific findings, and advances in food safety occur continuously. In undertaking a project of this scale and with the inevitably delays that occur during production, the editors acknowledge that some topics may have been omitted or insufficiently addressed. Therefore, the feedback of readers to point out any such errors or oversights will be greatly appreciated and will facilitate the development of future editions.

Acknowledgments

The lead editors would like to thank the Editorial Advisory Board members, section editors, and particularly, the authors who have generously contributed their time and talent to the development of this Encyclopedia. We are indebted to the Elsevier secretariat, which has assisted in the production of this work since its inception. Finally, a special note of thanks goes to our families whose patience and support are greatly appreciated.

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DEDICATION

This Encyclopedia is dedicated to our children, our grandchildren, and all the world's future generations who we hope will enjoy the benefits of a safe and nutritious food supply, produced with fair management of people working in industry and ethical treatment of animals.

FOREWORD I

Today's food system is one of the humanity's great achievements. It includes millions of commercial actors all over the world who produce, process, transport, store, market, and serve food that feeds billions of people daily. The complexity, diversity, and scope of the food system are almost beyond comprehension – ranging from small producers and processors serving local communities to vast global enterprises producing food for millions and managing extended international supply chains – all aimed at meeting high consumer expectations for safe, nutritious, and affordable food.

For all of its successes, the food system is full of challenges. Food insecurity and hunger remain major problems worldwide, and, for those with ready access to the foods of their choice, it is too easy to choose products high in salt, fat, and added sugar. Food safety – the task of avoiding chemical and microbiological contamination of food that can make people sick – is another persistent and dynamic challenge. In fact, new products in the marketplace, new patterns of production and supply, new consumer behaviors and new bacterial and chemical hazards – coupled with high consumer expectations – conspire to make food safety one of the central challenges of today's food system.

People working in the food system know this. Prominent illness outbreaks and contamination incidents take a toll on the public's health and cause a loss of confidence that can steer consumers away from healthy foods, like fresh fruits and vegetables, and impose big economic losses on food producers and processors. And the food system is responding with a heightened awareness of food safety at all levels of the food system and tremendous effort across the system to improve food safety. Much progress is being made.

One of the most important food safety developments of the last quarter century has been the emergence of a widely shared, science-based understanding of foodborne illness, its causes, and how it can be prevented. This begins with the understanding that the current burden of foodborne illness is unacceptable because it is largely preventable. It is preventable if we see food safety as a food system issue and recognize that microbiological and chemical hazards can enter the food supply at any point in the system along the pathway from the farm through processing, transport, storage, and retail sale. Likewise, opportunities to minimize hazards and help prevent food safety problems exist throughout the system, which means that everyone in the system shares responsibility for the safety of the food we eat.

Fulfilling this responsibility requires that we understand as much as we can about food safety hazards and their causes, devise the appropriate, science-based preventive controls for particular hazards and food production settings, monitor their effectiveness, and adjust the controls as needed based on experience. In short, progress on food safety depends fundamentally on a strong base of knowledge and continuous learning to systematically prevent food safety problems. And participants across the global food safety community are actively seeking and applying the knowledge needed to produce safe food and meet high consumer expectations.

This food safety encyclopedia provides a comprehensive overview of what we know about food safety hazards and control measures. We have more to learn, but the knowledge compiled in this encyclopedia demonstrates that we know a lot and that what we know can help empower participants in today's food system to fulfill their food safety responsibility. Although the food safety challenge is global and continuing, and may seem daunting, it can be met if all who share responsibility for food safety take advantage of the knowledge we have, participate in continuous learning, and place first priority every day on protecting the safety of food. That will be good for the food system – and for the consumers it serves.

Michael R Taylor
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FOREWORD II

Food is one of the most basic requirements to sustain life. However, the safety of food and water cannot be taken for granted. Owing to both manmade and natural processes, an array of chemical and microbiological disease-causing agents find their way into food through multiple routes. When contaminated, such food can endanger or even destroy life. Therefore, from time immemorial, humankind has waged a constant battle against foodborne disease. Over many centuries of human development, people invented technologies that helped them fighting this battle, such as cooking, smoking, sun drying, canning, and freezing, to mention but a few. But like any scientific advance, some of these technologies presented their own food safety issues.

In a number of holy books, religious proscriptions for handling food contributed to food safety. In addition, many centuries ago, some governments already recognized that they had responsibilities in this domain and many laws were enacted to ensure the purity of certain foods. But it was only at the end of the nineteenth century, following scientific developments in the field microbiology and other areas of food science, that 'modern' food regulatory activities started.

In 1948, the availability, accessibility, and affordability of food were recognized as a basic human right by the United Nations in its Universal Declaration of Human Rights (Article 25, 1948). Implicit in this concept is the assumption that the food is first and foremost safe to consume, i.e., absence of health damaging properties. It is therefore not surprising that in the same year, the World Health Organization (WHO) was established as a specialized agency of the United Nations with a broad health mandate that included the specific responsibility to "develop, establish and promote international standards with respect to food…". Subsequently in 1963, WHO together with the Food and Agriculture Organization of the United Nations established an intergovernmental body to develop international standards for food – the Codex Alimentarius Commission. Today Codex stands as a major achievement in the promotion of food safety worldwide with an extensive collection of health and safety recommendations for food that are international recognized and referenced by the World Trade Organization and its member countries.

Thirty years ago, in 1983, WHO, again jointly with FAO, convened an Expert Committee on Food Safety to review the global food safety situation and provide guidance for governments, the food industry and consumers on how to cope with the inherent hazards and risks of our food supply. Based on available data and evidence at the time, the committee concluded that "illness due to contaminated food is perhaps the most widespread health problem in the contemporary world and an important cause of reduced economic productivity."

Unfortunately, this rather alarming statement appears to be still true today. Recent studies examining the morbidity and mortality of foodborne diseases have confirmed the significant public health burden posed by these diseases. In developed countries, one-quarter to one-third of the population are made ill each year because of foodborne diseases. In the developing world, the burden is much more severe. For example, diarrheal diseases are now estimated to cause 2.43 million deaths a year. According to WHO statistics, this is the second leading cause of mortality in low-income countries and kills more people than human immunodeficiency virus infection/acquired immunodeficiency syndrome (HIV/AIDS), malaria, or tuberculosis.

In addition, the large number of food safety crises, which occur with increasing frequency, is contributing to the growing public demand for better health protection from contaminated food. This has prompted governments to strengthen their food safety legislation, improve capacities and infrastructure, and tighten control measures. Examples of governmental measures include the creation of European Food Safety Agency by the European Union in 2002, the Food Safety Modernization Act in the USA of 2011 and, most recently, 2013, the commitment of the Premier of the People's Republic of China, Mr. Li Keqiang, to act with an 'iron fist' to improve food safety.

These positive developments are, unfortunately, contrasted by the fact that in many other countries, mostly developing countries, food safety does not receive the attention it deserves. In this regard, the medical profession and public health community appear to be slow in accepting the role that contaminated food plays in the epidemiology of diarrhea, particularly in infants and young children. The treatment of hospitalized cases and outpatients is rarely seen as an opportunity for educating patients and their families on why foodborne diseases occur and how they can be prevented. Two publications published in WHO's Bulletin in 1993 and 2003 urged the health sector to take steps to correct this oversight. Yet even today progress has been disappointing. For example, in the 2009, United Nations Children's Fund (UNICEF) and WHO published a document entitled 'Diarrhea: Why children are still dying and what can be done,' that again overlooked food safety as one of the most important interventions for these diseases. Consequently, in a recent publication in a prestigious *Medical Journal of Gastroenterology*, the issue had to be raised again and omission corrected. It can only be hoped that the public health and donor communities will eventually adopt a more holistic approach for the prevention of diarrheal diseases, which includes essential food safety interventions.

It is for this and many other reasons that I enthusiastically welcome the initiative of Elsevier to publish this Encyclopedia of Food Safety under the editorial leadership of Drs. Yasmine Motarjemi and Gerald Moy (my former WHO colleagues) as well as Dr Ewen Todd, a world renowned expert in food safety. The laudable collaboration and support of the Editorial Advisory Board, Section Coordinators, and the many authors who have freely devoted their time to advance the cause of food safety through the development of this Encyclopedia is also acknowledged.

With such a collection of information, whoever needs first-hand, reliable, and authoritative information on food safety does not need to consult various books, periodicals, or

websites. All of what is presently known in this domain can be found in this comprehensive work. In particular, the Encyclopedia will be useful for decision-makers, managers, officials, and scientists working in government, the food industry, academia, and nongovernmental organizations.

This Encyclopedia may be particularly important for colleagues in developing countries to not only improve food safety for their people but also convince politicians and other policy makers of the pivotal role of food safety in health and development. Without this awareness, the ultimate goal of safe food for all cannot be achieved.

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Further Reading

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EDITOR-IN-CHIEF

Yasmine Motarjemi holds a Masters degree in Food Science and Technology from the University of Languedoc, Montpellier, France (1978) and a Doctoral degree in Food Engineering from the University of Lund, Sweden (1988).

After her research and academic career at the University of Lund, in 1990, she joined the World Health Organization in Geneva as Senior Scientist. In WHO, she was responsible for the surveillance and prevention of foodborne illnesses (including education of professional food handlers and consumers), the development of the food safety assurance systems (e.g., Hazard Analysis and Critical Control Point system), and for assistance to the WHO Member States in strengthening their national food safety programme. She also contributed to the development of the risk analysis process. She has served in the Secretariat of various sessions of the Codex Alimentarius Commission and its Committees.

From 2000 to 2011, she held the position of Assistant Vice President in Nestlé where she worked as the Corporate Food Safety Manager. In this capacity, she has, among others, developed the Nestlé Food Safety Management system and managed various emerging food safety issues and crises.

She is the author, co-author, or editor of numerous peer-reviewed articles, books, training manuals, and other publications. Her latest books are Food Safety Management: A Practical Guide for the Food Industry (Elsevier 2014) and Invisible Things (original in French under the title: Les Invisibles), a book on food safety for children.

CO-EDITORS BIOGRAPHY

Dr Gerald G Moy, since his retirement from the World Health Organization in 2008, is a scientific adviser with Food Safety Consultants International working with numerous governments and international organizations. His expertise includes assessment of national food safety programs, risk assessment of chemical hazards in food, total diet studies, food safety during mass gatherings, and food defense. Dr Moy is the editor-in-chief for recently published book Total Diet Studies (Springer) and is currently preparing a chapter on food and water protection for a book on key public health considerations during mass gatherings. He serves on the International Scientific Advisory Committee of the China National Center for Food Safety Risk Assessment, the Technical Advisory Group of the World Food Program Technical Advisory Group, and the WHO International Virtual Advisory Group on Mass Gatherings. He is the author of numerous book chapters, articles, and publications and serves on the editorial boards for several food safety journals.

He received his BS in chemistry from the University of Wisconsin and his PhD in physical organic chemistry from Oregon State University, followed by a post-doctoral fellowship in biophysics at the University of New Mexico. He is a Fellow in International Academy of Food Science and Technology and a recipient of the 2009 Great Wall Friendship Award for his contributions to food safety during the Beijing Olympics.

Ewen CD Todd is the President of Ewen Todd Consulting and the former Professor in the Department of Advertising, Public Relations and Retailing, and he is also the Adjunct Professor in the Departments of Food Science and Human Nutrition and Large Animal Clinical Sciences at Michigan State University (MSU). He was former directors of the Food Safety Policy Center and the National Food Safety and Toxicology Center at MSU. At both these centers, Dr. Todd coordinated research in microbiology, toxicology, epidemiology, risk assessment, social science, and policy in the area of food safety, distance education programs, and outreach in the community. Previously, he was in the Bureau of Microbial Hazards, Health Products and Food Branch, Health Canada, Ottawa where he was a research scientist for 33 years working on methods development for pathogens in foods, foodborne disease investigation and reporting, costs and surveillance of disease, illnesses caused by seafood toxins, and risk assessment of foodborne pathogens. He also helped develop risk management strategies for the Department including producing videos and pamphlets on food safety education. Some of his recent research has been working on *Listeria* and *E. coli* O157 transfer coefficient and modeling projects, hygiene in child care centers, schools, and elder care facilities. He has also collaborated with government agencies and academia in Spain, Kuwait, Saudi Arabia, Lebanon, Cambodia, Korea, Japan, and China on food safety issues, and is an expert witness in legal suits involving food safety. He has published extensively on many different aspects of food safety, including 11 recent papers on food workers and hand hygiene. He is active in the International Association for Food Protection (IAFP) and other organizations, and speaks and organizes symposia at national and international meetings. He is the associate editor for the Journal of Food Science and is a frequent reviewer of manuscripts submitted to several different scientific journals.

He has received the Government of Canada Distinctive Service Award for extraordinary teamwork and support to the Science and Technology Community; Recipient of the Excellence in Science Award for 1998 by Health Canada; Deputy Minister's Award of Team Excellence for the work done in promoting the Fight BAC! Campaign in Canada; the Professional Institute of the Public Service of Canada Gold Medal for Pure and Applied Science; and he is Fellow of the American Association for the Advancement of Science, the IAFP, and the MSU University Outreach and Engagement. He is also an honorary life member of the IAFP. He is a graduate of Glasgow University with a BSc in Bacteriology and a PhD in bacterial systematics.

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HOW TO USE THE ENCYCLOPEDIA

The material in this encyclopedia is organized into five broad sections, presented in four volumes. The sections consist of:

1. **History, science, and methods**: this section includes papers which help in the understanding of basic sciences underpinning food safety and foodborne diseases and their historical development.
2. **Hazards and diseases**: this section addresses the features of major foodborne hazards be they chemical, microbial, parasitical or physical and their health consequence.
3. **Food technologies**: this section explains the various food technologies and aspects related to their safety, or risks in their application.
4. **Foods, materials, and risks**: similarly, in this section, various groups of food products are described in terms of their risks and measures needed to ensure their safety.
5. **Food safety management**: finally, in this part, the building blocks of food safety management in the private and public sector are explained. The role of major international organizations is also reported.

To help realize the full potential of the material in the Encyclopedia the authors have provided five features to help you find the topic of your choice: a preface giving an overview of the encyclopedia and its objectives, a contents list by subject; an alphabetical contents list; cross-references to other articles; and a full subject index.

1 Contents List by Subject

Your first point of reference will probably be the contents list by subject. This list appears at the front of each volume, and groups the entries under subject headings describing the broad themes of quaternary science. This will enable the reader to make quick connections between entries and to locate the entry of interest. Under each main section heading, you will find several subject areas and under each subject area is a list of those entries that covers aspects of that subject, together with the volume and page numbers on which these entries may be found.

2 Alphabetical Contents List

The alphabetical contents list, which also appears at the front of each volume, lists the entries in the alphabetical order. This list provides both the volume number and the page number of each entry. On the opening page of an entry a contents list is provided so that the full details of any articles within the entry are immediately available.

3 Cross-references

All of the entries in the Encyclopedia have been extensively cross-references. The cross-references, which appear at the end of the entry, serve three different functions:

i. To indicate if a topic is discussed in greater detail elsewhere.
ii. To draw the reader's attention to parallel discussions in other entries.
iii. To indicate the material that broadens the discussion.

Example

The following list of cross-references appear at the end of the entry Characteristics of Foodborne Hazard and Diseases | Drug Resistant Pathogens.

> *See also*: Bacteria: *Listeria monocytogenes*; *Salmonella* Non-Typhi; *Salmonella* Typhi and *Salmonella* Paratyphi; Shiga Toxin-Producing *Escherichia coli* and Other Pathogenic *Escherichia coli*; *Shigella*; *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*. Characteristics of Foodborne Hazard and Diseases: Cost of Foodborne Diseases. Food Safety Assurance Systems: Cleaning and Disinfection. Public Health Measures: Food Safety in Hospitals and Other Healthcare Settings; Surveillance of Foodborne Diseases

Here you will find examples of all three functions of the cross-reference list: a topic discussed in greater detail elsewhere (e.g., *Listeria monocytogenes*; *Salmonella* Non-Typhi; *Salmonella* Typhi, and *Salmonella* Paratyphi; Shiga Toxin-Producing *Escherichia coli*), parallel discussion in other entries (e.g., Other Pathogenic *Escherichia coli*), and reference to entries that broaden the discussion (e.g., Food Safety Assurance Systems: Cleaning and Disinfection. Public Health Measures: Food Safety in Hospitals and Other Healthcare Settings).

4 Index

The index provides you with the page number where the material is located. The index entries differentiate between materials that is a whole entry, is part of an entry, or is data presented in a figure or a table. Detailed notes are provided on the opening page of the index.

5 Contributors

A full list of contributors is listed at the beginning of each volume.

GLOSSARY OF SELECTED TERMS

This Glossary of Selected Terms is a partial list of definitions for terms commonly used in the area of food safety. The terms selected are those that are important for communication among the various disciplines or are often subject to misunderstanding. Most of the definitions are taken from those recommended by international organizations or given by the authors contributing to this Encyclopedia. In cases where there are different definitions for a term, the Glossary presents the definition that is most consistent with usage by the majority of authors. Note that in some instances, slight differences between general definitions in this Glossary and those appearing in the individual articles may occur as the result of the specific context of the articles.

Acceptable daily intake The estimate of the amount of a substance in food or drinking water, expressed on a body weight basis, that can be ingested daily over a lifetime without appreciable health risk to the consumer.

Acute reference dose The estimate of the amount of a substance in food or drinking water, expressed on a body mass basis, that can be ingested in a period of 24 h or less without appreciable health risk to the consumer.

Adulteration (economic) A fraudulent action which is intended to omit a valuable constituent or substitute another substance, in whole or in part, for a valuable constituent; conceal damage or inferiority in any manner; or add any substance to increase its bulk or weight, reduce its quality or strength, or make it appear bigger or of greater value than it is (Note that in the US, adulterated food is generally defined as impure, unsafe, or unwholesome food.).

Antiseptic A substance that inhibits the growth and development of microorganisms. For practical purposes, antiseptics are routinely thought of as topical agents, for application to skin, mucous membranes, and inanimate objects, although a formal definition includes agents which are used internally, such as the urinary tract antiseptics.

As low as reasonably achievable A risk management approach that aims to keep exposure to a substance at the lowest level that is realistically achievable.

Asymptomatic shedder A person who does not exhibit the symptoms of an illness but excrete the pathogen (*see also* carrier).

Benchmark Reference point or standard against which performance or achievements can be assessed. A benchmark refers to the performance that has been achieved in the recent past by other comparable organizations, or what can be reasonably inferred to have been achieved in the circumstances.

Biomarkers Indicators of changes or events in human biological systems. Biomarkers of exposure refer to cellular, biochemical, or molecular measures that are obtained from biological media such as human tissues, cells or fluids, and are indicative of exposure to a substance. Biomarkers of effect refer to biological changes that represent an alteration in endogenous body constituents (e.g., depression of cholinesterase levels as an indicator of exposure to pesticides).

Carrier A person or animal that harbors a specific infectious agent without discernible clinical disease and serves as a potential source of infection. The carrier state may exist in an individual with an infection that is unapparent throughout its course (commonly known as healthy or asymptomatic carrier), or during the incubation period, convalescence and postconvalescence of an individual with a clinically recognizable disease (commonly known as an incubatory or convalescent carrier). Under either circumstance the carrier state may be of short or long duration (temporary or transient carrier, or chronic carrier) (*see also* asymptomatic shedder).

Case-fatality rate Usually expressed as the percentage of persons diagnosed as having a specified disease who die as a result of that illness within a given period. This term is most frequently applied to a specific outbreak of acute disease in which all patients have been followed for an adequate period of time to include all attributable deaths. The case-fatality rate must be clearly differentiated from the mortality rate (Compare with mortality rate).

Colony-forming unit A measure of viable bacterial or fungal cells in which a colony represents an aggregate of cells derived from a single progenitor cell.

Contaminant Any biological, chemical, or physical agent not intentionally added to food, which is present in food as a result of the production, manufacture, processing, preparation, transport, or holding of such food (Compare with hazard).

Control (noun) The state wherein correct procedures are being followed and critical criteria are being met.

Control (verb) To take all necessary actions to ensure and maintain compliance with criteria established in the Hazard analysis and critical control point system (HACCP) plan.

Control measure Any action and activity that can be used to prevent or eliminate a food safety hazard or reduce it to an acceptable level.

Corrective action Any action to be taken when the results of monitoring at the Critical Control Point (CCP) indicate a loss of control.

Crisis A predicted or unpredicted event which represents an immediate or future significant threat to an organization, its employees, consumers, and the public at large.

Critical control point A step at which control can be applied and is essential to prevent or eliminate a food safety hazard or reduce it to an acceptable level.

Critical limit (CL) A criterion which separates acceptability from unacceptability.

Detergent A chemical used to remove grease, dirt and food, such as washing-up liquid.

Disability adjusted life year (DALY) A metric used to express a health gap that extends the concept of potential years of life lost due to premature death to include equivalent years of 'healthy' life lost by virtue of being in states of poor health or disability. The DALY combines in one measure the time lived with disability and the time lost due to premature mortality. One DALY can be thought of as one lost year of 'healthy' life and the burden of disease as a measurement of

the gap between current health status and an ideal situation where everyone lives into old age free of disease and disability.

Disinfectant A chemical agent or a process that destroys, neutralizes, or inhibits the growth of pathogenic microorganisms (*see also* sanitizer).

Dose–response assessment The determination of the relationship between the magnitude of exposure (dose) to a chemical, biological, or physical agent and the severity and/or frequency of associated adverse health effects (response) in the exposed organism, system, or (sub) population in reaction to the agent.

Endotoxin A toxin present in intact bacterial cells and released when bacteria die or the cells are disrupted. A notable endotoxin is lipopolysaccharide, which is a major constituent of the outer cell membrane of Gram-negative bacteria and can cause toxic effect on lysis of bacteria. The term 'endotoxin' is to be differentiated from 'exotoxin', which is a toxin secreted in the surrounding medium and environment of the bacterial cell.

Enterotoxin A cytotoxin produced by bacteria that is specific for the mucous membrane of the intestine and causes diarrhea and/or vomiting associated with foodborne disease. Many infectious microorganisms produce enterotoxins in the gut, but some are produced external to the host (*see also* exotoxin and endotoxin).

Exotoxin A toxin that is secreted by bacteria. There are many different types of exotoxins. They can be released into the susceptible host (after infection and growth) or into the environment, including food (after contamination and growth). Those released into the intestines are typically heat labile (but some *E. coli* strains can produce both heat labile (HL) and heat stable (HS) toxins). *Clostridium perfringens* produces a HL enterotoxin after completion of sporulation in the host's intestines. *Staphyloccocus aureus* and *Bacillus cereus* enterotoxins produced in food are HS and cause vomiting and diarrhea, whereas toxins of *Clostridium botulinum* toxin, also produced in food, are HL and cause systemic neurological symptoms (*see also* exotoxin and endotoxin).

Epidemic The occurrence in a community or region of a group of illnesses which are similar in nature and clearly in excess of normal expectancy, and derived from a common or from a propagated source (Compare with pandemic).

Equivalence The situation where the application of two different food safety management measures lead to the same, or equivalent, public health outcomes.

Equivalence of sanitary measures (import–export of food) Equivalence is the state wherein sanitary measures applied in an exporting country, though different from the measures applied in an importing country, achieve, as demonstrated by the exporting country, the importing country's appropriate level of sanitary protection.

Exposure assessment The qualitative and/or quantitative evaluation of the likely ingestion of a biological, chemical, or physical agent in food as well as exposures from other sources if relevant.

Fecal–oral route A means of spreading pathogenic microorganisms from feces produced by an infected host to another host, usually via the mouth; for example, contact between contaminated hands or objects and the mouth.

Flow diagram A systematic representation of the sequence of steps or operations used in the production or manufacture of a particular food item.

Food Any substance, whether processed, semiprocessed, or raw, which is intended for human consumption, and includes drink, chewing gum, and any substance which has been used in the manufacture, preparation or treatment of 'food' but does not include cosmetics or tobacco or substances used only as drugs.

Food additive Any substance not normally consumed as a food by itself and not normally used as a typical ingredient of the food, whether or not it has nutritive value, the intentional addition of which to food for a technological (including organoleptic) purpose in the manufacture, processing, preparation, treatment, packing, packaging, transport, or holding of such food results, or may be reasonably expected to result, (directly or indirectly) in it or its byproducts becoming a component of or otherwise affecting the characteristics of such foods.

Food allergy A form of food intolerance in which there is evidence of an abnormal immunological reaction to the food (Compare with food intolerance).

Food establishment Any building or area in which food is handled and the surroundings under the control of the same management.

Food handler Any person who handles, prepares, or serves food, be they domestic food handlers, such as preparing family food, or professional food handlers, such as those working in food service establishments (cooks and waiters), retail stores, supermarkets, etc. (*see also* food worker).

Food hygiene All conditions and measures necessary to ensure the safety and suitability of food at all stages of the food chain.

Food industry The term includes primary manufacturing and processing industry as well as some other establishments involved in the food chain.

Food intolerance A reproducible, unpleasant reaction to a food or food ingredient, including reactions due to immunological effects, biochemical factors, such as enzyme deficiencies and anaphylactic reactions that often include histamine release (Compare with food allergy).

Food poisoning (or acute foodborne intoxication) A disease caused by a toxin or a chemical in food with symptoms usually appearing within 24 h after ingesting the agent. This term is commonly misused as a synonym for foodborne disease, which covers both infections and intoxications.

Food safety Assurance that food will not cause harm to the consumer, when it is prepared and/or eaten according to its intended use (Compare with food suitability and food hygiene).

Food safety hazard A biological, chemical, or physical agent in, or condition* of, food with the potential to cause an adverse health effect (Compare with contaminant). *Refers also to property of a food.

Food safety objective (FSO) The maximum frequency and/or concentration of a hazard in a food at the time of consumption that provides or contributes to the appropriate level of protection (ALOP) (Compare with Performance objective).

Food suitability Assurance that food is acceptable for human consumption according to its intended use (Compare with food safety and food hygiene).
Food worker Individuals who harvest, process, prepare and serve food, i.e., across the whole food chain to retail/foodservice; it is broader than that of a food handler, who typically works in foodservice establishments typically foodservice; however, the two terms are used interchangeably in the literature (*see also* food handler).
Foodborne disease Any disease of an infectious or toxic nature caused by or thought to be caused by consumption of contaminated food, including drink.
Foodborne pathogens Disease-causing microorganisms that are transmitted to humans via foods.
Generally recognized as safe Status of a substance that is generally recognized, among qualified experts, as having been adequately shown to be safe under the conditions of its intended use (used mainly in the USA).
Genomics The study of an organism via decoding the entire genetic sequence of the organism.
Genetic modification A process of altering the genetic makeup of an organism by techniques of modern biotechnology.
Genetically modified organism (GMO) AGMO or genetically engineered organism is an organism whose genetic material has been altered using genetic engineering techniques.
Good animal husbandry practice A system of management controls that need to be adopted at the level of primary producers to ensure production of food of animal origin that is safe for human consumption and acceptable for trade in the world market.
Good hygienic practice A system of management controls that need to be adopted at production, processing, storage, distribution, and preparation to ensure safety and suitability of products of consumption.
Good laboratory practice A system of management controls for laboratories and research organizations to ensure the quality, integrity, consistency, and reliability of results.
HACCP plan A document prepared in accordance with the principles of HACCP to ensure control of hazards which are significant for food safety in the segment of the food chain under consideration (see also HACCP).
Hazard A biological, chemical, or physical agent in, or condition*; of, food with the potential to cause an adverse health effect (Compare with contaminant). *Refers also to a property of a food.
Hazard analysis The process of collecting and evaluating information on hazards and conditions leading to their presence to decide which are significant for food safety and therefore should be addressed in the HACCP plan.
Hazard analysis and critical control point system A preventive system which identifies, evaluates, and controls hazards which are significant for food safety.
Hazard characterization The qualitative and/or quantitative evaluation of the nature of the adverse health effects associated with a biological, chemical, or physical agent which may be present in food. For a chemical agent, a dose–response assessment should be performed. For a biological or physical agent, a dose–response assessment should be performed if the data are obtainable.
Hazard identification The identification of the type and nature of adverse effects that a biological, chemical, or physical agent in food is capable of causing in an exposed population.
Incidence rate The number of new cases of a condition arising in a defined group within a given period or the number of new infections per unit of person–time at risk (Compare with prevalence).
In vitro In an artificial environment outside the living organism.
In vivo Within a living organism.
Lethal dose 50% The dose of a substance that would be expected to kill half of a population of exposed organisms.
Margin of exposure Ratio of the no-observed-adverse-effect level or benchmark dose lower confidence limit for the critical effect to the theoretical, predicted, or estimated exposure dose or concentration.
Maximum residue limit The maximum concentration of residues resulting from the use of a pesticide or veterinary drug that is acceptable in or on a food.
Minimum infective dose The lowest number of microorganisms required to cause an infection in the host.
Monitoring (CCP) The act of conducting a planned sequence of observations or measurements of control parameters to assess whether a CCP is under control.
Monitoring (general) Continuous or repeated observation, measurement and evaluation of health, and/or environmental or technical data for defined purposes, according to prearranged schedules in space and time, using comparable methods for sensing and data collection.
Morbidity rate An expression of the number of illnesses in a population at risk over a given period of time (usually one year).
Mortality rate An expression of the number of deaths in a population at risk over a given period of time (usually one year).
Nanomaterials Materials engineered at the nanoscale to have novel functionality or properties. Such properties will typically, but not exclusively, be demonstrated in the size range 1–100 nm, but this size range should be considered approximate.
Nanoparticles Particles with one or more external dimensions in the range 1–100 nm, but this size range should be considered approximate.
Nanotechnology The manipulation or materials at the nano level.
Notifiable disease A disease that must, by law or by ministerial decree, be reported to a government authority.
Outbreak A group of at least two cases of a single illness that are demonstrated by epidemiological investigation to have a single common exposure or source.
Pandemic Epidemic occurring over a very wide area, crossing international boundaries (often more than one continent) and usually affecting a large number of people.
Pasteurization A process involving heat treatment at a prescribed time–temperature combination to kill vegetative forms of pathogens that may be present, while causing minimal changes in the composition, flavor, and nutritive value of food. However, with advances and the development

of new food technologies, the term is sometimes used for nonthermal technologies leading to the same effect.

Pathogen An organism capable of causing disease.

Pathogenesis The course of a disease from its origin to its manifestation; more specifically it refers to the cellular events and reactions, and other pathologic mechanisms occurring in the development of the disease.

Pathogenicity Ability of a microorganism to cause disease in a host (Compare with virulence).

Performance criterion The effect in frequency and/or concentration of a hazard in a food that must be achieved by the application of one or more control measures to provide or contribute to a performance objective (PO) or an FSO.

Performance objective The maximum frequency and/or concentration of a hazard in a food at a specified step in the food chain before the time of consumption that provides or contributes to an FSO or ALOP, as applicable (Compare with Food Safety Objective).

Pesticide Any substance intended for preventing, destroying, attracting, repelling, or controlling any pest including unwanted species of plants or animals during the production; storage; transport; and distribution and processing of food, agricultural commodities, or animal feeds or which may be administered to animals for the control of ectoparasites. The term includes insecticides, herbicides, fungicides, rodenticides and algicides as well as plant growth regulators, defoliants, desiccants, and agents for thinning fruit or preventing the premature fall of fruit.

Prerequisite program Practices and conditions needed prior to and during the implementation of HACCP and which are essential to food safety.

Prevalence The number of persons in a population who have a disease at a specified point in time or over a specified period of time (Compare with incidence rate).

Primary production Those initial steps in the food chain up to and including, for example, harvesting, slaughter, milking, and fishing.

Processing aid Any substance or material, not including apparatus or utensils, and not consumed as a food ingredient by itself, intentionally used in the processing of raw materials, foods, or its ingredients, to fulfill a certain technological purpose during treatment or processing and which may result in the nonintentional but unavoidable presence of residues or derivatives in the final product.

Processing contaminant Undesirable contaminants that are formed during the treatment of food as a result of the interaction of their natural components or their ingredients.

Provisional maximum tolerable daily intake (PMTDI) The health-based reference value used for contaminants with no cumulative properties. Its value represents permissible human exposure as a result of the natural occurrence of the substance in food and in drinking water. In the case of trace elements that are both essential nutrients and unavoidable constituents of food, a range is expressed, the lower value representing the level of essentiality and the upper value the PMTDI.

Provisional tolerable monthly intake The health-based reference value used for a food contaminant with cumulative properties that has a very long half-life in the human body. Its value represents permissible human monthly exposure to a contaminant unavoidably associated with otherwise wholesome and nutritious foods.

Provisional tolerable weekly intake The health-based reference value used for food contaminants such as heavy metals with cumulative properties. Its value represents permissible human weekly exposure to those contaminants unavoidably associated with the consumption of otherwise wholesome and nutritious foods.

Quality management Quality management includes all the activities that organization use to direct, control, and coordinate quality. These activities include formulating a quality policy and setting quality objectives. They also include quality planning, quality control, quality assurance, and quality improvements.

Recommended dietary allowance The average daily dietary nutrient intake level that is sufficient to meet the nutrient requirements of nearly all (97–98%) healthy subjects in a particular life stage and gender group.

Reservoir An animal species that specifically harbors an infectious agent over long periods, often without harm to the host.

Risk A function of the probability of an adverse health effect and the severity of that effect, consequential to a hazard(s) in food.

Risk analysis A process of decision making (usually government) for managing food safety, consisting of three components: risk assessment, risk management, and risk communication.

Risk assessment A scientifically based process for evaluating risks associated with foodborne hazards, consisting of the following steps: (i) hazard identification, (ii) hazard characterization, (iii) exposure assessment, and (iv) risk characterization.

Risk assessment policy Documented guidelines on the choice of options and associated judgments for their application at appropriate decision points in the risk assessment such that the scientific integrity of the process is maintained.

Risk characterization The qualitative and/or quantitative estimation, including attendant uncertainties, of the probability of occurrence and severity of known or potential adverse health effects in a given population based on hazard identification, hazard characterization, and exposure assessment.

Risk communication The interactive exchange of information and opinions throughout the risk analysis process concerning risk, risk-related factors, and risk perceptions, among risk assessors, risk managers, consumers, industry, the academic community, and other interested parties, including the explanation of risk assessment findings and the basis of risk management decisions.

Risk estimate The quantitative estimation of risk resulting from risk characterization.

Risk management The process, distinct from risk assessment, of weighing policy alternatives, in consultation with all interested parties, considering risk assessment and other factors relevant for the health protection of consumers and for the promotion of fair trade practices, and, if needed, selecting appropriate prevention and control options.

Risk manager A person or an organization (usually government) with the authority to decide on the acceptability of risk and, if necessary, measures needed for their management.

Risk profile The description of the food safety problem and its context.

Safe (food) A level of risk that is deemed to be acceptable by some standard. The question of safety always involves the question of to whom the risk is acceptable, and by what criteria that party judges it so.

Sanitizer Type of antimicrobial (disinfectant) that kills or irreversibly inactivates microorganisms present on a surface, especially designed for use on food-processing equipment. The US Environmental Protection Agency further defines a sanitizer as providing at least 99.9% reductions of all microorganisms on a surface (*see also* disinfectant).

Shelf-life The predicted time at which a product will change from acceptable to unacceptable quality. It is influenced by factors such as raw ingredient quality, processing conditions, packaging practices, and storage conditions. Typically, shelf-life is determined by a combination of microbial, sensory, and chemical methods. 'Shelf-life' can be expressed on food labels by a variety of dates, including 'expiry', 'use by', 'sell by', 'best before', and 'consume by', depending on the applicable legislation.

Step (HACCP) A point, procedure, operation, or stage in the food chain including raw materials, from primary production to final consumption.

Strain An isolate of the same type of microorganism possessing different properties.

Surveillance The systematic, ongoing collection, collation, and analysis of data on specific diseases in a defined population, to guide public health decisions.

Surveillance (active) Public health surveillance that regularly reaches out to diagnostic laboratories or to clinicians to actively collect reports of specific diagnoses of infections.

Surveillance (passive) Public health surveillance that collects reports of specific diagnoses from clinicians or diagnostic laboratories, which they are required or requested to submit because of notifiable diseases regulations.

Time–temperature abuse A situation where food has not been cooked for long enough or at a sufficient high temperature to reduce contaminants to safe levels, or food has been stored for a time or at a temperature that permits bacteria to proliferate.

Traceability/product tracing The ability to follow, forward as well as backward, the movement of a food through specified stage(s) of production, processing, and distribution.

Uncertainty In risk assessment, imperfect knowledge concerning the present or future state of an organism, system, or (sub) population under consideration.

Validation (analytical methods) Practice undertaken to substantiate or confirm methods or procedures perform as expected and in a reliable manner and consistently meet expectations.

Validation (control measures) Obtaining evidence that a control measure or combination of control measures, if properly implemented, is capable of controlling the hazard to a specified outcome.

Validation (HACCP) Obtaining evidence that the elements of the HACCP plan are effective.

Variability Heterogeneity of values over time, space, or different members of a population. Variability implies real differences among members of that population.

Verification (general) The application of methods, procedures, tests, and other evaluations, in addition to monitoring and to determine whether a control measure is or has been operating as intended.

Verification (HACCP) The application of methods, procedures, tests, and other evaluations, in addition to monitoring and to determine compliance with the HACCP plan.

Veterinary drug Any substance applied or administered to any food-producing animal, such as meat or milk producing animals, poultry, fish, or bees, whether used for therapeutic, prophylactic, or diagnostic purposes, or for modification of physiological functions or behavior.

Virulence The degree of pathogenicity of a microorganism as indicated by case-fatality rates and/or its ability to invade the tissues of the host; the competence of any infectious agent to produce pathologic effects. The virulence of a microorganism is a measure of the severity of the disease it causes (Compare with pathogenicity).

Waterborne disease A disease resulting from the contamination of water either by pathogenic viruses, bacteria or protozoa, or by chemical substances. These agents are directly transmitted to people when the water is used for drinking, preparing food, recreation, or other domestic purposes.

Withdrawal period (veterinary drugs) The interval between the time of the last administration of a veterinary drug and the time of the collection of edible tissue or products from a treated animal that ensures the concentration of residues in food comply with the maximum residue limit for the drug.

Zoonosis A disease that can be passed directly or indirectly from animals, whether wild or domesticated, to humans. Also called zoonotic disease.

ABBREVIATIONS OF TECHNICAL TERMS

This is a nonexhaustive list of commonly used abbreviations in the area of food safety.

ADI	Acceptable daily intake.
ADME	Absorption, distribution, metabolism, and excretion.
AI	Adequate intake.
ALARA	As low as reasonably achievable.
ALOP	Appropriate level of protection.
ARfD	Acute reference dose.
BMD	Benchmark dose.
BMDL	Benchmark dose at lower confidence limit.
CCP	Critical control point.
CFR	Case fatality rate.
CFU	Colony forming unit.
CIP	Cleaning in place.
DALY	Disability adjusted life year.
DGGE	Denaturing gradient gel electrophoresis.
DNA	Deoxyribonucleic acid.
EAR	Estimated average requirement.
ED_{50}	Effective dose 50%.
ELISA	Enzyme linked immunosorbent assay.
EMRL	Extraneous maximum residue limit.
FSO	Food safety objective.
GAHP	Good animal husbandry practice.
GAP	Good agricultural practice.
GHP	Good hygienic practice.
GAqP	Good aquacultural practice.
GC	Gas chromatography.
GC–MS	Gas chromatography–mass spectrometry.
GHP	Good hygienic practice.
GLP	Good laboratory practice.
GM	Genetically modified.
GMO	Genetically modified organism.
GMP	Good manufacturing practice.
GPVD	Good practice in the use of veterinary drugs.
GRAS	Generally recognized as safe.
HAB	Harmful algal bloom.
HACCP	Hazard analysis and critical control point.
HPLC	High performance liquid chromatography.
HPLC–MS	High performance liquid chromatography–mass spectrometry.
HPP	High pressure processing.
HTST	High temperature short time.
HUS	Hemolytic uremic syndrome.
IEDI	International estimated daily intake.
IESTI	International estimated short term Intake.
LD_{50}	Lethal dose 50%.
LOAEL	Lowest observed adverse effect level.
LOD	Limit of detection.
LOQ	Limit of quantitation.
MFFB	Moisture on a fat free bases.
ML	Maximum level.
MLST	Multilocus sequence typing.
MLVA	Multiple locus variable number tandem repeat analysis.
MOE	Margin of exposure.
MRL	Maximum residue limit.
mRNA	Messenger ribonucleic acid.
MS	Mass spectrometry.
NEDI	National estimated daily intake.
NOAEL	No observed adverse effect level.
NOEL	No observed effect level.
OPRP	Operational prerequisite programme.
PC	Performance criterion.
PCR	Polymerase chain reaction.
PDCA	Plan do check act.
PEF	Pulsed electric fields.
PFGE	Pulsed field gel electrophoresis.
PMTDI	Provisional maximum tolerable daily intake.
PO	Performance objective.
PRP	Prerequisite program.
PrP	Protease resistant protein.
PTMI	Provisional tolerable monthly intake.
PTWI	Provisional tolerable weekly intake.
QPS	Qualified presumption of safety.
RDA	Recommended dietary allowance.
RNA	Ribonucleic acid.
SMEs	Small- and medium-sized enterprises.
SOP	Standard operating procedure.
SPS Agreement	Agreement on the Application of Sanitary and Phytosanitary Measures.
TBT Agreement	Agreement on Technical Barriers to Trade.
TDI	Tolerable daily intake.
TDS	Total diet study.
TEF	Toxic equivalency factor.
TEQ	Toxic equivalence.
TMDI	Theoretical maximum daily intake.
TSE	Transmissible spongiform encephalopathy.
UHT	Ultra high temperature.
UL	Upper limit.
UV	Ultra violet.

HISTORY OF FOOD SAFETY AND RELATED SCIENCES

Contents

History of Foodborne Disease – Part I – Ancient History

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Glossary

Alimentary toxic aleukia High mortality intoxication characterized by massive immune deficiency, ulceration of the digestive tract, and pulmonary hemorrhaging.
Coprolites Desiccated or fossilized feces or intestinal contents.
Coturnism Suite of symptoms resulting from quail meat poisoning.
Methylmercury Highly toxic organic form of mercury that causes irreversible damage to nerve cells resulting in overwhelming neurological damage.
Paleogenetics The study and characterization of deoxyribonucleic acid (DNA) from ancient remains in order to determine their genetic origins.
St. Anthony's fire Gangrenous ergotism characterized by intense burning pain, followed by fingers, toes, or limbs.

Prehistory

It is difficult to say precisely which foodborne diseases our Stone Age ancestors suffered from because there is little or no direct evidence or records available. However, based on our understanding of their environment and lifestyles, we can assume with some confidence that they regularly ate contaminated meat and fish, poisonous mushrooms, toxic plants and raw, indigestible grains. Notwithstanding their hardy constitutions and continual exposure to a variety of contaminating organisms, it is likely that they routinely suffered and occasionally expired from, gastrointestinal-related diseases.

Our forebears were opportunistic eaters and consumed whatever they could gather or kill, and carrion, left over by large carnivores, would not infrequently be a part of their food. Aside from large and small mammals, fish, amphibians, and reptiles, they ate insects, snails, shellfish, and a great variety of plants, tree nuts, mushrooms, fruits, grains, and roots. Tribal experience would have knowledge of the most toxic of these to be avoided such as certain berries and mushrooms, either at all times or during specific seasons. Like all other predators, they consumed the sickest or weakest animals because they were the easiest to kill. Because of the limited opportunities for regular kills, it would typically be feast or famine for tribes, and so the members also probably ate decomposing meat, stored after a large kill. With the advent of fire, cooking would eliminate many microbial and toxin risks but not completely. It would be logical to assume that these people had stronger constitutions than ourselves but nevertheless suffered regularly from the widest possible range of foodborne illnesses. Although certain societies may have grown more tolerant to certain toxic or other pathogenic challenges, the available evidence indicated our ancient prehistoric ancestors generally died in poor health at an age of 20–30 years.

In examining the history of foodborne diseases, we must occasionally take a small leap of faith because conventional evidence from ancient times can be quite rare. For the most part, foodborne disease organisms exert their effects on intestines and other soft organs, which are highly biodegradable and seldom survive for long in dead bodies; however, under natural conditions. But that does not mean there are no other clues scattered along the path of history. Much can be learned through ancient paintings, hieroglyphics or writings, which depicted ancient life in exquisitely animated and detailed ways. These primitive pictorial works, rock paintings, and carvings can be found on every continent and from the Arctic to the South Pacific. The images include local plants, animals, and people. From these records, we have learned what people ate and the experiences they had in obtaining food.

As time progressed, improved historical records provided much *in lieu* of physical evidence.

Accidental and intentional preservation of bodies have provided us with additional evidence of foodborne diseases. Mummification has resulted in bodies so well preserved that they continue to be useful for in-depth forensic examination in the present day.

Coprolites (desiccated or fossilized feces or intestinal contents) can be extremely valuable artifacts for the archeologist, particularly in the study of intestinal including foodborne diseases. Few, if any foods are digested completely, coprolites contain the residual contents of the diet as well as the remains of pathogenic organisms. Although soft tissue cannot generally survive through the ages, coprolites can, and the organisms they contain are often robust enough to remain intact throughout the centuries.

The new discipline of Paleogenetics seeks to characterize the deoxyribonucleic acid (DNA) of ancient remains in order to determine their genetic origins. As an example, the DNA from the bacteria responsible for tuberculosis was recently extracted from a 1000-year-old Peruvian mummy.

Ancient societies suffered from a great many food- and waterborne parasites. Egyptian mummies were found with beef and pork tapeworms, liver flukes, whipworms, guinea worms, huge intestinal roundworms, *Trichinella*, *Ascaris*, and many other food and waterborne parasites. Although Egypt of the Pharaohs is generally considered to be a highly structured and sophisticated society, the hygienic and sanitation practices were very rudimentary, resulting in continual fecal–oral reinfection cycles experienced by all classes of the population.

Legends, myths, religious prohibitions, and taboos were originally transferred orally and only later put into a readable form or a more lasting and extensive form of communication. Primitive pictorial works, rock paintings, and carvings can be found on every continent and from the Arctic to the South Pacific, created many tens of thousands of years ago up to the present. The images include local plants, birds, animals, and people. From these records, we have learned what people hunted and ate and the experiences they had in obtaining food. Pictographs and petroglyphs were reminders of key events in a community's history, and only later were ideas and words expressed through symbols, such as hieroglyphics in Egypt, considered to have originated approximately 6000 BCE. Other forms of primitive writing have been found in ancient Crete and Central America. Cuneiform writing, made up of thin, wedge-shaped strokes impressed on clay tablets, some opportunely baked and preserved through intense destructive fires, were developed by the Sumerians around 4000 BCE. Much of these texts are limited to royal proclamations, store inventories, and business agreements, but some include references to the contemporary diet, the way foods were prepared and the diseases that occurred.

By the time we get to the glory of ancient Greece, writings evolved to a highly detailed record. The ancient Greeks and Romans also described food consumption habits and food poisoning. Hippocrates, Horace, and Ovid all wrote about the poisonous effects of certain plants, and history records the tragic destruction of Euripides' entire family by poisonous mushrooms.

The Greek, Theophrastus, made many references to poisons in his book on plants, and Xenophon stated that intentional poisoning was so common that tasters were employed to check foods before they were presented to royalty – a practice that continued for another two millennia! The Greek and Roman writers also went to lengths to describe the practice of food adulteration – a trend first started from the time that foods and food ingredients became items of trade.

During the medieval period, records show that accidental and intentional food poisonings along with food adulterations were commonplace. Diseased animals were slaughtered together with animals that had previously succumbed to disease. Many were treated with adulterants to hide any evidence of their poor quality, and one of the most popular uses of the newly imported spice, nutmeg, was to hide the smell and taste of spoiled meat. The taste of nutmeg became so common and so closely associated with meat that up until in the present day, it is the overriding spice used in modern hot dogs and sausages!

From the sixteenth century onward, the origin and basis of diseases slowly began to become established on a scientific basis. It was a slow process, and the annals of science are littered with the remains of quack disease theories and equally useless treatments that were proffered up until those that passed objective scientific scrutiny were eventually discovered.

Taken together, the physical and historical evidence tells us that foodborne disease has been a constant companion of mankind throughout history. Indeed, these diseases and the elements that cause them have been associated with some of the most dramatic episodes of history, even though the victims were totally unaware of their presence.

This article has been written in order to give readers an idea of the incredible impact that foodborne and waterborne diseases have had on human history. As it would be impossible to describe all major events, only those events, which have had a powerful impact on shaping our great ages, have been highlighted.

The Biblical Period

The Bible's first texts were documented in the tenth century BCE, including Genesis, although often referring back to events almost 1000 years earlier, with the remaining books of the Old Testament completed over the next 800 years. The Old Testament has several references to foods, their consumption, prohibitions, and the pathological conditions that could result from improper consumption. Based on descriptions in the Old Testament, the foods available to the ancient Hebrews were generally similar to that of the ancient Egyptians. The main differences in consumption patterns between the two peoples resulted from the strict prohibitions enshrined in the Jewish dietary laws.

It is interesting that the very first law quoted in the Bible is a dietary law,

Genesis:

> ***2:16-17:*** *God gave the man a commandment, saying, 'You may definitely eat from every tree of the garden. But from the Tree of Knowledge of good and evil, do not eat, for on the day you eat from it, you will definitely die.*

The reference to 'every tree of the garden' makes it appear that God initially intended its inhabitants to be vegetarians. This is further confirmed by a previous passage:

Genesis:

> ***1:29-30:*** *God said, 'Behold, I have given you every seedbearing plant on the face of the earth, and every tree that has seedbearing fruit. It shall be to you for food. For every beast of the field, every bird of the sky, and everything that walks the land, that has in it a living soul, all plant vegetation shall be food. It remained that way.*

However, after the world was apparently destroyed in the Flood, God permitted Noah and all his dependants to eat the meat of animals, under certain conditions, where Genesis 9:4 sets out the first stricture on blood consumption that served as the moral basis of the Jewish dietary laws.

Genesis:

> ***9:3-4:*** *Every moving thing that lives shall be to you as food. Like plant vegetation, I have now given you everything. But nevertheless, you may not eat flesh of a creature that is still alive.*

Leviticus, chapter 12, describes in great detail the animals that God permitted to be consumed. Chapter 15 of the same book illustrates the phenomenon of contamination and goes on to describe the necessity to clean and wash those who are ill. The purpose for the kosher laws was to aspire to holiness and perfection by emphasizing certain moral and ethical obligations – they had nothing to do with hygiene – nevertheless, the dietary laws also happened to make good sanitary sense, and the ancient Hebrews who strictly followed them generally suffered from fewer gastrointestinal diseases than their neighbors. Subsequent Halal dietary laws were very similar and served to protect Islamic followers in the very same way. Much of this information can be used as a basic guideline for hygienic practices in the present day. Note that in the Book of Acts Peter is advised to give up on these dietary laws and eaten anything not sacrificed to idols; this explains why most of the western world see little limit to food choices.

Within the Bible, however, a number of instances where foodborne and waterborne poisoning incidents occurred are depicted. Even though much of the text is allegorical in nature, both the circumstances and symptoms are described in sufficient detail to allow a credible disease diagnosis to be made. Even more important is the fact that identical poisonings have occurred in the same geographical region for millennia and still occur in the present day.

Quail Poisoning

One of the greatest epics narrated in the Bible is the Book of Exodus, the flight of the Hebrews from Egypt and their wanderings through the desert to Mount Sinai in the 'Promised Land'. The story is a genuine classic with as much drama as any modern adventure can have. It describes not only the trials and tribulations of Moses and the Hebrews but also illustrates the many weaknesses of human character that surface under conditions of stress. The book is full of heavenly miracles from the plagues to the dramatic parting of the Red Sea. The story holds such fascination for us that we continually attempt to provide rational explanations for all these miracles.

The Book of Numbers describes how God was infuriated with the wandering Hebrews' desire for meat and promised to punish their animal passions!

> ***11:31*** *G_d caused a wind to start blowing, sweeping quail up from the sea. They ran out of strength over the camp, and (were flying) only two cubits above the ground for the distance of a day's journey in each direction.*

> ***11:32*** *The people went about all that day, all night, and the entire next day, and gathered quail. Even those who got the least had gathered ten chomers (A chomer is a little over six bushels).*

> ***11:33*** *The meat was still between their teeth when (the people) began to die. G_d's anger was displayed against the people, and He struck them with an extremely severe plague.*

What a series of events! A miracle is created in order to bring the Israelites the meat they craved, but after they set on the quails to eat them, they begin to die immediately – *while the meat was still between their teeth!* What actually happened? Was it a mass food poisoning?

This could not have been a foodborne infection like our modern *Escherichia coli* O157:H7 or *Salmonella* poisoning. Pathogenic foodborne bacteria take anywhere from 8 to 24 h to develop an infection sufficient for symptoms to be noticed. In this case, however, death occurred while the meat was still between their teeth – during or immediately after eating. There was no time for any type of infection to develop! The only thing that can make people ill that quickly is a foodborne toxin. How could quails flying in from the sea contain enough toxins to kill people?

Quail hunting has been a common practice in the Mediterranean region from time immemorial. The European migratory quail (Latin: *Coturnix coturnix*) is found in Europe, Africa and as far to the east as Pakistan and India. These little birds carry out a migratory flight twice every year. From August to October, as winter approaches, the birds leave Europe and fly south across the Mediterranean Sea and North Africa, all the way to equatorial Africa. After they fatten up over the winter, they begin to migrate back northward from late winter through early spring. The quail breed in Europe from late spring through summer and then begin the cycle all over again.

The ancient Egyptians caught large numbers of quail when they landed after crossing the Mediterranean. Fishermen spread their nets out on the ground and quickly trapped them as they landed on the shore, before the exhausted birds had an opportunity to lift off again. This tradition, carried out for millennia, still goes on in the present day despite the fact that the occasional food poisoning occurs.

The first intensive study of the phenomenon was carried out by Dr. Edmond Sergent, the Director of the Pasteur Institute in Algeria. Using the considerable facilities of his Institute, Sergent carried out several experiments to determine the effect of feeding quail with the hemlock seeds his literature studies suggested. He observed that the birds did not suffer

from the paralysis that was so typical of hemlock poisoning. He then fed the same quail to dogs, which immediately exhibited hind leg paralysis, the classical symptom of hemlock poisoning. In Sergent's mind, this experiment categorically solved the mystery of how the Israelites had died from consuming the quails.

Since the time of the original publication, Sergent's work was questioned by several other scientists and physicians. Some say the work was not conclusive and may have been technically flawed. They feel that other toxic plants or insects consumed by the quail were the materials responsible for making the birds poisonous. Based on recent studies carried out in Greece and the Mediterranean islands, still others felt that the ancient Israelites may possibly have had a genetic predisposition that made them particularly sensitive to some other type of quail toxin.

Regardless of the type of toxin involved or the genetic predisposition of the Israelites, none of the scientists dispute that the story in the Bible is a vivid description of a mass food poisoning incident.

A Serpent Returns

At a later time during their long sojourn in the wilderness, the Israelites find yet another excuse to complain as recounted in Chapter 21 of the Book of Numbers.

Numbers

> ***21:5*** *And the people spake against G_d, and against Moses, Wherefore have ye brought us up out of Egypt to die in the wilderness? For there is no bread, neither is there any water; and our soul loatheth this light bread.*

> ***21:6*** *And the LORD sent fiery serpents among the people, and they bit the people; and much people of Israel died.*

> ***21:7*** *Therefore the people came to Moses, and said, We have sinned, for we have spoken against the LORD, and against thee; pray unto the LORD, that he take away the serpents from us. And Moses prayed for the people.*

> ***21:8*** *And the LORD said unto Moses, Make thee a fiery serpent, and set it upon a pole: and it shall come to pass, that every one that is bitten, when he looketh upon it, shall live.*

> ***21:9*** *And Moses made a serpent of brass, and put it upon a pole, and it came to pass, that if a serpent had bitten any man, when he beheld the serpent of brass, he lived.*

This story is unique not only because it describes the disease but the cure as well. What were the fiery serpents and how could a brass representation of a serpent on a pole manage to cure this dreadful affliction?

Many of the signs point to the drinking of water infected with *Dracunculus medinensis* as a valid explanation for the fiery serpents mentioned in the Bible. With limited water resources for drinking and bathing, infections with Guinea worms, which are endemic to the region, could easily occur. It does not take a great leap of imagination to equate the long, slim Guinea worm with a serpent as vividly described in Verse 6. Incredibly, Verses 8 and 9 describe the curing of the disease by setting the serpent on a pole – the very same cure recommended in the present day by the US Centers for Disease Control and Prevention (CDC), the world's most advanced center for disease control – and the cure that originated the symbol of the Staff of Asclepius (Figure 1) – the icon of modern medicine.

A particularly nasty example of a long, slim parasitic worm common to the Bible region is appropriately named *D. medinensis*, Latin for the Dragon of Medina, and commonly known in the present day as the Guinea worm. A female of the species can measure from 3 to 8 feet in length but is less than an eighth of an inch in diameter. Males are only an inch or so in length. People become infected with these worms by drinking water containing minute *Cyclops* water fleas, which have consumed the *Dracunculus* larvae.

People who are infected with *Dracunculus* experience great pain and often try to relieve the fiery, burning sensation by soaking the infected part of their body in a pond or well. This stimulates the female to emerge and expel hundreds of thousands of larvae into the water, where they are consumed by the *Cyclops* to start the entire cycle over. The life cycle of *Dracunculus* is shown in Figure 2.

This disease continues to exist in many parts of the tropical world (mainly Africa) in the present day, and there still is no cure. If you visit the website of the US Centers for Disease

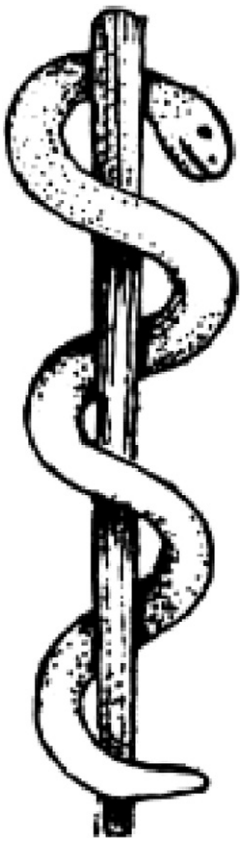

Figure 1 Staff of Asclepius.

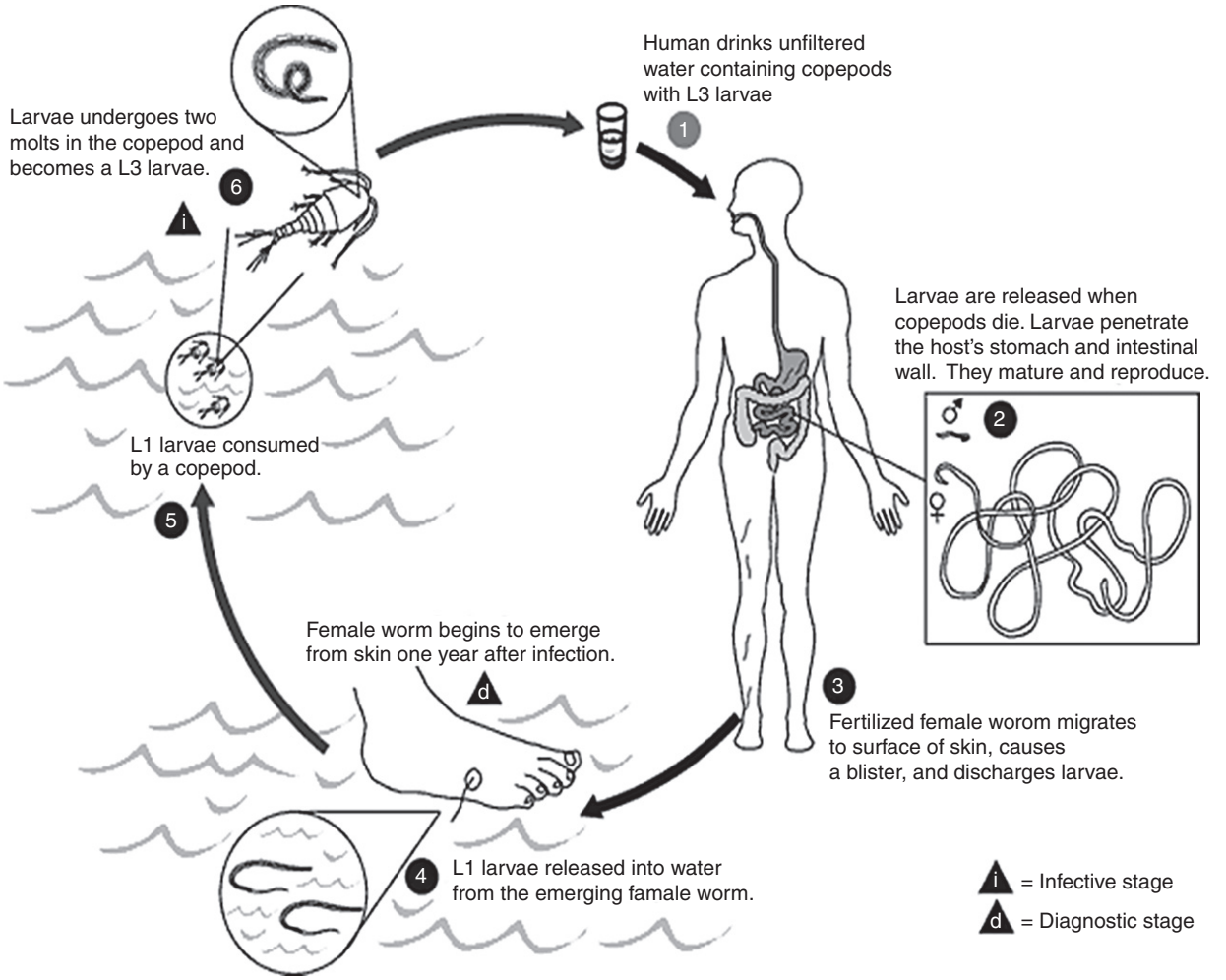

Figure 2 Life cycle of *Dracunculus*.

Control, you will find only a minor variation of the ancient treatment prescribed.

In this day and age, it seems an incredibly archaic remedy, but that is all that can be done. If the worm happens to break during the extraction, a severe secondary bacterial infection can occur. So, prevalent was the disease in ancient times, and so common the treatment that physicians advertised their services by displaying a sign with the worm on a stick or a serpent encircling a staff – a sign that eventually became the archetypal symbol of medicine.

Could the fiery dragons mentioned in Numbers be Guinea worms? We now know that the typical Guinea worm symptoms take many months to manifest themselves and deaths are rare with *D. medinensis*, but we also have a tendency to give the unnamed authors of the Biblical verses considerable poetic license. Let us leave the Dragon of Medina as a definite and very interesting possibility.

See also: History of Food Safety and Related Sciences: History of Foodborne Disease – Part II – The Greek and Roman Periods (1200 BCE–CE 500); History of Foodborne Disease – Part III – The Renaissance and the Enlightenment (CE 1300–1750); History of Foodborne Disease – Part IV – Modern Times (CE 1900–Present Day)

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History of Foodborne Disease – Part II – The Greek and Roman Periods (1200 BCE–CE 500)

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Glossary

St. Anthony's fire Gangrenous ergotism characterized by intense burning pain, followed by fingers, toes, or limbs.

Not-so-Funny Honey

In his chronicle The Anabasis, the Greek historian and army general Xenophon provided the Western world's first detailed eyewitness account of a great military campaign. He describes how, in 401 BCE, an army of 10 000 undisciplined Greek mercenaries traveled east to fight for Cyrus the Younger in the Persian prince's attempt to usurp the throne from his brother. The disastrous campaign ended in defeat, followed by a retreat characterized by the murder, rape, robbery, and enslavement of countless innocent villagers in the lands through which the Greek mercenary army passed.

The disheartened soldiers looted whatever they could from the local inhabitants, including food; honey in particular was considered a tasty prize. In the territory of Colchis, by the Black Sea, Xenophon's men raided the local supply of beehives. After gorging themselves on the honey, they became intoxicated and were seized with fits of vomiting and nausea. Xenophon finally caught a break because the pursuing Colchian army decided not to attack the mercenaries who were completely incapable of defending themselves. It took some days to recover, and the ragtag army moved westward as quickly as possible to get to more hospitable territory.

In 67 BCE, Rome, perceiving a threat to its territories, sent out the great general Pompey to conquer King Mithridates IV of Pontus. After a number of battles, Mithridates retreated until his army was forced to face off with the Romans near the city of Trabzon on the Black Sea coast of Turkey. Pompey, certain that Mithridates' retreat was chaotic and illogical, was totally unaware that it had been carefully planned.

All the forces were in honey country, and it did not take them long before Pompey's men plundered the regions' hives. After gorging themselves on honeycombs, the Roman army became intoxicated. This time, Mithridates did take advantage of the situation and massacred three squadrons of Pompey's troops while they were under the influence of the honey's toxins.

Despite this setback, Rome eventually gained full control of the area around the Black Sea but had never before come so close to 'sweet surrender.'

Back in Rome, the typical foods consumed were nearly all fresh, in-season, produce, stews or gruels, bread, and occasional meat. The facilities for saving food were limited to drying, smoking, pickling, or salting. The great expanse of the Roman Empire allowed it to import a wide variety of foods, spices, and herbs giving the population a taste for exotic dishes with highly flavored and spicy sauces. In high Roman society, feasts lasting many hours had many ready-to-eat delicacies, like dormice or lark's tongues prepared ahead of time, and probably many guests went home to suffer from some form of foodborne disease. It was the Greek and Roman bacchanalian love of food and particularly drink that served to initiate of the world's longest bout of chronic poisoning – one that plagued the Western world for 2 millennia.

Lead Adrift

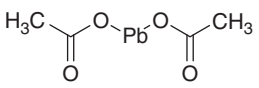
Of the seven famous metals of antiquity (gold, copper, silver, lead, tin, iron, and mercury), the ready availability of lead and the ease of casting it into useful products made it extremely widespread. Lead is normally found in nature as the lead sulfide ore called Galena, which was first used to make ornaments or as eye paint. Metallic lead is easily produced simply by heating Galena in a camp fire. By 3500 BCE, lead was used widely as a material for containers and lead pipes. The chemical symbol for lead is Pb from the Latin word plumbum, which also happens to be the root of the word for the tradesmen that worked with pipes – plumbers.

Most people believe that it was the lead pipes that carried their water supply that led to the downfall of the Roman Empire. Although there is no doubt that lead pipes slightly increased the risk of lead poisoning, pipes represented a relatively minor hazard to health. Both the Greeks and the

Romans discovered a more insidious application of lead that exposed them to a far greater risk.

They found that when they coated the interiors of their copper or bronze cooking pots with lead, many of the food and beverages they prepared tasted much better. Nowhere was this more evident than in the preparation of acidic products such as wines. Popular recipes of the day called for the boiling concentration of grape must in lead-lined vessels in order to prepare a liquid additive that enhanced the color, flavor, and shelf life of wine. The resultant thick, syrupy liquid was called 'sapa.'

When the must was boiled in lead vessels, there was a reaction between the acetic acid from the grape ferment and the lead of the pot to form a compound called lead acetate, also called 'lead sugar' because it is so sweet. This compound added just the right type of sweetness to bring out the best in wines and corrected many of the souring problems wine-makers routinely encountered. Sapa prepared using the ancient formulas contained between one-fourth and one gram of lead per liter! A single teaspoon of liquid sapa was more than enough to cause lead poisoning, but no one understood this at the time. In fact, because its effects were long term rather than immediate, the relationship between lead and disease were not well understood for another 2000 years!



Lead acetate

Hippocrates (460–377 BCE) was the first physician to link the excess consumption of food and wine with lead-based gout and Nicander of Colophon (197–130 BCE), a renowned botanist and physician of the time, wrote the first undisputed account of lead poisoning in the 2nd century BCE. He described symptoms of intense intestinal pains and tissue swelling. Several other ancient notables such as Pliny the Elder (CE 23–79) and Dioscorides (CE 40–90) specifically warned that the consumption sapa-corrected wine would result in severe nervous disorders but to no avail. The practice of consuming wine corrected with lead sugar continued unabated and was exacerbated by the consumption of several other foods that were sweetened with sapa.

This continual ingestion of lead by large segments of the population resulted in a series of epidemics throughout the Roman Empire. The average consumption of wine in ancient Rome was estimated to be more than a liter per day. The results were inevitable. A whole list of Roman Emperors was considered to be heavy drinkers and mentally disturbed enough to be considered as prime candidates for lead poisoning. For instance, Emperor Claudius limped, slurred his words, had fits, and slobbered. One publication goes so far as to suggest that more than two-thirds of the leading Roman aristocrats who served between CE 30 and 220 were most likely victims of lead poisoning. The disease eventually became endemic throughout most of Europe, particularly among the wealthy, who could afford to consume a good deal of sapa-corrected wine. When a rib bone of Pope Clement (who died in 1047) was analyzed, it revealed enough lead to conclude that he, too, had died of chronic lead poisoning.

During the course of these epidemics, chroniclers wrote of painful stomach colic, epilepsy, and paralysis as typical symptoms. The disease became variously known as the Huttenkatz of Germany (the foundry cat, because it tore at the entrails like a cat) and the Colic of Devonshire, however, the most common term used in the medical literature from the seventeenth century onward was the Colic of Poitou, so named in 1639 by Francois Citois, the personal physician of Cardinal Richelieu.

The disease proceeds when circulating lead ions are attracted and bound to the natural sulfhydryl groups that are common to most of our proteins. In the case of enzymes, this binding interferes with their normal action and results in a wide range of symptoms, the most important being those of the nervous system. Paralysis slowly develops with the greatest pain resulting from the inaction of the intestine causing cramps and constipation.

The first clues to uncovering the cause came in the German city of Ulm in 1694. Dr. Eberhard Gockel, a very astute observer, noted that monks who did not drink wine did not suffer from the colic, whereas those who did became sick. He spoke to a local manufacturer who described how the recent productions of wine were treated to overcome the sourness resulting from the past number of poor grape-growing years. Litharge or lead oxide was routinely added to the region's wines as a sweetening agent. Gockel successfully duplicated the process and was able to instantly turn an undrinkable acidic wine into the best of products. He deduced that the wine was the source of the colic problem and 2 years later published a medical paper describing the great harm that can befall anyone drinking 'corrected' wines.

Other physicians in the area confirmed Gockel's conclusions, and the matter came to the attention of Salomon Reisel, the personal physician of Duke Eberhard Ludwig. On 10 March 1696, the Duke issued an edict forbidding the adulteration of wine with litharge on pain of death – not only for the adulterators but also for anyone who knew of them but did not turn them in! A few years later Johanne Ehrni, a barrel maker from Eisslingen, was tried and convicted of the crime of adulterating wine with lead and publicly beheaded in Stuttgart!

In 1703, shortly after Gockel published his work on the colic of Poitou, a well-known British physician named Musgrave published a treatise on gout. It was Musgrave's contention that attacks of gout were precipitated by the colic. He was particularly concerned with the colic of Devonshire, the region that was well known for the conspicuous consumption of a rough and acidic cider and the region wherein the colic was endemic. He stressed that in those years when the apple harvests were small and manageable, the colic never appeared. However, he did not make the connection to the consumption of lead in the cider, even though his patients' symptoms were similar to the colic of Poitou.

John Huxham, a colleague of Musgrave's continued to work on the Devonshire colic and published a large essay on the subject in Latin in1739. Like Musgrave before him, Huxham did not associate the Devonshire colic with the colic of

Poitou, and hence the relationship to the consumption of lead was not made. Nothing further was done to mitigate the impact of the Devonshire colic until 1767, when Sir George Baker, the eminent court physician published a pamphlet warning his countrymen of the dangers of drinking the local cider. He kindly gave credit to the publications of Musgrave and Huxham but went on to say that he believed they missed the mark in discerning the cause of the disease. He stated that there was nothing wrong with pure cider, and that the cause of the colic was cider adulterated with lead.

Baker found out that the apple presses of Devonshire uniquely used lead sheeting as an inner lining to prevent leaking. Further, analytical experiments proved that cider from Devonshire was heavily contaminated with lead, whereas those from the other counties were not. The centuries old endemic colic of Devonshire was finally over.

Middle Ages (CE 500–1500)

The period of time between the fall of the Roman Empire and the middle of the fifteenth century is commonly referred to as the Middle Ages. The established society went through changes in law, culture, religion, and patterns of property ownership. The rule of Roman law (Pax Romana) with its guaranteed benefits was replaced by the rule of local warlords and kings, resulting in a dramatic change in the economic and social norms and infrastructure. This evolved to a feudal system wherein the overlord (usually a King), awarded land grants or 'fiefs' to his most important cronies, and the prelates of his church. At the bottom of society's ladder were the peasants or serfs who worked the lord's land, in exchange for his protection.

St. Anthony's Fire

Most people have never heard of ergotism, much less the mold that causes it, *Claviceps purpurea*. This fungus starts life out as a small, black rind-covered tube called a sclerotium. Barely, more than half an inch long and an eighth of an inch wide, this harmless looking tube is easily mistaken for a broken piece of stock and would likely go totally unnoticed lying on the winter ground. Within its thick walls, a compact mass of mycelium lies dormant awaiting the proper time to awake.

With the arrival of spring, the sclerotium awakes and sprouts a dozen or more stocks that looked like many tiny Enoki mushrooms. The heads of these stocks produce and discharge spores that are light enough to be carried by the passing winds. If these settle on cereals, they quickly colonize them and produce a new sclerotium at every infection site.

The word ergot is derived from the French for a rooster's spur on its foot, which the sclerotia resemble.

The cereals that are most commonly affected are wheat, barley, rye, and oats – all common staples of the Western diet. As can be seen from the illustration, it is quite easy for the sclerotium to be harvested along with the rest of the grain. According to agricultural records, in cold, damp periods, as much as a quarter of the harvest could be ergot sclerotia!

Manually culling out these contaminants is a very time-consuming job, and it is not surprising that a considerable amount of ergot eventually become mixed in with the rest of the cereal grains. What makes matters worse is that the *Claviceps* continues to thrive if the moisture content exceeds 14% – a situation not uncommon in grain storage. Once it is all removed from storage and milled into flour, it is very difficult to tell that a product is toxic. Aside from some very slight discoloration, ergot-contaminated flour looks exactly like normal flour.

Ergot toxins are alkaloids (nitrogenous plant chemicals) that have profound effects on the central nervous system. Many of them are very powerful hallucinogens, including lysergic acid diethylamide (more commonly known as LSD). Aside from hallucinations, these toxins can severely contract arteries (vasoconstrictor) and smooth muscles, causing numbness, extreme sensitivity, and irritability.

Ergotism manifests itself in two distinct ways. The first is called gangrenous ergotism, whereas the second is known as convulsive ergotism. Sometimes, both conditions can be found in the same incident.

Historically, gangrenous ergotism is more prominent, having been responsible for the infamous affliction, St. Anthony's fire. In this terrible manifestation of ergot-induced vasoconstriction, the limbs and their extremities (fingers and toes) becomes swollen and highly inflamed. Victims experience sensations of extreme heat (the 'holy fire'). Within a few weeks, gangrene sets in and the fingers, toes, or limbs become necrotic and fall off. As can be imagined, this whole process was agonizingly painful because the limbs felt like they were consumed by fire.

Ergotism has been a regular curse to rye- and other grain-eating populations for millennia. It was first described in an Assyrian tablet as a 'noxious pustule in the ear of grain.' The ancient Egyptians were aware of a disease caused by eating certain grains that produced both convulsions and hallucinations.

The first large European ergot epidemic appeared in the eleventh century and was christened ignis sacer (Latin for holy fire). Although less common in England than the rest of Europe, a number of major outbreaks of ergotism were recorded in 1762 and 1734. In Russia, ergotism was a major health hazard particularly in times of famine when little choice was left but to consume even-blighted grain.

Out of desperation, victims prayed to their various Saints for relief. One of the most popular Saints to appeal to was St. Anthony whose remains were interred in the Church of La Motte near Vienne, France. In 1089, there was a terrible plague of ergotism in the town of La Motte. A nobleman and his son were among those stricken but in time were miraculously cured by what they believed were the magical powers of the ancient relics of St. Anthony housed in their local church. The nobleman, Gaston, and his son, Girond, soon pledged themselves and their estate to establish a hospital near the Church. Since that time, gangrenous ergotism, previously called the holy fire, became commonly known as St. Anthony's fire.

More than 100 major outbreaks of St. Anthony's fire have been reported with as many as 40 000 deaths attributed to a single incident that occurred in the year 944 in France. Despite our knowledge of the disease and the toxins that cause it, outbreaks continue to occur in recent history; in

the twentieth century, at least four major outbreaks occurred (Soviet Union, 1926; Ireland, 1929; France, 1951; and Ethiopia, 1978).

See also: History of Food Safety and Related Sciences: History of Foodborne Disease – Part I – Ancient History; History of Foodborne Disease – Part III – The Renaissance and the Enlightenment (CE 1300–1750); History of Foodborne Disease – Part IV – Modern Times (CE 1900–Present Day)

Further Reading

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History of Foodborne Disease – Part III – The Renaissance and the Enlightenment (CE 1300–1750)

M Satin, Salt Institute, Alexandria, VA, USA

Glossary

Alimentary toxic aleukia High mortality intoxication characterized by massive immune deficiency, ulceration of the digestive tract, and pulmonary hemorrhaging.

The Constant Menace of Mold

During this period, grains supplied the basic carbohydrate calories and legumes (peas and beans) provided the complementary proteins required to sustain life. The two technological developments that allowed these commodities to be produced in large quantities to support the growth and development of a prosperous Europe were the moldboard plow and a new system of crop rotation, which significantly boosted agricultural output.

Unfortunately, this population explosion was cut short in the early fourteenth century as a result of several disastrous events. Political instability led to a European-wide recession toward the end of the thirteenth century. This was quickly followed by the onset of sustained poor climatic conditions in the opening years of the fourteenth century. Cold, damp weather dramatically reduced the yields of grain and initiated a series of calamitous famines from 1315 to 1317, which ravaged all of Europe.

The wet spring of 1315 saturated the soil, making it impossible to plow the fields that were ready for cultivation. To make matters worse, the heavy rains rotted a large portion of the seeding grains before they could germinate properly. Throughout the spring and summer, the rains continued and the temperature remained cool. Not surprisingly, the annual harvest was much smaller than usual, resulting in a rapid depletion of the very meager food reserves kept by most families.

To survive, peasants gathered whatever forest food they could find; roots, mushrooms, ground plants, grass, nuts, and even bark to supplement their meager and moldy grain supplies. Ravaged by malnutrition, the rural populations, who did almost all the work, were severely weakened. The following spring and summer of 1316 were once again cold and wet. Peasant workers, depleted of the sheer physical energy needed to till the heavy, wet soil, went through their food supplies to a point where it could no longer sustain them until the next harvest.

It has been estimated that more than 10% of the population perished during these famines. This was soon followed by the bubonic plague or Black Death, which broke out in 1347. Within 90 years, from 1340 to 1450, the population of Europe decreased by 30%.

Professor Mary Kilborne Matossian has constructed a well-documented and plausible theory that mold poisoning played a critical role in repressing population growth in England (and perhaps elsewhere in Europe) between the sixteenth and nineteenth centuries. Basing her research on factors such as population figures, child mortality, and fever epidemic, she convincingly argues that ergotism and alimentary toxic aleukia (ATA) were critical factors in child mortality and the consequent reduction in European population growth with all its consequent social impacts.

Another fungal contaminant of grain is the *Fusarium* mold, a filamentous fungi that is widely distributed in soil and closely associated with plants. Like *Claviceps*, *Fusarium* has a long and prominent place in the history of European morbidity and mortality. *Fusarium* species are responsible for blights, root rots, and cankers in legumes, grains, and grasses.

The role of fusaria as a potent producer of mycotoxins remained unsuspected until the 1970s. Since that time, however, research has firmly established its role in major episodes of human mycotoxicosis. Its prominent position in the fungal Rogue's Gallery is a result of the three different kinds of toxins it produces.

The first toxin, zearalenone, is a chemical that causes severe deformations to reproductive organs by imitating the estrogen hormone. Another toxin, fumonisin, can cause severe and irreversible damage to organs and has been implicated in esophageal cancer. Undoubtedly, the most deadly toxins produced by *Fusarium* mold are called trichothecenes, with one of them dubbed T-2, the most notorious of all.

If consumed by humans, the T-2 toxin can result in a condition first described by the Russians in 1943 as ATA. When infected by *Fusarium*, a wide range of grains including rice, rye, wheat, barley, corn, and millet can produce T-2 toxins capable of causing ATA. An epidemic in the Union of Soviet Socialist Republics (USSR) killed an estimated 100 000 people

between 1942 and 1948. It is now known that ATA also occurred in Russia in 1932 and before that in 1913, and there is little doubt that outbreaks occurred in earlier years as well.

The pathological course of ATA development in humans is both strange and terrible. When infected grain is consumed, victims first experience headache, vomiting, throat inflammation, and gastroenteritis. Then, the symptoms subside even if contaminated grain continues to be a part of the diet.

But the T-2 toxin acts like a time bomb. Victims can continue consuming infected grains without noticing overt symptoms for anywhere from 2 weeks to 2 months – all during that time, the T-2 toxins continue to exert their effects. The T-2 toxin kills bone marrow stem cells causing the destruction and shrinkage of bone marrow. The end result is a massive immune deficiency characterized by considerable ulceration of the digestive tract, as well as pulmonary hemorrhages. Once diagnosed, the body has suffered too much damage to be able to fight back. Mortality rates from ATA are very high.

During World War II, tens of thousands of Russians, most located in the Orenburg district, close to the Caspian sea, perished from ATA because they were forced to consume grain infected with *Fusarium* mold. Recently, in the Zhejiang Province of China, a small epidemic broke out after people ate rice contaminated with *Fusarium* toxins. As in all the outbreaks, it was rainy weather and poor storage conditions that led to the *Fusarium* contamination.

Silent, unobtrusive, and seldom the focus of our thoughts, the lowly fungus among us has had a far greater impact on our history and social evolution than most of our most famous historical figures and events.

Royal Parasites

Louis XIV (the Sun King) ruled France for 72 years (1643–1715) – longer than any other major European monarch. To get away from the swarm of courtiers and advisors at Versailles, he built a country retreat called Marly-le-Roi. The French Revolution destroyed Marly-le-Roi so completely that nothing remains to be seen. Recent archeological excavations managed to discover the site of Marly-le-Roi's latrines, originally constructed in 1680 and used until Louis XIV death in 1715. The unique construction of the latrines did not allow contamination from other sources, and so any parasite remains found in the sediments are specifically associated with the excrement of the King and his courtiers.

Parasitology researchers from the Faculty of Medicine at Reims, together with a team of experts from France's National Center for Scientific Research, investigated the remnants in Marly-le-Roi's latrines and their meticulous analyses provided an excellent insight into the parasitic diseases that the Sun King and his courtiers suffered from.

The two types of parasite eggs that were found to be most prevalent were the roundworms *Ascaris* and *Trichuris*, species that continue to be a problem in the present day. *Ascaris* is a large roundworm found in humans, pigs, and some other animals. They normally range from 6 to 12 in. in length and are usually approximately quarter inch in diameter. The spread of *Ascaris* is through the ingestion of their tiny eggs. Female *Ascaris* worms may contain as many as 25 000 000 eggs at any time and can eliminate as many as 200 000 per day into the host's intestine. The eggs are then spread in human and animal waste.

Trichuris trichiura (human whipworm), a roundworm that is fairly common in Europe, is notable for its small size compared to *Ascaris lumbricoides*. The worm derives its name from its characteristic whip-like shape, with adults reaching a length 1.5–2 in. The *Trichuris* buries its thin front end into the intestinal mucosa and feeds on tissue secretions, not blood. This worm does not migrate through the tissues like the *Ascaris* and therefore does not cause as much damage although heavy infections can result in similar symptoms.

Trichuris is found in other animals, which can serve as alternate sources of infection. The use of unsterilized 'night soil,' which contains both types of parasite eggs, as a fertilizer, is a definite cause of reinfection and the reason why this parasite is found on fresh vegetables. Humans are infected with this type of produce if it is consumed raw.

In addition to *Ascaris* and *Trichuris*, researchers found encapsulated tapeworm embryos but were unable to differentiate between beef- and pork-derived varieties (*Taenia saginata* and *Taenia solium*). Humans are usually infected by ingestion of undercooked or raw meat. The symptoms of infection with beef tapeworms (*T. saginata*) are variable. Many infections are completely asymptomatic, but in other cases they can be serious as a result of intestinal blockage.

Pork tapeworm (*T. solium)* infections are more problematic. The embryos hatch in the intestine and bore their way into the tissues to form cysts known as cysticerci. This cysticercosis state is the most dangerous form of tapeworm disease because of the tissues the larva invades and damages. The hatched tapeworm embryos burrow their way to the eyes, lung, liver, heart, and brain of the victim, wreaking inflammatory havoc and infection all along the way. The most serious form of this disease experienced is cerebral (neurocysticercosis), which often leads to epilepsy and death. The annual worldwide cysticercosis mortality has been estimated at approximately 50 000 cases.

Another parasite found, which infested the King's court was *Fasciola hepatica*, the liver fluke, most probably the result of consuming fresh watercress and dandelion greens. Adult parasites have a flat leaf-like body that is about 1 in. long and a 0.5 in. wide. After ingestion the cysts hatch in the small intestine and release the young parasite. They then penetrate the gut wall entering the peritoneal cavity. The *F. hepatica* then migrates directly to the liver where they penetrate and damage the tissue. The infection is rarely fatal and causes fairly nonspecific symptoms including an intermittent fever, mild jaundice, and in some cases anemia.

Fresh vegetables were the privilege of the wealthy and were very rarely consumed by the common people. The presence of *Ascaris*, *Trichuris,* and *Fasciola* are strong indicators of poor hygiene during the preparation of the fine vegetables and salads, not particularly surprising because the knowledge transmission of pathogenic organisms was very limited. Interestingly, human fascioliasis is a serious health problem in the present day in many countries and is acquired in the present day mostly by eating watercress, other vegetables, or by drinking water contaminated with metacercariae. The presence of *Taenia* cysts confirms the contamination of meat and consumption in an

uncooked or almost raw state. Poor people did not suffer in the same way because they had so little meat that it was usually boiled in a soup with coarse grains and starchy vegetables. As a result, the royal court and aristocracy were far more prone to chronic parasitic infections than the poor.

Part V: The Industrial Revolution (CE 1750–1900)

The Perils of Discovery

The beginning of the industrial revolution was characterized by the development of mechanized agriculture, which drove many rural laborers off the farm and into the squalid workhouses of the cities. The increased demand for food to feed urban workers and for raw materials to keep the factories running resulted in a total realignment of production and trade patterns. In particular, the concentration of agricultural production, processing, and trade migrated into the hands of fewer and larger enterprises. This established the conditions for the mass food poisonings that were to be regularly experienced over the next centuries.

The increasing demand for foods that would not readily spoil prompted the development of new processing technologies such as canning that remain in use in the present day. Initial efforts to preserve foods were accompanied by a great deal of food poisoning simply because no one understood the mechanism behind successful preservation. It was only in the last half of the nineteenth century that the scientific basis for food and beverage spoilage became understood, largely because of the work of Louis Pasteur. This knowledge quickly ushered in an era of understanding that led to new technologies that revolutionized food production and processing in the twentieth century.

Perhaps the most significant development in the history of food preservation was the process of canning. By the middle of the eighteenth century, it was generally accepted that it was possible to slow down the spoilage of certain foods through the use of heat. Unfortunately, no one had any idea why heat worked so well because the concept of microbial spoilage still remained undiscovered.

Toward the end of the eighteenth century, Napoleonic France was the powerhouse of Europe. The Society for the Encouragement of National Industry (Société d'Encouragement pour l'Industrie Nationale) was extremely influential in fostering practical developmental research and encouraged the public to submit their ideas and discoveries. They made sure to reserve a prominent place for deserving entries in their famous 'Bulletin.' This not only ensured the rapid dissemination of new ideas and information but also solicited an increasing input of more sophisticated experimental work.

Nicholas Appert (1749–1841), a Parisian confectioner, had developed a method to conserve certain foods in the 1790s. By the turn of the century, he had an operational factory at Massy (Seine-et-Oise) that employed 50 people. It was the world's first canning plant, but it did not use tin cans – it used glass bottles instead.

Using this technique, he produced the world's first canned foods and had even sent them around the world with the French Navy in 1806. His results were so impressive that the Société nominated a Special Committee just to review his process and products. This blue ribbon Committee passed Appert's work and products with flying colors, their only reservation being that glass containers might not be practical because they were so easily broken. Appert, however, insisted that glass was the purest material he could use. He was rewarded a prize of 12 000 Fr and asked to publish his work. In 1810, his now famous classic *Le Livre de tous les Ménages ou l'Art de Conserve pendant Plusiers Années Toutes les Substances Animales et Végétales* (The Art of Preserving All Kinds of Animals and Vegetable Substances for Several Years) was published.

In 1818, the French competition for food conservation by the Appert method sponsored by Ministry of the Interior's Board of Arts and Manufactures only allowed for products in metal containers ushering in the true age of canning.

Canned foods were a great boon to the increasingly prolonged voyages that were made to explore the vast reaches of the globe. The first of the Arctic explorers to bring canned goods along with him in 1815 was the Russian, Otto von Kotzebue who felt the new products 'too important not to be made use of for the expedition'. In 1818, the English company of Donkin, Hall, and Gamble supplied the British Admiralty with close to 30 000 cans of various meat, vegetable, and soup products and went on to supply Sir William Parry's famous voyage to discover the Northwest Passage in 1819.

Stephan Goldner was a businessman who set up canning factories near London and in Galatz (Moldavia, Romania) offering a great variety of canned foods including milk, soups, turtle meat, carrots, ox tongues, and several others. He claimed to be able to supply canisters ranging in size from 1 to 500 lbs each. (A 500 lb canister would be equivalent to a 60 gallon drum!)

The British Empire was at the zenith of its power and regularly demonstrated this in the arts, sciences, engineering, military, and commercial trade. The indomitable Navy wanted to discover a Northwest Passage from the Atlantic to the Pacific and sent out expeditions to achieve that goal. In 1819–20, Sir Edward Parry sailed further west than anyone had previously and further than anyone else would for the remainder of the century. At the same time, the British Admiralty sent John Franklin overland from Hudson Bay to the mouth of the Coppermine river on the Arctic ocean where he was to link up with Parry and then proceed together to Alaska. This did not work as planned, but Franklin did map a significant portion of Arctic coastline. Franklin ventured again to the Arctic in 1825 and carefully mapped a significant amount of territory and was knighted for his efforts in 1829.

Once again, in 1845, Sir John Franklin accepted the Admiralty's invitation to lead a new expedition to discover a Northwest Passage. No expense was spared, and by this time, canned foods had proven to be products that were highly acceptable to all mariners. Goldner's company obtained a major order to supply the canned foods for the Franklin expedition, but within 2 weeks of the expedition's departure had only supplied 10% of the contracted amount. Under tremendous pressure, Goldner promised that all the canned meat and soup would be ready on time. More importantly, he asked to be allowed to supply the soup in larger canisters than originally agreed and received approval. Two days before departure the products finally arrived.

On 19 May 1845, outfitted with 5 years of supply of food provisions, Franklin triumphantly sailed down the Thames river as head of the biggest, best-equipped, and most costly naval expedition ever mounted. Two months later, British whalers in the seas north of Baffin Island sighted the expedition ships. Neither other sighting was ever made nor a word heard from the expedition – a crushing blow to the Empire.

Because of the high number of faulty cans and spoiled products he delivered to the Navy, Goldner was refused further orders in 1849. A year later, the first traces of the Franklin expedition were found on Devon Island. Three graves were discovered lying in the shallow permafrost on nearby Beechey island together with stacks of abandoned tinned foods. Analysis carried out showed that the cans contained spoiled meat indicating that the expedition's supply of provisions had been critically compromised.

Several attempts were made to find the remains of the expedition without any success.

Charges were leveled at Goldner for selling the Navy substandard products making it clear that a major factor contributing to the demise of the expedition was the state of the canned provisions. Not only was a large proportion of their food supply unavailable but also it was possible that the limited consumption of the products resulted in widespread food poisoning.

It has been speculated that the cans were contaminated with botulism, which resulted in the ultimate demise of the expedition, but this premise, while theoretically possible, is unlikely.

Botulism poisoning symptoms usually begin to appear from 12 to 36 h after eating contaminated foods. The descending paralysis begins with blurred vision and dizziness, followed by difficulty in swallowing and speaking. The muscles experience weakness and breathing becomes difficult because the diaphragm can no longer compel the lungs to fill with air. Once this happens, death proceeds quickly.

Because they were considered to be superior to other provisions, canned products would have been consumed throughout the voyage from England to the Arctic. If that was the case, there should have been some record or note describing the death of sailors or officers during the first leg of the voyage. No evidence of any kind indicates that this occurred.

Finally, death from botulism is a rapid and dramatic process that can affect a great many people because of the potency of the toxin. Until the expedition unloaded their store of canned goods on Beechey Island, the only indication was that of a very limited number of deaths – certainly nothing compared to what one might expect from a large crew suffering from a botulism outbreak.

More than a century later, in 1981, Owen Beattie, an anthropologist at the University of Alberta, Edmonton, AB, Canada, found the skull and bones of two expedition crewmen and analyzed them. The results showed very high levels of lead. Three years later, he collected additional material for analysis from the body of the crewmen that had been well preserved in the permafrost. The hair samples (which can provide an excellent indication of what was consumed during the previous few months) showed lead levels that were 120 times above the normal.

Beattie also examined the tin cans that were found on Beechey Island. He noticed that the inside seams had large gobs of solder protruding – an indication of sloppy construction. The solder originally used to seal can seams contained more than 50% of lead. When the food made intimate contact with lead, the possibility of lead contamination increased. This increase would be dramatic if the food was acidic enough to actively leach the lead out of the solder – a common occurrence in spoiled products.

Heavy metal poisoning, such as that from lead, routinely result in symptoms of mental instability and lead to lapses in judgment (the former Roman Emperors being an example). Beattie's analysis showed that the lead solder in the cans and the lead content of the hair samples were the very same isotope – convincing proof that the lead poisoning the men suffered from came from the cans made by Goldner.

Although many factors played a hand in challenges faced by the Franklin expedition, there can be little doubt that food poisoning and canned products contributed a large part to the ultimate disaster of one of history's great expeditions.

See also: History of Food Safety and Related Sciences: History of Foodborne Disease – Part I – Ancient History; History of Foodborne Disease – Part II – The Greek and Roman Periods (1200 BCE–CE 500); History of Foodborne Disease – Part IV – Modern Times (CE 1900–Present Day)

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History of Foodborne Disease – Part IV – Modern Times (CE 1900–Present Day)

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Glossary

Methylmercury Highly toxic organic form of mercury that causes irreversible damage to nerve cells resulting in overwhelming neurological damage.

Based on the knowledge of food and beverage spoilage gained in the latter half of the nineteenth century, an explosion of technological improvements set the stage for a revolution in the production and processing that redefined the foods we eat and the manner in which we consume them. The twentieth century started with the average consumer eating home-cooked fresh foods that relied heavily on locally available products such as meat, potatoes, dairy, and seasonable vegetables. Rapid advances in food preservation technology made a wide variety of foods much more convenient and available throughout the year. Consumer acceptance of these products allowed very large quantities to be processed during a single growing season, which, in turn, encouraged mass production of agricultural products because of the obvious economies of scale. Likewise, the marketing of foods was carried out in much larger retail outlets that offered consumers convenience because of the great variety of goods one could buy at a single location.

By processing more and more foods and beverages in large production facilities, products became cheaper and more convenient. As processed foods proliferated, home cooking declined. The responsibility for safe foods passed from the homemaker to the commercial processor. Because of the shameful and corrupt behavior of several companies, new food safety legislation was promulgated and mechanisms were developed to enforce the new laws. Although this was a great leap forward, major scandals continued to occur.

Mercury in Minamata

One of the most touching images seen appeared as the 'Picture of the Week' in the 2 June 1972 edition of *Life* magazine. The black-and-white image by renowned photographer Eugene W. Smith portrays a mother holding up her daughter in a traditional Japanese chamber bath. The caption on the photograph reads:

> This is Tomoko Uemura. She was maimed by mercury poisoning in her mother's womb. Blind, speechless, crippled and deformed since birth, she must be bathed, fed and cared for like an infant. She is now 17.

The story of Tomoko Uemura is a tale of shame and disgrace. It involves the unconscionable behavior of a giant Japanese chemical corporation, the complicity of an uncaring government and an indifferent public that simply decided to look the other way.

The story unfolded in the tiny fishing village of Minamata on the western coast of southern Kyushu Island. Minamata Bay was blessed with a natural reef and the traditional village economy was based on fishing. At the start of twentieth century, the company Nippon Chisso (Japan Nitrogen) located a chemical factory to produce fertilizer there. In 1930s, the company perfected a process to make acetaldehyde by passing acetylene over a mercury sulfate catalyst. As the markets grew, so did Chisso and Minamata evolved from a quiet fishing village to an industrial city.

In Chisso's acetaldehyde process, a small amount of methylmercury is formed, and as this and other organic mercury compounds had no value to the company, they were dumped together with the rest of Chisso's waste products into the once pristine Minamata Bay.

During the Second World War, Chisso greatly prospered because it was a major supplier of strategic chemicals to the government. Unfortunately, Minimata Bay's stock of fish and shellfish dwindled dramatically making life very difficult for the local fishermen who felt that Chisso's practice of dumping its wastes into Minamata Bay was responsible. The local fishermen went to Chisso asking for compensation and were given a pitiful one-time payment on the condition that no other demands would ever be made in future.

At the close of the War, Chisso lost all its offshore assets but made an incredible comeback because it produced the fertilizer required for Japan's massive agricultural efforts to produce food. Right after it started up fertilizer production, it went back into the acetaldehyde. As Chisso grew, so did its toxic effluents, which it continued to dump into Minamata Bay

without any treatment. By the mid-1950s, schools of dead or dying fish could be seen floating on the Bay's surface. The reduced harvest impoverished the fishermen forcing them to eat more of the very products they caught. Out of desperation, they went back to Chisso asking for some type of compensation. The company, not wanting to incur any publicity, offered another pittance on condition that the fishermen promised to cease all further claims.

But Minamata's inhabitants began to notice a strange occurrence. Cats would get into a frenzied state and start dancing and jumping awkwardly toward the Bay where they threw themselves in and drowned. Although they were dubbed 'dancing suicide cats,' no one laughed because people felt it was a premonition of bad things to come.

In May 1956, Minamata City Hospital (owned by Chisso) admitted four patients exhibiting convulsions, agitated mental states, lapses into unconsciousness, coma, and death. The hospital's director, Dr. Hosokawa did a quick survey of the other medical facilities in the vicinity of the Bay and found 17 more fatalities, which had exhibited the same symptoms. The local medical community felt this was not a new disease but one that had been going on for some time. Hosokawa began a record of it and asked Kumamoto University in Kyushu to investigate. They very quickly came to the conclusion that the fish and shellfish in Minamata Bay were poisonous.

It was obvious to everyone – Chisso was poisoning the Bay with its industrial wastes, but how could anyone say anything against the chemical colossus that was the city's economic base? So, no one did. Kumamoto's medical research group determined that organic mercury in the waste stream was the primary cause of the disease, but Chisso quickly denied everything and severely criticized the quality of the University's research.

Some fishermen were so incensed, they broke into Chisso's facilities and destroyed a few pieces of office equipment. This type of behavior was unprecedented in Japan, and Chisso immediately brought in the police. For the first time the tragedy came to the country's attention, a full 4 years after the initial cases were admitted to Hospital.

The fishermen received very little sympathy from the public and even less from the government. No one had the right to criticize an industrial giant that played a critical role in Japan's new economy. Such things were unheard of and everyone immediately went into a form of denial speculating on all sorts of other causes that might be responsible for Minamata disease.

Dr. Hosokawa soon proved beyond doubt that Chisso's effluent was the singular cause of Minamata disease but as the Director of the Minamata Hospital, he was also the Chisso company doctor. When he presented his results to company executives, they instructed him to immediately stop all research and ordered him hide all information. As he was an employee in the old tradition, Hosokawa bowed his head and sealed his lips until his deathbed.

By October 1959, Chisso did not doubt its responsibility for the sickness and death of so many of the area's inhabitants and the collapse of the local fisheries, but this did not stop them from continuing to dump their toxic wastes as always.

Although metallic mercury (unless vaporized) and inorganic mercury salts have fairly low toxicities, organic mercury compounds are extremely dangerous. They are readily absorbed into the body and can easily cross the blood–brain and placental barriers. These two body mechanisms evolved to prevent toxins from entering our nervous systems and to protect a growing fetus. Unfortunately, methylmercury combines with one amino acid (cysteine) and tricks these defense mechanisms into thinking it is another amino acid (methionine). Once it crosses these defensive barriers, it is in the brain and central nervous system where methylmercury does its greatest damage through the destruction of nerve cells, resulting in devastating neurological problems.

In the fetus, methylmercury interferes with nerve cell development by binding to DNA. It also interferes with normal brain development. Exposure while in the womb can result in small misshapen heads, mental retardation, cerebral palsy, blindness, deafness, and seizures. As in the case of Tomoko, children can be born to mothers who exhibited no symptoms of methylmercury poisoning at all during pregnancy.

Methylmercury bioaccumulates as it moves up the food chain so that the levels in animals and people can be several million times higher than in the waters that the consumed fish were taken from. Like the rest of Japan, the diet of the people in Minamata depended largely on fish and shellfish for protein. The levels of mercury in their bodies climbed steadily because they had no other options but to eat the fish from the Bay.

So many people were affected and so many babies were born disabled that by the end of 1959, the Governor of the Prefecture had no choice but to intervene and ordered Chisso to pay out ¥100 million to the fishermen's associations. It sounds like a lot of money, but it was only $28 000 in 1959 rates. The Minamata Disease Association paid $830 for each confirmed death and $280 for survivors, regardless of how grievous their symptoms were.

Chisso insisted that their payments in no way reflected any responsibility on their part and added a clause prohibiting any further claims against the company. If anyone else got ill, the government would have to compensate them. In the meantime, Chisso's toxic effluent continued to pour into the Bay and surrounding waters.

Kumamoto University continued to study the subject and in 1962 demonstrated publicly that methylmercury was produced as a result of Chisso's acetaldehyde process – a fact that was long known by the company but kept secret. As soon as Kumamoto's results were made public, Chisso immediately objected and claimed that the university's research was bogus. Chisso managed to keep a lid on things until 1965 when a rash of new cases was reported in Niigata City, far from Minamata. Kumamoto University researchers quickly found the problem to be the toxic waste discharge from another acetaldehyde plant owned by the Showa Denko Company. As with Chisso, Showa Denko denied all responsibility.

In 1968, a dozen years after it was first uncovered, the Japanese government finally gave recognition to the direct relationship between methylmercury in the toxic waste streams and Minamata disease. After protracted negotiations,

the victims received a more reasonable compensation package, most paid by the government, not Chisso. The *Japan Times* recently reported on government plans to financially assist 3000 Minamata disease victims of the 1950s and 1960s. This decision was finally made in April 2005, 50 years after the first victims entered Minamata City Hospital.

On his deathbed, Dr. Hosokawa testified that all his research proving the link between methylmercury and Minamata disease was kept secret or destroyed by the Chisso Corporation. Tomoko Uemura died in 1977, 5 years after Eugene Smith produced the stunning image that is considered to be one of the 100 most influential photos ever taken.

Tainted Oil in Spain

On 1 May 1981, an 8-year-old boy, Jaime Vaquero Garcia, died on the way to Madrid's La Paz children's hospital. Soon afterward five of Jaime's brothers and sisters were examined at the same hospital and found to be very ill. They were initially diagnosed as having atypical pneumonia. The doctors immediately put one of the girls into intensive care and the other four children were transferred to the Hospital del Rey, Madrid's well-known institution for infectious diseases. Within days, the number of patients admitted to hospitals all over Madrid began to rise meteorically!

Because of the victims' symptoms, the hunt for some type of infectious agent was immediately commenced. The acute phase of the disease was characterized by noncardiogenic pulmonary edema with headaches, asthenia, itchy scalp, rash, abdominal pain, and fever. Severe muscle pain and cramps marked the end of the acute phase. After the first 2 months, patients began an intermediate phase, which seemed to last another 2 months. The clinical features of this phase were characterized by abnormalities of the sensory nervous system and intense muscle pain. Other findings were difficulty in swallowing, pulmonary hypertension, deposition of blood clots in the large vessels, marked weight loss, increased levels of peripheral blood eosinophils, and elevated triglycerides/cholesterol were also observed.

Almost 60% of the patients went on to a chronic phase with pathological thickening and hardening of the skin, motor and sensory nerve degeneration, carpal tunnel syndrome, and muscle pain and cramps. Problems such as memory loss and depression were also reported during the chronic phases.

A pediatrician at the Hospital Infantil de Niño Jesus, soon informed the government that he had finally determined the cause of the epidemic. He had investigated 210 of the children under his care and found that all had consumed cooking oil. This study established that consumption of oil sold in unlabeled 5-l plastic containers as the risk factor for developing this epidemic syndrome.

Following this finding, oils were collected from affected households and open-air markets and analyzed by the government laboratory. It was found to be rapeseed oil rather than the olive oil, the poor people who bought it thought it was.

Traditional rapeseed oil contains high levels of erucic acid, which has been linked to the formation of fatty deposits in heart muscle and the consequent negative cardiac events. It also contains high levels of glucosinolates, which are toxic antinutritional compounds. After extensive research, Canadian plant breeders were able to develop rapeseed oil that had extremely low levels of both these compounds – a product we now know as Canola oil.

But it was the old rapeseed oil that was implicated in the Spanish outbreak. In fact, erucic acid had excellent lubricating properties and made the traditional rapeseed oil ideal for industrial uses – but bad for food use. This was why the potent toxin, aniline, was added as a denaturant – so people would not use it in food. But, like the alcohol strippers of the prohibition days, there were operators who made a living at stripping the aniline out of rapeseed oil so that it could be used as a cheap food oil substitute.

The rapeseed oils that the Madrid medical researchers found were originally denatured with 2% aniline, but now contained other aniline-derived compounds, indicating they had been reprocessed and reheated. (It was later learned that the Spanish customs laboratory had known of the importation of aniline-denatured rapeseed oil for several months.) On 10 June 1981, exactly 40 days after the epidemic started, an official announcement was made by the Ministry of Health and Consumer Affairs on late-night television, informing the public that the widespread epidemic had been the result of contaminated, unlabeled cooking oil.

Referring to the outbreak as toxic oil syndrome or TOS, the announcement stated that the hospitals remained full of victims, but that new admissions had dropped precipitously. All suspected supplies of toxic oil would be recalled. Overnight, the panic that had gripped the country for weeks subsided.

The number of cases began to drop precipitously after the oil recall was instituted. By October 1981, almost no cases were reported. By the time the outbreak was quelled, more than 20 000 persons were diagnosed with acute TOS. Approximately 59% of those affected progressed to a chronic stage of the disease and were hospitalized with symptoms including neurological deficiencies, carpal tunnel syndrome, and muscle cramps. Approximately 375 deaths were reported, but that jumped to 1663 after further analysis.

Victims took the government to court and in 1997 the Supreme Court established indemnities at a cost that amounted to more than €300 million by 2002. Current regular payments to beneficiaries come to approximately €20 million per year.

A number of oil merchants were arrested, tried, and convicted in 1989, even though the judges stressed that the actual toxin was not fully known. Despite all the controversy, research continues into finding the most likely toxic compounds that might have caused the disaster – but there are no definite answers as yet.

The Birth of a Beast

Escherichia coli bacteria were first described by the German pediatrician and bacteriologist, Dr. Theodore Escherich, in an article he published in 1885. They were very common in the intestine (colon) and eventually called *E. coli* in his honor. For more than 100 years, these microorganisms were not

considered to be very harmful but were used as indicators of contamination in water, and in the food industry, it was considered a sign of poor employee sanitation.

The varieties of *E. coli* that are considered to be pathogenic to humans are those that cause diarrhea. The most common one is called enteropathogenic *E. coli* (EPEC) and is most often implicated in infant diarrhea – a condition that could end up being chronic unless great care is taken to ensure that the baby bottles, diapers, linens, and toys are kept scrupulously clean in order to prevent reinfection by passing or direct contact with a baby's feces. Two other types of pathogenic *E. coli* are known as enteroinvasive (EIEC) and enterotoxigenic (ETEC) forms.

Historically, the most well-known form of pathogenic *E. coli* is the enterotoxigenic type. This occurs most commonly in developing countries where sanitation remains a very low level. It is responsible for the range of diarrheal symptoms experienced by travelers such as Monteuma's Revenge, Delhi Belly, Rangoon Runs, Tokyo Trots, and the more generic Gringo Gallop.

The way bacteria evolve certain pathogenic characteristics depends on their nuclear structure. Unlike the cells of higher organisms, which carry their chromosomes within a well-defined nucleus, bacterial cells usually contain a large circular chromosome that is not enclosed by a membrane of any sort and is free to move around within the cytoplasm. In addition, bacterial cells may contain several smaller circular strands of DNA called plasmids that can operate independently from a microorganism's main chromosome.

A microbe's characteristics are defined by the DNA in both its large chromosome and its plasmids. Very often characteristics such as antibiotic resistance are carried in plasmids. It is a quirk of nature that bacteria, which may not be related to one another, are able to share characteristics through a process of plasmid transfer. That is why antibiotic resistance can spread so quickly between various species of bacteria.

Another way is through bacteriophage transfer. A bacteriophage is a small virus that can infect bacteria. Because all viruses are essentially DNA, bacteriophages infect bacteria and incorporate their own DNA into that of the bacteria. The bacteriophage begins to replicate itself until the bacterial cell bursts and disperses a cloud of young new bacteriophages ready to infect a great many more bacteria. At times, these bacteriophages are able to incorporate the DNA from one bacterium and pass it on to others that they infect.

E. coli's prevalence in the colon stems from its basic ability to attach to the inner surface of the intestines, causing the lining cells to rupture. This particular characteristic is carried in the bacteria's plasmids and can easily be transferred to other bacteria such as nonpathogenic *E. coli*. The results are greater number of *E. coli* types that can cause diarrhea.

In another case, EIEC inherited the characteristic of being able to actually invade the surface cells of the intestine by picking up genetic material from another bacterium called *Shigella dysenteriae*. Although they are from two completely different families of bacteria, both species exhibit the very same symptoms – because they share identical pathogenicities. In this case, the bacteriophage passed on the DNA that coded for the deadly Shiga toxin, and thereby created a new enterohemorrhagic (EHEC) *Escherichia coli* O157:H7.

It is thought that this bacteriophage-moderated DNA transfer may have been accelerated by the use of antibiotics in cattle feed, however, this has not yet been proven with any certainty. What is certain is that *E. coli* O157:H7 is one beastly, very deadly superbug. Our traditional perception of the quasibenign nature of *E. coli* changed dramatically in the 1990s when the first well-publicized cases of EHEC occurred.

It is thought that the *E. coli* O157:H7 originally evolved as early as 1955 because hemolytic uremic syndrome (HUS) – a typical characteristic of *E. coli* O157:H7 was first described by a Swiss pediatrician while examining a dairy-related outbreak. It occurred again in 1975, when doctors took a stool sample from California-based female naval officer who had a severe case of bloody diarrhea. They cultured a rare form of *E. coli* and sent it to the Centers for Disease Control (CDC) in Atlanta where it was promptly placed in storage. In December 1981, a severe outbreak of hemorrhagic diarrhea occurred in White City, Oregon. Local physicians were unable to identify the responsible organism and called Dr. Lee Riley, a California-based epidemiologist, who was associated with the CDC. No specific microbial agent was immediately found, but shortly thereafter, two simultaneous outbreaks occurred in Michigan and Oregon, both involving McDonald's restaurants. By this time, Joy Wells, a microbiologist at the CDC isolated *E. coli* O157:H7 from stool samples of the victims. However, it was not until two months later that investigators discovered the same *E. coli* O157:H7 in a processing plant that had supplied the suspected burgers to McDonald's. It was then that Dr. Wells took it one step further and canvassed the thousands of *E. coli* samples maintained at the CDC and found the one that had been responsible for the hemorrhagic diarrhea episode that took place in 1975. They were finally on to something conclusive and in 1982, they published the results in the *New England Journal of Medicine*.

When *E. coli* O157:H7 was first discovered, it was a true rarity among pathogenic bacteria because no one could accurately estimate the minimum infectious dose (the minimum number of organisms required to cause an infection). For most bacteria, the average infectious dose ranged from 100 to 1 000 000 000, however, for *E. coli* O157:H7, its infectivity (rather than pathogenicity) was so great that the ingestion of one single cell was thought to be sufficient to cause an infection. Even then, its true potential was not fully known because the outbreaks had been so limited.

In early January 1993, a Seattle pediatrician noticed what he thought was an unusual spike in the number of children coming down with bloody diarrhea. He immediately alerted the Washington State health officials about a possible foodborne outbreak.

In less than a week, the health department staff successfully identified hamburgers contaminated with *E. coli* O157:H7 bacteria from the Jack in the Box restaurant chain as the cause. More than 70 Jack in the Box restaurants located in the Washington, Nevada, California, and Idaho were involved in the outbreak, and their remaining ground beef inventories were immediately recalled.

Seven hundred people, many small children, became very ill and four children died. The staff epidemiologists at the

CDC quickly concluded that the outbreak resulted from errors in meat processing at the plant that made the burgers in addition to incorrect cooking at the restaurants that served them up. Ultimately, this incident turned out to be a defining moment in the history of food safety in the USA.

This was certainly not the first large-scale foodborne disease outbreak to be recorded. More than 10 years earlier in 1982, two large outbreaks of Norwalk gastroenteritis occurred in Minnesota. The first one involved 3000 cases and was related to eating bakery items with contaminated frosting, whereas the second implicated 2000 cases and was associated with eating bad coleslaw. In 1988, a large outbreak of *Shigella sonnei* infections occurred among the unfortunate individuals who ate what was supposed to be healthy, raw tofu salad at an outdoor music festival in Michigan. In that episode, 3175 persons became ill and 117 were serious enough to require hospitalization. In case numbers, the Jack in the Box paled in comparison to the national outbreak of *Salmonella enteritidis* infections from Schwan's ice cream that occurred a year later, where almost one quarter of a million people were infected.

What made the Jack in the Box incident a watershed event was that four children died an unusually cruel and agonizing death – from the simple act of eating a hamburger. Hamburgers are the quintessential icon of American fast food. By 1992, McDonald's alone had sold more than 80 billion hamburgers around the world. There is no other food as ubiquitous throughout America as the hamburger.

One of the saddest comments made following the outbreak was by Michael Nole of Tacoma, the father of one of the victims. "I don't care how long I live, I will never believe that my son died from eating a cheeseburger. Never." His son, Michael James, died at Children's Hospital and Medical Center in Tacoma. He died from complications resulting from HUS – a condition in which platelets start to clump up within the kidney's tiny blood vessels resulting in reduced blood flow and eventual kidney failure. The partial blockage of the blood vessels also leads to destruction of red cells. Michael was 25 months old.

Although it is unusual to take a very long quote from another source, the testimony of Michael Nole's mother appears so touching that it is reproduced in large part here, as it is no longer available on the Safe Tables Our Priority (STOP) website:

> …My son had bouts of diarrhea, which rapidly became runny, painful and eventually bloody…and later all blood.
>
> He was admitted to Mary Bridge Children's Hospital in Tacoma, Washington. I had no idea what was soon to follow.
>
> The bloody diarrhea continued throughout the night, every 3–5 min with screams of pain and terror with each one. We went through a diaper with each one because the blood burned his skin.
>
> In the morning, he was transferred to the pediatric I.C.U. unit. Unknown to us, there were already children there with *E. coli* O157:H7.
>
> By this time his kidneys had shut down and he was becoming very lethargic, his abdomen began to swell to an unbelievable size. He had hemorrhoids and was unable to eat or urinate.
>
> I remember the last time my husband and I saw our son responding and sitting up with our help. Due to his swollen tummy and tubes in his arms, he ate an orange Popsicle and I kept trying to tell myself he was going to be okay.
>
> Dialysis was needed and the decision was made to transport him to a children's hospital in Seattle that had the machines for this purpose.
>
> Before they transported him I had asked to rock him in my arms in a chair next to his bed. With the help of 3 nurses and his physician, they carried him over to me with all of his tubes, IV's and other monitoring devices and set him in my arms.
>
> I rocked him and sang our favorite songs together. One of our favorites was "Jesus Loves Me". To this day, I cannot bear to hear this song.
>
> This was the last time I held my baby in my arms.
>
> He was transported to Children's Hospital in Seattle, I rode in the ambulance with him and two other transport nurses. We made the hour trip in 22 min.
>
> …
>
> Michael had dialysis one or two times a day. There were so many other children arriving daily that needed dialysis that the machines were becoming very popular. Nurses, physicians and all other specialists were working around the clock, many sleeping at the hospital to provide the best possible care for our children.
>
> As Michael's meds increased and tried to help his pains, things got even worse.
>
> The physicians thought we might lose him at several different times, and several times we were rushed into say our last good-byes and prayers. This was so very painful.
>
> As our family members started to arrive to kiss his cheek and stroke his golden hair and say prayers I just sat back in complete helplessness, thinking "I'm his mommy…why can't I fix this? Make everything better? Trade him places?" This was such a hopeless, powerless feeling. After physicians noted he had red patches on his tummy, they thought something might have burst inside him. The suggestion was made to rush him into surgery to see if they could stop or identify the internal bleeding.
>
> Papers were signed and we kissed him good-bye one more time in the hallway on his way to surgery.
>
> I cannot remember how long it took…1, 2, 3 hours, seemed like 2 days.
>
> When he returned they said they "lost" him once during surgery but were able to revive him.
>
> When we were allowed to go in to see him he had an incision from his neck to his groin area. This was so difficult to see.
>
> He did not do well after this, he opened his eyes once, and we were able to see the blue of his eyes and barely a twinkle. I told him he was mommies big boy and that I would love him forever and someday would be with him forever in heaven. My husband and I spent several hours with him before he died. The nurses gave me a lock of his golden hair to cherish.
>
> I left the hospital with his blanket, shoes, choo-choo train, sweats I made for him, and a bag of toys.

As his father said, all Michael did was eat a cheeseburger.

Based on documents filed in the US District Court in Seattle, the Jack in the Box restaurant chain had made a decision to cook their hamburger patties at a temperature below that which was recommended by state regulations (155 °F). Had they done so, in all likelihood, the *E. coli* O157:H7, which contaminated their hamburgers, would have been killed. The Jack in the Box restaurant chain purchased this batch of frozen hamburger patties from Vons Companies Inc. Not surprisingly, the Jack in the Box parent company Foodmaker blamed and eventually sued Vons claiming that they were responsible for the outbreak.

The Jack in the Box outbreak in 1993 made *E. coli* O157:H7 a household word. The devastation it brought to families whose children died or ended up with lifelong disabilities, served as a rude wake-up call to a nation that took the safety of its favorite food for granted.

No sooner had the Jack in the Box outbreak subsided, than another national outbreak hits the press. This time, it was an upscale, unpasteurized apple juice that carried the deadly *E. coli* O157:H7 bacteria, and another child died. Although it was not difficult to understand how ground up hamburger could be contaminated, how was it possible that apple juice could carry the same bacteria? It was eventually linked to apples dropped from the trees contaminated with deer feces being squeezed to make the cider.

The stream of unanswered questions at the time began to mount up. What were we doing to allow such things to happen? What were the changes that have been made to standard quality control procedures? If it could happen in products differing as widely as hamburger and apple juice, where might it strike next? Were the laws that we worked under no longer sufficient to deal with this new threat? The increasing number of negative media articles dictated that some radical changes had to be made before the industry could restore the consumer's confidence in the food supply.

The National Food Safety Initiative began with President Bill Clinton's radio address on January 1997:

> We have built a solid foundation for the health of America's families. But clearly we must do more. No parent should have to think twice about the juice they pour their children at breakfast, or a hamburger ordered during dinner out.

The goal of the Initiative was to identify all possible contamination points along the farm-to-table continuum and implementing process controls for preventing problems that might affect our nation's food supply.

For the first time, representatives from Agriculture, Health and Human Services, the Food and Drug Administration, the Centers for Disease Control and Prevention, the Department of Defense, the Environmental Protection Agency, the Council of State and Territorial Epidemiologists, the Association of Food and Drug Officials, the Association of Public Health Laboratories, the National Association of City and County Health Officers, the Association of State and Territorial Health Officials, and the National Association of State Departments of Agriculture were put together to solve the problem. No one was to be left out.

The resulting Farm-to-Table approach clearly recognized that food safety began at the production level and included all the steps in the process from the farm to the ultimate consumer's table. Likewise, the key government agencies recognized the importance of a seamless interagency approach to food safety problems.

To test how effectively interagency cooperation worked, a joint program was set up for a method to be developed for the rapid detection of *E. coli* O157:H7. The Centers for Disease Control and Prevention published a list of all those diseases that could be transmitted by the food supply. Various state public health agencies began to publish their own food safety initiatives.

The National Food Safety Initiative began an overhaul of food safety programs that continues to the present. It has resulted in some of the most integrated food safety and surveillance systems in the world. In the present day, this pathogen is a major concern around the world and there is growing concern about closely related EHEC strains like O111 and O145, which have also caused outbreaks.

Conclusion

Food poisoning whether unintentional or deliberate has always had an impact on the course of human events. Going back to Biblical time, civilized society has been regularly plagued by it. In some cases, the very same diseases exist in the present day and strangely enough, the most up-to-date cure for one of them is take almost verbatim from the Bible.

Certain food and beverage poisonings have had a continual run of 2000 years and throughout that time have not only affected the daily lives of countless societies but have also had an impact on the thinking and behavior of influential leaders. Who knows how different the world would have turned out had lead poisoning not reduced the intellectual capacities of some of the great Greek and Roman leaders? Empires may have been lost over a few unsuspected molecules.

In every major historical era, foodborne diseases have altered the course of human events. It was not until the last quarter of the nineteenth century that we began to understand the nature of spoilage and disease. Even after we gained this knowledge, we were powerless against the forces of nature exerted through her tiniest beings.

Microorganisms, too small to be seen, have constantly evolved in order to survive. In pursuit of survival, they have evolved unique and opportunistic mechanisms that often exceed our technical abilities to control them.

The combination of industrialized food production, communal eating in fast food chains, and terrorists committed to using any means to destroy our lives and lifestyles resulted in a cocktail of risks such as we have never seen before. However, a risk is only a risk and not an outcome. Thus far, we have been reasonably effective or remarkably lucky in avoiding the consequences of these risks. Let us hope we continue to do so.

See also: History of Food Safety and Related Sciences: History of Foodborne Disease – Part I – Ancient History; History of Foodborne Disease – Part II – The Greek and Roman Periods (1200 BCE–CE 500); History of Foodborne Disease – Part III – The Renaissance and the Enlightenment (CE 1300–1750)

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History of Foodborne Disease in Asia – Examples from China, India, and Japan

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Introduction

With more than half of the world's population, the cultural, climatic, and geographical diversity of Asia is so vast that one might expect to find few commonalities among them. However, what is striking is that the basic commodities that make up the diet in India and China as well as of many other countries in the region are quite similar. Although diet will influence the nature and incidence of foodborne diseases, food-handling practices also play a role. For example, the consumption of raw or under-cooked fish and water plants is a tradition in many countries of Asia. Not surprisingly, many of the estimated 40 million people who are infected with foodborne trematodes live in Southeast Asia. In 1988 along the northern coast of China, inadequate cooking of 'hairy' clams contaminated with hepatitis A virus caused one of the largest foodborne disease outbreaks ever documented with more than 300 000 cases.

However, certain food safety practices have a long tradition in Asia although, as in the West, the underlying causes of foodborne illness were not understood. With the early development of large populations and urban centers in Asia, the problems of adulteration and fraud were among some of the first problems addressed by those societies. However, the concept that good wholesome food leads to good health was well appreciated even in the earliest times. This chapter discusses the history of food safety in China, India, and Japan, which begins with mythology and ends with modern times. Significant incidents involving foodborne disease are also discussed. It is recognized that many countries with long food safety traditions are not represented because much of the literature has been lost or in some cases, not translated.

Food Safety in Chinese Mythology

From the time of the first civilizations, the ancient peoples of Asia recognized that contaminated food can be the source of illness. Some of the earliest writings from China refer to food safety although from a traditional medicine perspective. In Chinese mythology, the legendary figure Shennong (*ca.* 3000 BCE) whose name means 'Divine Farmer' is said to have taught the Chinese a number of agricultural inventions that changed society, such as the axe, hoe, and plow and practices such as preserving seeds with boiled horse urine, irrigation and the weekly farmers markets. He is also considered the father of Chinese traditional medicine.

In about 200 BCE, the 'Divine Farmer's Herb-Root Classic' was the first compilation of Shennong's teaching that was based on oral traditions going back several millennia. Although the original text has been lost, it said to have been comprised of 365 entries on the safety and efficacy of medications that were divided into three categories. The first category included 120 foods and herbs with health-giving properties that were safe enough to be used as foods, including ginseng, jujube, orange, cinnamon, and liquorice. The second was comprised 120 herbs used for specific diseases, but which (were thought to have) possessed greater toxicity, such as ginger, peonies, and cucumber. The last category contained 125 medicinals that were acutely toxic and included rhubarb (oxalic acid) and the pits of certain fruits, such as peaches (cyanogenic glycosides). This insight into the relationship between toxicity and dose predated Paracelsus by several thousand years and laid the foundation for the development of traditional medicine in China.

Shennong is believed to have carried out much of his research by self-administering unknown plants and plant parts, and observing the effects. According to some accounts, his death was the result of consuming a yellow flower of a toxic weed (perhaps tansy). He died of intestinal hemorrhage before he had time to take the universal antidote, tea, which he also discovered. Shennong has been revered for his sacrifice and gifts to the Chinese people and even today many temples exist to honor him. While mythology is always a mixture of facts and fiction, the ancient heritage of traditional medicine continues to make important contributions to public health today. For example, the Chinese traditional medicine artemisinin (*Qinghoasu*) is now the standard treatment for malaria and saves millions of lives a year. Beyond traditional medicine, Shennong's influence on the Chinese culture can also be seen today in beliefs of many Chinese that foods have specific medicinal properties and perhaps explains why 'functional' foods are more widely accepted in Asia.

Food Safety in Ancient Times

China

One of the earliest accounts of food safety in the marketplace comes from the Zhou Dynasty (1046–256 BCE). 'The Book of

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Rites' codified a range of requirements for commerce. One of the rules for food trade prohibited the entry of unripe fruit into the market. During the Tang Dynasty (618–907), the Two-Year Laws required that foodstuffs that could cause food poisoning due to spoilage should be burnt immediately and the violators and associated officials should be severely punished. For example, the food owner would be sentenced to imprisonment for one year if a condemned food was not destroyed. If a food that was known to be harmful was sold and was the cause of death, the food owner would be hung. In the context of consumer education, it should be noted that in about 500 BCE, the Chinese philosopher Confucius warned against eating 'sour rice.'

The Song Dynasty (960–1279) witnessed a period of prosperity and a doubling of the population made possible by a great expansion in rice cultivation and the use of short-ripening rice cultivars from Southeast Asia. This was also a time of scientific and cultural advancements, including the discovery of the compass, gunpowder, and movable wooden type. With the great expansion of the food trade, the government adopted the Famous Sages of the Capital, which were specific laws that required all trade associations to be registered. The law carried on the regulations of Two Year Laws, which included severe punishment for merchants who sold spoiled food. The exceptional growth of the economy also gave rise to many fake and inferior commodities being sold in the market. According to the record of Xi Hu You Lan Zhi Yu, unscrupulous traders in Hangzhou adulterated food using such practices as putting ash into wine and sand in chickens, filling geese and sheep with air and injecting water into fish.

During the Ming and Qing Dynasties (1368–1911), the government decreed that when deaths are caused by food safety problems, crime would be considered equivalent to a homicide and assault resulting in severe penalties, including death.

India

During the Vedic period in India (1700–500 BCE), a sophisticated culture and cuisine developed in the Indus Valley. The Vedic Indians believed that mental health was linked to the purity of food and washing of the hands, feet, and mouth was undertaken before consuming morning and evening meals. Ancient texts also prescribe that chefs should bathe before preparing food and refrain from speaking, coughing, or spitting when facing the food. They should also wash their hands with water after touching their hair, limbs, or clothes. Other food handlers in the kitchen were also required to bathe as well as to shave their hair and beards and cut their nails. In regard to food handling, it was required that rice should be well washed before boiling. Indo-Aryans were also particular about the cleanliness of utensils. For example, a previously used earthenware plate had to be baked in a fire before it would be used as a plate to serve meals. Similarly, an iron plate had to be scrubbed with ashes before it could be reused. Various metals were used for utensils and for cooking, including iron, copper, and even gold. While lead utensils were also used, these did not appear to have had a significant impact on health.

As in China, food also plays a role in the system of traditional medicine in India, called Ayurveda. The basic texts date from around 300 BCE, but are thought to have been authored by the physician Sushruta who lived in the sixth century BCE. Although little is known about him, later Ayurvedic texts present him as a descendant of Dhanvantari, who was the physician of the gods in Hindu mythology. The text includes reference to 700 medicinal plants, 64 mineral preparations, and 57 preparations made from animal products. However, it should be noted that in modern times, many Ayurvedic herbal preparations have been found to contain heavy metals, including lead and mercury compounds.

One of the most famous cases of foodborne disease occurred in India in 483 BCE. According to tradition, Gautama Buddha died near the town of Kushinagara in Northern India near the border with Nepal allegedly after consuming contaminated food that was offered to him by a blacksmith named Cunda. Different texts do not agree on the content of the meal. However, given the short time to onset, one possible cause could be *Bacillus cereus* toxin, which is a commonly present in cooked rice that was subject to time–temperature abuse. In some countries today, Buddhist monks and initiates still receive offerings of food from the local inhabitants in the morning, which can pose risks if the food is stored at ambient temperatures.

Japan

In Japan, a new study has added more evidence to the theory that the decline of the samurai class during the Edo period (1603–1867) was due to mental and physical developmental problems in the warriors' children caused by lead contamination of their mother's breast milk. Studies show that the bones of young children who died near the end of the Edo period had extremely high levels of lead. Those children that survived would be expected to suffer from the known adverse effects of lead, including learning disabilities, behavioral problems, and mental retardation. It has been suggested that the cause of the problem was fashion for women to wear white make-up, which contained white lead ($(PbCO_3)_2 \cdot Pb(OH)_2$). Although it may be argued that the ruling Tokugawa family was already in financial and political decline, historical evidence indicates that weak leadership was a factor bringing the Tokugawa dynasty to an end. If this is substantiated, it would have an eerie resemblance to the fall of the Roman Empire, which was also partially attributed to the consumption by the elite of wine sweetened with lead acetate.

Even into the twentieth century, cases of lead poisoning in young children in Japan have been documented although its toxicity was well known. During the early Showa period (1926–89), some Japanese women were still using white lead make-up, which resulted in adverse effects in their children. However, it should be noted about the same time, the USA introduced the use of tetraethyl lead in fuel for automobiles and began what was probably the most significant and widespread lead pollution in history.

From 1910 to 1945 cadmium from mining waste contaminated irrigation water used in rice cultivation. Illness known as Itai-itai disease (which translates to 'pain-pain') was estimated to affect more than 20% of women over the age of 50 years. In 1968, another outbreak of Itai-Itai disease was again traced to cadmium-contaminated rice.

Food Safety in Asia in Modern Times

The danger of food-related outbreaks is particularly acute in Asia because of the instances in which animals and people live in proximity and the way in which some food is produced and distributed. The avian influenza epidemic, as the most recent example of a disease linking animals and human health, has been historically unprecedented and of great concern for human health as well as for agriculture. First reported in Hong Kong in 1998, the epidemic has now spread to every continent, which now appears to be due to both legal and illegal trade in poultry rather than wild bird migration as was initially thought. Hundreds of human cases in Cambodia, China, and Vietnam have been caused by direct exposure to infected birds and by consuming inadequately cooked poultry. Although transmission to humans is low, the disease is extremely deadly in domestic flocks and hundreds of millions of birds have died or been culled.

However, in the region, more than a million people die and many more are debilitated every year from single cases and small outbreaks of foodborne disease that are most often downplayed by governments and overlooked by the international media. Even though disruptions in food exports due to problems in food safety have been on the increase and are attracting greater attention. Since 2001, unacceptable pesticide residue levels in fruits and vegetables, chloramphenicol and other antibiotic residues in seafood and poultry, pathogens in seafood, and mycotoxins in crops and peanuts have been the cause of rejection of food exports from the Asian region. After one incident, a ban on fish imports into the European Union (EU) cost one Asian country US$335 million in lost export opportunities. The export of peanut meal by another Asian country to the EU dropped by more than US$30 million per year since the EU introduced new mycotoxin regulations in the early 1980s.

China

With a greatly expanding economy and the mass migration of over 100 million people from the western provinces to the lucrative jobs of its eastern seaboard, China has experienced phenomenal changes in the past three decades. With all of the societal benefits, including the lifting of millions out of abject poverty, the social costs in terms of environmental degradation and food safety problems have also grown. The following incidents are cited as only examples of the food safety problems in China that have resulted. In 2003, to meet the growing demand, producers of Jinhua hams operated out of season during warmer months and to prevent spoilage, hams were soaked in dichlorvos, which is an acutely toxic organophosphate insecticide.

In April 2004, an incident involving infant formula that was deficient in protein occurred in Anhui Province. An estimated 60–70 infants died of protein malnutrition and another 100–200 were hospitalized but survived. Local officials arrested 47 people who were responsible for making and selling the substandard formula, but further investigation revealed 45 other brands of infant formula from over 140 factories also failed to meet the standard. By mid-April, officials had closed many of the factories and seized most of the substandard products. In May 2004, the State Food and Drug Administration ordered an investigation and the government agreed to cover the medical costs for affected families.

In June 2004, the government monitoring data in Chengdu report that three-quarters of the pickled vegetables produced in the city had levels of toxic chemicals that exceeded maximum limits. On investigation, many factories in Sichuan Province were found to be using industrial-grade salt in the pickling process and some were spraying dichlorvos onto their products before shipment.

In 2005, the presence of Sudan I in food products in the UK resulted in a global search for contaminated products, as well as in China. However, the investigation in China resulted in the discovery of the widespread use of Sudan I in Chinese food products despite a ban that had been in place since 1996. This lapse of enforcement was the beginning of the recognition that the Chinese food control system needed reform.

In 2006, pigs were illegally fed clenbuterol, an anabolic steroid, to enhance fat burning and muscle growth. Over 300 persons consuming the contaminated pork became ill. Clenbuterol is not approved for use in China, but remains a persistent problem.

In late 2006, Shanghai chemists from the Shanghai Food and Drug Administration found traces of carcinogen nitrofuran in turbot fish and at the same time, Beijing authorities found other antimicrobial drugs, including malachite green, in the fish. Other cities, including Hangzhou, have begun testing turbot fish and banning turbot shipped from Shandong Province.

In 2008, one of the most notorious cases of food adulteration occurred in China resulting in about 300 000 infants being affected with 52 000 hospitalizations and 6 deaths. The cause was infant formula made from powdered milk containing melamine. The melamine was added to milk by producers to cover up their dilution of milk with water. The standard test used for monitoring milk only measures total nitrogen and melamine, which is high in nitrogen, was used to falsify results. Later in 2008, melamine was also found in eggs and other food, which was traced to contaminated animal feed. Melamine contaminated wheat gluten used in pet food had earlier caused the death of many cats and dogs in the US raising the international profile of this problem.

In 2009, an outbreak involving clenbuterol occurred in Guangzhou where 70 persons were hospitalized with stomach pains and diarrhea after eating contaminated pig organs. In 2010, snakes used for food became contaminated with clenbuterol when fed frogs treated with the substance. Thirteen people who consumed the contaminated snakes required hospitalization. Overall, there were 113 prosecutions in 2011 relating to illegal use of clenbuterol with sentences ranging from 3 years imprisonment to death.

Also in 2009, a company in Qingdao was found to be marinating duck meat in goat or sheep urine to give the duck the smell and taste of lamb. The duck was then sold as lamb to customers. In Wuhan inspectors discovered that most of the pork blood pudding sold in the local markets contained little actual blood, but was made with formaldehyde, corn starch, industrial grade salt, and food coloring.

In 2011, 11 people died in Xinjiang and 120 became ill after consuming vinegar stored in tanks that contained antifreeze made with ethylene glycol.

India

Like China, India is a large populous country (1.2 billion) experiencing rapid economic growth. In particular, a growing middle class (approximately 15% of the population) is demanding more and better food. However, the problems faced by domestic consumers appear to be more serious as India has many more micro-, small-, and medium-size enterprises that generally pay less attention to food safety. The following are examples of some of the problems encountered. Many of these examples are the result of findings by importers of Indian food products, but serve as indicators of the types of problems that may be encountered in the domestic economy.

India has had many food safety problems with exports. In 1997, the European Commission found Indian fish processing industry to be noncompliant in maintaining hygiene standards. Because of continued detection of salmonella, all exports of fish and fishery products to the EU from India were banned. Problems of heavy metals and other contaminants are emerging issues that could further threaten trade. Other rejections by the EU include: raw peeled shrimp, prepared Indian breads (paratha and roti), basmati rice, sesame seeds, pepper, and coriander and chili powder for salmonella and filth; lentils for excessive pesticide residues; and processed food products with undeclared or unapproved coloring matter. From 1998 to 2000, Indian dry chili exports to Germany, Italy, Spain, and the UK were rejected due to the presence of aflatoxin.

In 1998, adulteration of edible mustard seed oil with the highly toxic oil from the seed of the Mexican poppy caused an epidemic of dropsy that affected thousands of people in New Delhi even though the level of contamination was only 1%. Over 3000 people were hospitalized and 60 persons died. Even after this incident, further similar epidemics were reported in Gwalior in 2000, Kannauj in 2002 and Lucknow in 2005.

In 2003, Indian grape exports suffered a setback when Dutch authorities discovered the insecticides methomyl and acephate on Indian grape samples. However, the more serious problem for India is spices because India is the world's largest producer, consumer, and exporter of spices. One of the most widely publicized food safety incidents occurred in 2005 when Indian chilies containing the banned coal tar dye Sudan I was imported into the UK and used to make Worcester sauce that was subsequently added to over 600 food products. This resulted in a massive global recall of contaminated products and a major loss of confidence in food safety controls in India by both domestic and foreign consumers.

In July 2007, the European Commission issued a health warning after high levels of dioxins were found in guar gum from India, which is a thicken agent used in many processed foods. In 2011 adulterated alcohol in West Bengal sold illegally resulted in an estimated 126 deaths. The alcohol was thought to have contained methanol or nitrite, but it was not confirmed.

Japan

One incident of particular sensitivity occurred in 1954 when a Japanese tuna fishing boat was operating near the Bikini Atoll. Subsequently, tuna in Tsukiji fish market was found to contain extremely high levels of radionuclides. In 1955, another incident involving arsenic-contaminated infant formula caused illness in 11 718 patients and 113 deaths. Then in 1956, the infamous Minamata disease outbreak occurred as a result of methyl mercury contamination of fish harvested in Minamata Bay. By 2010 over 14 000 victims had received some type of financial compensation. This outbreak, which resulted in irreversible neurological damage to the developing fetus and nursing infant gained international attention and raised awareness of the potential impact of environmental pollution on health and food safety. For a more detailed discussion of this outbreak, see other article. It should be noted that in 1965, a second cluster of Minamata disease was identified among people living along the Agano River.

In July 1996, a massive outbreak of *Escherichia coli* O157:H7 infection occurred among schoolchildren in Sakai City and was associated with consumption of white radish sprouts. Of the 12 680 cases, 121 children developed hemolytic uremic syndrome and 3 children died.

On 11 Mar 2011, an earthquake and tidal wave struck the Fukushima Daiichi nuclear facility resulting in the release of radioactive materials. Atmosphere releases were estimated to be significant, but still only about one-tenth of that released during the Chernobyl disaster. Significant amounts of radioactive material were released into the marine environment. The sale of food grown in affected areas has been banned. Fishing in the waters around the site is prohibited and fish caught for monitoring purposes still show high levels of cesium-134 and -137, indicating that marine contamination is still occurring.

Food Safety Legislation in Modern Times

As the world's food supply became global, most countries of Asia have moved to adopt modern food safety legislation, which often conforms to the standards, guidelines, and other recommendations of the Codex Alimentarius Commission. Certain countries are making great strides in raising the priority of food safety as an essential public health function. For example, the Korean Food and Drug Administration, which was established in 1998, is due to be elevated to the ministerial level under direct supervision of the Prime Minister. More detailed descriptions of the development of food safety legislation in China, India, and Japan are provided in the following sections.

China

With the establishment of the People's Republic in 1949, food safety became the responsibility of the Ministry of Health. Initially, the focus of the government was on recovering from the war and ensuring food security. However, in 1965, the first comprehensive food hygiene law and regulations at the national level were enacted as 'Food Hygiene Management – Proposed Regulations'. In 1982, 'Food Hygiene Law of the People's Republic of China (Trial Implementation)' was promulgated in China, which gave the main responsibility for the

management of food hygiene to Health Administrative Departments at various levels of government (local, provincial, and national).

In 1995, 'The Food Hygiene Law of the People's Republic of China' became effective, which was China's first food safety law (FSL). Since 1998, the Chinese government adopted a food safety regulatory model composed of multiple regulatory authorities based on the existing administrative structure. The responsibilities of overseeing food safety were shared by the following authorities: Ministry of Agriculture was responsible for the production and processing of farm products, General Administration for Quality Supervision, Inspection, and Quarantine for the production and processing of food, State Administration for Industry and Commerce for food in markets, and Ministry of Health (MOH) for food catering services. However, this posed some problems in that there was an overlap of management activities in some area, unclear responsibilities in other (gaps) and a general lack of coordination that hampered response to food safety emergencies.

Subsequently in 2003, the State Food and Drug Administration (SFDA) was established under the State Council with a broad remit for the coordination and supervision of the management of food safety, including the investigation of serious outbreaks of foodborne disease. In 2004, the SFDA was given additional instructions concerning the food safety regulatory system. While improved, the system still suffers from overlapping responsibilities and lack of effective means of coordinating.

At the provincial/municipal level, food safety controls have been improving. Beginning in 2005 the Beijing Olympic Food Safety Committee made great efforts to strengthen food safety in the city in preparation for the 2008 Beijing Olympic Games. Eight new technological measures were put in place, including a food tracking and monitoring systems, as well as mobile laboratories. As a result, not a single incident of foodborne disease was reported during the Olympic Games. Moreover, the residents of Beijing have continued to benefit from this legacy of improved food safety.

In 2009, the FSL of the People's Republic of China was enacted by the Standing Committee of the Eleventh National People's Congress in 2009 to replace the Food Hygiene Law of 1995. The FSL takes an evolutionary approach in adopting a legislative framework that gradually modernizes approaches to food safety. As China's first law was devoted solely to the subject of food safety generally, the new FSL takes a broader perspective by providing principles and guidance for food safety regulators on how to evaluate risks and conduct inspections.

In 2010, the State Council has established the Food Safety Committee as the highest level of coordination and supervision for implementing food safety efforts of the different authorities as well as provincial and local governments. The oversight system was reconfigured and expanded to include the Ministry of Industry and Information Technology and Ministry of Commerce. This increased the effectiveness for coordination and reduced overlap and gaps in management.

In October 2011, the MOH established the China National Center for Food Safety Risk Assessment (CFSA) to provide risk managers with sound scientific evaluations of chemical and microbiological hazards in foods, including assessment of new chemicals that are proposed for use in food in China. CFSA is expected to play a major role in building up the country's food safety research and scientific regulatory capacity, enhancing food safety quality, protecting public health, and strengthening international cooperation and communication in this field.

India

With increasing income, urbanization, literacy, and awareness of international trends, the people of India are giving greater attention to food safety issues. India is the world's leading producer of milk, sugarcane, cashew, and spices, and the second largest producer of rice, wheat, pulses, fruits, and vegetables. Consequently, the domestic food market with 1.2 billion consumers is one of the largest, and the potential for food exports is enormous. However, the problems of assuring food safety in this society undergoing rapid transition have proved to be a formidable challenge.

With the adoption of the Food Safety and Standards Act in 2006 and the establishment of the Food Safety and Standards Authority of India (FSSAI), India now appears to be rising to this challenge. The act consolidates various pieces of legislation that were implemented by different departments and ministries. Under the Ministry of Health and Family Welfare, FSSAI has been designed to provide a harmonized food safety environment using science-based requirement for food and modern 'inprocess' approaches for the manufacture, storage, distribution, sale, and import of food.

Although adoption of the latest technology, farming practices, and modern breeding techniques is essential for assuring food security and promoting exports, the FSSAI will focus on assuring food safety in the context of the Indian climatic and other relevant physical and cultural conditions. One area of particular concern is developing basic requirements for street food vendors because of the significant growth in this sector. Street foods are an accessible and affordable option for a sizeable percentage of our working population. The requirements must not be too complex or burdensome, but must be a channel for assisting street vendor to recognize their food safety problems and for finding practical solutions to these.

For example, one major problem is the use of calcium carbide to ripen fruit. The practice is ingrained in the food supply system because the poor transportation system makes it nearly impossible to market ripe fruit. Consequently, unripe fruit is picked and shipped. Although calcium carbide is a known carcinogen, vendors do not see any obvious health effect on their customer nor do they see any alternative to the practice itself.

Japan

Immediately following the end of World War II, the population faced shortages of many foods items. This resulted in a many cases of fraud, which in some cases caused illness and death. For example, a number of fatal cases were associated with the consumption of methyl alcohol, which was being

sold as ethanol. In 1947, the Food Sanitation Law was published and the following the administrative structure, including an inspectorate, was established. In 1969, Japan banned the production of benzene hexachloride and DDT and in 1973 banned the use of polychlorinated biphenyls (PCBs). The 1971 Amendment of the Agricultural Chemicals Regulation Act enhanced the regulation of pesticides by requiring toxicological testing and establishing limits for residues in food. In 1972, approval of irradiation for potatoes to prevent germination was granted. In 1974, furyl furamide (AF2) was banned as a food additive after reports of its potential carcinogenicity were published. In 2001, the first BSE case in Japanese cattle, which resulted in the mandatory testing for bovine spongiform encephalopathy (BSE) for all the slaughtered bovine. In 2002, the Food Safety Basic Act was established, and in 2003, the Food Safety Commission was established as the government's food safety risk assessment body. In 2013, Japan reduced its restrictions on imported beef.

Conclusion

In many countries of Asia, awareness of the importance of safe wholesome food has long traditions. Some of the earliest civilizations in Asia established standards and norms for assuring the safety of the food supply. Although many of these measures contributed to food safety, the true nature and impact of foodborne diseases were not recognized. As a result, governments were slow to act and problems became acute during the period of industrialization. In the modern era, globalization of the world's food supply has meant that all countries are involved in the international food trade in some capacity, either as food exporters or importers, and often both. At the same time, the 24/7 news cycle and the access to information and social networks by many consumers, including those in developing countries, has significantly raised awareness of food safety problems. Consequently, the response of many governments has now become swift and decisive, not only to promote their trade but, most importantly, to protect their people.

Acknowledgments

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See also: History of Food Safety and Related Sciences: History of Foodborne Disease – Part I – Ancient History; History of Foodborne Disease – Part II – The Greek and Roman Periods (1200 BCE–CE 500); History of Foodborne Disease – Part III – The Renaissance and the Enlightenment (CE 1300–1750); History of Foodborne Disease – Part IV – Modern Times (CE 1900–Present Day)

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DISCIPLINES ASSOCIATED WITH FOOD SAFETY

Contents

Food Microbiology

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Introduction

Food microbiology is concerned with the activity of microorganisms in the production, preservation, and safety of foods and encompasses topics as diverse as the molecular basis of pathogenesis through to the development of food quality management systems. It is, by its very nature, an applied subject and one of, enormous economic and social importance.

Unfortunately, food microbiologists can take little credit for the many great advances in food safety, preservation, and fermentation prior to the mid nineteenth century. Methods of conserving food based on drying, salting, smoking, acidic fermentation, and combinations of these were developed empirically thousands of years ago. Even more recent developments such as canning preceded knowledge of the thermal stability of microorganisms and their role in food spoilage. Seeking to explain Appert's original heat preservation process, eminent scientists of the day such as Gay-Lussac held the view that the exclusion of oxygen was responsible for the preservative effect. It was not until much later, and an extensive applied research program in the 1920s, sponsored by the National Canners Association in the USA, that microbiologists established the safe practices in food canning that have been applied with considerable success for nearly a century. In fact, it is only since the twentieth century that newly developed food processing techniques such as food irradiation and high hydrostatic pressure have been able to benefit from the food microbiologist's input from their inception.

Foodborne Illness

It is clear that an association between foods and the transmission of some illnesses was noted long before the advent of the germ theory of disease and may well account for some of the longstanding dietary taboos and religious priscriptions relating to food. Diarrhea and vomiting have been observed and reported since the earliest times and, though they are not invariably the result of microbial activity, associating them with the activities of eating and drinking would be natural. The link between foods and illness would be particularly apparent in those illnesses that are more severe or more distinctive in their symptoms, such as botulism.

Botulism or sausage poisoning was a distinctive syndrome recognized by German physicians in the eighteenth century with the first recorded case in 1735 and others in 1755 and 1789. The first well-recorded outbreak was in Wurttemberg in 1793 when 13 people were affected and 6 died. This was caused by *Schweinsmagen* or *Blunzen*, a sausage consisting mainly of blood packed in a pig's stomach which was then boiled, smoked, and stored at room temperature for weeks giving an ideal opportunity for surviving *C. botulinum* spores to germinate, grow, and produce toxin. Alarm at the increasing number of cases of sausage poisoning led to some early explicit attempts to control foodborne illness. Justinius Kerner, as district medical officer in Wurttemberg, promoted a law requiring all cases of sausage poisoning to be registered and in 1802, a warning was issued against eating spoiled sausages, describing the correct method for preparing sausages and the symptoms of botulism. It was correctly believed to be an intoxication, although it was not known that the toxin was microbial in origin. Subsequent investigations by Kerner established a range of conditions that were necessary for botulism to occur, all consistent with establishing conditions suitable for the survival of spores, their germination, anaerobic growth, and toxin production.

With increasing knowledge of microorganisms and their role in disease, an early theory on foodborne illness, developed in the late nineteenth century, ascribed pathogenicity to microbial production of ptomaines or cadaveric alkaloids during putrefaction of organic material: In effect making all foodborne illness the result of an intoxication. This theory has long since been superseded in the light of our increasing knowledge of the different mechanisms of pathogenesis

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involved in foodborne illness, although there are certainly echoes of the ptomaine theory in our modern understanding of scombroid fish poisoning.

Food Fermentation

Appreciation of the more positive aspect of food microbiology, the use of microorganism in the production of food also increased in the second half of the nineteenth century. Like traditional food preservation techniques, fermented foods had been developed empirically, without any knowledge of the presence or activity of microorganisms. They did, however, make an important early appearance in the development of microbiology. In 1680, Leeuwenhoek described brewing yeast in his 32nd letter to the Royal Society but made no allusion to its living nature or involvement in the fermentation process, although he does describe his work as "observations of the yeast from which beer is made."

The nature of yeast and its role in alcoholic fermentation became embroiled in the frequently told tale of the, sometimes acrimonious, controversy over spontaneous generation featuring such luminaries as Pasteur and Liebig: A dispute which also included a negative aspect of food microbiology: Spoilage and putrefaction. Pasteur expounded the view that contaminating bacteria were responsible for defects (diseases) in wine and beer and he developed methods to improve brewing and winemaking by reducing the incidence of bacteria. Success was only partial however and it needed the later seminal work of Hansen at the Carlsberg laboratories where he demonstrated the role of wild yeasts in beer fermentations and developed the technique of producing pure yeast cultures from a single cell to lay the foundations modern brewing microbiology.

The Advent of Food Microbiology

It is clear that the growth of microbiology as a subject was based primarily on investigation of what microorganisms did rather than what they were. In that context, an early focus of food microbiology was on the safety, cleanliness, and perishability of one particular food commodity, milk, an area of continuing importance to this day. Many of the leading learned and professional societies currently active in food microbiology started life as organizations concerned with the safety and quality of milk but have since broadened their remit to encompass all food commodities. For example, around 1910 a number of US cities and states had passed laws regulating dairies, to protect the consumer from problems such as adulteration but also to protect their health as it had been shown that if a city supervised the production and sale of milk efficiently, the infant mortality rate could be greatly reduced. This led in 1911 to the formation of the International Association of Dairy and Milk Inspectors which eventually became the International Association for Food Protection. Similarly in the UK, in 1925, the Ministry of Agriculture started to appoint Advisory Dairy Bacteriologists who in 1931 recommended the foundation of the UK's first microbiology society, the Society of Agricultural Bacteriology, which later became the Society for Applied Microbiology. The importance of animal products both as a perishable food product and as potential carriers of disease also led to the early involvement of veterinarians in the development of food microbiology – a theme that was most marked in continental Europe.

Analytical Techniques in Food Microbiology

Progress on understanding microorganisms was for a long time constrained by the limited practical techniques available. Early light microscopy revealed the presence of the microbial world in the seventeenth century when van Leeuwenhoek, working with simple microscopes and magnifications up to 200 ×, famously described, in letters to the Royal Society, his observations of protozoa, yeast, and what may well have been bacteria. It was not until the 1880s, however, with the introduction of lenses corrected for both chromatic and spherical aberration by Abbe, the use of aniline dyes for staining and the development of methods for isolating and culturing organisms by Koch, that the pace of advance quickened. This still centered largely on medical microbiology where a number of pathogens were isolated and identified. These included some bacteria that were later recognized as food poisoning organisms, although they were first identified as the causative agents of very different conditions. For example, *Clostridium perfringens* was described as a cause of gas gangrene in 1892 but was not unequivocally associated with foodborne illness until 1943; *Staphylococcus aureus* was described as a pyogenic (pus forming) organism in 1882 but its role in food poisoning was not discovered until 1914. The identification of food and waterborne microbial hazards has been a continuing process throughout the last 130 years with *Cryptosporidium*, *Campylobacter jejuni*, norovirus, and verotoxin producing *E. coli* first being reported in 1976,1977, 1978, and 1982, respectively.

Compared to other analytical sciences, the techniques of food microbiology have changed relatively little in the century following the introduction of cultural techniques and solid media. Though there have been great strides in some areas such as molecular and serological detection methods, it is probably true to say that most of the methods used in a routine practising food microbiology laboratory today would not be too unfamiliar to Robert Koch.

As an analyte, microorganisms have the unique property of self amplification; given the right conditions, a single organism can multiply exponentially over time and produce a detectable signal, traditionally a visual indication such as a colony on solid media or turbidity in broth media. Our improved understanding of the biochemistry and physiology of microorganisms has allowed an increasing range of organisms to be cultured (although molecular studies suggest that we are still some way from growing all microorganisms) and for growth media to be formulated so that growth gives additional signals, such as color changes, or fluorescence, that are of diagnostic value.

To be able to distinguish not only between microbial species, but also between strains of the same species is a powerful tool in tracing pathogens through the food chain and in the investigation of outbreaks of foodborne illness. To do this requires focusing on properties that differ between closely related organisms rather than those they share in

common and techniques have been developed in recent years to high levels of refinement. Procedures based on phenotypic characteristics such as antimicrobial resistance (antibiotyping, phage typing), metabolic activities (biotyping), and antigenic properties (serotyping) have been around for almost a century now. The Kauffman–White serotyping scheme for *Salmonella* based on somatic (O) and flagellar (H) antigens was originally established in 1929 and phage typing schemes were developed shortly after for the same genus. In the ensuing 80 years numerous phenotypic schemes have been produced for a range of other organisms (usually pathogens).

The development of molecular (nucleic acid based) techniques employing restriction enzymes and the polymerase chain reaction (PCR) has revolutionized microbial typing – improving strain differentiation, enhancing reproducibility, and standardization. Although techniques such as gene probes were used previously, their utility was limited by the lack of material available and it was not until the advent of the PCR technique, patented in 1985, that molecular approaches to the identification and typing of bacteria took off. We now have a battery of techniques, described in detail elsewhere in this book, that focus on small differences in genetic composition of cells to achieve high levels of discrimination such as pulsed field gel electrophoresis (PFGE), ribotyping, random amplification of polymorphic DNA (RAPD), and amplified fragment length polymorphism (AFLP).

The use of instrumental methods based on electrical detection of microorganisms either directly using the principles of flow cytometry or as a result of changes in electrical properties of a medium produced by their growth has proved particularly valuable in handling large numbers of samples of particular foods. The huge progress made in understanding the molecular basis of microbiology has led to molecular and immunological techniques which have had a dramatic effect improving the accuracy and precision of diagnostic techniques.

Microbiological Criteria

Once analytical techniques were available, it seemed reasonable to use them as a tool to determine and thus control the microbiological quality of foods. This approach was similar to that adopted with chemical contaminants but suffered from a number of drawbacks. Microorganisms do not behave as most chemical contaminants in foods: Their levels are subject to change as organisms die, grow, or simply survive, depending on the properties of the food, its processing and storage. Their behavior will vary depending on their location in an inhomogeneous food matrix so, as a result, microbes will not be distributed randomly. This means that taking a representative sample, one that gives an accurate picture of the quality of the whole lot, is either practically infeasible or hobbled by a high degree of uncertainty in the results from a limited number of samples. The need for sound statistically based sampling plans rather than casual sampling prompted the establishment of a leading international body in food microbiology, the International Commission on Microbiological Specifications for Foods (ICMSF). This was set up as a standing commission of the International Union of Microbiological Societies in 1962 in response to the need for international microbiological specifications for foods. It is made up of leading food microbiologists from around the world and has expanded from its initial focus on methodology and sampling plans to produce a series of high-quality publications on food safety management including Hazard Analysis and Critical Control Point (HACCP) (see below).

Quality Control at Source and HACCP

Recognition of the limitations of relying on product testing as a control measure led to more effective strategies based on intervention at source, well summarized by Sir Grahame Wilson at a meeting on quality control held in 1969:

> Bacteriologists are better employed in devising means to prevent or overcome contamination than in examining more and more samples…. Control of processing…is of far greater importance than examination of the finished article.

Initially this philosophy involved the production and propagation of codes of good manufacturing practice by bodies such as the Codex Alimentarius Commission, trade associations, and national regulatory bodies. These described the equipment, layout, and operating procedures and testing that would deliver high-quality products on a regular basis. However, to achieve wide applicability they tended to lack specificity in terms of their requirements and recommendations. This deficiency was later remedied by the development of what became known as the HACCP concept. HACCP originated by the effort of the National Aeronautical and Space Administration (NASA) and the Pillsbury Corporation in the US to apply the same kind of zero defects approach used in engineering the hardware of the US space program to the foods that the astronauts would eat. Its value was seen beyond the immediate concerns of space travel and was taken up by the food industry, national and international regulatory bodies. As a result, it was refined and codified into the now internationally agreed approach based on the seven principles:

1. Hazard Analysis
2. Critical Control Points
3. Critical Limits
4. Monitoring Procedures
5. Corrective Actions
6. Record Keeping
7. Verification

Application of a full HACCP approach can be a time consuming and costly exercise and has found its greatest application in larger scale food production and processing, but the emphasis of HACCP on identification of where and how hazards can be controlled has now become the universal basis for food control/inspection down to the smallest scale of food operation.

The Physiology and Ecology of Foodborne Microorganisms

Control of microbiological quality at source, during production, required a sound appreciation of how microorganisms

behave in different foods and under different conditions of processing and storage. This knowledge, which makes it possible to understand the effect of control measures and to establish effective critical limits for them, had developed over the years since cultural techniques were first developed. Using them it was possible to analyze the microbial flora associated with foods and determine how population levels changed under different conditions. This work did not confine itself to pathogens but examined all components of the microflora including spoilage organisms.

It was an early observation by Beijerinck in 1908 that a specific type of spoilage arises in a food under its normal conditions of storage, anticipating the current concept of specific spoilage organisms (SSOs) by many years. This observation that food materials serve as an ecological niche wherein particular groups of organisms are able to thrive led to an ecological approach to microbial growth and survival in foods, applicable to pathogens and spoilage organisms alike. This was formulated by Mossel and Ingram in the 1950s in a seminal article in the *Journal of Applied Bacteriology* where they outlined the different factors that determine the microbial association that develop within a particular food.

1. The initial infection of the substrate.
2. Factors depending on the properties of the food material (intrinsic factors): nutrient content, pH, a_w, redox potential…
3. The conditions of storage (extrinsic factors): temperature, relative humidity, and gaseous atmosphere.
4. The properties of the organisms (implicit factors): physiological properties such as growth rate, K_s…
5. Processing factors: washing, comminution, heating, freezing, irradiation…

A huge published literature developed describing the various factors affecting the growth and survival of microorganisms, particularly pathogens, and the limit values of various parameters beyond which growth or survival is not possible. It was an early observation that these factors often act in concert; microbial behavior being determined by the aggregate effect of a combination of factors. This idea was formulated as the hurdle or multiple barrier concept of food preservation. It had been applied unwittingly in the past in a whole range of traditional foods such as cured meats and conserves but now also gave guidance to the developers of new food products for the market.

Predictive Microbiology

Although the mass of published experimental data available helped support the formulation of expert opinion as to the likely fate of a pathogen in a particular food, it was rarely possible to make quantitative predictions and there was frequent recourse to challenge trials where the fate of an organism was followed in the situation under investigation. Quantitative predictions were possible where a simple mathematical model was available as in the case of thermal death, but models predicting growth were not a practical proposition until the advent of modern accessible computing power. This new potential soon stimulated research programs around the world generating mathematical models that predict microbial growth (principally pathogens) over a whole landscape of conditions, combining factors such as pH, temperature, and water activity. Much of this activity has now been consolidated within a single program – the ComBase initiative. This is a collaboration between the Institute of Food Research in the UK, the USDA Agricultural Research Service in the USA, and the Food Safety Center in Australia which has used a massive library of quantitative data as the basis for ComBase Predictor – freely available web-based predictive microbiology software.

The availability of such models and the predictions they generate are an invaluable tool for food microbiologists. They do however remain models of microbial behavior. They are formulated so that their predictions err on the side of safety, i.e., overpredicting growth or survival. The predictions give the microbiologist useful guidance though ultimately some limited challenge testing remains the gold standard for confirming how a pathogen will actually behave in a particular situation.

Microbiological Risk Assessment

Sophisticated tools such as ComBase bring us nearer to a position where we can assess accurately the risks associated with foodborne microbial hazards. They support what has been described as the third wave of food safety – microbiological risk assessment: a scientifically based process that produces an estimate of risk. This is performed in four steps

- Hazard identification
- Hazard characterization
- Exposure assessment
- Risk characterization

Risk assessment takes place within the broader framework of what is known as Microbiological Risk Analysis and brings food microbiology into the sphere of public policy. In addition to risk assessment, the other aspects of risk analysis are risk communication – the interactive exchange of information on risk between interested parties such as consumers and risk managers (regulators) and risk management – the weighing of different policy options with regard to public health in the light of the risk assessment and its communication. Recognizing the impossibility of eliminating risk entirely, the outcome of the risk analysis would be an agreed Appropriate Level of Protection (ALOP): The level of risk (incidence of illness) a society is prepared to accept. In order for this to link with food safety management programs it has to be translated into a Food Safety Objective, defined as the maximum frequency or level of a hazard in a food at the time of consumption that gives the appropriate level of protection. It is then up to good manufacturing practice (GMP) and HACCP systems to deliver this objective in practice.

Conclusion

Food safety is not a goal that can be achieved and maintained without continuing effort to improve our knowledge and keep pace with changes in the food chain, microorganisms, and

consumer behavior. In the foregoing, the author presented a concise picture of the development and scope of food microbiology and shown it to be a discipline that draws on many areas in pursuit of safe, stable, reliable, and nutritious food. The author hopes that it provides some setting for the numerous topics that will be explored in greater depth elsewhere in this volume.

See also: Disciplines Associated with Food Safety: Epidemiology; Food Virology. Food Safety Assurance Systems: Hazard Analysis and Critical Control Point System (HACCP): Principles and Practice; Microbiological Testing, Sampling Plans, and Microbiological Criteria. History of Food Safety and Related Sciences: History of Foodborne Disease – Part I – Ancient History; History of Foodborne Disease – Part II – The Greek and Roman Periods (1200 BCE–CE 500); History of Foodborne Disease – Part III – The Renaissance and the Enlightenment (CE 1300–1750); History of Foodborne Disease – Part IV – Modern Times (CE 1900–Present Day). Risk Analysis: Estimating the Burden of Foodborne Disease; Risk Management: Application to Biological Hazards

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DISCIPLINES ASSOCIATED WITH FOOD SAFETY

Parasitology

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Glossary

Cercaria An immature trematode, usually free-swimming, produced by an intramolluscan larval stage.
Definitive host The host in which a parasite reaches sexual maturity.
Geohelminth A helminth parasite transmitted via soil.
Intermediate host A required host in a parasite's life cycle in which morphological change in the parasite will occur.
Metacercaria An immature trematode, usually encysted, in the open or in an intermediate host; develops from a cercaria.
Microfilaria The larval stage of a nematode (round worm) produced by an adult female that temporarily resides in the blood or another tissue of its vertebrate definitive host.
Reservoir host A host in which a parasite can mature and reproduce, then pass infective stages to hosts that are typically not part of the normal life cycle.
Trophozoite The active, feeding stage of a protozoan or myxozoan parasite.
Vectorborne The mechanism for transmission of a parasite into its host via a blood-sucking insect.
Zoonosis A disease produced by a parasite in a human that would normally occur in another animal host.

Historical Perspectives and Introduction

To most working parasitologists, a parasite is any eukaryotic organism that lives on, off, and at the expense of its host. Generally, this means that viruses, bacteria, and even the eukaryotic fungi are excluded from consideration by traditional parasitologists. Unfortunately, this approach was created irrationally. It is largely based on historical convention begun in the seventeenth century when Antonie van Leeuwenhoek began grinding glass lenses and using them to look at just about anything he could find, ranging from his own semen, to his own feces (and, in the latter case, he discovered and described *Giardia lamblia*, a widespread, and particularly unseemly, water- and foodborne protozoan parasite). Although Leeuwenhoek also observed bacteria, he did not know what they were, referring to them as 'animalcules.' The serious study of bacteria as causes of infectious disease was begun by Robert Koch and Louis Pasteur in the late nineteenth century.

Accordingly, conventional parasitology focused on eukaryotic organisms and essentially stayed that way until the parasite population dynamics models created by Roy Anderson and Robert May in 1970s. These models shaped an approach that eliminated the historic and artificial barriers separating the epidemiologic qualities of virtually all infectious pathogens. During an interview (by Gerald W. Esch) with Roy Anderson, he was adamant that the artificial distinction between virology, for example, and traditional parasitology was just that, artificial! Anderson was asked if he and May had to make an adjustment in their thinking when they moved from traditional parasitology to include viruses and bacteria in their models. He replied, "No, studies on infectious diseases at that time were quite Balkanized. There were departments of virology, microbiology/bacteriology, and parasitology." Continuing, he said, "Despite this, the ecological concepts and evolutionary issues faced [in any of these areas] were identical. They are all identical." Accordingly, it is important to understand that the ideas and views presented here regarding the ecology of parasitic organisms transcend the synthetic separation created by approximately 350 years of bad practice.

Parasites, whether eukaryotic or otherwise, have most likely been with humans since the appearance of *Homo sapiens* some 200 000 years ago in Africa. However, it would be difficult to say which of these parasites predominated in early humans, i.e., soil-, food-, water-, or vectorborne. Based on early Chinese writings, however, it is known that humans were definitely affected by malaria, a vectorborne disease, more than 4500 years ago. It is also known from the Ebers papyrus (1550 BC) that people in Egypt were infected with at least one species of *Taenia*, a foodborne tapeworm. The Old Testament forbade Jews from consuming pork, an agent for transmitting *Taenia solium*, as well as the foodborne nematode, *Trichinella spiralis*, although it is unclear if these parasites actually had anything to do with the ban. Numbers 21: 6–9 tells of the Fiery Serpent being sent among the Jews during the long migration with Moses out of Egypt. The Fiery Serpent is most likely *Dracunculus medinensis*, a waterborne parasite transmitted by microcrustaceans.

It was not until the nineteenth century that substantive discoveries were beginning to be made in understanding the

basic biology of parasitism. In part, this progress was due to the development of better microscopy. However, the significant forward movement in the nineteenth century was also made because the ideas of evolution and natural selection displaced the notion of spontaneous generation in 1859 with the publication of Darwin's, On the Origin of Species. With these changes also came the concepts of alternation of generations and of parasite life cycles. Louis Pasteur, Robert Koch, and others, promoted the notion of the germ theory for many diseases. In fact, Koch won a Nobel Prize in 1905 for his work on the germ theory. A great deal of the parasite research accomplished in the late nineteenth century, and even into the middle of the twentieth century, can be considered within the context of natural history.

Parasite life cycles are complicated, with many species requiring more than one host to complete. In definitive hosts, parasites reach sexual maturity. Intermediate hosts are required for the life cycle to be completed, and, generally, morphological change in the parasite will also occur in intermediate hosts. For many parasite species, insects that feed on blood are required to complete the life cycle. Insect vectors, in fact, play a significant role in the transmission of some of the world's most dangerous diseases, for example, malaria, African sleeping sickness, American trypanosomiasis (also known as Chagas disease), leishmaniasis, etc. Some parasites also employ paratenic hosts, an intermediate host in which the parasite does not change morphologically and which is used by the parasite to bridge a trophic gap between an intermediate and a definitive host. Paratenic hosts, accordingly, carry foodborne parasites.

Among the first parasite life cycles resolved was that of the foodborne cestode, *T. solium*. The German parasitologist, Friedrich Küchenmeister, fed cysticerci, a larval stage of the tapeworm, to the prisoners who were to be executed. Following their demise, the corpses were autopsied and adult tapeworms were found. The life cycle of *Taenia saginata*, the so-called beef tapeworm (another foodborne tapeworm) came soon after. Rudolf Leuckart and A.P.W. Thomas, working independently, are both credited for resolving the comparatively more complex life cycle of *Fasciola hepatica*, a plant- or foodborne trematode, which infects the liver and bile ducts of humans and livestock, primarily sheep. Life cycles for many of the important helminths infecting humans, for example, *Ascaris lumbricoides*, the hookworms, *Wuchereria bancrofti*, schistosomes, etc., were determined during roughly the same time frame. The zenith of the life cycle efforts came with Ronald Ross' studies on malaria and his pronouncement to the world in 1897 that he had worked out the *Plasmodium* life cycle. So significant was his discovery that he received the Nobel Prize for Medicine and Physiology in 1902.

Studies on the ecology of parasitic organisms began in natural history, most of it associated with life cycles, and was then followed with more sophisticated aspects of epidemiology and epizootiology. The main emphasis at first was on infectious diseases affecting humans and domesticated animals. Eventually, however, the scope of parasitology/ecology began to change. At first, the research was focused on the diversity of parasites that were to become the models for later work, for example, cestodes such as *Hymenolepis diminuta* and *Echinococcus granulosus*, trematodes like *F. hepatica* and *Echinostoma revolutum*, and nematodes such as *T. spiralis* and a tiny free-living species named *Caenorhabditis elegans*. *Toxoplasma gondii* and *Trypanosoma cruzi* were described and would eventually become protozoan models. As time passed into the twentieth century, parasitology began to take on a new face. Most of this change was related to rapidly developing technology in the last half of the century that was to lead into so many new fields of biology and a complete diversification of research efforts, for example, biochemistry, molecular biology, immunology, quantitative systematics (cladistics), etc.

One of the fields to emerge early in the twentieth century dealt with the ecology of animal parasites, which can be appropriately associated with species that infect humans (epidemiology) and those that infect other animals (epizootiology). As noted earlier, parasite transmission to humans can occur in a number of ways, and may, or may not, involve an intermediate host. Thus, parasite life cycles that involve humans and other animals can be direct (monoxenous) or indirect (heteroxenous). Immersed within these categorizations are a number of conceptualizations that are concerned with the dynamic qualities of host–parasite relationships, i.e., the regulation of parasite population dynamics (and predictive models), the distribution of parasites with host populations (and dispersion), transmission strategies, etc. Each of these concepts may, in one way or another, be linked to parasites that are foodborne.

Obviously, the consumption of food is basic to life, and parasites have exploited this necessity beautifully. A number of eukaryotic protistan species, commonly known as protozoans, access humans directly, via food/water consumption. Several parasitic flatworm species, commonly known as trematodes (flukes) or cestodes (tapeworms), have evolved indirect life cycles that require one, or more, intermediate hosts to successfully complete their life cycles. In many instances, humans also acquire these parasites via consumption of food. In most of these cases, the food is almost always raw, or poorly cooked. Finally, there are numerous parasitic nematodes (roundworms) that have either direct or indirect life cycles. In the former species, the infective vehicle is an egg, which is accidentally ingested, usually in soil. In the latter, the infective stage is called an L3, or filariform larva, and is usually associated with the tissues of an intermediate host. As with the trematodes and cestodes, flesh of the intermediate host for the nematode is almost always eaten raw, or poorly cooked.

Parasite infective stages that are acquired directly by the human hosts are almost always resistant to desiccation, and can usually remain viable for extended periods of time, ranging from several days for protozoans, to months for cestodes, trematodes, and nematodes, depending on the species. A large number of helminths employ vertebrate intermediate hosts, and some can survive in these hosts for many years. In almost all parasite species using a vertebrate animal as an intermediate host, the larval stages will be sequestered inside a cyst of host origin. The taeniid cestodes, for example, *T. saginata* (beef), *T. solium* (pork), and *E. granulosus* (a wide range of hoofed mammals) are all foodborne and possess larval stages that are sequestered inside cysts. The nematode, *T. spiralis*, and metacercariae of many trematodes are also encysted in vertebrate hosts, and all are foodborne parasites.

Transition Parasite Ecology

Parasite ecology was to parallel the changes in other disciplines of biology but there was a transition period. Probably the first significant breakthrough in the ecology of animal parasites came with the publication in 1964 of *General Parasitology*, written by the Russian, V.A. Dogiel. The content of the book was largely sophisticated natural history. However, it was also the first major effort to codify ecological parasitology. It represented a way in which the ecology of parasitism could be developed as a 'stand-alone' subdiscipline of parasitology.

Perhaps one of the most significant pieces of transitional work cited by Dogiel was W.L. Wisniewski's (1958) research dealing with eutrophication and parasitism in Lake Druzno, Poland. This effort was fundamental in food chain biology, with indirect, but real, meaning for the ecology of many foodborne parasites in aquatic ecosystems. In effect, what Wisniewski proposed was that with cultural eutrophication, a rapid and man-made form of ecological succession, significant changes in hypolimnetic oxygen concentration in lakes could affect food web dynamics simply through the addition of excess phosphorus, a limiting factor in aquatic habitats. Accordingly, the parasite fauna would be significantly altered and assume a characteristic quality, i.e., dominance by allogenic parasites. A number of investigators became involved in similar research in the rest of Europe and North America. Collectively, their efforts supported the central thrust of Wisniewski's thesis regarding man-made environmental changes and their consequences for parasite ecology.

Modern Quantitative Ecology

The first breakthrough in modern parasite ecology was purely quantitative and came with the publication of two papers by Harry Crofton of the University of Bristol, Bristol, UK. Most people working in the area of parasite epidemiology and ecology consider these papers as seminal. He ascribed four basic qualities to the notion of parasitism. First, he asserted that parasites are physiologically dependent on their hosts. Second, he said that heavily infected hosts would be killed by their parasites. Third, the reproductive capacity of parasites is always greater than that of their hosts. Finally, parasites exhibit what he termed an overdispersed frequency distribution within their host populations (it should be noted here that this concept only applies to helminth parasites that do not reproduce asexually in their hosts).

So, what is overdispersion? If one examines the manner in which free-living populations are distributed in space, there are basically three patterns of dispersion, each of which can be described mathematically simply by statistically comparing the mean population density per unit area with the variance of the mean. If the variance equals the mean ($S^2 = X$), then the distribution is said to be random. About the only time this frequency distribution can be seen is early, during parasite recruitment, which also means that this kind of frequency distribution is always ephemeral. A second kind of frequency distribution occurs when the variance is significantly less than the mean population density per unit area ($S^2 < X$). This distribution can also be described as regular, but it is rarely observed in host–parasite systems. Finally, most organisms, whether free-living or parasitic, exhibit overdispersed frequency distributions, where variances are greater than population means per unit area ($S^2 > X$). These distributions are also described as being aggregated, clumped, or contagious.

Now then, another question can be asked, what makes overdispersion unique because the distributions of most organisms, whether parasitic or not, are overdispersed? The answer to this question is simple. If one examines the distribution patterns of, let's say, enteric helminths in humans, most of the parasites of a given species within an African village will occur in relatively few hosts. For example, roughly 80% of all of the helminth parasites within the village may occur in just 20% of the human hosts. This kind of frequency distribution has a number of important consequences, and several are crucial for understanding the nature of host–parasite interactions. For example, most of the potential for morbidity/mortality in a population will be associated with just a few hosts. This implies a differential effect by parasites on host fitness. There is some evidence, albeit somewhat ambiguous, that aggregation can also affect sex ratios among some parasite populations. Despite the overriding nature of overdispersion, Poulin argued that, "The variance in [parasite population sizes] appears a much more important characteristic than their average size." He continued, "The failure of many studies to address this [issue] will need to be corrected if parasite evolution is to be placed in the proper context of highly variable" sizes of parasite populations within individual hosts.

The second major quantitative contribution to the study of parasite ecology came with the publication of two papers by Roy Anderson and Bob May. In these two papers, they merged several ideas and concepts, including those of Crofton regarding frequency distributions and dispersion, the classic ideas of Lotka and Volterra dealing with predator–prey interactions, and the notion that viruses, bacteria, protozoans, and helminths can be bundled together, under a single umbrella. In other words, their model applies to viruses and bacteria, as well as the classic eukaryotic protozoans and helminths.

Nonquantitative Contributions

Population Ecology

In general terms, a population can be defined as a group of organisms of the same species occupying the same space at a given point in time. It is not believed that anyone would strongly object to this definition as it applies to free-living organisms. As noted by Esch, Gibbons, and Bourque in a paper published in 1975, however, there are problems with defining just exactly what represents a parasite population. For example, should a population of parasites correspond to all individuals of the same species within a single host? Or, should it include all individuals of the same parasite life cycle stage in all hosts within a given habitat, or all life cycle stages of a parasite species within a given habitat? In other words, the definition of a free-living population does not fit the structure of a parasite population. For example, gene flow within a contiguous free-living population has no real physical

restrictions. However, consider the adult nematodes of the same species within the intestines of sunfishes in a single pond. Within a single host, these parasites can freely interact with each other, but not with adult nematodes of the same species in another sunfish in the same pond. So, the question is, what constitutes a population of these parasites?

To resolve this enigmatic problem, Esch *et al*. suggested that all of the individuals of a given species of parasite within a single host should be considered as an infrapopulation and that all of the parasites of a given species, including all life cycle stages, within a given ecosystem should be considered as a suprapopulation. Subsequently, Bush *et al*. introduced the idea of a component population as representing all of the parasites of a specified life history phase at a particular place and time, i.e., the adults of a given nematode species in all of the hosts within a single habitat.

It is clear that each individual within a host population is unique in terms of its age, size, genetic makeup, immunocompetence, etc. It is known that these factors can potentially influence the infrapopulation dynamics of a parasite species differently within various individuals of a given host population in a single habitat. Another of the important factors that must be considered when undertaking any sort of study on the population biology of parasites is the phenomenon of overdispersion. Why do just a few individuals in a population possess 80% of the parasites, whereas the majority has just 20%? Moreover, if the villagers are dewormed and a year later the same host population is examined again, the parasites will be overdispersed again, but are the same villagers wormy the next time around? Some investigators say yes, and some say no. The implications here are clear, but the reasons for discrepancies in understanding the manner in which overdispersed frequency distributions are not clear.

In the very first study using Crofton's ideas regarding overdispersion as applied to the epidemiology of human parasites, Croll and Ghadarian examined the frequency distributions of *Ancylostoma duodenale*, *Necator americanus*, *A. lumbricoides*, and *Trichuris trichiura* in a series of Iranian villages. They found that all four species of geohelminths were overdispersed. They dewormed the residents, returned a year later, and examined the same villagers. The parasites were overdispersed again, but the second time, a new set of persons was 'wormy.' Their conclusion was that certain people in these villages were not predisposed to infection. They contended that, "in this population of mixed ages, sexes, and social histories, the 'wormy' persons before treatment were not reliable predictors of subsequent intensities of infection." Continuing, they stated, "Risk factors are much more subtle than the classic categories we are considering or that 'wormy' persons in these communities result from superimposition of otherwise random events."

In contrast to the results and conclusions of Croll and Ghadarian as well as Schad and Anderson, working on hookworm disease in several villages in West Bengal, India before drug treatment, asserted that genetic predisposition to infection was important because the same people were found to acquire heavy infections after treatment. The subsequent literature regarding this aspect of epidemiology and overdispersion is mixed with respect to the influence of predisposition and the transmission of geohelminths in humans. Genetic differences between component parasite populations and host populations in separate geographic localities may be reflected by disparities in life cycle variations, population dynamics, and other biotic, as well as abiotic phenomena, whether dealing with human parasites, or not.

Antibody titers for *T. gondii* in human populations are highly variable on a worldwide basis, ranging from as low as 4% in areas of Korea to 92% in parts of Brazil. Cats are the primary reservoir hosts for the parasite in nature. This protozoan can have a fecal–oral route of infection (soil- or waterborne), or it may be acquired by consumption of poorly cooked meat (foodborne); it is even known to have a transplacental path of recruitment by a fetus still in the womb. Fortunately, only rarely clinical manifestations of toxoplasmosis can be seen. Interestingly, New World monkeys and Australian marsupials are highly susceptible to toxoplasmosis, whereas Old World monkeys, rats, cattle, and horses are very resistant. Dubey, perhaps the world's foremost expert on the parasite, suggests that a combination of evolution, genetics, and ecology are in many ways involved in this apicomplexan's epidemiology/epizootiology.

Community Ecology

Bush and Holmes adapted the approach of Esch *et al*. by applying the latter's hierarchical organization of parasite populations to parasite communities. The Bush and Holmes study of parasites in lesser scaup thus employed the notion of infra-, component, and supracommunities, which are parallel to analogous conceptualizations at the population level.

The Crowding Effect (Competitive Exclusion)

In 1951, Read published a paper that dealt with the crowding effect of parasites in a host. Since then, this paper and another published by Read in 1959, have become two of the most frequently cited parasitology papers ever published. The premise of both papers is based on what is commonly termed the Gaussian principle, or the Principle of Competitive Exclusion. In essence, the idea is a simple one. It says that no two species can occupy the same niche at the same time. If a conflict between two species should arise in this regard, then the outcome will fall into one of three categories, i.e., one of the species must move, one species must become locally extinct, or one species must change (evolve).

Read initially asserted that the options for the crowding effect among parasites were dictated by a single limiting factor, i.e., oxygen. Subsequently, he altered his position and stated that the limiting factor in crowding was more likely involved with the quantity and quality of dietary carbohydrates, which directly influenced parasite growth. Roberts as well as Bush and Lotz published summary commentaries on the crowding effect as it involved parasites. The latter authors noted that the seminal research on the crowding effect from an ecological parasitology perspective was published by Holmes in 1961 and 1962, using the cestode *H. diminuta* and the acanthocephalan *Moniliformis dubius* as models for his investigations. He was able to clearly demonstrate competitive exclusion of *H. diminuta* with respect to its normal attachment location

during simultaneous infections with both species of helminth. These results were so apparent that Bush and Lotz placed the work of Holmes into the same class as Gause's classic 1934 experiments using two species of *Paramecium* and Park's equally impressive study using two species of *Tribolium*.

Parasite/Host Behavior

Parasite behavior cannot be divorced from their ability to successfully locate and infect their hosts. Furthermore, the routes for parasite transmission are deeply rooted in several ecological traits, for example, the nature of environmental and host cues, parasite and host abundance, population structure, and evolutionary processes. MacInnis noted that host-finding techniques employed by macroparasites (ecto and endoparasitic insects, nematodes, trematodes, cestodes, and parasitic crustaceans) will include either active or passive means. In active transmission, either the parasites respond to cues from their environment or host, or hosts respond to the parasite. In either case, parasite behavior is oriented toward increasing their chances of encountering (and subsequently infecting) a host. In passive modes of transmission, no response by the parasite or the host is seen. This mode of transmission can occur via several methods, for example, randomly, or in response to a variety of environmental cues, through food web dynamics, predation, or accidental ingestion. Thus, parasite behavior can, at the very least, be attributed to indirectly influencing transmission in some passive modes, for example, by maintaining a high reproductive output, or by infecting intermediate hosts that are likely to be consumed by the definitive host in the parasite's life cycle. As with ecological aspects of parasite transmission, evolutionary processes will ultimately increase the fitness of the parasite by enhancing direct and indirect parasite behaviors that amplify the probability of increased transmission success.

Site and Host Specificity

One of the most remarkable attributes of most parasites is their very strong fidelity for both their host(s) and their site of infection in a host. *Dicrocoelium dendriticum* and *Fasciola hepaticum* are both trematodes (flukes) of sheep, are distributed worldwide, and are occupants of the bile ducts of sheep as adults. However, the similarities between the two parasites stop here. *Dicrocoelium dendriticum* is acquired inadvertently. Although a sheep forages in the pasture, it may accidentally ingest *Formica fusca* (an ant), where *D. dendriticum* metacercariae reside in the hemocoel. On arrival in the sheep's duodenum, the metacercariae are freed by digestion from the ant and excyst; the parasites then move directly into the bile duct where they mature sexually into adults. *Fasciola hepatica* is acquired when succulent emergent vegetation on which cercariae have encysted (metacercariae) is consumed as the sheep forages. Metacercariae excyst in the duodenum just like those of *D. dendriticum*. However, the excysted larvae of *F. hepatica* penetrate the gut wall of the sheep, then locate the liver and penetrate it from the outside. After wandering in the liver for 50–60 days, consuming tissue in the liver parenchyma, and generally causing significant tissue damage, the parasite leaves the liver and takes up residence in the bile duct. Thus, even though the two species occupy the same site of infection, they get there in completely different ways.

The remarkable point regarding the two parasites and their migration inside sheep is that initially they are both exposed to precisely the same set of environmental conditions. However, they respond in completely different ways to specific, but unknown, physical and/or chemical cues. It can, therefore, be said that everything that occurs inside the sheep with respect to migration by the two parasites operates within the context of the proverbial 'black box.' The same thing can be said for virtually every parasite species in terms of site location behavior. The nature of the stimuli to which parasites respond in order to find a given site of infection is largely unknown.

Most importantly, successful internal migration is almost always dependent on the parasite being in the correct host. If the wrong host becomes involved, one of the two things will happen to the parasite. On the one hand, the parasite will be killed immediately, or it will be killed in short order, by the host's immune response while migrating. On the other hand, there are many examples of a parasite evading, or at least surviving, the host's immune response for an extended period. In these cases, the parasite will almost always induce severe pathology, sometimes even death of the host.

Host specificity is another constant for most species of parasites. For some parasites, specificity is broad, for example, *E. revolutum* is known to successfully infect some 40 species of birds and mammals. In contrast, there are some species of parasites that will infect a single host species, and no more. In some of these cases, where specificity is narrow, parasites can actually be used as 'markers' as a way of understanding the origins of host stock. Along the northwest coast of North America, for example, certain species of helminth parasites are used to 'mark' (identify) specific migrating salmon in the northern Pacific Ocean and, thereby, can be employed to recognize the geographic origins of various fish stocks.

Host specificity translates into certainty with respect to a parasite's life cycle. For many human parasites, this predictability is related to cultural mores of the human host. If one wanted to find a human population with a high seroprevalence of *Trichinella spiralis*, a foodborne nematode found in pork, the Middle East would not be a good place to look. Why? Because both Jews and Muslims are forbidden to eat pork. (It should be noted here that this does not necessarily preclude the presence of *T. spiralis* in these areas. It has been reminded that relatively large outbreaks of trichinosis have occurred in both Lebanon and Turkey in recent years, and that the presence of *T. spiralis* has even been noted in pigs raised for foreign trade in Cairo, Egypt!)

Nonetheless, a high degree of host predictability by parasites has been honed over many thousands of years. Some of this predictability is related to parasites adapting certain behavioral qualities that match those of the host. Thus, for example, if you wanted to find a certain species of snail in eastern Africa that is shedding infective cercariae of *Schistosoma haematobium* (a waterborne pathogen that infects humans by skin penetration), you need not look between 18.00 and 06.00 h, because cercariae shedding by this trematode occurs between 06.00 and 18.00 h. Similarly, if you want to see shedding of *Schistosoma rodhaini* cercariae, check the snails

between 18.00 and 24.00 h. The explanation for these shedding patterns rests with adaptive characteristics of the parasite developed over long periods of coevolution by both the parasite and host. The definitive hosts are most active during these periods of cercariae shedding, and the probability of success in infecting their hosts is enhanced if cercariae are shed during periods of activity on the part of their hosts.

Another example of a biological clock is displayed by the appearance of *W. bancrofti* microfilariae (mf) in peripheral blood of humans in many areas of eastern Asia. The mf begin circulating in the peripheral blood around 22.00 h and stop around 02.00 h, moving deep into the blood vascular system. This 4-h sequence in peripheral blood coincides with the time during which the correct mosquito vectors take their blood meals.

Most host-matching behavioral phenomena are also parsimonious, i.e., the direction of coevolution over time ensures that the encounter probability between the two hosts is enhanced, not diminished. These behavioral characteristics represent just a few of the many examples that increase the probability of a parasite's success in completing a complicated step in its life cycle, as well as help in identifying an important aspect of host specificity by the parasite for a given host.

Emerging Infectious Disease

Over the past 30 years or so, certainly a rather short period of time considering the evolutionary history of the human race is at least 200 000 years old, a spate of new infectious disease problems have emerged. Clearly, the leader of this group must be acquired immune deficiency syndrome (AIDS). Approximately 30 million people are infected with the human immunodeficiency virus (HIV), greater than 1.7 million deaths occur per year, and nearly 75% of the cases are in sub-Saharan Africa. Although this is among the leading infectious diseases affecting mortality and morbidity in humans, it is not considered as a 'classic' parasite, and it is certainly not foodborne, so it will not be considered further from this point.

A rapidly spreading emergent disease of humans is cryptosporidiosis, caused by *Cryptosporidium parvum* that infects humans and other mammals, and *Cryptosporidium hominis* that infects primarily humans (both parasites are water- or foodborne). Massive outbreaks of this disease were, for example, reported in Carroll County, GA, USA in 1987, with an estimated 13 000 cases of diarrhea, and in Milwaukee, WI, USA in 1993, where it is variously estimated that 300 000–400 000 cases of the disease occurred, virtually all at the same time. In both localities, there were functional water treatment facilities present, yet the outbreaks still took place (chlorine treatment of infective oocysts is ineffective). Transmission in the latter case was traced to a cattle feedlot that overflowed into the primary water source for the city. Tragically, there were many hospitalizations and even several deaths among immunocompromised individuals. Even though this parasite is primarily a waterborne problem, it can also be transmitted via oocysts present on contaminated vegetables.

Cyclosporosis is strictly a human pathogen that produces a mild and self-limiting form of diarrhea. Originally, it was thought to be a blue–green alga, or perhaps a large form of *Cryptosporidium* sp. Presently, *Cyclospora cayetanensis* is the only cyclospora species known to infect humans. Cyclosporosis is clearly a newly emergent disease, having been recognized for the first time in 1977 in Papua New Guinea. Since 1990, outbreaks have been reported several times in North America, usually in a large gathering of people in conjunction with a special luncheon/dinner. On more than one occasion, the source of the parasite was traced to raspberries imported from Guatemala. According to Mansfield and Gajadhar, *C. cayetanensis* may also be transmitted via contaminated and untreated water.

As noted above, a significant recent problem has been the near pandemic associated with HIV-induced AIDS, an increase in the number of immunocompromised people, and the resulting acquisition of certain parasites that might not otherwise be a serious problem. Included among these parasites are *Entamoeba histolytica*, *C. parvum*, and *T. gondii*, all of which may produce lethal diseases in people who have AIDS, or those who may be taking immunosuppressive drugs for the treatment of cancer, for example.

Several species of what are normally considered as free-living amoebae, i.e., *Acanthamoeba* and *Naegleria*, are known also to infect humans, causing granulomatous amebic encephalitis and amebic keratitis. The former condition is frequently fatal and the eye problem is considered a progressive disease that can ultimately cause permanent blindness. The free-living stages of these parasites occur as trophozoites or as cysts in both soil and water. Keratitis is an emergent disease because there is an increase in the use of contact lenses on a worldwide basis. The disease is usually associated with a corneal abrasion and/or contaminated water in the lense storage case.

Food Webs and Trophic Dynamics

Food webs are used by ecologists to track energy flow in ecosystems. Parasites have been shown to play an integral role in these food webs, even to the point of acting as an indicator regarding the health of an ecosystem. Although there is a relatively small amount of energy transfer in parasite life cycles, they may have a significant impact viz-a-viz their effect on host life histories and behavior. Their influence on energy flow in an ecosystem is enhanced by the fact that at least half of all animals in an ecosystem are infected by parasites, and that close to 100% of all species present are parasitized. Accordingly, parasitism can have a significant impact on community structure, even to the extent that some parasites may act as keystones species in certain ecosystems. Within food webs, species in the highest trophic levels (including humans in this group) are most vulnerable to infection by parasites due to accumulation of parasites in the food of top predators.

In general, parasite life cycle dynamics can involve direct or indirect, and active or passive, action by the parasite. There are some species and life cycle stages in which the parasite can actively penetrate the surface of a host, for example, a schistosome cercaria, thereby requiring an expenditure of energy by the parasite. The venue for schistosome cercariae is water and these kinds of parasites are described as waterborne (although unlike most waterborne parasites, they are not ingested, they

penetrate the skin directly). In some cases, an inactive form of the parasite, for example, an egg of *A. lumbricoides*, will be directly ingested by the host, and not require an expenditure of energy by the parasite for transmission purposes. These sorts of parasites are usually soilborne, or have a fecal–oral route of infection. It must be emphasized, however, that many of these so-called soilborne parasites of the fecal–oral kind may also be transported into one's body through ingestion of contaminated food or water, or via unclean food handlers. In many cases, the parasite thus 'catches a ride' and is carried into the host via trophic, or feeding, activity of the host. These constitute the foodborne group of parasites.

Virtually any foodstuff that passes through the mouth into the digestive system can be a source of infection by a eukaryotic parasite. Some of these parasites are associated only with the human host. Others are more closely associated with other animals, so-called reservoir hosts, but humans 'get in the way' and can acquire them, usually in the same manner as reservoir hosts. These species are also referred to as zoonotic parasites and the diseases they produce as zoonoses. Variety in this latter group is thought to be increasing (in the form of emerging, or reemerging, diseases). The explanation for the expansion can be traced to the increasing exposure to reservoir host habitats as humans expand their reach into new habitats. Others claim that climate change is causing some species to expand their natural range and intrude on the normal human habitat. Finally, as travel by humans becomes more frequent, they are exposed to a much wider array of 'exotic' food experiences in Third World countries and, as a result, to a great many new and different parasites.

When it is spoken of foodborne parasites and foodborne disease, what are the possibilities of acquiring parasites that cause these problems? Somewhere, it has been said that, 'you are what you eat,' from a parasite's perspective. Accordingly, there is virtually nothing that can be eaten that is not capable of carrying a parasite, or parasites. For example, depending on where one goes in the world, parasites may be associated with leafy vegetables or salads, for example, metacercariae of *F. hepatica* or *Fasciolopsis buski* may be present on watercress or water caltrop. Consumption of this sort of vegetation in the Far East may thus lead the unsuspecting traveler to severe digestive problems or liver disease. Ingestion of lettuce in third world countries may also lead to dysentery caused by *E. histolytica*. Similarly, a special kind of intestinal problem, called steatorrhea, is induced by another protozoan, *G. lamblia*. The infective cysts of the latter parasite can occur on vegetation, or will more likely be in water contaminated with infective cysts of the parasite. In fact, giardiasis is a rather common parasitic disease in several so-called advanced countries, where it can spread from reservoir hosts, for example, beaver, to the unsuspecting hiker or picnicker who might be enticed to drink the cold and clear water of a nearby mountain stream.

The parasites briefly highlighted to this point are mostly protozoans, and most have alternative transmission routes, i.e., fecal/oral (soil or water), or foodborne, pathways. There are also a substantial number of helminths that infect humans, many of which are strictly foodborne. They almost always involve complex life cycles, with two, or more, hosts necessary for completion. Most, but not all, require consumption of raw, or poorly cooked, meat, or fish. Several are transmitted via vegetation of some sort, whereas others employ crabs, crayfish, lobsters, etc.

Both gnathostomiasis and anisakiasis are caused by parasitic helminths and are also typical emerging diseases; they should be mentioned together because their similar life cycles are connected with the consumption of sushi or sashimi, which really means the same thing, i.e., eating raw fish. Both of these diseases in humans are caused by eating raw fish containing larval nematodes, which should normally infect felids/canids, or marine mammals, respectively. In each case, the larval parasite in humans will wander 'aimlessly' in the host's body cavity/subcutaneous tissues, seemingly searching for the appropriate site of infection, which is the stomach in the normal definitive host (fish-eating marine mammals). One or both are common in parts of the world where raw fish are consumed on a regular basis, for example, Scandinavia, the Far East, and coastal areas of Central and South America.

As previously mentioned, *F. hepatica* and several closely related zoonotic trematode species employ primarily aquatic vegetation, for example, watercress/water caltrop, on which metacercariae of the parasite will occur. On ingestion, metacerariae excyst in the small intestine and the parasites migrate into the liver, gall bladder, liver, or bile ducts, where they develop into relatively large adults. *Opisthorchis* (*Clonorchis*) *sinensis*, the so-called Chinese liver fluke, and *Opisthorchis viverrini* are frequently (estimated at 10 million infections) seen in the bile ducts of humans in southeastern Asia. The latter has even been associated with cancer of the bile duct epithelium (cholangiocarcinoma). The intermediate hosts are freshwater/marine fishes in which metacercariae occur in the flesh. Several other zoonotic trematodes are also known to infect humans. These species, for example, *Metagonimus yokogawi*, *Heterophyes heterophyes*, *Watsonius watsoni*, *Haplorchis* spp. etc., are mostly associated with the gastrointestinal tract and employ a number of different fish species as second intermediate hosts. These parasites, along with the liver flukes mentioned above, commonly occur in association with the flourishing aquaculture industry. Their numbers within a given host can be quite high, for example, from several hundred to several thousand, and are capable of inducing gastric pain, typically accompanied by diarrhea.

Human paragonimiasis is another zoonotic disease caused by at least 10 species of *Paragonimus* in several parts of the world, but primarily in Central and South America and the Far East. The parasites become encapsulated in the lungs, usually in pairs, and cause tuberculosis-like symptoms. Eggs are coughed up in sputum and swallowed. Ectopic sites of infection, for example, brain, spleen, subcutaneously, etc., confirm that humans are not the normal definitive hosts. Intermediate hosts include marine and freshwater crabs, crayfish, and shrimp. Because these hosts are frequently eaten raw in many parts of the world, the disease is fairly common (estimated at 22 million cases) in endemic areas.

As noted earlier, foodborne parasites in humans are frequently 'cultural' in character. However, most of these same parasites are also closely related to poor socioeconomic conditions, i.e., poverty. Some say that parasites cause poverty, whereas others believe that parasites and poverty simply go hand-in-hand. The cestode, *T. solium*, is quite unusual in that humans can serve as both the definitive and intermediate

hosts for the cestode. The normal life cycle should include pigs as the intermediate host where bladder worms, technically known was cysticerci, encyst in skeletal and cardiac muscle, as well as subcutaneously. When humans consume inadequately cooked pork, they will acquire the cysticerci, which will develop into a robust tapeworm, reaching 10–12 feet in length. Segments (proglottids) that can enclose up to 10 000 eggs drop-off of the tapeworm strobila and are shed in the feces. Once outside, proglottids dry up and disintegrate, releasing the eggs. Foraging pigs then ingest the eggs by accident. The problem is that in many impoverished countries, humans and their pigs live in very close proximity to one another, which makes it likely that humans can also accidentally consume the eggs. If this happens, then the eggs will hatch and cysticerci will develop in the same sites of infection as in pigs, as well as in the brain (neurocysticercosis) where they can cause epileptic-like seizures.

Foodborne parasites are a bane for mankind. They can be exotic and limited in geographic dissemination, or they can be common and have a near worldwide distribution. Many are dependent on cultural mores, involving the choice of food, or the way it is prepared for human consumption, or both. Once eating habits have been established for a particular group of humans, and these eating habitats have become ingrained for many hundreds, if not thousands, of years, cultural tradition is difficult, if not impossible, to change.

Generally, most of these parasites and the diseases they cause are well known. For many of these parasites, there is adequate treatment or prophylactic drugs. Adequate cooking, or freezing, will take care of most species. A change in food consumption behavior would take care of others. Nonetheless, the likelihood of their total eradication, especially those with reservoir hosts, is very small. Depending on the parasite, humans can be the definitive host, or a dead-end intermediate host. Although most foodborne parasites rarely kill human hosts, morbidity is common. Although effective drug treatment is presently available for most helminth parasites, the risk of eventually developing resistance by parasites is real. For most of the protozoan parasites affecting humans, the same thing can be said. However, as was just noted, because so many foodborne parasites ultimately come from reservoir hosts, the likelihood of forced extinction is virtually nil. Moreover, the probability of severing their association with humans is near zero because of entrenched cultural mores.

See also: Foodborne Diseases: Overview of Biological Hazards and Foodborne Diseases

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DISCIPLINES ASSOCIATED WITH FOOD SAFETY

Food Virology

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Glossary

Hepatitis A virus A 27–32 nm, nonenveloped, positive-sense single-stranded RNA virus with a 7.5-kb genome and a capsid with icosahedral symmetry. This virus is classified in the Picornaviridae family under the genus *Hepatovirus*. The virus causes a classic hepatitis syndrome that is less severe than hepatitis A and B and does not result in subsequent chronic disease. Nonetheless, it is one of the more severe of the foodborne viral illnesses.

Hepatitis E virus A positive-sense single-stranded RNA virus, hepatitis E virus is transmitted by the fecal–oral route and is endemic in the developing world. Clinical symptoms are very similar to those of hepatitis A virus infection, but with more severe consequences to infected pregnant women. Its increasing recognition in industrialized countries suggests that hepatitis E virus may be an emerging foodborne pathogen.

Human enteric viruses A functional, rather than taxonomic, group of viruses that infect humans by ingestion of infectious particles. Many virus families are represented by the enteric viruses, which are most commonly transmitted by the fecal–oral route.

Norovirus One of four genera within the Caliciviridae family. Human noroviruses are the most common cause of acute viral gastroenteritis in industrialized countries, and also the leading cause of foodborne illness. These are nonenveloped icosahedral viruses approximately 27 nm in diameter and having a genome consisting of a single strand of positive-sense RNA of 7.4–8.3 kb in length. Based on amino acid homology of the viral capsid protein, the *Norovirus* genus can be further subdivided into five genogroups.

Positive-sense RNA Single-stranded RNA molecule that can be translated directly, without need for modification.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) A modification of the DNA amplification method qPCR intended to be applied to RNA. This is accomplished by preceding the qPCR with a RT step that produces a double-stranded DNA copy of the RNA. RT-qPCR is a commonly used method to detect the genomes of human enteric viruses, as these typically consist of single-stranded RNA.

Virus A small infectious agent that can only replicate in a live host cell, i.e., an obligate intracellular pathogen. Viruses consist of one or more nucleic acid molecules (genome) surrounded by a protective protein coat (capsid); some viruses are further surrounded by a lipid bilayer or envelope.

Introduction

Human enteric viruses are the most common cause of foodborne illness worldwide. These viruses comprise a functional, rather than taxonomic group, almost all of which are nonenveloped (lacking a lipid outer layer) and transmitted by the fecal–oral route. From an epidemiological perspective, human noroviruses (HuNoV) are now considered the most important of the foodborne viruses, causing 58% of foodborne illnesses of known etiology in USA. Hepatitis A virus (HAV) is also important as it causes perhaps the most severe of the viral foodborne diseases. There are other important viruses that can be transmitted by foodborne routes, although their significance is less well characterized.

Viruses are obligate intracellular parasites that require the metabolic machinery of the host cell to replicate. They are tissue tropic (enteric viruses infect the gastrointestinal tract) and species specific, so human enteric viruses infect humans but not other animals, and vice versa. Enteric virus particles are shed in extremely high numbers in the feces of infected individuals, and hence their transmission is usually due to the fecal–oral route. However, epidemiological evidence suggests that NoV particles are also shed in vomitus and this may significantly increase transmissibility of these viruses, especially if virus particles become aerosolized during vomiting events. In general, enteric viruses are highly infectious and typically disease can be caused by a small number (perhaps 10–100) of infectious virus particles.

Human enteric viruses are spread in a number of ways: by person-to-person contact; through contact with contaminated inanimate objects (fomites); or by consumption of contaminated food or water. Most cases of foodborne viral disease are caused by consumption of foods that have been handled or prepared by infected food handlers who have not practiced adequate hygiene, particularly after using the restroom. Contamination can also occur preharvest, as a consequence of exposure to human feces (sewage) during irrigation or fertilization of fresh produce, or during production of molluscan shellfish.

Although the disease caused by the common foodborne viruses is rarely life threatening, the sheer volume of cases places severe burden on the public health system. This article will summarize the early days of food virology, outline key characteristics of important and emerging foodborne viruses, and discuss epidemiological surveillance and control of these important agents of foodborne disease.

The History of Food Virology

Poliovirus was the first enteric virus to be widely recognized, causing foodborne disease outbreaks in the early 1900s associated with the consumption of contaminated raw milk. Raw milk outbreaks of poliomyelitis continued until the 1950s, when an effective vaccine was introduced. Molluscan shellfish-associated outbreaks of hepatitis were also reported in the 1950s and 1960s. The cause of this disease was HAV, which was identified as an enterically transmitted infectious agent when Dr. Saul Krugman intentionally fed mentally disabled children purified fecal extracts prepared from symptomatic patients; the children subsequently developing classic hepatitis symptoms. During the same era, foodborne outbreaks of nonbacterial gastroenteritis were recognized and although a viral etiology was suspected, it remained difficult to confirm.

This changed when, in 1972, Dr. Albert Kapikian identified the first virus to be associated with acute nonbacterial gastroenteritis. Appropriately named 'Norwalk virus' because of its association with an outbreak in Norwalk, OH, in USA, this virus was the first of many that would ultimately be identified as members of the genus *Norovirus* (*NoV*) within the Caliciviridea family. Electron microscopy was used to identify the Norwalk virus, and this method, which requires virus loads $>10^6$ particles per g of stool, is still used to this day.

The absence of culture methods for both HuNoV and HAV was a major impediment to further study of these agents. In 1987, Dr. Theresa Cromeans developed a method to propagate HAV, ushering in a new era for studying this virus. This method, as well as cultivation methods for the vaccine strain of poliovirus, eventually allowed for the quantification of infective virus plaque forming units and facilitated studies on detection and control of enteric viruses in water and foods, with a particular focus on molluscan shellfish. Dr. Dean Cliver, who served as a professor at the University of Wisconsin in Madison and at the University of California in Davis, provided much of the foundation on which current principles in food virology are based.

In 1990, Dr. Mary Estes team at Baylor College of Medicine sequenced the full Norwalk virus, and many other NoV genomes have been characterized since then. With knowledge of nucleic acid sequence and the rapid adoption of molecular biological methods, scientists were suddenly presented with new tools to study human enteric viruses. Particularly important was the nucleic acid amplification method polymerase chain reaction (PCR), which was readily adapted to the detection of RNA viruses by preceding PCR with a reverse transcription (RT) step, hence the designation RT-PCR. Conventional RT-PCR methods, followed by Southern hybridization were quickly developed for the detection of these viruses in clinical (fecal) samples and foods, particularly molluscan shellfish. These were later replaced by more rapid and sensitive quantitative real-time RT-PCR (RT-qPCR) methods.

Although promising, the utility of these molecular amplification methods for virus detection in food and environmental samples was limited by low levels of contamination; high levels of matrix-associated inhibitory substances that interfered with nucleic acid amplification; and the lack of broadly reactive primers and probes for HuNoV. In addition, the development of an *in vitro* cultivation system for HuNoV has remained elusive. Cultivable surrogate viruses (e.g., feline calicivirus and murine norovirus) have been used in a variety of studies but their behavior does not always mimic that of HuNoV. These impediments to the study of HuNoV continue to this day.

The epidemiological importance of HuNoV came to the forefront in 1999 when Mead *et al.* (1999) suggested that viruses, not bacteria, were the leading cause of foodborne illness of known etiology. This study prompted expanded epidemiological surveillance activities in Europe. The Mead *et al.* (1999) publication, along with some high profile outbreaks in the late 1990s and early 2000s, also focused attention on foods other than molluscan shellfish as significant causes of foodborne viral gastroenteritis. In fact, we now know that foods such as salads, bakery products, and sandwiches are responsible for more NoV and HAV infections than are molluscan shellfish. Fresh produce (berries and green onions) have also emerged as important vehicles of infection. The association of disease with foods such as these suggest that the hands of infected food handlers are arguably the most common source of the virus contamination for many if not most foods. Recent epidemiological data continue to support the fact that viruses, particularly HuNoV, are the most common cause of foodborne disease of known etiology in USA.

HuNoV

HuNoV are the most common cause of acute gastroenteritis in industrialized countries. Scallan *et al.* (2011b) estimate that these viruses are responsible for approximately 58% of foodborne disease of known etiology in USA. This amounts to 5.5 million infections per year, resulting in approximately 50 000 hospitalizations and 300 deaths. These numbers reflect only those diseases of known etiology; if foodborne disease of unknown etiology were included, as well as other transmission routes, these estimates would be staggeringly high. For example, of the more than 170 million cases of gastroenteritis that occur in USA each year, the etiology of only about one-fifth of them is confirmed, leaving an estimated 140 million cases of gastroenteritis caused by unspecified agents. Owing to the frequency of NoV infection, it is likely that a large proportion of these are also caused by HuNoV. Similar data are available from Europe.

The taxonomy of NoV has changed substantially over the past 15 years, and what was originally called the 'Norwalk-like' virus group is now classified as the *NoV* genus, one of four genera within the Caliciviridae virus family. The other genus in this family causing disease in humans is *Sapovirus*, also shown to cause gastroenteritis. HuNoV are a nonenveloped icosahedral

viruses approximately 27 nm in diameter and having a genome consisting of a single strand of positive-sense RNA of 7.4–8.3 kb in length. The genome encodes three open reading frames (ORF); ORF1 encodes a nonstructural polyprotein that contains the genes for p48, NTPase, p22, VPg, protease, and RNA polymerase; ORF2 encodes the viral capsid protein; and ORF3 encodes a small basic structural protein of unknown function.

Based on nucleic acid sequence analysis, the *NoV* genus can be further classified into five genogroups, designated GI, GII, GIII, GIV, and GV, based on >60% amino acid homology of the viral capsid protein. Human infections are caused almost exclusively by genogroups GI and GII, with the vast majority caused by GII strains. Each genogroup can be further classified into genotypes, based on a >80% amino acid homology in the capsid protein. Currently, 8 GI strains and 19 GII strains have been identified. The genetic cluster GII.4 is the most significant of the genotypes, having predominated in outbreaks around the world for over a decade. However, strains other than GII.4 are most often the cause of foodborne disease outbreaks.

Outbreaks of NoV have involved food products including molluscan shellfish, fresh fruits and vegetables, and 'ready-to-eat' (RTE) foods. Epidemiologically speaking, it appears that RTE foods are the most common cause of foodborne NoV outbreaks, although high-profile outbreaks have occurred in produce items such as berries. In healthy adults, the incubation period for NoV infection ranges from 24 to 48 h, with symptoms lasting 12–72 h. The disease is gastrointestinal in nature, typically presenting with vomiting (hallmark symptom), diarrhea, and abdominal cramps that may or may not be accompanied by fever. In certain at-risk groups, particularly the elderly, NoV infection can result in a much more severe disease, with symptoms lasting as long as 6 weeks. The hospitalization rate for HuNoV infection is estimated to be 0.03% and the mortality rate is less than 0.1%. Nonetheless, the shear numbers of cases make these viruses one of the leading causes of foodborne disease hospitalizations and deaths. No antiviral strategy exists for prevention or treatment of NoV illness. As is the case for all enteric diseases, infected individuals should be treated to maintain hydration and electrolyte balance.

Although some immunologically based commercial methods are available for detection of HuNoV in clinical samples, they are licensed in only some parts of the world. This is largely because of poor sensitivity due to the lack of broadly reactive antibodies that will detect all HuNoV strains. The high degree of genetic diversity for the NoV has historically complicated the development of broadly reactive RT-qPCR methods. Four 'regions' of the NoV genome have been used for primer design (designated regions A, B, C, and D). These regions are generally conserved among HuNoV strains of the same genogroup. The ORF-1–ORF2 junction (just downstream of region B) seems to be the most conserved and is frequently used for genogroup-specific detection. For strain comparison (as might be appropriate in outbreak investigation), primers corresponding to the NoV capsid region (region D) are usually used. Commercial RT-qPCR method detection methods are available for use in the food and environmental sector, but in all cases, substantial sample preparation to concentrate the viruses and remove the sample matrix is required before the application of RT-qPCR for detection. Taken together, HuNoV detection in clinical, food, and environmental samples is not done routinely and there are wide regional variations in protocols. This highlights the need for standardized methods of detection and consistency of surveillance and disease reporting across countries.

HAV

HAV is a 27–32 nm, nonenveloped, positive-sense single-stranded RNA virus with a 7.5-kb genome and capsid having icosahedral symmetry. This virus is classified in the Picornaviridae family under the genus *Hepatovirus*. Unlike other RNA viruses, the HAV genome is highly conserved, with only 1–4% amino acid variation. Human isolates of HAV comprise a single serotype, but sequence heterogeneity within the VP1/2A can be used to differentiate HAV into seven unique genotypes. Of these seven genotypes, genotypes I and III predominate in human disease. Transmitted primarily by contact with the blood of infected individuals or through male homosexual relations, only approximately 5% of HAV cases are foodborne, with transmission almost always in keeping with the fecal–oral route. Once infected, the disease incubation period averages 4 weeks (range of 2–6 weeks). Disease initially presents with a prodrome that includes fever, headache, nausea, vomiting, and diarrhea. These symptoms progress 1–2 weeks later into inflammation of the liver and jaundice. Hospitalization occurs in 31.5% of cases, with mortality rates estimated at 2.4%. Infected young children are frequently asymptomatic, while disease severity increases with age. Hepatitis A infection is endemic in developing regions of the world, and foods imported from third world countries where sewage treatment and hygiene advancements are still developing pose an increased risk to naive consumers in developed countries.

Like NoV, outbreaks of HAV have involved food products including molluscan shellfish, fresh fruits and vegetables, and RTE foods. For example, in 1988 approximately 300 000 people in China contracted HAV from consuming partially cooked clams that had become contaminated by release of raw sewage in the proximity of the harvest area. Within the fresh produce category, products such as raspberries, strawberries, lettuce, and green onions have caused outbreaks, some quite sizable. A good example is the 2003 US outbreak when green onions served at a single Pennsylvania restaurant resulted in more than 600 HAV cases, with 124 hospitalizations, and 3 deaths. Fresh produce most likely becomes contaminated by the use of human sewage-contaminated irrigation water, because of human defecation in production fields, and/or from the hands of infected food handlers during harvest or preparation phases of the farm-to-fork continuum. Still, the most common cause of HAV foodborne outbreaks in general is poor personal hygiene of infected food handlers. Fortunately, an effective vaccine that provides lifelong immunity is now available suggesting that as the immunized population grows the disease will eventually go the route of poliovirus, although it may take decades before this is realized.

Because HAV has a degree of genetic and antigenic homogeneity, its detection is much easier than that of HuNoV. Clinical assays that detect antibodies against HAV are commercially available, and for food and environmental samples,

RT-qPCR using primers targeting the highly conserved VP1/2 A junction or 5′ untranslated region of the viral genome are used. As is the case for NoV, substantial preanalytical sample processing must be done when applying these methods to complex sample matrices and this remains the limiting factor in the routine detection of HAV in foods.

Rotaviruses

Rotaviruses are the leading cause of infantile diarrhea worldwide and are responsible for more than 500 000 deaths annually, the majority of which occur in developing countries. These viruses are 70–75 nm in diameter and contain 11 segmented double-stranded RNA molecules encased in a double-layered protein coat of icosahedral symmetry. The 11 genome segments range in size from 667 bp (segment 11) to 3302 bp (segment 1), for a total of 18.5 kb. Each of the segments encodes a single protein, with the exception of segments 9 and 11 which encode two proteins. A total of six structural viral proteins form the virus particle (termed VP1, VP2, VP3, VP4, VP6, and VP7), whereas a further five nonstructural proteins (termed NSP1–NSP5) are responsible for RNA replication, packing, and other functions. Interestingly, NSP4 is an enterotoxin that induces diarrhea. At least seven different rotavirus groups exist (A–G) based on VP6 reactivity with monoclonal antibodies.

Waterborne and person-to-person transmission are the most common causes of rotavirus infection; however, transmission via food products has been documented, with infant populations under 5 years of age most often affected. The incubation period preceding disease is approximately 2 days, and symptoms of disease include vomiting, diarrhea, and fever; dehydration is the most common cause of death. Hospitalization rates in USA are estimated at 1.7%, with a mortality rate of less than 0.1%. The World Health Organization (WHO) now recommends the use of second generation rotavirus vaccines worldwide in an attempt to reduce the burden of disease in developing countries.

Emerging Foodborne Viruses

A number of enteric viruses have the capacity to be transmitted by foodborne routes, although this is rarely documented or has not yet occurred. A few of these are described briefly in this section.

Hepatitis E virus (HEV)

HEV is a positive-sense single-stranded RNA virus that is transmitted via the fecal–oral route, generally through the consumption of water and sometimes food that has become contaminated with human feces. This virus is endemic in developing countries, particularly those locations having hot climates. Large waterborne outbreaks have been recorded in many countries, and it is also possible that the virus is transmitted by the consumption of raw or undercooked pork and deer meat. Interestingly, cases of HEV have recently been reported in USA, UK, and Japan, leading to speculation that the geographic range of this virus is increasing and that HEV may become an emerging food and waterborne pathogen in the developed world.

The incubation period for HEV infection ranges from 3 to 8 weeks, with disease symptoms generally lasting several weeks. Frank disease occurs more often in adults, with children often spreading the infection without displaying symptoms. Clinical symptoms are very similar to those of HAV infection, but unlike HAV, the disease is quite severe in pregnant women for whom mortality is approximately 20%. Clinical diagnosis of HEV involves detecting antibodies specific to the virus in the blood of patients displaying the symptoms of hepatitis, or by RT-PCR.

Other Human Enteric Viruses

Other human enteric viruses also have the potential to be transmitted by contaminated food products, although their epidemiological significance is not well understood. The human enteroviruses, including poliovirus, coxsackie, and echoviruses, are nonenveloped particles containing single-stranded positive-sense RNA, with particle diameter of approximately 27 nm. Poliovirus has been all but eradicated in the developed world, coxsackie and echoviruses, which cause a variety of symptoms that can range from gastroenteritis, neurological, and skin manifestations, can be transmitted by foodborne routes, albeit infrequently. Like HuNoV, they are resistant to harsh conditions, making them environmentally persistent.

Astroviruses are 28 nm single-stranded RNA viruses with a star-shaped capsid structure. These viruses cause sporadic disease and outbreaks of diarrhea in children and the elderly, particularly in venues such as day care centers and hospitals. Transmission of these astroviruses by food is uncommon but has been documented for molluscan shellfish. Human adenoviruses are nonenveloped double-stranded DNA viruses that range from 80 to 110 nm in diameter. They can cause gastroenteritis, conjunctivitis, and most frequently, respiratory symptoms. The presence of these viruses in environmental samples such as wastewater, sludge, and drinking water has been reported, and for this reason, their use as an indicator for the presence of human enteric viruses has been proposed. However, the transmission of adenoviruses by foodborne routes has not yet been documented.

Nipah Virus

Nipah viruses belong to the genus *Henipavirus* in the family Paramyxoviridae, and are relatively large (120–150 nm diameter), enveloped, single-stranded RNA viruses. The Nipah virus was first recognized in 1999 in Malaysia in association with pig farmers who contracted the disease by contact with infected animals. It can also be transmitted by person-to-person contact and can be carried by fruit bats. There have been recorded outbreaks where the vehicle of infection was fruits and vegetables contaminated with the saliva of bats. Other outbreaks have involved direct contact with contaminated pigs or their tissues. Disease symptoms usually include

fever, headache, muscle pain, vomiting, and sore throat. These can progress to pneumonia and other respiratory illnesses. In severe cases, seizure and encephalitis can occur, often resulting in death. Currently, there is no vaccine or treatment for Nipah virus infection, so control of foodborne disease relies on cleaning and disinfection of pig farms, culling animals suspected of being infected, and controlling bat populations.

Highly Pathogenic Avian Influenza (HPAI) Viruses

HPAI viruses are large (300 nm diameter), negative sense RNA viruses having a segmented genome and belonging to the Orthomyxoviridae family. Domestic and wild birds are the major reservoir for these viruses. There are virtually hundreds of HPAI strains, however, only four have been shown to cause infection in humans; H5N1, H7N3, H7N7, and H9N2. Disease in humans is typically mild, except for the H5N1 virus, which has been responsible for a number of human deaths following outbreaks. The potential for spread of this virus through the food chain has been of concern because the virus appears to survive on imported meat. There is also concern for the risk of fecal contamination of water that is subsequently used in production agriculture or even for food preparation. However, it has been documented that HPAI is susceptible to thermal processes, meaning that the consumption of properly cooked food poses little risk for HPAI infection.

Coronavirus

Coronaviruses are enveloped viruses with positive-sense single-stranded RNA that belong to the family Coronaviridae. The coronavirus that causes sudden acute respiratory syndrome (SARS-CoV) was first recognized as a human pathogen in 2002 in association with an outbreak in China. The virus was subsequently detected in more than 30 other countries, infecting more than 8500 people with a 10% mortality rate. Although generally thought to be spread exclusively by respiratory routes, there is evidence that SARS-CoV can replicate in the small and large intestines, causing diarrhea that results in fecal shedding of the virus. Thus, the fecal–oral transmission route cannot be excluded. However, SARS-CoV are sensitive to fairly mild heat treatment and commonly used disinfectants, suggesting that attention to proper food handling and preparation measures should control foodborne transmission of the virus. Taken together, the spread of SARS-CoV via contaminated food products remains possible but is now considered unlikely.

Lassa Virus (LV) and Hantavirus (HV)

LV and HV are RNA viruses that belong to the Arenaviridae and Bunyaviridae families, respectively. LV, which is endemic in sub-Saharan Africa, causes a viral hemorrhagic fever; HV initially presents as a flu-like syndrome that can progress to severe pulmonary disease. The natural reservoir for both of these viruses is the mouse, and although rare, food can potentially be a vehicle of infection if it becomes contaminated with urine and/or feces of mice.

Epidemiological Surveillance and Burden of Disease

The US Centers for Disease Control and Prevention (CDC) conducts surveillance of foodborne disease outbreaks, including those caused by HuNoV and HAV, through the National Outbreak Reporting System (NORS). Because the program relies on individual states reporting their data, which is quite variable, the database is incomplete. The NORS system provides some important information that can be used relative to epidemiological attribution estimates, but it only reports on epidemic disease. In the absence of routine clinical diagnostics, it is very difficult to get estimates for endemic HuNoV disease. The CDC has also recently launched CalciNet, a national HuNoV sequence database that may eventually have utility similar to that of the PulseNet system for bacterial foodborne pathogens.

Surveillance for HuNoV illness in Europe has been done by the European Centre for Disease Control and Prevention (ECDC) and the Foodborne Viruses in Europe (FBVE) Network. In Australia, the notification of individual NoV infections is not required, however, the reporting of two or more infections having a time, place, and/or person association, suggesting an outbreak, is required.

In many other countries, foodborne viral disease surveillance is not conducted, making it difficult to estimate the global impact of disease. In a UK study, it was estimated that only 1 in 1562 HuNoV infections was actually reported. This is not surprising due to the mild nature of most infections and the lack of routine clinical diagnostics. Of particular interest is the proportion of HuNoV infections that are caused by contaminated foods as compared to other transmission routes. Getting at these estimates will undoubtedly require targeted epidemiological efforts such as active surveillance and/or case control studies, both of which are expensive and time-consuming. Further, until standardized reporting at the country and international level is established, the true burden of foodborne viral disease will remain unknown.

Control of Foodborne Viruses

As obligate intracellular parasites, enteric viruses cannot replicate in foods or water. In general, if wastewater is adequately treated through primary, secondary, and tertiary steps, and including chlorination, the risk of viral contamination is minimal. When it comes to foods, many measures classically applied to control bacterial growth are not very effective against viruses. For example, exposure to extremes of pH (2.0–10.0) and water activity will have little or no effect on the infectivity of enteric viruses. Refrigeration and freezing actually help to preserve virus infectivity. The efficacy of standard thermal inactivation treatments is dependent on the food matrix and virus studied. As a general rule of thumb, NoV and HAV appear to be less sensitive to heat than are typical Gram-negative bacteria, and more sensitive to heat than spores. High hydrostatic pressure has been recognized as an emerging processing technology for inactivating viruses in molluscan shellfish, however, similar to thermal inactivation, the effectiveness of the treatment is variable and virus specific. Enteric viruses are also notably resistant to ionizing radiation.

Many studies have demonstrated the ability of enteric viruses to survive on abiotic surfaces commonly found in food processing and preparation environments, including stainless steel, aluminum, and polystyrene. Viruses also persist in foods, having been found to survive in shellfish for weeks or months and on the surface of fresh produce for days to weeks. To make matters worse, it is well documented that many common sanitizers used in food processing environments have poor efficacy against nonenveloped enteric viruses, at least when used at manufacturer-recommended concentrations.

It is for this reason, current control measures focus on the prevention of contamination, rather than treatments to inactivate viruses after a contamination event has occurred. The central tenets of control are good hygiene practices in food processing and handling environments, and prevention of contamination in the preharvest environments. Because the decontamination of hands and surfaces in food processing and preparation is critical to preventing virus contamination, an important effort is assuring effective hand decontamination. The best method remains the traditional soap and water wash followed by towel drying. Commercial alcohol-based hand sanitizers should not be used in place of adequate hand washing. Of course, hand washing compliance in retail, institutional, and home settings remains a challenge and must be continuously advocated. There has been interest in further promoting the need for infected food handlers to report illness symptoms and abstain from work during periods of time in which they are actively shedding virus. However, the latter may be difficult in light of emerging evidence suggesting that shedding of HuNoV may persist for weeks after symptom resolution. Adequate cleaning and disinfection of surfaces is also important to preventing virus contamination of foods, but the availability of disinfecting agents with specific activity against the nonenveloped enteric viruses is a pressing need. Also needed are better microbiological indicator systems that have a more direct correlation with virus contamination of water in production environments, as the fecal coliforms and *Escherichia coli* remain poor indicators for this application. This is particularly relevant for molluscan shellfish and fresh produce production.

Conclusion

Food virology is a relatively young field, at least from the perspective of food safety. The development of molecular techniques during the 1990s, in combination with increased epidemiological surveillance, have raised awareness of the importance of viruses to foodborne illness. Although methodological advancements have been made, much still remains unknown. The lack of a culture system for HuNoV is probably the single most important limiting factor to studying and controlling these viruses. With availability of such a method, or in its absence, availability of better cultivable surrogates or more sensitive detection methods for complex sample matrices, scientists would be able to tackle the challenges associated with trying to control foodborne viruses. Likewise, the availability of routine clinical assays would result in greater awareness of these diseases and improvements in reporting and epidemiological surveillance. The field of food virology is set to grow rapidly in the coming years as scientists tackle these problems with the aid of developing technologies.

See also: Food Safety Assurance Systems: Personal Hygiene and Employee Health. Organisms of Concern but not Foodborne or Confirmed Foodborne: Foot-and-Mouth Disease Virus. Viruses: Hantavirus; Hepatitis A Virus; Hepatitis E Virus; Lassa Fever Virus; Nipah Virus; Norovirus

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DISCIPLINES ASSOCIATED WITH FOOD SAFETY

Epidemiology

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Glossary

Agent A factor that is essential for a disease to occur.
Endemic A disease that is habitually present within a given geographic area.
Epidemic The occurrence of a group of illnesses in a community or region, which are of similar nature and clearly in excess of normal expectancy and derived from a common or from a propagated source.
Epidemic curve A histogram that shows the course of an outbreak or epidemic by plotting the number of cases of a disease by the date or time of illness onset.
Epidemiologic triad The traditional model of infectious disease causation, which has three components: an external agent, a susceptible host, and an environment that brings the host and agent together so that the disease occurs.
Host A person or other living organism that is susceptible to an infectious agent under natural conditions.
Pandemic A worldwide epidemic.
Portal of entry A pathway by which an agent enters the host allowing it to multiply or act.
Portal of exit A pathway by which an agent can leave its source or reservoir.
Prospective study An epidemiologic study in which the groups of individuals (cohorts) are selected on the bases of factors that are to be examined for possible effects on some outcome.
Retrospective study An epidemiologic study in which participating individuals are classified as either having disease or other outcome (cases) or not having the disease or outcome (controls) and the persons' histories are examined for specific factors that might be associated with that outcome.

Introduction

Epidemiology is the study of patterns of disease in the population. Foodborne diseases often have seasonal and geographic patterns, are reported disproportionately among people with certain demographic characteristics, or are associated with eating specific foods. By understanding these patterns, we can learn about the factors that affect the disease occurrence. This understanding provides critical information that can help direct prevention and control efforts. This article describes the science of epidemiology and its importance to food safety and the study of foodborne disease.

Disease Transmission

Epidemiologists view disease as the result of complex interactions between an agent, a host (person or other living animal), and an environment, which includes food and water, as illustrated by the epidemiologic triad (Figure 1). The agent can be a bacteria, virus, parasite, or a toxin that causes disease by infecting or injuring the host. The presence of the agent is essential but whether disease occurs also depends on characteristics of the agent, host, and environment. For example, the amount of toxin or number of microorganisms ingested (infective dose), the ability of the agent to produce disease (pathogenicity), and the degree of pathogenicity (virulence) will influence the occurrence and severity of disease. Host factors, such as age and ethnicity, influence exposure to disease, whereas other factors, such as the host's immune status, can influence host susceptibility and disease severity. Environmental factors are extrinsic factors that affect both the agent and the opportunity for exposure and may include: physical factors, such as climate, geology, and physical surroundings (e.g., childcare center and nursing home); and

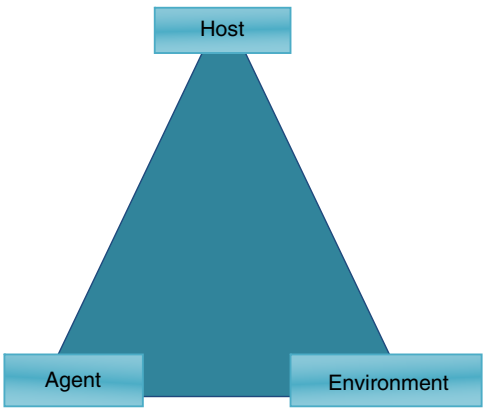

Figure 1 Epidemiologic triad.

socioeconomic factors, such as crowding and sanitation. When studying the distribution and causes of a disease in a population, it is important to consider all the three components of the epidemiologic triad and how they interact with the disease under investigation. Some key epidemiologic concepts related to disease transmission are described in the following sections.

Modes of Transmission

The mode of transmission is the mechanism by which an agent interacts with the host. Infectious agents can be transmitted directly from one person to another or indirectly by a vector (e.g., a fly or other insect) or through a vehicle, such as food or water. Foodborne diseases are by definition transmitted by a food vehicle. However, it is important to note that many agents that cause foodborne illness may also be transmitted by other modes. Infectious foodborne agents must have a human, animal, or an environmental reservoir to live. Because many involve the gastrointestinal tract, causing diarrhea or other gastrointestinal symptoms, they typically leave the human or animal reservoir through the feces (portal of exit) and are ingested (portal of entry) by the host via contaminated food.

Incubation Period

An incubation period is the time between infection or contact with the agent and the onset of symptoms or signs of infection. The incubation period for foodborne diseases can range from hours for bacterial food intoxications (e.g., *Bacillus cereus* and staphylococcal enterotoxins) to days for infectious agents (e.g., *Salmonella* and *Escherichia coli* O157) or weeks (e.g., Hepatitis A virus, *Listeria monocytogenes*, and *Toxoplasma gondii*) and even years for cancer-causing chemicals or toxins. Infectious diseases typically require an incubation period that allows for the multiplication of the agent to a threshold necessary to produce symptoms. Variation in the incubation period may occur for a variety of reasons, including the dose and rate of replication of the organism.

Disease and Carrier States

People who are infected often show clinical symptoms, such as diarrhea or vomiting, whereas some may be asymptomatic showing no obvious sign or symptoms of disease. The term 'carrier' is used to describe a person who is infected by a pathogen and is capable of disseminating that pathogen but shows no signs of clinical disease. Asymptomatic carriers infected with a foodborne pathogen who handle food present an important threat to public health and may shed organisms intermittently over a long period of time.

Patterns of Disease

Epidemiologists classify three patterns of disease – endemic, epidemic, and pandemic. A disease is endemic if it is habitually present within a given geographic area. An epidemic is the occurrence of a group of illnesses in a community or region, which are similar in nature and clearly in excess of normal expectancy and derived from a common or from a propagated source. A pandemic refers to a worldwide epidemic.

Disease Outbreaks

The term outbreak is synonymous with the term epidemic, although the former is used more frequently and can refer to more localized epidemics. The term cluster may also be used when describing a group of cases that are clustered in time or place but require further investigation to determine if they constitute an outbreak. If a group of people become ill after eating the same contaminated food, the outbreak is referred to as a 'common-vehicle exposure.' In these outbreaks, there is a sudden and rapid increase in the number of cases among the people exposed, followed by a more gradual decline. A histogram, called an epidemic curve, is used to show a disease outbreak by plotting the number of cases by the time of symptom onset (Figure 2). The index case is the first person who indicates the existence of an outbreak, whereas a primary case is any person who acquires the disease directly from the contaminated food. Secondary cases acquire the disease from exposure to a primary case and may result from a foodborne

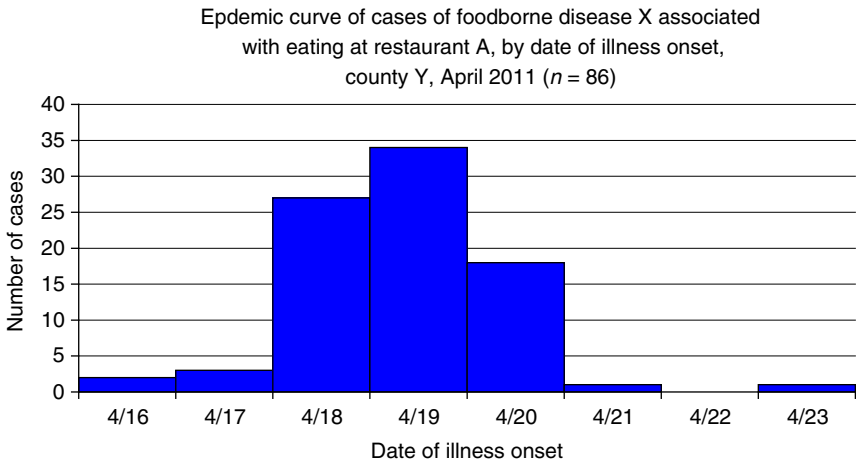

Figure 2 Example of epidemic curve.

outbreak if the pathogen can also be transmitted from one person to another (e.g., *E. coli* OI57).

Measures of Disease Occurrence

To describe the patterns of disease, epidemiologists use standard measures of disease occurrence. Each measure is calculated by dividing a numerator (counts of cases) by a denominator (population at risk), where the population at risk is defined as the population considered capable of acquiring the particular disease within the population being studied. A clear case definition and a good understanding of the population at risk are essential when calculating measure of disease occurrence. Cases of foodborne disease are usually defined using laboratory-based criteria (e.g., culture-confirmed cases of *Salmonella enteritidis*), although clinical criteria (i.e., signs and symptoms, such as diarrhea) may be used.

Incidence Proportion

The incidence proportion (also called cumulative incidence) measures the average risk of disease in a population (ranging from zero to one) and is defined as the number of persons who develop disease divided by the total number of persons at risk. For example, if we study 1000 people (population at risk) over a 2-year period and two people are diagnosed with *Campylobacter* spp. (cases), then the incidence proportion is 2 in 1000 or 0.002 (0.2%). To get an accurate measure of the incidence proportion, it is important that all persons at risk are monitored for the entire time for which the risk is being measured. To interpret the measure correctly, it is also important to know the length of the time period over which the risk applies (e.g., 3 months or 2 years).

$$\text{Incidence proportion} = \frac{\text{Number of persons who develop disease during a time period}}{\text{Number of persons followed for this time period}}$$

The incidence proportion is called an attack rate when used in the context of infectious disease outbreaks. An attack rate may be calculated for all persons exposed during an outbreak. For example, if 30 of 100 people became ill with *E. coli* O157 after attending a church supper, the overall attack rate would be 0.30 (30%). Attack rates can also be calculated for groups with different exposures, for example, attack rates among people who ate a certain food. Another version of incidence proportion is the case-fatality rate. Here, the numerator is the number of deaths and the denominator are people who have already developed the disease, or population at risk. For example, if 2 of the 30 people with *E. coli* O157 infection die, the case-fatality rate would be 0.07 (7%).

Incidence Rate

Similar to the incidence proportion, the numerator for the incidence rate is number of persons who develop a disease. However, the denominator is not the number of persons initially followed but the total time experienced by the people being followed (person-time) during which the disease or event being studied could have occurred. Person-time can be calculated by summing the time that each person is followed for every member of the group (e.g., 100 persons followed for 2 years equals 200 person-years). The incidence rate assumes that the risk on an event is constant over the observed time interval.

$$\text{Incidence rate} = \frac{\text{Number of persons who develop disease}}{\text{Total time experienced for persons followed}}$$

The incidence rate is used for reporting data on the number of individual cases of disease (e.g., from foodborne diseases surveillance) over a specific period of time. Here, the population at risk is often estimated using census data. It is common to find incidence rates expressed as the number of cases per 1000 or per 100 000 persons in the population. Incidence rates are typically described as an annual basis. For example, in 2009, the annual incidence of culture-confirmed nontyphoidal *Salmonella* infections in USA was 14 per 100 000 population.

Prevalence

The incidence proportion and the incidence rate measure the frequency of disease onset. Prevalence (also called prevalence proportion) is a measure of disease status at a given moment in time. For example, the prevalence of nontyphoidal *Salmonella* infections is the number of people ill at a specified time point. For acute diseases of short duration such as nontyphoidal *Salmonella*, the prevalence of disease will be low even if the incidence of disease is high because most people recover from the disease within a week and are therefore no longer in a diseased state. For chronic infections, such as the parasite neurocystercercosis, the prevalence of disease is greater than the incidence. The greater in incidence or duration of disease, the more people will be effected.

$$\text{Prevalence} = \text{Incidence rate} \times \text{Average duration of illness}$$

Measures of Excess Risk

To determine if there is an association between exposure and disease, epidemiologists measure the excess risk of disease among people exposed to a certain risk factor, where risk is defined as the probability of a disease occurring. This can be done by comparing the risk of disease (e.g., culture-confirmed *Campylobacter* spp. infection) between people who were exposed to a specified risk factor (e.g., chicken) and those who were not by subtracting the risk of disease in the unexposed group from the risk of disease in the exposed group (risk difference) or calculating the ratio of disease risk among exposed and unexposed people (e.g., relative risk and odds ratio).

Relative Risk

The relative risk (also called risk ratio) is a ratio of the incidence proportion. The relative effect of a risk factor is measured by dividing the incidence proportion (or average risk of disease) in the exposed group by the incidence proportion in the unexposed group (Table 1). For example, if 23 people

Table 1 Calculating relative risks in a cohort study

Exposure (or risk factor)	*Number of people*	
	Ill persons	*Well persons*
Present	a	b
Absent	c	d
Total	$a+c$	$b+d$
Relative risk $=\dfrac{\text{Incidence proportion in exposed}}{\text{Incidence proportion in unexposed}}$		$\dfrac{(a/(a+c))}{(b/(b+c))}$

Table 2 Calculating odds ratios in a case–control study

Exposure (or risk factor)	*Number of people*	
	Cases	*Controls*
Present	a	b
Absent	c	d
Odds ratio $=\dfrac{\text{Odds of being exposed among cases}}{\text{Odds of being exposed among controls}}$		$\dfrac{(a/c)}{(b/d)}=\dfrac{ad}{bc}$

became ill with campylobacteriosis after attending a church supper, including 20 of 50 people who ate chicken and 3 of the 30 people who did not eat chicken, then the relative risk of illness among chicken eaters (exposed) compared with nonchicken eaters (unexposed) is $(20/20+30)/(3/3+27)$ or 4, meaning that the people who ate chicken were 4 times more likely to become ill than the nonchicken eaters. A relative risk of 1 means the exposed and unexposed people were equally likely to develop disease, whereas a relative risk less than 1 indicates that the exposure was protective against the disease. The rate ratio is a similar measure that uses the incidence rate rather than the incidence proportion.

Odds Ratio

The odds ratio is a relative measure of association typically used in case–control studies. It compares the odds of being exposed among cases (persons with disease) with the odds of being exposed among controls (persons without disease) (Table 2). For example, if we interview 50 people with campylobacteriosis (cases) and 20 confirm that they ate chicken in the week before illness onset compared with only 10 of 50 controls, then the odds ratio is $(20/30)/(10/40)$ or $(20\times 40)/(10\times 30)$ or 2.7. Therefore, cases were 2.7 times more likely than controls to have eaten chicken. Like a relative risk, an odds ratio of 1 indicates no association and less than 1 indicates that the exposure was protective.

Causal Inference

It is important to note that because most epidemiologic studies are observational rather than experimental, an association between an exposure and a disease does not necessarily indicate that the exposure caused the disease. The observed association may be due to chance (random error appearing to cause an association between an exposure and a disease), bias (systematic error in the design, conduct, or analysis of a study that results in a mistaken estimate of an exposure's effect on the risk of disease), or confounding (error in the interpretation because of a third factor that is associated with the exposure and an independent risk factor for the disease). These factors need to be carefully considered in the design and analysis of any epidemiologic study before we can draw conclusions about causality.

Types of Epidemiologic Studies

Epidemiologic studies can be descriptive or analytical. Descriptive studies focus on describing people who develop disease in terms of their personal characteristics and where and when they were exposed to the agent causing disease – person (e.g., sex, age, and race/ethnicity), place (e.g., location and geography), and time (e.g., season and year). Descriptive studies have no fixed hypotheses about the relationship between exposure and disease, although the results of these studies can suggest hypotheses. Most investigations of foodborne disease begin with a descriptive study and may use existing data from surveillance, surveys, or medical records. For example, surveillance summaries often describe cases of illness by person, place, and time. In an outbreak setting, counts of illnesses are frequently organized in a line list (Table 3), which allows for a counting of cases by their various characteristics (Figure 3).

Analytical studies, unlike descriptive studies, start with a hypothesis (or hypotheses) about the association of disease with possible exposures or risk factors and the purpose of the study is to test the hypothesis. To measure excess risk, we would have to make comparisons between groups of people with and without the exposure of interest. The study design

Table 3 Example of line list

ID	*Date of birth*	*Sex*	*Ill*	*Date of illness onset*	*Fever*	*Diarrhea*	*Chills*	*Abdominal cramps*	*Vomiting*	*Headache*
1	08 April 1990	Male	Y	18 April	N	Y	Y	N	N	N
2	26 September 1962	Male	Y	17 April	Y	Y	Y	Y	N	N
3	25 November 1980	Female	Y	18 April	Y	N	Y	Y	N	N
4	04 February 1961	Male	Y	18 April	N	Y	N	Y	N	Y
5	05 December 1970	Female	Y	19 April	N	Y	Y	Y	N	Y
6	05 June 1963	Female	Y	19 April	Y	Y	Y	Y	N	Y
7	08 November 1959	Male	Y	19 April	N	Y	N	Y	N	N
8	05 August 1957	Male	Y	20 April	N	Y	Y	Y	N	Y
9	29 May 1985	Male	Y	20 April	N	Y	N	Y	Y	N
10	17 June 1984	Female	Y	21 April	N	Y	N	N	N	N

Y, yes; N, no.

Figure 3 Mary Mallon (1869–1938), nicknamed 'Typhoid Mary,' was the first person in USA to be identified as an asymptomatic carrier of typhoid fever. Over the course of her career as a cook, she infected 47 people, three of whom died from the disease.

must compare exposed and unexposed groups with similar risk characteristics and minimize the opportunity for bias or confounding. The major analytical studies used in the investigation of foodborne disease are cohort studies and case–control studies; other types of analytical studies include cross-sectional studies, case crossover, experimental, and randomized controlled trials.

Cohort Studies

Cohort studies evaluate the occurrence of disease in a carefully defined group of people. Cohort studies can be prospective or retrospective. Prospective cohort studies monitor a group or groups of persons over time for the development of disease in the presence or absence of suspected risk factors that are measured at the start of the study. The cohort is followed until the effect of the exposure occurs or until the end of the study period. Conversely, retrospective cohort studies identify a group of persons at some point in time in the past when they were presumably free of the disease under investigation. The cohort is then followed to the present. Large population-based cohort studies are typically time and resource intensive but have been used to estimate the incidence of foodborne pathogens in the community and to investigate the risk factors for the development of sequelae from foodborne disease. Cohort studies are frequently used in outbreak investigations when the outbreak has been linked to a specific group of people (e.g., wedding party or church supper). Statistical methods used in a cohort study involve the calculation of a relative risk.

Case–Control Studies

Unlike cohort studies, case–control studies begin with people who have the disease and compare their characteristics with a control group free from the disease for the presence or absence of potential risk factors. Because total population at risk is not known, case–control studies cannot be used to estimate the incidence of the disease. Advantages of case–control studies include the relatively small number of subjects and the shorter time frame needed to complete the study, which increases their suitability for the study of low-incidence diseases. Nested case–control studies are a variation on case–control studies and encompass the concept of a cohort study. In a nested case–control study, a large cohort is prospectively or retrospectively followed for the occurrence of a specific disease. These cases are then matched by age and sex with persons in the original cohort who did not develop the disease. This

approach is useful for diseases of low frequency, where analyzing the entire cohort would be overwhelming. Case–control studies have been used to examine risk factors for sporadic foodborne pathogens and are frequently used in foodborne disease outbreak investigations.

The main biases that occur in case–control studies are selection bias and recall bias. Selection bias means that cases or controls have different probability of being selected according to exposures or outcomes of interest, creating a biased measure of association. To minimize selection bias, cases should be representative and, optimally, include all diagnosed cases. Controls are selected from the same population group as cases in some symptomatic fashion (e.g., reverse directory or random digit dialing) and are usually matched to cases by age, sex, or other characteristics. Sometimes people diagnosed with a foodborne disease other than the one under study may be used as controls. Recall bias can occur when cases or controls recall exposures differently. For example, cases may try harder than controls to recall everything that they ate in the week before onset of the illness because they think what they ate might be related to their disease.

Conclusions

Epidemiology is a cornerstone of public health. It is only by gaining a rigorous and systematic understanding of patterns of disease that we are able to design rational interventions to reduce foodborne infections and improve human health. The basic tools of measuring disease occurrence and quantifying risk are through epidemiologic studies core skills with applicability to nearly all facets of foodborne disease.

See also: Public Health Measures: Foodborne Disease Outbreak Investigation; Surveillance of Foodborne Diseases. Risk Analysis: Estimating the Burden of Foodborne Disease

Further Reading

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DISCIPLINES ASSOCIATED WITH FOOD SAFETY

Food Safety Toxicology

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Glossary

Contaminant Any substance not intentionally added to food that is present in such food as a result of the production (including operations carried out in crop husbandry, animal husbandry and veterinary medicine), manufacture, processing, preparation, treatment, packaging, transport or holding of such food or as a result of environmental contamination. The term does not include insect fragments, rodent hairs and other extraneous matter.
Dose–response assessment Analysis of the relationship between the total amount of an agent administered to, taken up by or absorbed by an organism, system or (sub)population and the changes developed in that organism, system or (sub)population in reaction to that agent, and inferences derived from such an analysis with respect to the entire population. Dose–response assessment is the second of four steps in risk assessment.
End-point Qualitative or quantitative expression of a specific factor with which a risk may be associated as determined through an appropriate risk assessment.
Exposure assessment Evaluation of the exposure of an organism, system or (sub) population to an agent (and its derivatives). Exposure assessment is one of the steps in the process of risk assessment.
Hazard Inherent property of an agent or situation having the potential to cause adverse effects when an organism, system or (sub)population is exposed to that agent.
Risk The probability of an adverse effect in an organism, system or (sub)-population caused under specified circumstances by exposure to an agent.
Risk analysis A process for controlling situations where an organism, system or (sub)population could be exposed to a hazard. The risk analysis process consists of three components: risk assessment, risk management and risk communication.
Risk management Decision-making process involving considerations of political, social, economic and technical factors with relevant risk assessment information relating to a hazard so as to develop, analyze and compare regulatory and non-regulatory options and to select and implement appropriate regulatory response to that hazard.
Uncertainty factor Reductive factor by which an observed or estimated no-observed-adverse-effect level or other reference point, such as the benchmark dose or benchmark dose lower confidence limit, is divided to arrive at a reference dose or standard that is considered safe or without appreciable risk.

Introduction

Food safety is a public health issue, with the microbiological and chemical contamination of food being important causes of diseases. In addition to improving public health, effective food safety systems are also essential in maintaining consumer confidence in the food system, as well as to provide the basis for food trade regulation, which supports economic development. Food safety is a responsibility of everyone involved in the food chain. However, governments are responsible for providing an institutional and regulatory framework of food control. The Food and Agriculture Organization (FAO) of the United Nations, along with the World Health Organization (WHO), has played a leadership role in the development of risk analysis in food safety, which has demonstrated its capacity to improve the processes of decision making regarding food safety and the development of improvements in public health. Nevertheless, it should be mentioned that the paradigm of risk analysis is only part of an effective food safety system. Risk analysis, as defined by the Codex Alimentarius Commission is a process consisting of three components: assessment, management, and communication of risk (Figure 1).

Risk assessment is a scientific process intended to characterize the nature and likelihood of harm resulting from human exposure to agents in the environment, and provides the scientific basis for the decisions that may be taken at the stage of risk management necessary to protect human health, based on all relevant scientific data (including toxicological data), as well as identifies the uncertainties inherent to the food safety evaluation. For food, the focus is on the nature and likely harm related to the ingestion of food-associated toxicants. It consists of four steps (Figure 2).

The assessment of risk to human health resulting from exposure to food toxicants is one of the central activities of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the Joint FAO/WHO Meeting on Pesticide Residues (JMPR).

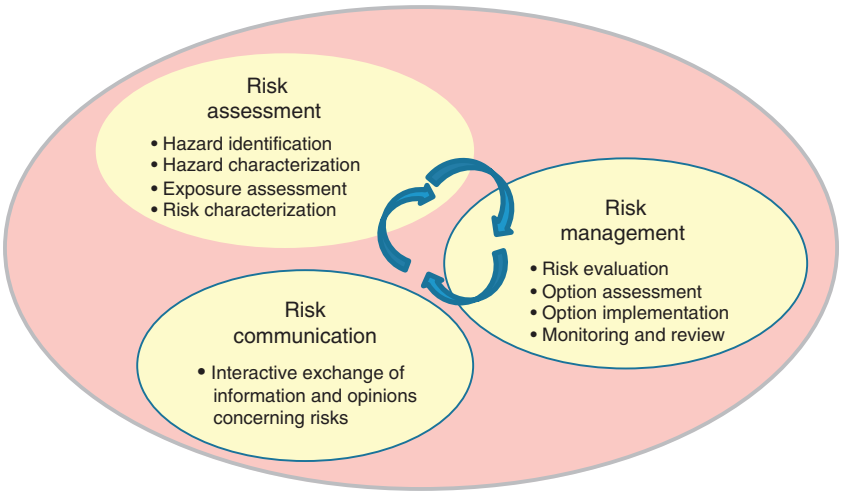

Figure 1 Components of risk analysis. Adapted from FAO/WHO (2009b).

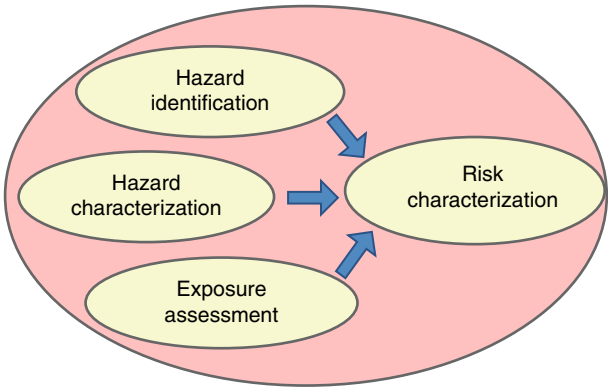

Figure 2 Steps of risk assessment. Adapted from FAO/WHO (1995) Application of risk analysis to food standards issues. *Report of the Joint FAO/WHO Expert Consultation*. World Health Organization, Geneva, CH, 13–17 March 1995. (WHO/FNU/FOS/95.3). Available at: http://www.who.int/foodsafety/publications/micro/en/march1995.pdf (accessed on 14 April 2013), and FAO/WHO (2005) *Food Safety Risk Analysis. Part I. An Overview and Framework Manual*. Rome, Italy. Available at: http://www.fsc.go.jp/sonota/foodsafety_riskanalysis.pdf (accessed on 14 April 2013).

Classification of the Main Groups of Foodborne Toxicants

The main groups of potentially hazardous chemicals in food, including some representative examples, are shown in Table 1.

Analytical Methods and Development of Specifications

Validated analytical methods are needed for determining the concentration of food toxicants and its biotransformation products, in pharmacokinetic and toxicokinetic studies; residue depletion; and for the determination of additives, contaminants, and residues of veterinary drugs and pesticides in foods. The specifications for identity, purity, and chemical and physicochemical characteristics are essential in safety assessment of food toxicants. It must be ensured that any substance that is to be evaluated is well characterized for the presence of any impurity. It also should describe the manufacturing process of the target substance. It is worth mentioning that the safety assessment is only valid for products that do not differ significantly in their identity and quality profile used to generate confident data.

When necessary the form of application and distribution of residues that result from each application mode (e.g., pesticides and veterinary drugs) should be determined. For veterinary drugs the depletion of residues should be studied in each species, as well as, in the edible tissue and food of animal origin.

Toxicological Preclinical and Clinical Studies

The toxicological studies for hazard identification and characterization can be divided into:

- *In silico* studies: performed to denote computer simulations that model a laboratory or natural process.
- *In vitro* studies: performed in laboratory cultured cells, tissues, or organs of animals or humans.
- *In vivo* studies: performed in laboratory animals or humans.

These studies are applied in hazard identification, definition of the exposure conditions necessary to observe the adverse effects, and in the dose–response evaluation (hazard characterization). Both JECFA and JMPR consider the data from *in vitro* and *in vivo* studies for risk assessment. However, despite the recent advances in *in vitro* studies, these approaches do not currently allow the replacement of animal testing for most of the adverse effects of concern (endpoints). No animal species is an ideal experimental model; there are evidences that preclinical studies generally provide an effective evaluation of the toxicity potential of the toxicants, as well as provide data for a critical interpretation. Further, all studies used in risk assessment of food toxicants shall be conducted in accordance with the principles of good laboratory practice.

Table 1 Main groups of foodborne toxicants

Agricultural chemicals: insecticides, herbicides, fungicides, and fertilizers
Veterinary drugs and feed additives: antibiotics, hormones, and other veterinary drugs
Environmental and industrial pollutants: arsenic, selenium, fluorine, mercury, radionuclides, polychlorinated biphenyls, polychlorinated dibenzodioxins and dibenzofurans, and polybrominated biphenyls
Chemicals migrating from packaging materials and containers: lead, tin, copper, and zinc
Unintentional toxicants from food processing: polycyclic aromatic hydrocarbons; alcohols; bacterial toxins; biogenic vasoactive amines; nitrates, nitrites, and nitrosoamines; acrylamide; chlorinated propanols; phthalates; and bisphenols
Mycotoxins: aflatoxins, ochratoxins, sterigmatocystin, zearalenone, fumonisins, trichothecenes, patulin, citrinin and citreoviridin, ergot toxins, and many other mycotoxins
Bacterial toxins: staphylococcal enterotoxins, *Clostridium botulinum* toxins, *Bacillus cereus* toxins, Bongkrek acid
Endogenous plant toxicants: lectins or hemaglutinins, ricin, enzyme inhibitors, alkaloids, cyanogenic glucosides, phytoestrogens, glucosinolates, coumarin, thujones, anisatin, toxic amino acids, toxic lipids, oxalates, fluoroacetates, bracken toxins, saponins, grayanotoxin, and mushroom toxins
Animal endogenous toxins: prions, phytanic acid, avidin, and tetrodotoxin (puffer fish poisoning)
Food additives: natural and artificial colorants, artificial sweeteners, acidulants, anticaking agents, antimicrobial agents, antioxidants, emulsifying agents, sequestrants, stabilizers, flavorings, and others

The safety evaluation is not a standardized process. Thus, not all toxicological tests must compulsorily be conducted. In general, pharmacokinetic studies (absorption, distribution, metabolism, and excretion) of a substance are important to the extent that they assist in the selection of species to be tested and the choice of doses for toxicity studies. The short- and long-term tests are performed to assess systemic toxicity. They identify target organs for toxicity and may indicate the need for additional or more specific tests (e.g., neurotoxicity or immunotoxicity). The effects of the test on a range of parameters indicative of toxicity are assessed, including observational, functional, biochemical, and pathological parameters. Generally, studies are conducted in two species, one rodent and one nonrodent, or in two rodent species, and in both the sexes to maximize the chance to verify any effect (hazard identification). Often, the tests also include long-term carcinogenicity tests in two species of rodents. Preferably, testing should be conducted in a manner that best relates to the scenarios of human exposure. The choice of dose should take into account anticipated human exposure, and the frequency and duration of exposure. For food toxicants, their administration in repeated doses in experimental animals is usually done through diet, by gavage, or via drinking water.

The doses have to be chosen so that toxic effects are produced at the highest dose level tested, with lower dose levels producing graded responses, and with no adverse effects at the lowest level. The study should be adequate to allow for determining a reference point for hazard characterization, also known as point of departure (POD), such as a no observed adverse effect level (NOAEL) or a benchmark dose (BMD) which is a dose that produces a low but measurable adverse response (**Figure 3**).

In the studies of carcinogenicity and developmental toxicity, comparison of the data obtained in the test with historical control data may be needed to understand the meaning of a particular result. Positive results in carcinogenicity studies in rodents require careful interpretation in relation to mode of action, the possible interspecies differences, and to extrapolate from high doses to low doses. Genotoxicity studies can provide information about the mode of action of substances that are carcinogenic.

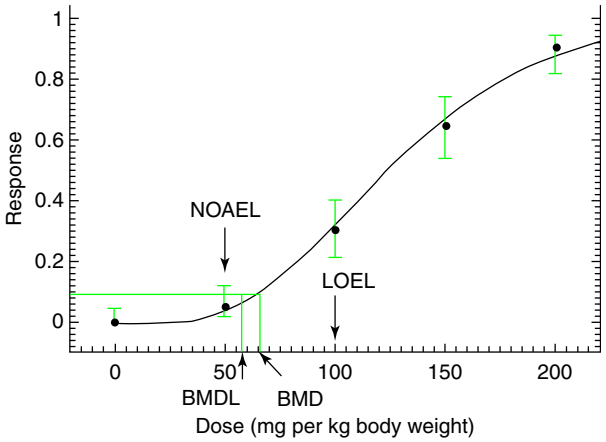

Figure 3 PODs in toxicological studies. Adapted from FAO/WHO (2004) *Technical Workshop on Residues of Veterinary Drugs without ADI/MRL*. Bangkok, Thailand, 24–26 August 2004. Available at: http://www.fao.org/docrep/008/y5723e/y5723e00.htm#Contents (accessed on 14 April 2013).

Acute toxicity studies as a result of short exposure, for example, metals, mycotoxins, veterinary drugs, and agricultural chemicals, may be useful. The JMPR establishes the acute reference dose, which is an estimation of the quantity of a specific substance in the food, normally expressed on body weight basis, which can be ingested in a period equal to 24 h or shorter without appreciable health risk for the consumer.

Information obtained from clinical studies in humans are of great importance in risk assessment of food toxicants. These information may be obtained from epidemiological or clinical studies. The crucial points of the clinical studies in humans are ethics, professional and legal controls for human studies, and the circumstances in which the study should be adequately developed.

Dose–Response Assessment

The dose–response evaluation is an essential part of hazard characterization. It is used to derive health-based guidance

values, such as the acceptable daily intake (ADI) or tolerable daily intake (TDI), which are levels considered to be 'without appreciable health risk.' The interpretation of dose–response data usually requires knowledge of exposure levels that do not produce a measurable adverse effect, and of the relationship between the increase in incidence, severity, or the nature of the effect and increased exposure.

Extrapolation is a essential part in all risk assessments. In most cases, data from the dose–response evaluation results from preclinical experiments using doses that significantly exceed human exposure. For the dose–response analysis, two aspects of extrapolation need to be considered:

1. Extrapolation from animal exposure data to humans (interspecies extrapolation).
2. Extrapolation to allow for possible differences in response between humans (intraspecies extrapolation).

The methods employed in the extrapolations range from the use of uncertainty factors to models based on differences in toxicokinetics and toxicodynamics between humans and experimental animals, as well as variability among different individuals.

Derivation of Health-Based Guidance Values

The setting of these parameters provides quantitative information of risk assessment used for risk management. These values arise from the dose–response evaluation for the most relevant endpoint, on the more relevant species in the preclinical studies.

Usually, the first step is to set the NOAEL (which is the highest concentration of a toxicant expressed as mg per kg body weight per day, which does not cause dysfunctions in animals) or, sometimes, the lowest observed adverse effect level as the POD.

The BMD has also been used as the POD to derive health-based guidance values. BMD is defined as the lower confidence limit of the dose calculated to be associated with a given incidence (e.g., incidence of 5% or 10%) of an estimated effect based on all toxicological data concerning the effect in the study.

The dose–response evaluation can also be used to establish a dose associated with a negligible increase of an adverse response (e.g., one in a million) relative to its background. Also, for substances that do not exhibit a safe threshold of exposure to adverse effects (e.g., genotoxic and carcinogenic substances) the calculation of a margin of exposure (MOE) is conducted, which is the ratio of POD (lower one-sided confidence limit of the BMD) with the theoretical or estimated exposure level (Figure 4).

ADI values are established for food additives, pesticides, and veterinary drugs used in food production. It is established based on the lowest relevant POD in the most sensitive species, and it is defined as the amount of a substance, expressed in mg per kg body weight and as a range of zero to an upper limit, which may be ingested daily for even a lifetime without any damage to human health, based on toxicological data available at the time of the evaluation.

$$\text{MOE} = \frac{\text{Carcinogenic dose for 10\% of rodents (mg kg}^{-1}\text{ day}^{-1})}{\text{Average human exposure (mg kg}^{-1}\text{ day}^{-1})}$$

Figure 4 Example of calculating the MOE for genotoxic and carcinogenic substances.

TDI values are established for unavoidable contaminants in foods. The principles in establishing TDI values are the same as those used for the ADI. Both NOAEL and BMD approaches can be used as POD to establish the health-based guidance values for tolerable contaminants.

Assessment of Exposure to Foodborne Toxicants

For the assessment of exposure to toxicants via diet, the data on food consumption and the concentration of the food toxicant are considered. These data on exposure are compared with the ADI or TDI value, or with the POD (NOAEL; benchmark dose lower confidence limit (BMDL)) of the chemical of interest present in the food. Evaluations can be made for either acute or chronic exposures. Assessments of exposure via diet should cover the general population, as well as critical groups who are vulnerable, or whose exposure is expected to be significantly different from that of the general population (e.g., infants, children, pregnant women, the elderly, or vegetarians).

Characterization of Risk

This stage of the risk assessment integrates information from hazard characterization and their exposure assessment. Historically, different approaches have been used for risk characterization of food toxicants that have a threshold for adverse effects observed, and those that do not have a threshold. The health-based guidance values (ADI and TDI) are established for substances producing adverse effects that exhibit a threshold of toxicity. In the risk characterization for these types of substances, the ADI or TDI values are compared with the estimated or determined human exposure.

Where exposures exceed the health-based guidance values, it does not allow to infer the extent of the risk to those individuals exposed to these higher levels, as the ADI and TDI values incorporate uncertainty factors. An exposure that occasionally exceeds the ADI or TDI value does not necessarily imply that adverse health effects will occur in humans. The risk characterization shall consider the uncertainty and variability. Uncertainty refers to the limitations of the information available to the risk assessor about the data and models used. The variability reflects the inherent biological heterogeneity, either in exposure or response. The uncertainty can be reduced when the quantity or quality of the information is improved. The characterization of exposure variability via diet may be improved by better information, but the variability cannot be eliminated.

For toxicants that are genotoxic and carcinogenic, the traditional hypothesis is that there is no threshold dose and that some degree of risk may exist in any exposure level. Thus, ADI or TDI values are not established for this type of compounds. The substances that are both genotoxic and carcinogenic

are not accepted for use as food additives, pesticides, or veterinary drugs.

Principles Related to Specific Groups of Substances

Many toxicants evaluated by JECFA are present in food in low concentrations, such as flavorings, extraction solvents, enzymes, and others. In these cases, the concept of Threshold of toxicological concern (TTC) is applied. The TTC concept considers that toxicity is a function of both the chemical structure and exposure time. Thus, by knowing the chemical structure and low exposure via diet it is possible to provide guidance based on scientific evidence, when there is negligible probability that damage will be induced. This concept does not propose the replacement of risk assessment used for toxicants for which complete toxicity data are available.

The TTC approach uses threshold values of exposure for which there is a very low probability of any appreciable risk to human health. These TTC values are derived from existing data on toxicity of toxicants classified into three classes – I, II, and III – for 1800, 540, and 90 mg per person per day, respectively. As the threshold values for human exposure are compared with known or anticipated exposure, the TTC approach requires adequate estimates of human exposure.

The safety assessment of substances that are consumed in relatively large amounts, such as modified starches, nutrients and related substances, as well as nontraditional food, presents additional problems. In assessing the safety of these substances, secondary constituents or processing impurities may assume a greater importance than usual.

Interactions of Toxic Substances

Interactions between chemicals administered to humans at high doses have been known for many years in the field of pharmacology. However, these experiences may not be directly useful for predicting toxic effects of mixtures of food toxicants given that exposure levels of the general population are relatively low. However, the main emphasis has to be laid on the identification of the basic principles for combined actions and interactions of toxicants and on the current knowledge on effects of exposures to mixtures of food additives, feed additives, veterinary drugs, agricultural and industrial chemicals, and environmental contaminants.

There are many possibilities for interaction between toxicants because of the complex structure and functions of the cellular systems. Recently, a number of examples of endocrine-disrupting chemicals, reproductive toxicity, and neurotoxicity have been described. The strongest interaction found in the literature was a 5-fold increase in the neurotoxicity of hexane when methyl isobutyl ketone was coadministered. It is therefore evident that interaction might be a real phenomenon and not just a theoretical possibility.

Although an expressive number of studies have concluded that toxicants can act additively, very few quantitative studies have been performed. Interaction has not been studied systematically, and the present state of knowledge does not allow general conclusions.

Conclusions

The use of Codex standards as a reference for the construction of national standards assists in harmonizing the overall implementation of food safety measures. Thus, it is suggested that countries consider the Codex criteria of risk analysis as an important normative reference. However, despite the adoption of harmonized standards, in many countries the main limitations for risk analysis is the lack of information regarding the quality of food products, resulting in deficiency of available data necessary to assess the risk.

The risk assessment of exposure to toxicants should make optimal use of the toxicological databases, in order to assess the safety of foods. The POD should be based on studies using the oral route of exposure.

Despite the difficulties and limitations that still exist, in our perception, there is an ongoing virtuous progress of risk analysis in food, both the global harmonization of the regulatory framework as well as the technological capacity and consciousness of the health authorities for the consumer needs for safer and quality food.

Further Reading

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RISK ANALYSIS

Contents

Risk Analysis of Hazards in Food: An Overview

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Glossary

Hazard A biological, chemical or physical agent in, or condition of food with the potential to cause an adverse health effect.

Risk A function of the probability of an adverse health effect and the severity of that effect, consequential to a hazard(s) in food.

Risk analysis A process consisting of three components: risk assessment, risk management and risk communication.

Risk assessment A scientifically based process consisting of the following steps: (i) hazard identification, (ii) hazard characterization, (iii) exposure assessment, and (iv) risk characterization.

Risk assessment policy Documented guidelines on the choice of options and associated judgements for their application at appropriate decision points in the risk assessment such that the scientific integrity of the process is maintained.

Risk characterization The qualitative and/or quantitative estimation, including attendant uncertainties, of the probability of occurrence and severity of known or potential adverse health effects in a given population based on hazard identification, hazard characterization and exposure assessment.

Risk communication The interactive exchange of information and opinions throughout the risk analysis process concerning risk, risk-related factors, and risk perceptions among risk assessors, risk managers, consumers, industry, the academic community and other interested parties, including the explanation of risk assessment findings and the basis of risk management decisions.

Risk management The process, distinct from risk assessment, of weighing policy alternatives in consultation with all interested parties, considering risk assessment and other factors relevant for the health protection of consumers and for the promotion of fair trade practices, and, if needed, selecting appropriate prevention and control options.

Risk profile The description of the food safety problem and its context.

Introduction

Risk analysis is a logical framework that underlies decision-making concerning all kinds of risks and not just those that pertain to food safety. The origins of risk analysis can be traced back to the era of atmospheric nuclear testing when health physicists in the United States first used risk analysis to assess the potential adverse health impact posed by the presence of

radionuclides in food and water. In 1983, the National Research Council of the US National Academy of Sciences in a report entitled *Risk Assessment in the Federal Government: Managing the Process* described the approach.

One of the most important advantages of the risk analysis approach is that it provides a clear and transparent framework for objective and structured decision-making in food safety. It delineates the responsibilities of governmental authorities and, in particular, fosters the separation of risk assessment from risk management functions. It also facilitates communication and interaction between risk assessors and risk managers as well as among all stakeholders, including industry, consumer organizations, and civil society.

Each of the four components of risk assessment and management are presented. However, only a cursory mention of risk communication is given because a full discussion would go beyond the scope of this article. Risk communication is an essential function that encompasses the whole process, but is particularly important between the risk manager and risk assessor. Although the principles and process of risk analysis are similar, the differences between chemical and microbial risk analysis are great enough to warrant separate discussions.

Background

Growing public concern for food safety and challenges posed by the modern food supply system, including globalization and new technologies, have underlined the need for more objective and transparent methods for decision-making based on the concept of risk analysis. Subsequently, governments are increasingly adopting the risk analysis framework as a tool for managing potential risks to human health posed by hazards in food. Risk analysis is a process that consists of three main components as shown in **Figure 1**.

The ever-increasing global trade during the past few decades in both raw commodities and processed foods has also led to the need for an intergovernmental body to protect the health of consumers and ensure fair trading practices. In 1963, the World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO) established the Codex Alimentarius Commission (Codex), which has 184 member countries, including all of the main food exporting and importing countries. Codex has become a model for international cooperation and has adopted 186 food standards, 46 codes of practice and related texts, and maximum limits covering 292 food additives, 218 pesticides, and 49 veterinary drugs. These are collectively referred to as the *Codex Alimentarius*. The work of Codex relies basically on risk assessments performed by WHO and FAO scientific committees, such as the Joint FAO/WHO Expert Committee on Food Additives (JECFA), Joint FAO/WHO Meetings on Pesticide Residues (JMPR), and the Joint FAO/WHO Meetings on Microbiological Risk Assessment (JEMRA) as well as other *ad hoc* expert meetings. Although compliance with Codex Alimentarius was voluntary, much of the international trade in food met Codex requirements. However, trade disputes over health and safety requirements continued. In some cases, such requirements constituted nontariff barriers to trade.

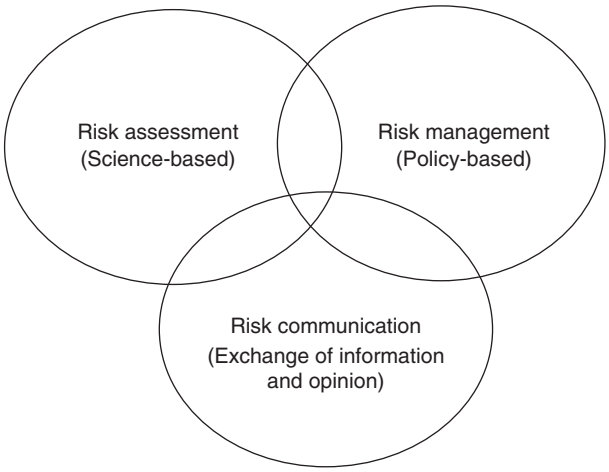

Figure 1 Components of risk analysis.

Under the General Agreement on Tariffs and Trade (GATT), countries negotiated reductions in tariff barriers to trade, including food, and it was recognized that nontariff barriers based on reputed health and safety requirements could undermine such negotiations. To address this concern, the World Trade Organization (WTO), which succeeded GATT in 1995, adopted as part of its founding texts the Agreement on the Application of Sanitary and Phytosanitary Measures (often referred to as the SPS Agreement). This agreement sets forth principles regarding the establishment of health and safety regulations for food and, in particular, that they be based on sound scientific assessment of the risk.

In addition, the SPS Agreement imposes other disciplines on countries in developing their food safety requirements, including nondiscrimination, transparency, and acceptance of equivalent approaches to achieve the same level of health protection. In this regard, WTO referred to the standards, guidelines, and other recommendations of the *Codex Alimentarius* as representing the international consensus regarding health and safety requirements for food and countries in compliance with Codex Alimentarius were assumed to meet the requirements of the SPS Agreement.

The impact of the SPS Agreement on the Codex Commission and countries would be game changing. Countries would be obligated to follow the Codex Alimentarius unless they could scientifically justify their need for their divergence using risk analysis. The effect was twofold, namely that *Codex Alimentarius* became essentially mandatory and that risk analysis became the foundation for standard setting at both the international and national levels. In response, Codex has made the use of risk analysis explicit in its work and has developed several guidance documents to promote its application at the national level.

Currently, risk analysis has become the internationally agreed model for the formulation of policies and legislation to manage foodborne hazards. The risk analysis process is now used to guide the development of a range of risk management options, including both mandatory and voluntary measures. Risk analysis has had a significant impact on the regulation of the food industry as well as on the information and advice provided to consumers. In many countries, consumers have

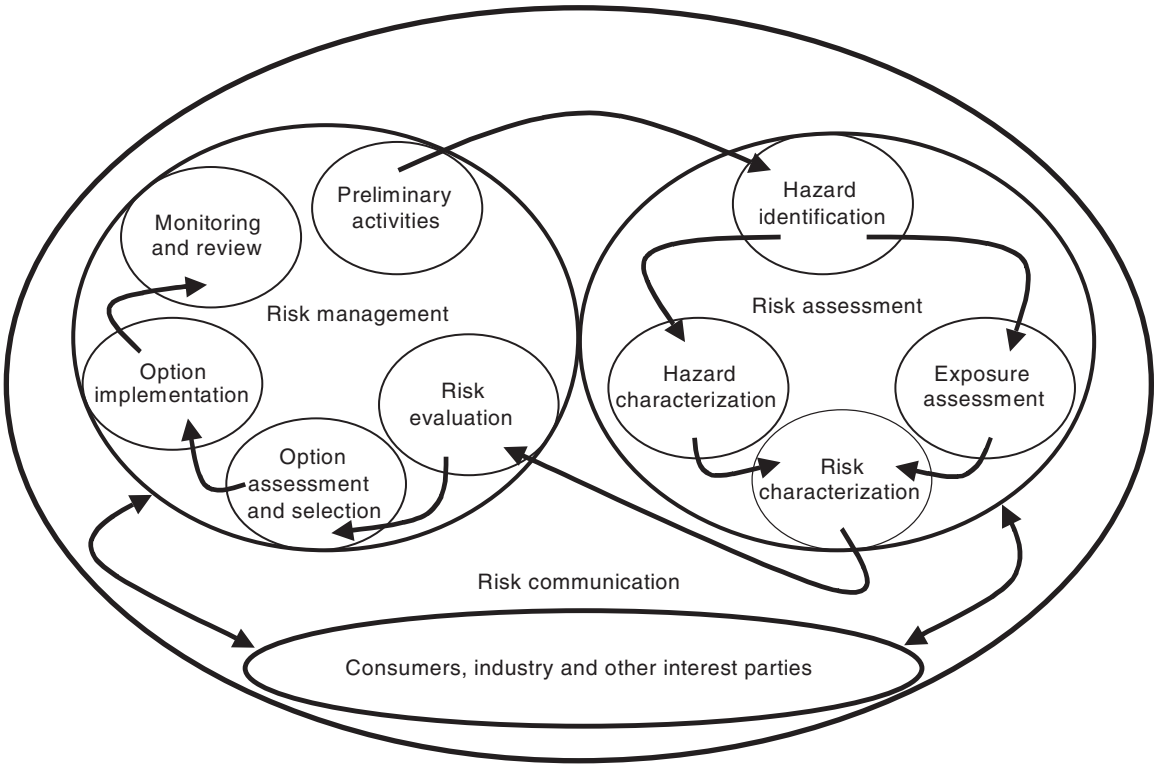

Figure 2 Risk analysis process.

benefited from greater protection from hazards in food afforded by the application of risk analysis. However, harmonization of risk analysis procedures has not been fully achieved and many developing countries are only now beginning to routinely apply it. In addition, the socio-economic and cultural differences among countries often lead to different consumer opinions about safety. The SPS Agreement recognizes the right of countries to establish their appropriate level of protection (ALOP) provided that it does not unjustifiably impede trade.

Risk Analysis Process

Risk analysis is a tool used by the risk manager in making complex decisions concerning the safety of the food supply. Risk analysis can also be used in preventing fraud and unfair trading practices based on contrived health and safety claims. As the legal authority designated to protect the food supply, risk managers in government have the overall responsibility for the risk analysis process. In many countries, more than one risk manager may be designated to cover different parts of the food supply network, such as primary production, processing and manufacturing, wholesale and retail markets, and food service establishments. As food safety is an essential public health activity, conflicts of interest by the risk manager should be avoided in order to assure integrity and confidence in the process.

Each of the components of risk analysis, namely risk assessment, risk management, and risk communication, has been the subject of an FAO/WHO expert consultation in 1995, 1997, and 1998, respectively. Each consultation elaborated further details of each component. For example, the risk assessment process has been defined as consisting of four elements, namely hazard identification, hazard characterization, exposure assessment, and risk characterization. Similarly, risk management also consists of four elements, namely risk evaluation, option assessment, option implementation, and monitoring and review. However, risk communication links all of the processes together from the critical exchange between risk assessors and risk managers to the important on-going dialog with industry, consumers, and other stakeholders. Figure 2 provides a schematic overview of the risk analysis process with the steps in the process shown with arrows.

Note that the risk assessment process is initiated by preliminary activities carried out by the risk manager. Although these activities normally precede the risk analysis process, they are particularly important in crisis situations where the experience and judgment of the risk manager is necessary in the face of uncertainty. Based on available information of the risk posed by hazard–food combination, the risk manager may (or may not) decide to commission a risk assessment. The purpose and scope of the risk assessment, including relevant risk assessment policy and the preferred form of the output, need to be communicated clearly to the risk assessor by the risk manager. Although the function of risk management and risk assessment should be separated to the extent possible, maintaining effective and continual communication between the risk manager and risk assessor is essential during all steps of the risk analysis process.

Risk Assessment

Hazard Identification

The first step in a risk assessment process is hazard identification, which identifies agents capable of causing adverse health effects, which may be present in a particular food or groups of foods. In this regard, epidemiological, biological, and other pertinent information and expert knowledge are used to link potential hazards and their sources in food to illness in consumers. Obviously, a chemical that produces toxic effects shortly after ingestion of small amounts can be easily identified as a hazard. Similarly, biological agents that produce disease in humans can be identified as potential hazards. However, for both chemicals and biological agents, the long-term adverse health effects may be difficult to determine. This is particularly a problem for chemicals that can produce adverse health effects, such as cancer, after long-term low-level exposure. Physical hazards, such as stones, glass, or metal fragments, can produce serious injuries, but are usually not routinely found in food.

Hazard Characterization

Hazard characterization considers the dose level at which a specific adverse effect or disease can occur in order to establish an exposure level considered to be acceptable or tolerable. For example, the standard health reference value used at the international level to indicate the safe level of intake for a chemical, such as a food additive, pesticide, and veterinary drug, is the acceptable daily intake (ADI), which is the estimate of the amount of a substance in food and/or in drinking water, expressed on a body weight basis, which can be ingested daily over a lifetime without appreciable health risk to the consumer.

For microbial hazards, the hazard characterization consists of estimating the lowest infectious dose for the general population and for any vulnerable groups.

Exposure Assessment

For intentionally added chemicals, methods have been developed to predict the likely exposure to populations based on the intended use of the chemical. Other methods to assess short-term exposure have also been developed for those chemicals that pose acute risks. For contaminants, monitoring data on such chemicals in the food supply are used in conjunction with food consumption patterns to estimate exposure.

For biological hazards, exposure assessment attempts to model the level of contamination at the various steps in the food production to consumption continuum, i.e., farm to fork.

Risk Characterization

Risk characterization is the last of the four steps of risk assessment. It is defined as an estimation of the probability of occurrence and severity of known or potential adverse health effects in a population based on the preceding steps of hazard identification, hazard characterization, and exposure assessment. It comprises the results of the risk assessment in the form of risk estimates and risk descriptions and provides the best available science-based evidence to support food safety management. If requested by the risk manager, it may also provide information on a range of possible decisions for ensuring consumer safety.

Risk Management

As discussed earlier, the risk manager will prepare a preliminary risk profile based on available information. After the risk assessment is completed, the risk manager will weigh policy alternatives in the light of the risk assessment and other factors and, if necessary, select and implement an appropriate risk management measure. The risk manager will also establish relevant surveillance and monitoring activities so that the effectiveness of the risk management measure can be assessed and, if necessary, modified. These risk management activities are part of the four-step risk management process, namely risk evaluation, option assessment and selection, option implementation, and monitoring and review (refer to Figure 2). Each component is briefly discussed in the following sections.

Risk Evaluation

After the risk has been characterized, the risk manager will use this and other information to complete the preliminary risk profile, which is the main task of risk evaluation. Typically a risk profile consists of a description of the food safety situation, including the hazard, the product or commodity involved, how and where the hazard enters the food supply, frequency, distribution and levels of the hazard in food, pathways by which consumers are exposed, possible adverse health effects, the quantitative (or qualitative) estimate of the risk, the distribution of the risk in the population, perception of consumers of the risk and values involved, and information on the prevention and control measures undertaken by other various countries, including recommendations from Codex. The evaluation may also consider other factors, such as the nature of the hazard or food that might affect the availability and feasibility of risk management options.

Option Assessment and Selection

Option assessment includes the identification of risk reduction measures and the selection of the preferred option based on the policies, goals, and priorities of risk management. Risk management measures may range from mandatory to voluntary and may be implemented by the government, the food industry, or consumers. The efficiency and effectiveness of an option and its cost to the industry and the government are important considerations in option assessment and selection.

The establishment and enforcement of maximum limits or levels is the most common risk management option. For certain other hazards, guideline levels are recommended for use by industry. Setting a ‘zero’ or ‘negligible’ tolerance for a hazard in food appears to be a simple option, but the method of analysis used to determine ‘zero’ must be specified. If not, ‘zero’ or ‘negligible’ will continual go down as methods of

analysis become more sensitive. These limits are often used in the absence of an agreed-upon acceptable risk as in the case of ALARA, which stands for 'as low as reasonably achievable'.

In considering possible health and safety requirements for food, countries are obligated to use Codex standards, guidelines, and other recommendations unless they can justify that a more stringent standard is necessary to protect their populations based on a sound scientific assessment of the risk. WTO members are also required to observe other disciplines, including transparency and consistency. Furthermore, the option selected should not be unnecessarily restrictive to trade. Decisions on appropriate levels of protection should be determined primarily by human health considerations even when consideration of other factors (e.g., economic costs, health benefits, technical feasibility, and societal preferences) may be relevant in some risk management contexts.

In other cases, codes of practice at various levels of the food supply network may be appropriate. Some risk management options involve changing consumer behavior through product labeling or public education programs. Education programs may be targeted to specific subgroups of the population, such as pregnant women, and may include specifically tailored risk management messages. As such, they rely to a great extent on professional risk communicators. Risk managers need to be good risk communicators given their overall responsibility for maintaining a dialog with all stakeholders.

Option Implementation

Once a decision is taken, the option should be implemented in a uniform and consistent manner within the country and with regard to both domestic and imported products. As the primary responsibility for the safety of food rests with the food industry, the implementation of food safety measures is often carried out by industry. For example, the levels of contaminants in food must conform to the limits prescribed by food safety regulations. To meet these requirements, the food industry will often incorporate appropriate prevention and control measures into their codes of practice and quality management systems. In particular, the application of the Hazard Analysis and Critical Control Point (HACCP) system should use maximum limits and levels as the basis for setting critical limits for hazards in the HACCP plan.

It should also be noted that both the government and the food industry have major roles to play with regard to consumer education. Obviously, product labeling is important for the safe handling of specific packaged foods. For broader food safety concerns, the government and consumer organizations can promote safe food practices in the home through a number of channels, which can be targeted toward various members of the family, especially school children.

Monitoring and Review

Finally, risk managers need to establish means for monitoring the effectiveness of the selected option in reducing the risk. For an adverse health effect that occurs shortly after exposure, disease surveillance and related epidemiological techniques may be used to assess the disease burden for a particular hazard. For chemicals, however, adverse effects resulting from long-term, low-level exposures are often expressed only after long induction periods, in some cases decades. Although epidemiological studies may reveal the current disease burden posed by previous exposures, they are insensitive to recent exposures. Therefore, monitoring of chemicals in food and total diet is the most practical way of assuring the safety of the food supply. In some special cases, biomonitoring can be useful in assessing exposure and the impact of risk management measures. Based on the review of monitoring information, the risk manager may commission a further risk assessment or consider alternative management options.

Risk Communication

Risk communication is the interactive exchange of information and opinions throughout the entire risk analysis process concerning risk. It is important not only between risk assessors and risk managers, but also with consumers, the food industry, and other stakeholders. Providing meaningful, relevant, and accurate information in clear and understandable terms tailored for specific audiences is usually the responsibility of the risk manager. Obviously, good risk communication skills are important for obtaining cooperation and support of stakeholders, particularly in the implementation of a risk management measure. Risk communication is also important in crisis situations and the risk analysis framework provides a useful structure when there is uncertainty about the extent and nature of the risk.

Conclusion

The introduction of risk analysis in food safety has provided governments with a powerful tool for assessing and managing risks posed by potentially hazards in food. The approach provides a logical framework for making complex public health decisions in context of economic, social, and political considerations. The risk analysis approach can foster better and more interactive communication among all parties involved in risk analysis, including industry and consumers. Food safety risk analysis has been recognized by global health and trade organizations as the primary basis for establishing health and safety requirements for foods. As a consequence, food safety authorities at the international, national, and local levels should make greater efforts to understand and apply the principles and procedures of risk analysis, particularly those used by relevant international organizations.

See also: Risk Analysis: Risk Assessment: Microbiological Hazards; Risk Assessment: Principles, Methods, and Applications; Risk Assessment: Chemical Hazards; Risk Communication: Biological Hazards; Risk Communication: Chemical Hazards; Risk Communication; Risk Management: Application to Biological Hazards; Risk Management: Application to Chemical Hazards; Risk Communication: Diet, Nutrition, and Health

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World Health Organization: Food Safety.

Risk Assessment: Principles, Methods, and Applications

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Glossary

Appropriate level of protection (ALOP) A metric advocated by the World Trade Organization reflecting the level of public health protection achieved in a country; competent authorities may establish a similar metric on the basis of relevant policy decisions on public health protection (then more appropriately referred to as a public health goal).
Exposure assessment Exposure assessment is an activity within risk analysis that establishes the dynamics of a hazard in a food and food environment up to the point of consumption of the food and assesses exposure of a consumer population to that hazard through the food.
Food Safety Objective (FSO) A metric advocated by Codex Alimentarius and established by a competent authority that represents the maximum level of a hazard in a food at the point of consumption that may be tolerated in the light of an ALOP or relevant policy decisions on public health protection.
Hazard characterization Hazard characterization is an activity within risk analysis that establishes the inherent properties of a hazard and the consequences for a consumer population being exposed to that hazard.
Hazard Identification Hazard identification is an activity within risk analysis that evaluates the likely association between a particular hazard and a food and the causal relationship of this association with the concerned risk to a consumer population.
Risk Risk is a measure of an undesirable outcome; in food safety, risk is a combination of the impact that a hazard may have on consumers following exposure and the probability of actual exposure.
Risk analysis Risk analysis is a systematic framework advocated by Codex Alimentarius to determine the level of risk to a consumer population due to hazards possibly associated to foods.
Risk characterization Risk characterization is an activity within risk analysis that combines knowledge derived from hazard characterization and exposure assessment into a measure of the level of risk to a consumer population due to a particular hazard possibly associated to a food.
Sensitivity analysis Sensitivity analysis is a systematic assessment of the impact of individual input parameters used in risk characterization for the purpose of determining the influence of such parameters on the risk characterization outcome relative to insight in their robustness.
Uncertainty Uncertainty of input parameters may relate to either their natural variability (aleatory uncertainty) or lack of specific knowledge on the side of the risk assessor (epistemic uncertainty). The latter may be reduced by gathering more knowledge.
Variability Variability (aleatory uncertainty or natural variability) relates to inherent variability in the physical world; it can be better described but not reduced by gathering more knowledge.

Introduction

The management of product safety can be performed using a number of different approaches, falling in one of two categories: hazard-based or risk-based decision making. In hazard-based decision making, the mere occurrence of a hazard in a food product is the basis for decision making, whereas in risk-based approaches, the basis for decision making is likely the exposure of a consumer to a hazard present in a food combined with the degree of severity of the impact of exposure on a consumer. Although there is possibly a place for both types of approaches when managing food safety, in the public context, there is a move toward the consistent use of risk-based management within an overall framework referred to as risk analysis. Risk analysis consists of risk management, risk assessment, and risk communication. In this framework, risk managers are responsible for making the ultimate decision about product safety and risk assessors are responsible for bringing together the scientific information in support of decision making. This article discusses risk assessment as the science-based process within risk analysis, explaining the concept of risk, the principles underlying risk assessment, and the generic process followed in risk assessment. Examples of the application of risk assessment for different potential hazards, such as chemicals and microorganisms in food, are given where it serves to illustrate key differences.

Risk

Risk is a measure of the probability and consequence of uncertain future events. It is the chance of an undesirable outcome.

That outcome could be a loss or a potential gain that is not realized. It is often described by the expression:

$$\text{Risk} = \text{Probability} \times \text{Consequence}$$

This conceptual model is not conveying the complexity of models with which risks are actually calculated; clearly, few are this simple. Instead, it conveys the fact that both elements must be present for a risk to exist. If an event of any consequence has no probability of occurring, there is no risk. Likewise, when there is no unfavorable consequence, there is no risk no matter how likely the event is. Risk assessment tends to focus on assessing the probability and consequence of specific events.

In food safety, the risk model can for instance be translated as follows:

$$\text{Risk} = \text{Exposure to hazard} \times \text{Consequence of exposure}$$

The probability is reflected in the likely exposure of a consumer to a hazard in food, with exposure being determined by the hazard concentration and prevalence in the food as well as the amount and frequency of the food consumed. The consequence is characterized by the resulting outcome of exposure for the consumer, which usually is an adverse health effect, i.e., illness. There are many opportunities for potential gain concerning food, but they tend to be more properly considered as nutritional concerns rather than food safety concerns.

Risk Assessment

Risk assessment is the science-based component of risk analysis that concerns itself with characterizing the probability of exposure to a hazard and the consequences of exposure for the consumer. It is a set of logical, systematic, evidence-based, analytical activities designed to gain an understanding of specific risks and to measure and describe them to the fullest extent possible. A risk assessment intends to answer risk manager's questions about the identified risks and provides the objective information needed for decision making. It describes and addresses uncertainty in intentional ways and then characterizes the relevant uncertainty encountered in the assessment that could influence the decision or change decision outcomes.

Risk assessments may address all relevant dimensions of risks in a decision problem. These may include:

- Existing risk
- New risks
- Future risk
- Risk reductions
- Residual risk
- Historical risk
- Geographical risk
- Transferred risk
- Transformed risk.

Informally, risk assessment is the work needed to answer the following questions:

- What can go wrong?
- How can it happen?
- How likely is it?
- What are the consequences?

In food safety, these questions help risk assessors (often referred to as analysts) to identify hazards and the sequence of events by which consumers become exposed to them, as well as the likelihood and consequences of exposure.

Risk Assessment Principles

The main principles underlying a good risk assessment include: following a systematic and structured approach, being objective and transparent, providing appropriate documentation, allowing for peer review and communication suitable for different stakeholders, and ensuring that the responsibilities of risk assessors and risk managers are separated. A good risk assessment meets a risk manager's information needs for decision making. It provides an objective, unbiased treatment of the available evidence and clearly links the evidence to its conclusions. It is also transparent, which relates to clearly detailing the rationale for the evidence selected and being honest about what is not known and how that affects an assessment's conclusions. Risk assessment is a structured and systematic process. It follows a methodology consisting of specific steps that provides for a thorough and consistent approach to the assessment of risks. Good risk assessment documents the details of the work to support current decision making as well as for future consideration. Documenting the assessment is important to peer and stakeholder review of the assessment and decisions based on it. In food safety, stakeholders include the risk manager, risk managers in other countries, consumers or consumer organizations, industry, and academia. A good risk assessment requires substantial interactions among risk managers and risk assessors, whereas the responsibilities associated to these two roles are kept separate. The functional separation of responsibilities is a key principle, which is difficult to adhere to where resources are limited and the two roles are only found in a single person. However, generally, the risk assessors should not influence the risk manager's decision for instance by altering the scope of the work, specific selection of evidence, or through the way that the results of the risk assessment are presented. Conversely, risk managers should not influence the integrity or objectivity of the risk assessment.

Defining Risk Assessment

No definition is going to meet the needs of the many and diverse uses of risk assessment, given the wide range of hazards found in the world and the many differences in the context that risk assessments can be utilized. Even within the food safety community of practice, multiple definitions can be found. The first formal attempt to provide a description of the risk assessment process was for cancer risk associated with the chemicals in the environment and it is usually traced to *Risk Assessment in the Federal Government: Managing the Process*, a report of the National Research Council in 1983. Although originally setup for chemicals, the principles in this early work have proven

robust for a wide range of chemical, microbial, and physical risks. It defined risk assessment as follows:

"Risk assessment can be divided into four major steps: hazard identification, dose–response assessment, exposure assessment, and risk characterization."

The steps were defined by the National Research Council as follows:

- Hazard identification: the determination of whether a particular chemical is or is not causally linked to particular health effects.
- Dose–response assessment: the determination of the relationship between the magnitude of exposure and the probability of occurrence of the health effects in question.
- Exposure assessment: the determination of the extent of human exposure before or after application of regulatory controls.
- Risk characterization: the description of the nature and often the magnitude of human risk, including attendant uncertainty.

The spirit of these definitions is also found in the Codex Alimentarius definition of risk assessment as it applies to a food context:

"Risk Assessment: A scientifically based process consisting of the following steps: (1) hazard identification, (2) hazard characterization, (3) exposure assessment, and (4) risk characterization."

The risk assessment steps, noticeably expanded to a wider range of hazards, have been defined by Codex as follows:

Hazard identification: The identification of biological, chemical, and physical agents capable of causing adverse health effects and which may be present in a particular food or group of foods.

Hazard characterization: The qualitative and/or quantitative evaluation of the nature of the adverse health effects associated with biological, chemical, and physical agents which may be present in food. For chemical agents, a dose–response assessment should be performed. For biological or physical agents, a dose–response assessment should be performed if the data are obtainable.

Exposure assessment: The qualitative and/or quantitative evaluation of the likely intake of biological, chemical, and physical agents via food as well as exposures from other sources if relevant.

Risk characterization: The qualitative and/or quantitative estimation, including attendant uncertainties, of the probability of occurrence and severity of known or potential adverse health effects in a given population based on hazard identification, hazard characterization, and exposure assessment.

Notably, these definitions use the term 'hazard' similarly for chemicals and microorganisms, namely to specify the chemical or the microbial agent (infectious cell or toxin). However, in toxicology, the term 'hazard' may rather be used to describe the adverse health effect to consumers, for instance which specific illness is caused or which particular organ is affected.

Hazard Identification

Hazard identification is primarily a qualitative process that describes the association of hazards to foods. The main hazards of concern are microorganisms or chemicals. The chemicals can be naturally occurring; food additives or colorants; or contaminants, such as pesticide residues. When construed broadly, food safety may include hazards, such as antimicrobial-resistant microorganisms, genetically modified (GM) organisms, too little or too much of a nutrient, nanotechnology hazards, and even plant and animal diseases. This discussion focuses on microbial and chemical hazards.

Why is hazard identification needed? In some cases, it is not known if the agent in question is indeed a hazard, i.e., capable of harm. For example, does a specific chemical cause cancer in humans? In other cases, the potential for harm is well established, as for well-known human pathogens for which there is ample epidemiological evidence. In any case, the purpose of the hazard identification step is to confirm that there is an association of the hazard of concern to the food(s) or food group considered. In formulating the risk manager's questions and in compiling the necessary scope and background for the risk assessor (which often is done by providing a so-called risk profile), risk managers may already bring together key details for hazard identification. The hazard identification step is an opportunity for risk assessors to signal to risk managers that the association assumed by the risk manager may not be correct or that there are other hazards to be considered. Every risk assessment begins with hazard identification, but hazard identification need not result in taking further steps in risk assessment. For instance, where the results of hazard identification lead to the conclusion that the hazard–food association is incorrect or that other hazards are more significant, the risk manager may decide to discontinue the particular risk assessment.

Hazard Characterization

Hazard characterization describes the adverse health effect or effects to the consumer that may result from exposure to the hazard. This step therefore considers demographics and health status information among other characteristics of the consumer or specific consumer groups in the population related to their vulnerability to the hazard. For chemical hazards, this step develops detailed information regarding the nature of the chemical and how it causes adverse health effects, for example, which organ(s) might be affected. For microbial hazards, hazard characterization considers characteristics of the pathogen related to adverse health effects, such as infectivity and toxin production, as well as possible impacts of the food matrix on their ability to cause adverse health effects. When possible, this step includes quantitative information in the form of a dose–response relationship between hazard concentration and the level of consumer health effect as well as probabilistic estimates of adverse outcomes. Data sources for hazard characterization include animal toxicity studies, cell line studies, clinical human exposure studies, and epidemiological data from investigations of outbreaks and illness.

The relationship between the magnitude of exposure (dose) to a chemical, biological, or physical agent and the severity and/or frequency of associated adverse health effects (response) defines the dose–response relationship. This relationship is often represented as a curve, such as the stylized dose–response curve shown in Figure 1. The dose–response

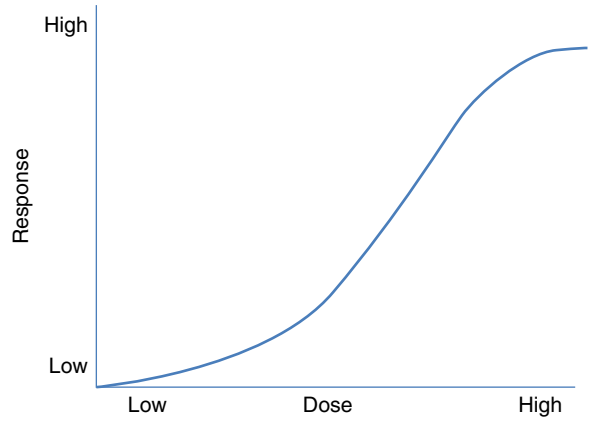

Figure 1 Conceptualized dose–response relationship.

curve is the primary health-consequence model used to characterize the adverse human health effects of chemicals, toxins, and microbes in food, but specific data may not always be available. When data are available, they are often characterized by variability and uncertainty. Exposure to chemicals typically leads to chronic adverse health outcomes, whereas exposure to microorganisms most often may cause acute effects. This difference has a major impact on the way dose–response relationships can be constructed and the data required.

A dose–response relationship may be used to describe the effect of a hazard on a typical individual or a large population. In the first case, a low response might be a low probability of illness, and a high response might be a high probability of illness. In the latter case, the response may vary from no detectable effects at low doses to subtle effects (such as tumors) in some subjects, severe effects in some to many subjects to death at high doses. The dose may be milligram per kilogram of bodyweight daily for a lifetime in the case of a chemical, such as a pesticide or the number of colony forming units (CFUs) ingested in the case of a pathogenic microorganism. Dose–response relationships involve consideration of the elements or factors related to the pathogen or chemical, the host and the matrix, insofar as they may modulate the response to exposure.

It is important to categorize the adverse health effects to meet the requirements of risk managers. Adverse health effects include such possible responses as infection, illness, hospitalization, fatalities, and/or long-term sequelae. Although individual sensitivity to a chemical hazard can vary, the types of adverse effects are generally similar for all members of a population. This is less the case for microbial hazards, for which significant differences have been noted in health responses of individual consumers to different hazard levels, and also specific subpopulations of particularly vulnerable consumers may exist within the overall population. Dose–response curves for subpopulations are highly desirable, but rare due to a general lack of data to develop these relationships.

In the absence of dose–response data, it is sometimes possible to relate the incidence of disease or other adverse health effects to eating occasions. This method requires extensive surveillance data that are also not always available. Qualitative characterizations are acceptable when there are insufficient data to support quantitative work.

Exposure Assessment

The exposure assessment step provides an estimate of the level of a hazard the consumers may be exposed to through food consumption. The exposure assessment integrates information about the hazard (e.g., prevalence and/or concentration, growth, and survival characteristics for microbial hazards), the food product (e.g., production methods, presence, ability to support growth of microbes, and food chain handling), and the consumer (e.g., the quantity and frequency of consumption, and handling practices) to produce the required estimates of the exposure in units that vary from per serving or per (sub)population for microbial hazards, to average daily intakes and lifetime exposures for chemical hazards.

Exposure assessments vary significantly between chemical and microbial risk assessments not only because chemicals are often concerned with lifetime exposures and microbial hazards with a single acute exposure but also because the microbial hazard is alive. Growth, attenuation, death, and cross-contamination possibilities over the life of a food product make microbial exposure assessments challenging. Chemical levels in foods often change little throughout the production process. Exposure assessments may be quantitative or qualitative. Quantitative assessments are preferred when practical. Exposure assessments and hazard characterizations can be done in any order, including concurrently.

Risk Characterization

In the risk characterization step, risk assessors integrate the evidence gathered in the previous steps. A characterization often includes one or more estimates of risk, risk descriptions, evaluations of risk management options, and estimates of changes in risk attributable to the management options.

A risk estimate is an estimate of the likelihood and severity of the adverse health effects, with attendant uncertainties, for a given hazard–food combination in a specified population. It may be quantitative or qualitative. The risk description is a narrative that bounds and defines a risk for decision-making purposes. The evaluation of risk management options requires the estimation of 'baseline' risks, understanding the public perception of the hazard, as well as inequities in the distribution of benefits and risks. The evaluation should include the effects of substitution risks (i.e., transformed and transferred risks) as well as an estimate of expected risk reductions under alternative mitigation strategies. The practical feasibility and monetary costs of alternative mitigation strategies must also be taken into account, although some consider this to be a part of risk management, whereas others consider it to be a part of risk assessment.

Roles and Responsibilities in Risk Assessment

The risk manager has a critical role in the risk assessment process. Although the management and assessment tasks are to be kept functionally separated, regular communication, cooperation, and collaboration between these two functions

are essential for success. Some specific examples of the risk managers' involvement in the assessment process include:

- assembling the risk assessment team,
- identifying the questions to be answered by risk assessors,
- providing sufficient resources and time to complete the risk assessment,
- understanding the risk assessment results and using them appropriately, and
- seeing that the purpose statement, scope, conduct, results, and use of the risk assessment is properly communicated to all interested parties.

Qualitative and Quantitative Assessments

Risk assessments can be qualitative or quantitative. When sufficient data and resources are available, a quantitative assessment is preferred, except where the risk manager's questions can be adequately answered in a narrative or categorical fashion. Quantitative risk assessment relies on numerical expressions of risk, which are generally more informative than qualitative estimates. They can be deterministic or probabilistic. The choice depends on the risk manager's questions, available data, the nature of the uncertainties, the skills of the assessors, the effectiveness of outputs in informing and supporting decision makers, and the number and robustness of the assumptions made in the assessment.

When quantitative risk assessment is not possible or necessary, nonnumerical qualitative risk assessment can be a viable and valuable option. It is especially useful:

- for noncontroversial and routine tasks,
- when transparency and consistency in handling risk are desired,
- when theory, data, time, or expertise are limited,
- for broadly defined problems, where quantitative risk assessment is impractical, and
- as a first iteration of a risk assessment, when uncertainty is great.

Qualitative risk estimates rely primarily on ratings (high, medium, and low), rankings (first, second, and third), and narrative descriptions. There is no internationally agreed approach on how to conduct a qualitative risk assessment. However, the better qualitative assessments include both a narrative descriptions of the risk and a qualitative risk estimate, which together provide a descriptive or categorical treatment of risk information in a formal, organized, and reproducible manner. Much of the relevant evidence in any given risk assessment is not numerical. Thus, a qualitative assessment compiles the available evidence and combines it in a logical and transparent manner that supports a statement of risk. Qualitative assessments reveal data gaps and can be useful in directing resources to productive areas of research. Their value stems from the ability to inform and support risk management decision making in complex situations.

Uncertainty and Variability

Risk analysis is for making decisions under uncertainty and in the face of variability. Risk assessors lack information because there are facts that they do not know, data that they do not have, the future is fundamentally uncertain, and because the universe is inherently variable. Uncertainty about the probability and consequence of a risk may be due to either or both 'epistemic uncertainty' (knowledge uncertainty) and 'aleatory uncertainty' (natural variability).

Epistemic uncertainty is due to a lack of knowledge on the part of the observer. Epistemic uncertainty arises from incomplete theory, incomplete understanding of a system, modeling limitations, and/or limited data. It reflects the risk assessors' level of knowledge about the components of the risk assessment. Assessors can be uncertain about what a true value is (e.g., the specific prevalence of *Salmonella enteritidis* in shell eggs). They may be uncertain about risk scenarios, i.e., the sequence of events that produce the risk. The model(s) used to estimate the risk may also be uncertain. Epistemic uncertainty is reducible in principle; more study, research, finding someone who knows what the assessors do not, and expert elicitation are common means of reducing this uncertainty. In some circumstances, it may be expensive, difficult, or even impossible to do so.

Aleatory uncertainty deals with the inherent variability in the physical world. Eggs are not all the same size, they may carry a varying numbers of *Salmonella enteritidis* cells, and people eat varying quantities of eggs prepared in a variety of ways. Natural variability is often attributed to a random process that produces variability among members of a population or of a quantity over time and/or space. It is, in principle, irreducible. In other words, natural variability cannot be altered by obtaining more information. More data may improve the characterization of the variability, but the variability will not be reduced.

Ideally the effects of uncertainty and variability should be separated in a risk assessment so that their effects on the risk estimate(s) and the answers to the risk manager's questions can be explicitly described for the risk manager. Risk assessment should address the potential for uncertainty to affect the outcomes of risk management options. It is the risk assessors' job to address uncertainty in models and their inputs and the risk manager's job to address uncertainty in the risk assessment outputs. To do this effectively, risk managers must understand the significant uncertainties and their implications for the risk assessment and the efficacy of risk management measures.

Sensitivity Analysis

Sensitivity analysis is an essential part of every risk assessment, quantitative and qualitative. The gaps in our knowledge are bridged by assumptions, probability distributions, expert opinion, best guesses, and a variety of other techniques. Sensitivity analysis is a systematic investigation of the means by which assessors bridge these uncertainty gaps. It includes 'what if' analysis of uncertain model parameters and inputs, as well as all significant assumptions. Sensitivity analysis seeks to learn such things as how sensitive model outputs are to changes in inputs and how that sensitivity might affect decisions. A good sensitivity analysis increases overall confidence in a risk assessment.

Risk Assessment Applications

The first risk assessment methodologies were developed for chemical risks. Chemical hazards may be naturally present in

foods, they can enter into food products via raw materials and ingredients, can be formed during certain processing steps (e.g., acrylamide), or enter in later stages of the food supply chain (e.g., packaging migrants). The amount of a chemical present in a food product is often the result of a choice made along the food chain, for example, food additives, and residues of veterinary drugs and pesticides used on crops.

Microbial hazards are markedly different because they are alive and biologically active. They can enter foods at any point in the farm-to-table food chain. The prevalence and concentration of a hazard can change dramatically as the food moves along that food chain. Despite intentional efforts to manage the risk, the hazard is often present at the point of consumption. Individual consumers exhibit a wide variety of health responses to different levels of the hazard. Microbial hazard health risks tend to be acute and may result from consuming a single portion of contaminated food.

There are a growing number of different areas for application of risk assessment, covering microbial, chemical, antimicrobial resistance, nutrient, functional food components, biotechnology, and nanotechnology. Generally, in all application areas, analysts keep to the four basic steps in risk assessment, although tailored methodologies have been developed for individual areas.

A good source for risk assessments conducted around the globe is foodrisk.org (http://foodrisk.org/rarepository/), and the following examples of qualitative risk assessments and risk assessment procedures can be found there:

- Opinions on the geographical risk of bovine spongiform encephalopathy in various countries.
- Evaluations of the safety of new antimicrobial animal drugs with regard to their microbiological effects on bacteria of human health concern.
- Regulatory procedure for health risk assessment of GM foods.
- Safety assessment of GM food.
- Example of a qualitative exposure assessment in a microbial risk assessment.
- Qualitative risk assessment of the risk of introduction and transmission of H5N1 HPAI virus for 1-km buffer zones surrounding compartmentalized poultry farms in Thailand.

Safety Assessment

Safety assessments are a commonly used tool in the assessment of acceptable levels of risk. These assessments generally compare an estimated level of a hazard to a science-based safety standard. Hazard levels in excess of the standard are considered unacceptable and are subject to risk management measures aimed at reducing the hazard levels below the standard.

Traditionally, chemical risks, such as food additives and pesticide residues, have been subjected to safety assessments. These safety assessments were considered risk assessments by many in the early days of food safety risk assessment. It is perhaps more appropriate today to consider that they are a combination of risk assessment because they are based on science, and risk management because the technique relies on the subjective derivation of a 'safe level of exposure,' which in current terms would be a risk management responsibility.

In the process of a safety assessment for a food additive, the potential hazard is characterized by establishing an acceptable daily intake (ADI), which reflects a level of exposure to the hazard that is considered to be without undue public health effect. This is done by first establishing a no-observed-adverse-effect level (NOAEL) of exposure to a chemical, often through animal feeding studies. To extrapolate from animal feeding studies to humans, an overall factor of safety or uncertainty factor is used. For example, a factor of 10 may be used for extrapolation from animals to humans, another factor of 10 to account for variable susceptibility among humans, and a third factor of 10 to account for sensitive populations. The product of the selected factors $10 \times 10 \times 10 = 1000$ yields the overall safety factor. The NOAEL, say 5 mg per kg of bodyweight daily for a lifetime, is divided by the safety factor to yield the ADI (0.005 mg per kg of bodyweight daily for a lifetime) for the human population of interest.

The estimated daily intake (EDI) of the substance is then estimated and compared with the ADI. An EDI/ADI > 1 is considered unacceptable and levels of the toxic substance must be reduced. Other levels are considered safe.

For chemical contaminants, a so-called tolerable daily intake (TDI) is derived following a comparable process. In the case of pesticide residues, the exposure assessment relies on identification of a suitable index of residue levels that can be used to predict residue intake. This level is usually the maximum residue limit (MRL). The MRL is used to estimate the dietary intake of the residue, which is compared with the TDI.

Linking Risk Assessment to Food Safety Management

On the basis of a risk assessment, risk managers make decisions in the light of public health protection policies. They may decide to implement particular risk management options to mitigate a risk they deem unacceptable. They may also determine a tolerable number of adverse health effects in their population that can result from a specific hazard associated with a particular food. Such a level of public health effects may be called a 'public health goal' stipulated in regulatory policy for the protection of public health in a country. In the context of international trade in food, it is often referred to as the 'appropriate level of protection' (ALOP). A public health expectation is normally determined for a sovereign nation by a competent authority. It may be based on a decision made considering epidemiological evidence in a country; but in the international context, it is advocated to be set using the risk analysis framework. In the latter case, a risk assessment is commissioned by the risk manager in the responsible regulatory body to provide the required estimate of prevailing risk from which to derive the public health goal or the ALOP. The ALOP then states the number of illnesses per year from a particular pathogen–food pair that can be tolerated; for the

purposes of providing an example, say, the ALOP is 100 illnesses per total population per year. Working backward from this number of illnesses using the exposure assessment data of the risk assessment, now suppose that 100 or fewer illnesses can be expected to occur if the probability of illness is 10^{-6} or less. The ALOP translated into this probability of illnesses is shown in Figure 2.

To realize a probability of illness of 10^{-6} or less, the dose–response relationship of the figure indicates that the food at the time of consumption must have 10^5 or fewer pathogenic cells per gram (or more practically: CFUs of the pathogen per gram, CFU g^{-1}) at the time of consumption. Considering the results of the risk assessment, such as the vulnerability of subpopulations, or variability and uncertainty associated with the dose–response data, the risk manager may wish to lower this value to a level deemed more appropriate. In any case, this value would then be a target to be met by the food industries involved in the food supply chain for the particular food product. Codex Alimentarius refers to this target value as the food safety objective (FSO) and defines it as 'the maximum frequency and/or concentration of a hazard in a food at the time of consumption that provides or contributes to the ALOP.' If the food industries manage to meet the FSO, then the ALOP will be realized. Codex Alimentarius provides guidelines on the ALOP and FSO concepts specifically for risks due to microbial hazards. Both the ALOP and FSO are determined at the national level by competent authorities.

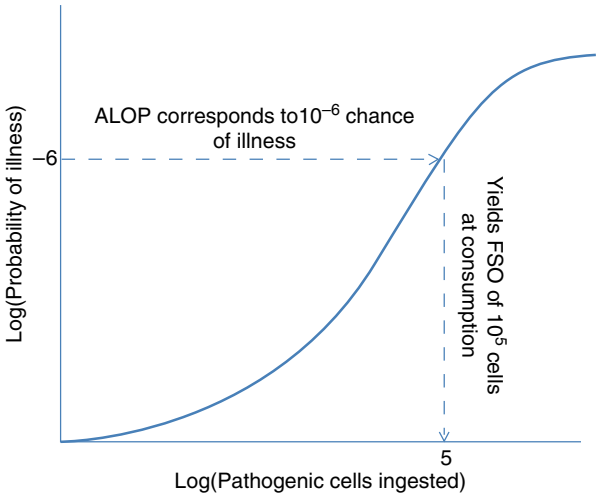

Figure 2 From ALOP to FSO with a dose–response relationship.

Armed with this information, the individual firms can establish the appropriate design of their food safety management systems, which generally are based on employing good practices (such as good agricultural practice, good hygienic practice, and good manufacturing practices) and following the principles of hazard analysis critical control points. Figure 3 shows a simplified food supply chain. Codex Alimentarius advocates ALOP and FSO to drive the design of food safety management systems targeting microbial hazards.

Raw ingredients are received and processed, there is a kill step of some kind and the product is stored until sold to the consumer. Notice that the ALOP influences health outcomes by limiting the number of illnesses. The FSO is set at the point of consumption. As one of the risk management options, a performance objective (PO) may have been issued by the competent authorities to aid industries that lack resources to design appropriate food safety management systems to meet the FSO. Other industries may be setting PO values by their own means. The concept of a PO is identical to that of the FSO, but it occurs at an earlier stage in the food chain, not at consumption. The PO expresses the level of a microbial hazard at a particular point in the food chain that must not be exceeded in order to meet the FSO at the point of consumption. To meet the PO, industries must consider in detail their production and/or handling of the food products and arrange their food safety management systems accordingly. In particular, industries will have to determine how much of the hazard is coming into the food they produce through for instance raw materials, ingredients, and processing environment. They will then know how much they will need to change the hazard level in order to meet the PO. This change is called a performance criterion (PC) by Codex Alimentarius and it relates to the complete stage in the food chain for which an industry is responsible. Codex Alimentarius defines the PC as the effect in frequency and/or concentration of a hazard in a food that must be achieved by the application of one or more control measures to provide or contribute to a PO or an FSO.

Suppose that a PO is set at 10^3 CFU g^{-1} of a particular pathogen at the time of storage and that this will deliver the FSO set for the particular food–pathogen combination.

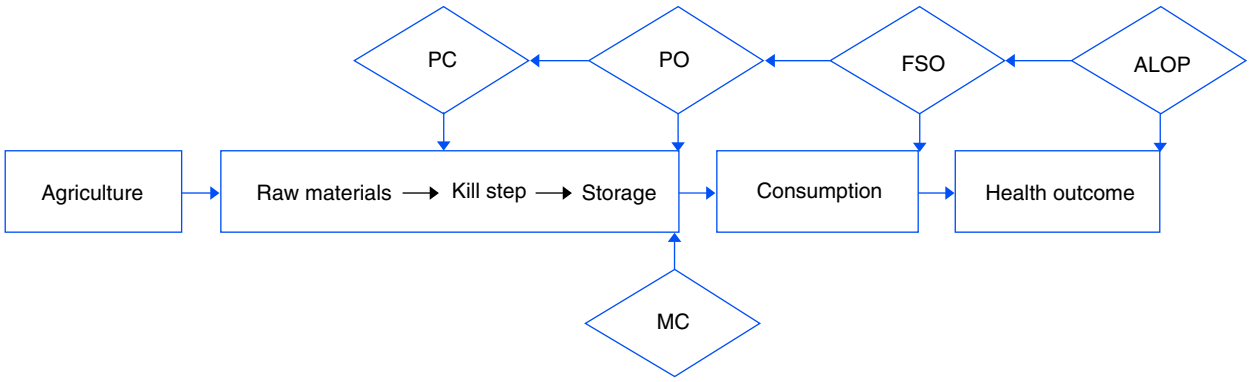

Figure 3 Linking risk assessment to food safety management.

However, raw materials, ingredients, and the production environment may all be sources of the hazard that together bring in a level of the hazard to the food at 10^5 CFU g^{-1}. To meet the PO of 10^3 CFU g^{-1}, there is then a need to reduce the hazard level before storage of the finished product 100-fold, which is the PC value. To achieve a 100-fold reduction, the kill step (for instance pasteurization or other thermal process) will be essential, but also limiting hazards coming into the food through ingredients added after the kill step or from the food production environment will be required to ensure that the PO is not surpassed. For enforcement purposes, competent authorities may establish microbiological criteria (MCs) specifically related to the FSO they have set or to a PO they derived from it. An MC is defined by Codex Alimentarius as the acceptability of a product or a food lot, based on the absence or presence, or number of microorganisms, including parasites, and/or quantity of their toxins/metabolites, per unit(s) of mass, volume, area, or lot. When industries meet the MC, this provides evidence to the regulatory body that they are meeting the PO thus, ultimately the FSO and the ALOP.

Although no competent authority has implemented the FSO approach to date, a significant advantage of this approach for food safety, and the reason why it is advocated by Codex Alimentarius and considered by national competent authorities, is that it does not prescribe how industries should meet food safety expectations without necessarily an integrated view across the food chain, as has been done in the past by governments issuing performance standards for specific operations such as pasteurization. It empowers individual industries to design their products and the associated food safety management systems to their own capabilities. It fosters innovation and helps meet public health expectations in the most efficient manner possible for both governments and industries.

Who Does Risk Assessment?

Many sovereign nations have central food authorities that conduct their own risk assessments. A variety of ministries and agencies, like the US Food and Drug Administration and the US Food Safety Inspection Service conduct food-safety risk assessments. Where such expertise is not available in competent authorities, risk managers in regulatory bodies may have access to specific risk assessment experts and expertise from other relevant public organizations, such as Universities. Academicians have certainly contributed to the growing literature on risk assessment. At the regional level, dedicated risk assessment bodies have been established, such as the European Food Safety Authority of the European Union, which functions as the risk assessment and risk communication body. At the international level, Codex Alimentarius represents the risk management function taking advice from risk assessment specific committees set up under the auspices of FAO and WHO. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) is an international scientific expert committee that has been meeting since 1956. They began by evaluating the safety of food additives. That work has grown to include the evaluation of contaminants, naturally occurring toxicants, and residues of veterinary drugs in food. The Joint FAO/WHO Meeting on Pesticide Residues (JMPR) is an international expert scientific group that has been meeting regularly since 1963. JMPR establishes TDIs and acute reference doses for pesticides they evaluate. They use scientific evidence to recommend MRLs for pesticides in food commodities that follow good agricultural practice. The Joint FAO/WHO Expert Meetings on Microbiological Risk Assessment (JEMRA) began their work in 2000 with the objective of conducting risk assessments for the Codex Committee on Food Hygiene and member countries. JEMRA focuses on providing pathogen commodity combination risk assessments for selected pathogens and on developing guidelines for risk assessment of microbiological hazards in food and water.

See also: Public Health Measures: Health Education, Information, and Risk Communication. Risk Analysis: Risk Analysis of Hazards in Food: An Overview; Risk Assessment: Chemical Hazards; Risk Assessment: Microbiological Hazards; Risk Communication: Biological Hazards; Risk Management: Application to Biological Hazards

Relevant Websites

http://www.codexalimentarius.org/codex-home/en/
: Codex Alimentarius information on standards, guidelines and procedural manuals.

http://foodrisk.org/
: Foodrisk: website on many aspects of food safety risk analysis with datasets, tutorials, and tools, including a repository for completed food safety microbiological risk assessments.

http://www.ilsi.org/Europe/Pages/Publications.aspx
: ILSI Europe Publications on chemical and microbiological risk assessment.

http://www.fao.org/ag/agn/agns/jemra_index_en.asp or at http://www.who.int/foodsafety/micro/jemra/guidelines/en/index.html
: JEMRA (the Joint FAO/WHO Expert Meetings on Microbiological Risk Assessment) risk assessment publications and guidelines.

http://www.nationalacademies.org/nrc/
: National Research Council's website for expert reports.

http://www.who.int/ipcs/food/principles/en/index1.html
: WHO.

Estimating the Burden of Foodborne Disease

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Glossary

Burden of disease A measure that combines prevalence or incidence of health states with indices of severity, disability from long-term complications, and duration.
Cost of illness Monetary value of the effect of illness on individuals and society.
Disability adjusted life year A lost year of 'healthy' life. The sum of these across the population is a measurement of the gap between current health status and an ideal health situation.
Disability weight A value between 0 and 1 reflecting the relative desirability of a health state, where 0 indicates perfect health and 1 worst possible health status; assigned by a panel of judges including medical and/or lay people, used in the calculation of disability adjusted life years.
Foodborne Disease Burden Epidemiology Reference Group (FERG) An expert group advising the WHO initiative to estimate the global burden of foodborne diseases.
Global burden of disease (GBD) A series of studies producing comprehensive estimates of the burden of diseases, injuries and associated risk factors.
Reporting pyramid A quantitative or semiquantitative depiction of the underascertainment of cases at each step of the pathway (general practitioner (GP), clinical laboratory, notifiable disease system) leading to a case that is reported to a disease surveillance system (the top of the pyramid). The base of the pyramid represents the total number of cases which occur in the population.

Introduction

Making choices about the allocation of resources to address health issues requires a consistent quantitative assessment of the relative magnitude of diseases. Burden of disease estimates provide such a metric, which can act as a transparent criterion for the relative importance of specific diseases. This provides opportunities not only to allocate resources and to target interventions, but also to monitor their effects.

Burden of disease is defined as a measure that combines prevalence or incidence of health states with indices of severity, disability from long-term complications, and duration.

Foodborne disease encompasses acute and chronic syndromes caused by a multitude of hazards. In this article, the authors describe the application of burden of disease estimates as a tool to prioritize or rank food safety risks. There are numerous publications that discuss burden of disease concepts and alternate metrics. The authors will focus on issues specific to the burden of foodborne disease and summarize existing and 'in progress' estimates.

Burden of disease metrics represent one component of analysis for (regulatory) decision making. Health policy analysis takes the perspective of maximizing population health (rather than individual preferences), so summary measures of population health are required.

Burden of Disease Metrics

A number of metrics to quantify the burden of disease as a summary measure of public health have been put forward. These relate to differing approaches to the economic evaluation of health care programs. In the context of foodborne disease, such evaluations compare the costs of an intervention to improve food safety, with the consequences of the intervention, in terms of illnesses avoided.

Cost–Benefit Analysis

Cost–benefit analysis is an economic evaluation that attempts to value the consequences or benefits of an intervention in monetary terms, rather than in 'units of health'. This approach requires monetary values for the effect of illness on individuals. Although this approach has difficulties, it does have the advantage of being able to compare health programs with nonhealth programs.

There are two main approaches to the monetary valuation of health outcomes. The 'cost of illness' (COI) approach sums the medical and nonmedical costs of the disease along with productivity losses from associated morbidity or premature mortality. Direct costs are those for medical services such as visits to a general practitioner (GP) or physician, specialist,

and costs associated with medicines. Nonmedical direct costs are incurred by the case in accessing medical services (e.g., travel costs).

Indirect costs are those resulting from the inability of a case (or a caregiver) to perform normal life activities. This principally concerns an inability to perform paid work, valued as lost income (usually on a daily basis, and regardless of whether the case actually loses that income). For some analyses, the period during which a case is unable to perform normal activities is valued, regardless of whether the case is employed, i.e., the cost includes both work and leisure activities. For illnesses where patients die prematurely, or are unable to return to work, the lost productivity may be represented by estimates of foregone earnings.

The second approach, 'willingness to pay' (WTP), attempts to measure the value an individual places on reducing risk. In the absence of market pricing mechanisms, several alternatives have been developed to provide WTP values. The most common approach is 'contingent-valuation studies' in which individuals are surveyed in the context of a hypothetical market situation. Examples related to food safety include WTP, over and above current expenditure, for poultry meat treated to eliminate the risk of foodborne illness, and experimental auctions to reduce the risk of campylobacteriosis from consuming poultry. For illnesses where patients die prematurely, or are unable to return to work, WTP estimates of the value of a statistical life can be used.

Inherent in the analysis of this COI is a choice of 'perspective,' which guides the costs that are included. The most common choice is to take a societal perspective, which includes all costs to the individual case and society as a whole, in providing medical services and lost production through inability to work. However, alternative perspectives, such as the individual patient, or the medical system, could be chosen.

Additional nonhealth costs may be borne by the food industry and governments. Industry costs include control measures, recalls, and loss of product and brand name credibility; whereas for regulators there are costs associated with the generation of standards, monitoring, and surveillance. However, these costs are estimated infrequently.

Cost–benefit analysis is discussed in more detail in another article.

Cost–Utility Analysis (Cost-Effectiveness Analysis)

Cost–utility analysis incorporates changes in the quantity of life (mortality) and changes in the quality of life (morbidity) into a single 'unit of health' metric. The concept of health adjusted life years (HALYs) has been developed by health economists, and the two most common metrics of this type are the quality adjusted life year (QALY, broadly useful as a measure of health gain from interventions) and the disability adjusted life year (DALY). The DALY is a measure of the gap between an existing health state and a hypothetical ideal, and thus is a metric suited to burden of disease.

To incorporate both the quantity and quality of life into a common unit of measurement, it is necessary to use a set of values or weights, sometimes called utilities. These reflect the relative desirability of the health state, with perfect health and death representing the opposite extremes. There are a variety of methods for eliciting these preferences from individuals or health experts, including scaling methods (rating scales category scale, visual analog scale, and ratio scale) and choice methods (time trade-off, paired comparison, equivalence, person trade-off, and standard gamble).

DALYs were originally developed by the World Health Organization (WHO) for the Global Burden of Disease (GBD) Study. Since then, they have been extensively used for burden of disease estimation, including foodborne disease.

The fundamental calculation for DALYs is:

$$\mathrm{DALY} = \mathrm{YLL} + \mathrm{YLD}$$

YLL is the number of years of life lost due to mortality and YLD is the number of years lived with a disability, weighted with a factor between 0 and 1 for the severity of the disability.

The YLL due to a specific disease in a specified population is calculated by the summation of all fatal cases (n) due to the health outcomes (l) of that specific disease, each case multiplied by the expected individual life span (e) at the age of death.

$$\mathrm{YLL} = \sum_{l} n_l \times e_l$$

YLD is calculated by accumulation of overall health outcomes (l), the product of the number of cases (n), the duration of the illness (t), and the severity weight (w) of a specific disease. It should be noted that the calculation for YLL implicitly includes a severity weight factor. The severity weight or disability weight factors are in the range 0–1, with the severity weight for death being equal to 1.

$$\mathrm{YLD} = \sum_{l} n_l \times t_l \times w_l$$

An example calculation, for an infection with a microbial foodborne hazard that has a variety of outcomes, is illustrated in Table 1. In this example, $\mathrm{YLD} = 55 + 200 + 8 = 263$ and $\mathrm{YLL} = 607$, hence $\mathrm{DALY} = 263 + 607 = 870$.

Table 1 Example DALY calculation for a specific disease

Burden calculation	*Outcomes*			
	Do not visit a GP and recover	*Visit a GP and recover*	*Hospitalized and recover*	*Fatal*
Cases per year	59 000	18 300	570	46
Duration	5 days (0.014 years)	10 days (0.028 years)	14 days (0.038 years)	13.2 years[a]
Disability weight	0.067	0.39	0.39	1
Disease burden	55	200	8	607

[a]Fatality occurred on average 13.2 y before normal life expectancy.

A number of methodological choices have to be made when calculating DALY values for burden of disease.

Although a prevalence approach may be taken to the calculation of DALYs (i.e., calculating the current burden of disease in a population, considering previous events), more commonly an incidence approach is taken. This includes both current and future health outcomes. Future outcomes include sequelae and mortality resulting from the initial infection with pathogens within a defined time period.

To calculate YLL, life expectancy must be defined. Some studies have used life expectancy tables for the population being studied. A more universal approach would be to use life expectancy that reflects an ideal of human potential. The greatest potential for the life-span of humans is currently the life expectancy of the Japanese population (82.5 years for females). This ideal is used to choose appropriate values from the Coale–Demeny regional model life tables (West level 26 for females, and 2.5 years less for males, using the West level 25 for females).

When calculating YLL, the 1990 GBD study weighted a year of healthy life lived at younger and older ages lower than years lived at intermediate ages. This was a reflection of societal choices, particularly in nonindustrialized countries where individuals at intermediate age tend to be the main breadwinners for dependents among several generations within one family. However, the concept of such weighting (and the appropriate weighting to be applied) has been controversial. On equity grounds, it has been argued that every year of life should have equal value.

The discounting of future benefits is a standard practice in economic analysis. However, opinion is divided as to whether future health, such as future DALYs avoided, should be discounted.

The 2001 GBD Study results were generally presented without age weighting, but using a 3% discount rate, although the effect of varying these settings was also discussed. A Dutch study of foodborne pathogens presented both undiscounted and discounted estimates (using a discount rate of 4%), whereas a New Zealand study applied no discounting to DALY estimates.

Comorbidity, when multiple conditions coexist in an individual, presents particular difficulties. This is particularly relevant for foodborne disease, because a preexisting condition (e.g., diseases affecting the immune system) or a risk factor (e.g., old age) may make a person more susceptible to infection, or at greater risk of more severe consequences. Questions arise of how a comorbid condition might affect the disability weight for the disease of interest and how the burden of disease can be apportioned, particularly when mortality occurs. These issues have yet to be addressed on a population health basis by existing foodborne disease burden estimates, although national burden of disease studies have examined the issue.

Methodological Choices Particular to Estimation of Foodborne Burden of Disease

Foodborne diseases are those commonly transmitted through food and include illnesses caused by microbial pathogens, parasites, chemical contaminants, and biotoxins. They also include food allergies. There are a number of particular methodological issues which must be resolved for foodborne burden estimates to be generated. These are not entirely specific to foodborne disease; similar challenges would apply to estimating the burden of waterborne disease.

Incidence

One approach to estimating the burden of disease for foodborne hazards is to estimate the incidence of specific relevant syndromes or outcomes (as clinically defined by International Classification of Diseases codes), such as acute gastrointestinal illness (AGI), which is important for foodborne microbial pathogens. This is sometimes called the 'top down' approach. A number of studies estimating the incidence of AGI have been published including prospective studies in England and the Netherlands, as well as retrospective telephone surveys in Australia, Canada, Ireland, the US and New Zealand. Global estimates of the incidence of diarrheal illness have been published for children younger than 5 years of age as well as older children and adults.

The next step in this approach is to assign proportions of the incidence to etiologic agents relevant to foodborne transmission. This may be achieved by direct analysis of clinical samples provided by surveyed cases, as in the prospective infectious intestinal disease study in England. Alternatively, proportional estimates may be extrapolated from surveillance data generated by clinical laboratories, with an additional proportion assigned from the remaining cases of unknown etiology.

A complicating issue in assigning such proportions is that asymptomatic carriage of pathogens may occur in a proportion of people. In a standard approach to estimating the etiological fraction, the prevalence in symptomatic individuals should be corrected for the prevalence in asymptomatic individuals. However, for infectious diseases this approach may not be applicable because asymptomatic carriage may be associated with acquired immunity and less virulent subtypes of the pathogenic microorganisms.

An alternative approach is to estimate the incidence of illness from agent-specific diagnoses. This so-called 'bottom up' approach uses the concept of reporting pyramids, where data may be obtained from a variety of levels. The base of the pyramid is all community cases, with higher levels being those that present to the health system, those that provide samples and an agent is diagnosed, with those that are reported to surveillance being the top of the pyramid. Data may be provided by notifiable disease surveillance systems, hospitalization, and mortality records, and incidence at other levels of the pyramid can be estimated using 'multipliers.' Well-known examples of such studies include estimates of the incidence of foodborne illness in the USA, which were updated in 2011.

To capture all the relevant outcomes associated with a particular agent, 'outcome trees' or 'disease models' are usually constructed, which describe the progression of health states potentially resulting from ingestion of a hazard through food. For microbial infections, the most common outcome is an acute disease followed by recovery. A small proportion of cases

will go on to develop sequelae, and a smaller proportion will result in mortality. The conditional probabilities of each branching pathway are assigned in the tree as far as possible.

The development of these trees requires choices about which outcomes to include and exclude. An international consensus on these choices has not yet been reached; links between initial infections and sequelae are often difficult to establish and quantify. Frameworks such as those developed by the Institute of Medicine to assess scientific evidence for causation can be helpful.

By including not only the initial illness caused by the ingestion of the hazard in food, but also longer term effects as sequelae, this approach integrates the burden from a number of syndromes. In doing so, it is important that the outcome burden components fit within the syndrome-specific burden envelopes estimated by overall public health burden of disease estimates. For example, the burden of disease assigned to Guillain-Barré syndrome (GBS) as a sequel of campylobacteriosis would be a component of the overall burden from this syndrome within a population.

Attribution for Foodborne Transmission

Hazards relevant to foodborne disease usually have multiple pathways of exposure for humans. Once a burden of disease estimate has been generated, a proportion needs to be assigned to foodborne transmission. The GBD Study assigns burden of disease estimates to risk factors using a comparative risk assessment approach (CRA). The CRA approach estimates the burden of disease and injuries due to risk factors based on a counterfactual exposure distribution that would result in the lowest population risk, irrespective of whether this is currently attainable in practice. The counterfactual is referred to as the theoretical minimum-risk exposure distribution. Reviews of the literature are used to provide suitable counterfactual exposure distributions, and the stratified estimates for existing exposures.

The corresponding counterfactual exposure distribution for foodborne hazards would be zero exposure. To estimate current exposure requires knowledge of food consumption, hazard prevalence, and concentration. However, it is often not possible to conduct exposure estimates for foodborne hazards, either because of limited data, or the dynamic nature of the hazard (i.e., microbial hazards, which may grow or decline depending on factors through the supply chain). A number of alternative approaches exist for attribution of the burden of illness from hazards that may be foodborne, including the analysis of microbial subtyping data, epidemiological studies, outbreak analysis, and expert elicitation.

Severity and Duration: Disability Weights Relevant to Foodborne Disease

The disability weight is a value elicited from a panel of judges that reflects the relative desirability of particular health states. The value ranges from zero (best possible health state) to one (worst possible health state). The members of the panel may be persons suffering from the illness in question, their carers, medical experts, or lay people. Numerical values may be elicited by asking panel members to simply provide a score across a range, or else specify a period of healthy life that would be sacrificed to avoid the illness (a 'time trade-off' approach).

The health state needs to be described to the panel. This may be done using generic tools that describe a range of health states using sets of specific attributes describing how the disease affects a person. The panel assigns values to a number of health conditions with different combinations of these health attributes. These are then disaggregated (e.g., by regression modeling) to produce a formula to calculate disability weights for any combination of attributes. This approach has been used to generate the large number of disability weights needed by the GBD Study.

Alternatively, disease-specific descriptions may be used to indicate both the cause and the health effects of the condition. Such descriptions may adopt a period profile approach, where the health state is considered to be constant over time, and duration information is not included (i.e., duration and disability are independent). Alternatively, the annual profile approach is taken, where the duration of the disease is indicated in the disease presentation (as a portion of a year), which removes the need to estimate duration of acute, self-limiting diseases for DALY calculations.

The annual profile approach has been applied to short-term AGIs and sequelae, which are particularly relevant to foodborne disease, in a Dutch study using lay person panels to assign disability weights. This study also considered the use of a relevance criterion. Using the 'time trade-off' method to generate disability weights, panel members had the option of refusing to sacrifice any days of healthy life to avoid the illness. A threshold was established that half the panel had to be willing to sacrifice a period of healthy life, in order to consider the illness relevant. If the threshold was not achieved, then the illness was considered trivial in terms of burden and excluded from the DALY estimate. For example, fewer than half the panel members were willing to sacrifice any period for mild gastrointestinal illness (one day). Excluding these cases reduced the burden estimate for all pathogens, particularly norovirus for which the burden was reduced by 94%.

Global Burden of Disease

The global burden of disease project grew out of work by the World Bank to prioritize control of specific diseases in developing countries. Cost-effectiveness analysis for this purpose required an assessment of the relative magnitude of diseases, injuries, and risk factors. The original GBD Study was commissioned by the World Bank in 1991 to provide a comprehensive assessment of the burden of 107 diseases and injuries and 10 selected risk factors for the world and 8 major regions estimated for the year 1990. A major update, for the year 2001, was published in 2006. In 2008, WHO published a further update, for the year 2004.

Since 2007, the Global Burden of Diseases, Injuries, and Risk Factors Study (the GBD 2010 Study) has been working to produce comprehensive and comparable estimates of the burden of diseases, injuries, and risk factors for two time periods, 1990 and 2005. The core team for this initiative

includes scientists from the University of Washington, Harvard University, the University of Queensland, Johns Hopkins University, and the WHO, and will use improved methods and data that have been developed since the original GBD 1990 Study. The project is expected to produce its estimates in 2011.

Global Burden of Foodborne Disease

In 2006, the Department of Food Safety and Zoonoses (FOS) in WHO conducted an international consultation on estimation of the burden of foodborne diseases. This consultation resulted in the launch of the WHO Initiative to Estimate the Global Burden of Foodborne Diseases and a strategic framework to guide the task. An external advisory group was required, so the Foodborne Disease Burden Epidemiology Reference Group (FERG) was set up, under the leadership of WHO/FOS, with the following specific functions:

1. Assemble, appraise, and report on the current, the projected, as well as the averted burden of foodborne disease estimates.
2. Conduct epidemiological reviews for mortality, morbidity, and disability in each of the major foodborne diseases.
3. Provide models for the estimation of foodborne disease burden where data are lacking.
4. Develop cause attribution models to estimate the proportion of diseases that are foodborne.
5. Use the FERG models to develop user-friendly tools for burden of foodborne disease studies at country level.

A dedicated website for the FERG initiative has been developed by the WHO.

FERG has established five task forces to address elements of the global burden. Three of these are hazard based (Enteric, Parasitic, Chemical) whereas a fourth is concerned with Source Attribution. Through the commission of systematic reviews addressing specific hazards, global estimates of incidence will be gathered and used for DALY calculations.

The fifth task force, Country Studies, was established in 2008 and is intended to foster a series of national foodborne disease burden studies in specific countries, particularly developing countries. The studies will be conducted according to a specific protocol, developed partly through adaptation of the National Burden of Disease Studies protocol published by WHO. These studies will have several objectives, in addition to filling data gaps for the FERG global estimate. Besides capacity building within each country, the task force has also taken on the challenge of providing tools for the translation of burden of disease information into food safety policy. This novel objective is being addressed by a knowledge translation subgroup of the Country Studies Task Force.

FERG has a 5-year mandate, with reporting to begin in 2012. The Country Studies will begin in 2011 and are likely to be completed in 2013–2014.

A number of publications have already emerged from this project, including global estimates of the incidence and etiology of diarrheal morbidity and mortality in older children and adults and estimates of the global incidence of specific parasitic diseases.

National Burden of Microbial Foodborne Disease Studies

Economic Estimates

Cost of illness estimates have been extensively investigated in the US. A series of estimates have been published by the USDA Economic Research Service (ERS), beginning with a 1996 estimate of the cost of foodborne illnesses from six bacterial pathogens of $2.9–6.7 billion (US). This estimate was based on a COI approach, including foregone earnings of affected individuals, and productivity losses. More up-to-date estimates have been provided at the ERS website.

A 2009 estimate of the cost of foodborne illness for six major foodborne enteric diseases in New Zealand of $162 million (NZ$) has been published. The estimate of the value of personal and lifestyle costs (pain, suffering and disruption, including the possibility of premature death) was based on a WTP estimate for the value of a statistical life. This estimate also included costs for treatment and lost production, as well as industry and regulatory costs.

An Australian estimate of the cost of foodborne illness, published in 2006, also incorporated industry and regulatory costs, although these were modest compared with productivity and lifestyle costs. The overall cost was estimated as $1249 million with productivity and lifestyle costs being $772 million (AU$), largely based on WTP by individuals to avoid illness.

DALY and Cost of Illness Estimates

The Netherlands

A series of studies performed by the National Institute for Public Health and the Environment (RIVM) for the Dutch government have generated both burden of disease and COI estimates for a range of pathogenic microorganisms that may be foodborne. The first study, for the year 2004, described the burden of disease for norovirus, rotavirus, thermophilic *Campylobacter* species, *Salmonella* spp., Shiga toxin-producing *Escherichia coli* O157, *Listeria monocytogenes*, and *Toxoplasma gondii*. For the first four of these, COI was also estimated. A second study published in 2007, applied the same methods to estimate the disease burden and COI of *Giardia lamblia* and *Cryptosporidium* spp.. Most recently, an update for 2006 has been published. In addition to using updated data, this report expanded the range of pathogens to include hepatitis A and E virus, and the toxin-producing pathogens *Clostridium perfringens*, *Staphylococcus aureus*, and *Bacillus cereus*.

A significant change in the DALY estimates in 2006 for some pathogens was due to the addition of postinfectious irritable bowel syndrome (IBS) as a sequel for infectious diarrhea. The effect of this added sequel was to increase the DALY estimates by 86, 92, and 151%, respectively, for *Campylobacter*, *Salmonella*, and *Shigella*. This illustrates the potential importance of choices about sequelae for burden of disease estimates.

The attribution component of this analysis was taken from an expert elicitation study. A strength of this study was that it asked experts to estimate environmental, human (person-to-person), and animal contact proportions of the

attributable fraction, as well as the foodborne proportion, providing a total of 100%.

The DALY burden estimated for 2006 in the Netherlands was highest for infections with *Campylobacter* spp., followed by *Salmonella* spp. and norovirus.

New Zealand

Estimates of the burden of foodborne disease caused by six selected enteric pathogens in New Zealand have been reported. Illnesses were selected based on considerations of incidence and clinical severity in the New Zealand context:

- Campylobacteriosis.
- Salmonellosis.
- Listeriosis (invasive, perinatal, and nonperinatal).
- Infection with Shiga toxin-producing *E. coli*.
- Yersiniosis.
- Infection with norovirus.

The estimation of disease burden closely followed the approach taken in the Netherlands. New Zealand specific attribution estimates were derived from an expert elicitation process. The elicitation was carried out as a two-pass modification of the Delphi method. The base year for these estimates was 2005, although some data (especially for rare outcomes like mortality) were taken from a wider time span.

In addition to the DALY burden, COI estimates were generated for direct and indirect medical costs. Direct health-care costs included GP consultations and medication for AGI, GP and specialist consultations for sequelae, hospital, and rehabilitation costs. Indirect costs included lost production resulting from illness. For fatalities and long-term disability, lost production was estimated using discounted future income (instead of the friction cost approach used in the Netherlands estimate).

As would be expected for New Zealand, having a high reported incidence of this illness compared with other developed countries, the greatest burden of foodborne disease estimate was for campylobacteriosis and sequelae. The DALY estimates ranking for the remaining illnesses (and relevant sequelae) were at least four-fold lower. The reported incidence of campylobacteriosis in New Zealand has declined by approximately 50% over the period 2006–2010. This will substantially reduce the burden estimate for this disease.

After the production of these estimates, alternative disability weights for AGI outcomes were published. The New Zealand burden estimates were revisited using these new weights. The revised estimates, using a relevance criterion, provided the same ranking of the bacterial pathogens. However, when the relevance criterion was not applied, then the DALY estimate for the foodborne burden of infection with norovirus was higher than that for campylobacteriosis and sequelae.

National Burden of Chemical Foodborne Disease Studies

Some estimates of the foodborne disease burden from chemical contaminants in food have been published. In the Netherlands, estimates of the burden attributable to chemical contamination (mycotoxins, nitrates, processing contaminants) and allergens in food have been reported by RIVM, although these were described as probably overestimates. Estimates of the disease burden from neurodevelopmental toxicity of methylmercury have also been published by WHO, based on seafood consumption.

Future Challenges

Several aspects of the estimation of the burden of foodborne disease are well established and dependent on the availability of sufficiently robust incidence data and the ability to attribute a proportion of that incidence to foodborne transmission. This is the case for most of the relevant bacterial, viral, and parasitic microbial pathogens.

The estimation of the burden of disease caused by chemical hazards in food is the most pressing area for methodological development. Like the linkage between microbial infections and potential sequelae, linkages between exposure to chemicals and health effects are often circumstantial. Another complicating factor is the multiplicity of exposure routes for many chemicals.

As described above, the use of age weighting and discounting presents important methodological choices. Adjustment for comorbidity also remains to be addressed by foodborne disease burden estimates.

There is a need to standardize the outcome trees used by agent-specific foodborne disease burden estimates, so that results can be compared across countries and regions. As illustrated by the inclusion of IBS in the Netherlands burden estimates, these decisions can have a major effect on the size and ranking of burden.

The extrapolation of disability weights developed for a single country to other countries or regions is an issue that requires further study. This is particularly important for extrapolation between developed and developing countries, where illness caused by the same agent may have different courses of disease (e.g., campylobacteriosis). The GBD Study 2010 has set up an interactive website with the aim of soliciting value judgments on disability weights from a large number of individuals around the world.

Finally, there is a need to consider how burden of disease information might be used by decision makers, particularly governments and other regulatory organizations. The quantification of the disease burden itself, and ranking of individual hazards, could assist in the allocation of food safety resources. However, the DALY metric is probably less familiar to policy makers, and arguments based on economic value may be applicable in the wider policy arena. WHO is mindful of this issue and has asked the FERG to advice on this through the Knowledge Translation and Policy Group within its Country Studies Task Force.

Where a range of interventions to improve food safety are available, a cost-effectiveness analysis would be useful. Use of nonmonetary burden of disease metrics for cost-effectiveness analysis requires a weighting of costs (in $) against improvements in public health (usually in some other metric, often DALYs). In a ranking situation, where there is a requirement to

rank options for risk management, a league table of costs and benefits/effectiveness could be sufficient (ranking based on cost-effectiveness ratio). However, if the choice is whether to take action or not, then some threshold of the amount of expenditure for a unit of health gain is required. Exploration of this issue can probably only be achieved after a number of cost and burden estimates have been produced. It is possible that such criteria may emerge after ranking exercises that encompass a variety of transmission routes (especially if options to reduce waterborne disease can be included).

See also: Characteristics of Foodborne Hazard and Diseases: Cost of Foodborne Diseases. Disciplines Associated with Food Safety: Epidemiology. Public Health Measures: Foodborne Disease Outbreak Investigation; Surveillance of Foodborne Diseases. Risk Analysis: Risk Management: Application to Chemical Hazards

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Relevant Websites

http://www.foodsafety.govt.nz/science-risk/risk-assessment/risk-ranking.htm
New Zealand: The Ministry of Agriculture and Forestry has a risk ranking website with burden of disease reports.

http://www.globalburden.org/index.html
The Global Burden of Diseases, Injuries, and Risk Factors Study 2010.

http://www.rivm.nl/bibliotheek/
The Netherlands: several burden of foodborne disease reports are available.

http://www.ers.usda.gov/Briefing/FoodSafety/economic.htm
United States: The USDA Economic Research Service Briefing Room on economic costs of foodborne illness.

http://www.who.int/foodsafety/foodborne_disease/ferg/en/index.html
WHO Project on Global Burden of Foodborne Disease.

RISK ANALYSIS

Risk Assessment: Microbiological Hazards

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Glossary

Equivalence The demonstration that one set of food safety management measures leads to the same public health outcome as another, and are accepted as such by both jurisdictions.

Exposure assessment The qualitative or quantitative evaluation of the likely intake, both amount and frequency, of a defined hazard in a specified food by a defined population.

Farm-to-fork A term to describe the conditions of processing, storage, distribution, etc., of a food from the point of harvest to the point of consumption.

Harmonization (of law) The process of creating common standards across diverse jurisdictions.

Hazard A thing that could cause harm; in microbiological food safety risk assessment, the hazard will be a microbial pathogen, parasite, or toxin produced by a microorganism that could be present in food.

Hazard characterization Description and evaluation of the adverse health effects associated with ingestion of a pathogen or microbial toxin that may be present in food; it should include a dose–response assessment, i.e., a (quantitative) description of the relationship between the amount of the hazard ingested and probability and/or symptoms of illness caused.

Hazard identification The identification of known or potential health effects associated with a particular microbial hazard in a particular food.

Risk A function of the probability of illness to a consumer(s) and the severity of the illness, and number of people affected, due to a hazard in food.

Risk characterization Integration of the hazard identification, exposure assessment, and hazard characterization stages of risk assessment to produce an estimate of the likelihood and magnitude and severity of adverse health effects in a defined population due to a specified hazard in a specified food.

Stochastic simulation modeling A form of mathematical modeling in which variables in the calculation are described by distributions of values, rather than a single representative value. Because calculations involving distributions can become very complex, it is usually easier to use simulation modeling approaches, involve computer software to automate the repetitive calculation of the model for many thousands of combinations of different values of each variable, to generate a distribution of the possible results.

Introduction

Historically, regulations governing the microbiological quality and safety of foods were developed empirically, and independently, by nations. Over time, this leads to various problems.

During the 1980s and 1990s, increased awareness of microbial foodborne disease led national governments to seek better ways to manage food safety, and prioritize the allocation of resources for food safety management to the greatest risks, and where most benefits might result. The systematic analysis of the sources of risk that occurs in formal risk assessment approaches was promoted as a way to optimize food safety outcomes from the (often) limited available resources by focusing regulatory actions on the control of those steps in a 'farm-to-fork' food chain that most contribute to consumer risk. Related to this were concerns about appropriate sharing of responsibility for food safety along the 'farm-to-fork' chain so as to ensure the safety of the product at the time of consumption. Responsibility for food safety had usually been imposed on food processors despite their efforts being thwarted by others, for example, by mishandling their products further along the supply chain. Food safety management approaches based on risks, rather than perceived hazards, were proposed to be more rational, equitable, effective, and defensible.

Similarly, increasing focus in some nations on the overall impost (on industry and national productivity) of government regulation led to the idea that such regulations should be justified, i.e., by proving that their benefits outweighed the costs (to government, industry, and consumers) of their implementation. Concurrently, with increasing international trade in foods, inconsistencies in the food regulations of trading nations began to cause tensions between trading partners: Exporting nations considered that the importing nations sometimes imposed unfair, or unrealistic or unnecessary regulations on the imported products (including food) to protect their domestic industries from international competitors able to supply products of equivalent quality and integrity at lower cost. The ability to compare the efficacy of

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Box 1 Applications of microbial food safety risk assessment (MFSRA)

At its most basic level, risk assessment is a systematic analysis of the likelihood and severity of some undesirable event. Although the most extensively documented MFSRAs have generally arisen from large national or international projects to establish and justify new food safety regulations, the approach is useful for many applications including:

- assessing equivalence of food safety management systems in different jurisdictions,
- assessing the risk from different foods to establish priorities for risk management and for the allocation of resources to the management of that risk,
- comparing the public health burden of foodborne diseases to other diseases for the apportionment of resources,
- attribution of the burden of foodborne microbial illness to different foods (ranking of relative risk of foods for different pathogens),
- to better understand the relative contributions of various factors that affect a specific foodborne microbial risk and thereby to identify and select the most optimal risk management options including sharing of responsibility for food safety at all points along the farm-to-fork food chain,
- assisting in the development of effective hazard analysis critical control points (HACCP) plans for food businesses,
- establishing relevant microbiological criteria for food that achieve an appropriate level of protection (ALOP) to consumers,
- verifying that a Food Safety Objective (FSO), related to an ALOP, is achievable, and
- evaluation of the relevance/efficacy existing regulations

different risk management approaches to food safety in different trading nations, and to have objective means of setting food safety standards for international trade in foods, respectively termed equivalence and harmonization, were also advocated as benefits of formalized food safety risk assessment (see Box 1).

Until the 1990s, however, microbial food safety risk assessment (MFSRA) had been considered a daunting task because of the apparent randomness of microbial contamination of foods and the subsequent consequences, i.e., because microbial contamination levels can increase or decrease dramatically (due to microbial growth and inactivation) according to the handling of foods along the farm-to-fork chain. With the advent of predictive microbiology, the ability to monitor temperature continuously throughout the food supply/distribution chain, and quantify the probability (and severity) of illness from different doses of pathogens, the ideas of risk analysis have begun to be applied to microbial food safety management and food safety standard setting.

Many MFSRAs have now been conducted and presented. As discussed in the section Methods for Risk Characterization, they differ in scale and complexity and the risk questions addressed. They include academic examples from diverse researchers and organizations in various nations and presented in the published scientific literature, studies conducted for industry organization in various countries and presented as internal reports, those initiated by provincial or national governments, and others undertaken by international organizations such as the European Food Safety Authority, Codex Alimentarius Commission (CAC), International Life Sciences Institute, etc. They consider a variety of pathogens and foods; for some food–pathogen combinations, the risk has been assessed by different organizations. Results from these risk assessments have been translated into government policy concerning priorities in public health management, or changed food safety management strategies and regulations that have affected industry practices and technologies, have resulted in changed international regulations and guidelines (including relaxation of standards in some cases), and changes to recommended industry practices. The list of useful websites at the end of this entry provides a guide to many of these reports. The Joint Institute of Food Science and Applied Nutrition (JIFSAN) site is particularly useful because it provides access to a wide range of published food safety risk assessments, resources and related information, and is actively maintained and updated.

This article describes the principles of microbiological risk assessment, and development of 'best practice' methods for microbiological risk assessment.

Risks and Hazards

Humans have used various forms of empirical risk assessment since antiquity, and continue to do so on a daily basis. The development of formalized, objective, and science-based assessment of risks may be traced to the need to improve, and defend, various regulations introduced by government agencies, particularly in the US. Catastrophic accidents in the space program, failures in nuclear power facilities, and unforeseen environmental and health effects of industrial developments all accentuated the need for a more wholistic analysis of the likelihood and consequences of possible failures in complex systems. In the frameworks for risk analysis that arose, a hazard represents the potential for harm, i.e., the thing that could 'go wrong' to cause an undesirable effect, but risk considers both the likelihood and consequences of the hazard occurring. In the context of microbial food safety, risk involves:

- the potential presence of a microbial hazard, i.e., a pathogen or microbial toxins in foods at levels that could cause harm to the consumer,
- the likelihood that the hazard will arise, i.e., how often consumers are exposed to the hazard at disease-causing levels, and
- the severity and magnitude of that exposure, which will depend on the severity of the illness caused by the specific pathogen, the number of people exposed, and to a lesser extent, the dose ingested.

Risk Assessment Within the Risk Analysis Paradigm

Microbiological risk assessment is one aspect of the 'risk analysis' paradigm proposed by the CAC, an international body

convened under the United Nations (UN). The other two elements are risk management and risk communication.

Risk analysis involves:

1. objectively determining risks and sources of risk ('risk assessment'),
2. conceiving appropriate actions and strategies based on the risk and societal expectations to limit the risks to acceptable levels ('risk management'), possibly including imposition of food safety regulations, and
3. understanding the interests, concerns, and level of tolerance of the stakeholders to risks to which they are exposed ('risk communication') and explaining the costs and consequences of alternative risk management options to them and why the selected risk management strategies are the most appropriate options.

Food safety risk assessment is intended to objectively assess (as quantitatively as possible) the risk faced by a defined group of consumers of a specific food, or group of foods. Determining whether that risk is acceptable, and defining an acceptable level of risk from a food product, are societal decisions and require 'risk communication.' Risk communication is described as an interactive exchange of information and opinions concerning the risk between those likely to be affected by the risk and/or risk management decision. Notably, risk communication is not only limited to explain (to those affected by the risk) what will be done to manage the risk but also to understand their concerns about the risk, tolerance of the hazard, preferences for potential risk management options, etc.

Determining the most appropriate action, or actions, to ensure that the risk remains below some tolerable level is the function of 'risk management,' and must include consideration of cost versus benefit, technical feasibility, cultural acceptability, the willingness of consumers to accept some responsibility for management of the risk, etc. Thus, risk assessment is intended to be as objective and quantitative as possible, whereas risk management must consider subjective aspects to determine the best options for managing the risk in the context of the culture in which they arise. The risk analysis paradigm and risk management are considered in greater detail in other entries.

Within this overall risk analysis framework, the main function of risk assessment is to provide support to risk managers for decisions that they are required to make.

Principles of MFSRA

The CAC is a UN-based organization established under the joint Food Standards Program of the UN's Food and Agriculture Organization (FAO) and World Health Organization (WHO). The main purposes of the Food Standards Program are protection of the health of consumers, ensuring fair trade practices in the international food industry, and promoting coordination of all food standards work undertaken by international governmental and nongovernmental organizations, i.e., to achieve 'harmonization' of regulations so as to facilitate international trade in foods. An underlying ideal of this work is to assist developing nations to build their economies through the development of export agriculture, considered as an important first step along the path to national development.

Codex Principles and Guidelines for the Conduct of Microbiological Risk Assessment

Development of internationally agreed principles of microbial food safety risk analysis, including those for risk assessment, fell to CAC (see Box 2). In practice, most of the work has been coordinated by FAO/WHO's Joint Food Standards Program and undertaken not only through a series of expert consultations that have become known as Joint FAO/WHO Expert Meetings on Microbial Risk Assessment (JEMRA) but also through the Codex Committee on Food Hygiene. Arguably, the most fundamental of the CAC risk assessment documents is Guideline CAC/GL 30 (1999): "Principles and Guidelines for the Conduct of Microbiological Risk Assessment" which establishes high-level desiderata for the conduct of transparent, defensible, science-based estimates of the magnitude and severity of foodborne microbiological risks. Importantly it does not specify methods for risk assessment, but describes the qualities of defensible and informative risk assessments. The guidelines (summarized in Box 3) reflect principles articulated in 1990 by Morgan and Henrion as the 'Ten Commandments' of risk assessment in the context of policy decision making.

It is useful to elaborate on some of the principles articulated in CAC/GL (1999), as discussed further below.

Functional Separation Between Risk Assessment and Management

The need for functional separation between risk assessors and managers is to maintain objectivity in the risk assessment process. Risk managers, due to their familiarity with the risk and its potential sources, may have preconceived ideas about the magnitude of the risk and how it is best (or most pragmatically) managed. A risk assessor should be more 'removed' from the situation, and also be able to view the risk from different, and more holistic, perspectives and to evaluate it from more diverse sources of information, (e.g., a simple approach to risk assessment is to review illness incidence data and reports to quantify the magnitude of the risk; however, that information is often not systematically collected and is often biased toward known problems or pathogens for which established test methods are available). The intention is that a systematic risk assessment approach will lead to more reliable estimates of actual risk, rather than perceived risk, and thereby to better risk management strategies and actions.

Any Constraints that Impact Upon the Risk Assessment … Should be Identified and Their Possible Consequences Described

As noted in the section on Transparency, risk assessment should be 'transparent,' meaning that the data, methods and calculations used, and assumptions made, should be thoroughly

Box 2 International development of formalized microbial food safety risk assessment

The General Agreement on Tariffs and Trade (GATT) was an international forum established in 1948 by the UN to liberalize and 'harmonize' international trade. GATT was established as an interim measure due to inability of the UN at that time to agree on the establishment and operation of an 'International Trade Organization' (ITO). The ITO was envisioned as a UN-based body that would establish equitable rules for international trade.

From 1948 to 1995 GATT met periodically as a body of national representatives at various locations around the world. The Uruguay Round of GATT, initiated in 1986 and finalized in 1995, led to the creation of the World Trade Organization (WTO) to fulfill (at least some aspects of) the role originally conceived for the ITO and also because GATT had become effectively unworkable due to changes in the world economy, especially 'globalization,' as it was first convened.

In 1995, the WTO introduced the Sanitary–Phytosanitary Agreement and Technical Barriers to Trade Agreement that:

1. mandated (in effect) demonstrable, and unacceptable risk to human, plant, or animal populations in an importing nation as the only basis on which import of foods from other nations could be restricted,
2. sought to establish the 'equivalence' of efficacy of disparate (food) safety regulatory systems in trading nations by comparing the level of protection that each system afforded. The aim was to facilitate international trade without being prescriptive about methods and strategies to achieve required levels of consumer, agricultural, and ecological protection.

Both agreements required internationally accepted methods to assess risks and the efficacy of diverse risk management approaches. Thus, science based, risk assessment methods were proposed by the WTO as the means for evaluating risk from imports and 'equivalence' of risk management systems. However, whereas several nations had begun to adapt risk assessment methods to microbial foodborne hazards for the establishment of domestic regulations and food safety management strategies, at the time of introduction of the WTO agreements there were no internationally accepted methods for the objective assessment of foodborne microbial risks.

The task of developing those methods fell to Food and Agriculture Organization (FAO) and World Health Organization (WHO), through the Codex Alimentarius Commission. FAO and WHO have convened a series of international expert consultations, which have become known as the Joint FAO/WHO Expert Meetings on Microbial Risk Assessment (JEMRA), to develop agreed principles for food safety risk analysis. JEMRA was intended to become a dynamic body analogous to the Joint Expert Committee on Food Additives (JECFA), established in 1956. Well-defined procedures for assessing the safety of chemical food additives, and for determining tolerable levels, had been established by JECFA but were considered to be inappropriate to microbiological hazards. The work of JEMRA has included the development of guideline documents and the conduct of a number of risk assessments employing those guidelines for specific hazards and food combinations. The JEMRA risk assessments have usually been done in response to questions from Codex committees for the purposes of establishing international guidelines and standards. Further information and copies of documents can be found at: http:www.who.int/foodsafety/micro/jemra/en/

Box 3 Principles for the conduct of microbiological risk assessment (CAC/GL, 1999)

1. Microbiological risk assessment should be soundly based upon science
2. There should be a functional separation between risk assessment and management
3. Microbiological risk assessment should be conducted according to a structured approach that includes hazard identification, hazard characterization, exposure assessment, and risk characterization
4. A microbiological risk assessment should clearly state the purpose of the exercise, including the form of risk estimate that will be the output
5. The conduct of a microbiological risk assessment should be transparent
6. Any constraints that impact the risk assessment such as cost, resources, or time, should be identified and their possible consequences described
7. The risk estimate should contain a description of uncertainty and where the uncertainty arose during the risk assessment process
8. Data should be such that uncertainty in the risk estimate can be determined; data and data collection systems should, as far as possible, be of sufficient quality and precision that uncertainty in the risk estimate is minimized
9. A microbiological risk assessment should explicitly consider the dynamics of microbiological growth, survival, and death in foods and the complexity of the interaction (including sequelae) between human and agent following consumption as well as the potential for further spread
10. Wherever possible, risk estimates should be reassessed over time by comparison with independent human illness data.
11. A microbiological risk assessment may need reevaluation, as new relevant information becomes available

documented so that the reliability and limitations of the assessment can be evaluated by others. If there were resource constraints, the risk assessment may not be based on all, or the best, data available, or not using the most complete and objective methods. For example, if time is limited, simpler methods for data analysis might be employed than would be possible with more time, or computing power. These limitations might affect the generality of the results, or might not reveal all aspects of the sources of risk. As such, any constraints that might limit the validity or generality of the assessed risk should be documented and, preferably, their potential consequences discussed. Simple risk assessments do not necessarily produce results significantly different than those derived from more complex ones if they are based on the same data and knowledge, but complex analyses are likely to provide more subtle insights about the 'range' of

risk, its sources, and the sets of circumstances under which unacceptable levels of risk arise.

Sources of Uncertainty Should be Documented and Their Influence on the Assessed Risk Estimated

Similar to that discussed in section Any Constraints that Impact Upon the Risk Assessment…, if data or knowledge required to estimate the risk is unavailable or incomplete, then the influence of that uncertainty (or those 'data gaps') on the estimated risk needs to be evaluated and documented. This principle is important because MFSRA is used to support decisions about the most effective ways to manage and minimize risk, and consequent risk management actions can affect individuals, companies, organizations, or nations, i.e., the 'stakeholders.' Risk management decisions derived from results of a risk assessment that is based on incorrect or inadequate data could adversely affect some 'stakeholders' without producing a real, net benefit.

Transparency

Related to the above two sections a risk assessment should be presented in a way that enables its validity to be critically evaluated by others, i.e., to enable expert or 'peer' review. Risk assessments are often complex in structure, with voluminous supporting data, and can produce nonintuitive results. Given the complexity of the systems being modeled (see section A Microbiological Risk Assessment Should….) and the possibility of correlations (either positive or negative) between factors influencing risk, logical errors in risk assessment models can occur. A common example is to forget to include the negative correlation between shelf life of foods and the storage temperature of foods – if this correlation is not included, pathogen growth to very high levels can be predicted and can skew the risk estimate (i.e., in practice, foods that are held at higher than recommended temperature spoil earlier and are usually discarded without eating; this can ameliorate the probability that high pathogen loads will be encountered by consumers if storage temperatures are inappropriately high). These considerations require comprehensive documentation of data sources, methods of data synthesis and analysis, calculations, assumptions, inferences, etc., so that errors and oversights, and incomplete understanding of limitations in the risk estimate do not lead to inappropriate risk management decisions. As discussed above, incorrect decisions can adversely affect some stakeholders without any real benefit being achieved.

A Microbiological Risk Assessment Should Explicitly Consider the Dynamics of Microbiological Growth, Survival …

Microbial contamination of foods is often a more-or-less random event, but the consequences of such contamination can be magnified enormously by microbial growth or eliminated completely by microbial inactivation processes. Microbial populations present in foods respond dynamically over time to processing conditions, product formulation, and storage and distribution conditions. It was not until the advent of 'predictive microbiology' that microbiological risk assessment became feasible, because predictive microbiology enabled those dynamic responses to be quantified. Microorganisms are living, selfreplicating entities. Severe conditions can kill microbes but, because some can reproduce asexually, even a single surviving cell can recover and grow to produce sufficient cells or toxins to cause illness in a consumer. There is considerable variability in virulence and tolerance of stressful conditions between strains of the same species. Moreover, microbial susceptibility to inimical conditions can be modified by earlier exposure to stressful conditions, as can microbial virulence. Equally, microbes are discrete entities and less likely (than chemical contaminants) to be homogenously distributed in foods. Thus, at low levels of contamination in a batch of foods, some units of the food may harbor a cell that could grow to disease-causing levels although most of the units remain free from risk. To fully characterize the risk of microbes in foods requires a detailed knowledge of microbial ecology and physiology in foods and particularly the dynamics of the microbial ecology of foods.

Microbiological Risk Assessment Should be Conducted According to a Structured Approach that Includes Hazard Identification, Hazard Characterization, Exposure Assessment, and Risk Characterization

Risk characterization describes the process of estimating the magnitude of risk by assessing:

1. how often consumers experience the hazard (termed 'exposure assessment'), and
2. the relationship between the magnitude of the hazard (e.g., numbers of pathogens in the food at the point of consumption, or levels of toxin) and the effect on the consumer (termed 'hazard characterization' or 'dose–response assessment').

Hazard identification involves (1) presentation of the evidence for a relationship between the presence of the hazard in food and human illness, (2) information about the hazard that will assist in determining exposure, and (3) identification of other data that is available to support the risk assessment.

In more established fields of risk assessment, for example, toxicology and/or environmental health, often the major focus of the hazard identification step is to determine if there is sufficient evidence to implicate a substance (e.g., a chemical) as the cause of an adverse health effect (e.g., cancer). In contrast, the hazard in microbial risk assessment is often already known to be capable of causing human illness before the initiation of the risk assessment. The cause-and-effect relationship for microbial hazards can often be measured over short periods of time (hours, days, or weeks) due to the acute nature of foodborne infections or intoxications. Conversely, the consequences of chemical hazards are often only manifest after prolonged exposure, often in the order of years or lifetimes, and leading to chronic disease. Some microbial toxins, for example, aflatoxins, lead to chronic effects after prolonged, low level, exposure.

In the following sections, the four elements of MFSRA are discussed in greater detail.

Hazard Identification

In MFSRA, hazard identification involves the presentation of evidence that a microbe is capable of causing adverse health effects if present in a particular food or group of foods, and that it can be present in those foods. Unlike chemical hazards in foods, the evidence for pathogenicity of foodborne microbes is often stronger because microbes usually cause acute disease (infections or intoxications) and there is often clear epidemiological evidence linking the food, pathogen, and human illness. Because pathogens, or their toxins, are usually able to be isolated from victims, Koch's postulates provide a mechanism for demonstrating cause and effect. There are, however, exceptions. Not all pathogens can be readily cultured and identified from the patient's intestines, stools or their blood, and from the food implicated in illness. Foodborne enteric viruses, for example, are difficult to cultivate. Similarly, *Mycobacterium paratuberculosis* causes Johne's disease, a wasting disease, in sheep and cattle. Crohn's disease is associated with similar symptoms in humans and it has been suggested that Crohn's disease may be caused by *M. paratuberculosis*, possibly acquired from meat or milk from animals affected by Johne's disease. The onset of symptoms is very slow however and, at the time of writing, the causative link between *M. paratuberculosis* and Crohn's disease is not established, let alone whether infection can be acquired from food. A hazard identification would present the evidence that such links may exist as the justification for undertaking a risk assessment. Sometimes MFSRA is undertaken to test the strength of the evidence implicating a relationship between human disease, a suspected pathogen, and food.

Hazard identification in MFSRA also describes the severity of the outcomes of exposure. For example, the consequences of intoxication with staphylococcal enterotoxin (i.e., vomiting for several hours) are far less severe than the consequences of infection of a pregnant women with *Listeria monocytogenes* (i.e., potentially fetal death or miscarriage). A useful measure of the severity of consequences is the disability adjusted life years (DALY) concept, which is a measure of the time that the symptoms of the disease persist (measured in years of fractions of year) and the extent to which the victim's normal activities are compromised, assessed as proportional loss of normal life activities. For example, if a person were infected with *Salmonella* for 3 days, and it prevented them from attending their normal work, the loss of function might be assessed as 50% for 1/120th of a year. In this case the DALY would be 0.0042. Conversely, if an adult were intoxicated by botulin from *Clostridium botulinum* in a food, and required hospitalization involving life support for 12 months, the DALY might be calculated as 90% loss of function for 1 year, i.e., the DALY would be 0.9. If a pregnant woman contracted listeriosis and lost her unborn child, the DALY would be 100% times the life expectancy of the unborn child, for example, 80 years. In this case the DALY would be 80. In this way systemic listeriosis in a pregnant women would be considered to be tens of thousands of times more severe than salmonellosis, and 90 times more severe than nonfatal botulism.

Exposure Assessment

'Exposure assessment' in MFSRA is the qualitative and/or quantitative evaluation of the likely intake (both frequency and amount) of pathogens or their toxins via food as well as exposures from other sources if relevant. In other words, it aims to answer the questions: How often, and at what levels, is the hazard ingested in a specific food or group of foods of interest?

To assess exposure one needs to consider:

- how much of the food is eaten, by whom, and how often,
- how often the food is contaminated and how heavily contaminated it is,
- how the contamination arose, for example, at what point in the farm-to-fork pathway, and
- changes in the hazard level between the point of harvest or processing and consumption, and by how much it changed.

To answer these questions, data from many sources are combined.

Questions about the route of contamination and change in contamination level usually require the development of a 'conceptual model' that describes in text or mathematical equations the 'farm-to-fork' pathway, including all points where contamination could occur. It also includes detailed description of the factors that could change the hazard level, such as processing operations and conditions, and times and temperature of storage and transport. In the case of bacterial and fungal hazards this will also include knowledge of the food's composition (pH, water activity, preservatives, etc.) so as to be able to estimate microbial growth (or death) and toxin production, i.e., using predictive microbiology models. Processes such as:

- mixing, leading to dilution;
- drying, leading to concentration or decreased water activity; and
- separation of fatty components from water components that might lead to concentration or reduction in the levels due to preferential partition of the hazard into the oil or aqueous phases, etc.,

also need to be described.

For consumption estimates, data sources include national nutrition/diet surveys, production figures for various food industries, import and export statistics, data from supermarkets or market survey companies that estimate the sales of various types of products and foods, etc. National dietary surveys also often include breakdown of consumption into different age and gender classes and geographic regions. Food categories used in those surveys, however, are often based on nutritional considerations, rather than categories that relate to the probable presence of foodborne hazards.

Prevalence and concentration of pathogens (or their toxins) in foods at the time of consumption are required to estimate risk. Available data describing levels and frequency of the hazard in the food, however, almost always relate to times in the farm-to-fork chain much earlier than the point of consumption, requiring the application of predictive microbiology as discussed below. Sources of data relating to prevalence, and

concentration, of microbial hazards at selected points in the farm-to-fork chain include industry organizations, and hazard analysis critical control points (HACCP) and quality assurance records of individual companies. Some government agencies also conduct routine and random monitoring of selected hazards in foods. Additionally, there are many small studies published in scientific journals.

Predictive microbiology (*q.v.*) models facilitate the estimation of change in pathogen levels between various stages in the farm-to-fork chain and point of contamination. Essentially, predictive microbiology models summarize the ecology of microbial hazards in foods. To use predictive microbiology for estimating hazard levels at the time of consumption requires knowledge of food-processing operations, physicochemical properties of the food, and times and environmental conditions (particularly temperature) experienced by the food from harvest and during processing, usually available from companies that process foods. Formulation data may also be available from the published literature. In addition, several large studies have been undertaken in recent years that determined temperatures of foods during storage, transport, retail display, and home storage. Some data are available from industry groups and others have been published in the public domain.

Hazard Characterization

Hazard characterization describes and, ideally, quantifies the adverse health effects associated with the various microbial hazards that may be present in food. This requires that a 'dose–response assessment' be performed if the data are obtainable. In practice, the relationship between pathogens and consumer illness is better described as a dose versus probability of infection or probability-of-illness relationship because, unlike chemical contaminants, the symptoms of infection do not usually change qualitatively as a function of dose although they may change in severity or duration.

Data to define human microbial 'dose–response' relationships are not readily available, for ethical reasons, although limited older data from volunteer trials do exist. These are usually derived from healthy, young adults, however, and do not reflect the susceptibility of the general population accurately; it is known that the very young and the elderly are more susceptible to foodborne infections because of their undeveloped or waning immune responses. Equally, in many nations there are increasing numbers of consumers whose immune system is weakened by underlying illness or medical treatments (e.g., immunosuppressive therapies for organ transplant recipients). Given these limitations, many of the available dose–response relationships for foodborne pathogens are derived from the analysis of collations of outbreak data. The limitations of outbreak data (e.g., difficulty in determining doses ingested from foods possibly ingested days earlier) are also recognized and acknowledged. Animal models have also been used to gain information about dose–infection relationships for some pathogens but animal models do not always reflect accurately infection processes in humans, or the consequences of toxin ingestion by humans, due to the specificity of the pathogen–host interactions leading to infection and differences in biochemical pathways in different species.

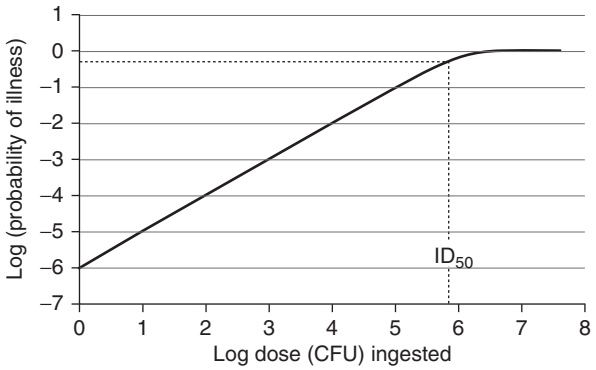

Figure 1 An illustration of the predictions of an exponential 'dose–response' model. The exponential model is often used in MFSRA to relate the probability of illness to a given dose of a pathogen. The intercept of the model with the *y*-axis gives the '*r*' value of the exponential model, which is effectively the probability that one cell of the pathogen could cause illness (i.e., in this example, 1 in a million). The dotted lines illustrate the calculation of the ID_{50}, i.e., that dose that would be expected to lead to illness in 50% of the population which, in this example, is approximately 70 000 cells.

There is also debate concerning the nature of the infection process and how this can be modeled mathematically. A JEMRA consultation concluded, however, that models that are based on the single-hit hypothesis of infection are the most biologically plausible. Of this class of models the 'exponential' and 'Beta-Poisson' models are the most widely used, with the exponential model being simpler and relying on fewer assumptions. The Beta-Poisson model includes the possibility of variability in infectivity of individual cells in the dose ingested and that for some organisms some proportion of the exposed population will never become no matter what the dose. Both models predict that the probability of infection is a simple function of the dose of cells ingested, up to some upper limiting dose, above which the probability of illness does not increase further. An example of a dose versus probability of illness relationship is shown in Figure 1.

Risk Characterization

Risk characterization synthesizes the exposure assessment with the dose–response relationship to generate an estimate of human health risk. To do so, however, one has to understand all the factors that contribute to, or affect, the risk to the consumer, and the interrelationships between these factors. This involves the articulation of our understanding of how the risk arises, and changes in the level of the hazard from the point of contamination to the point of consumption. This description, whether expressed in words or as mathematical equations, may be referred to as the 'conceptual model.' An example is shown in Figure 2.

The estimated risk can be expressed in many ways including risk per serving, risk to an individual or to an entire population and over some defined time interval, etc., and may be expressed in absolute or relative terms. The use of relative measures of risk can often simplify the risk assessment process

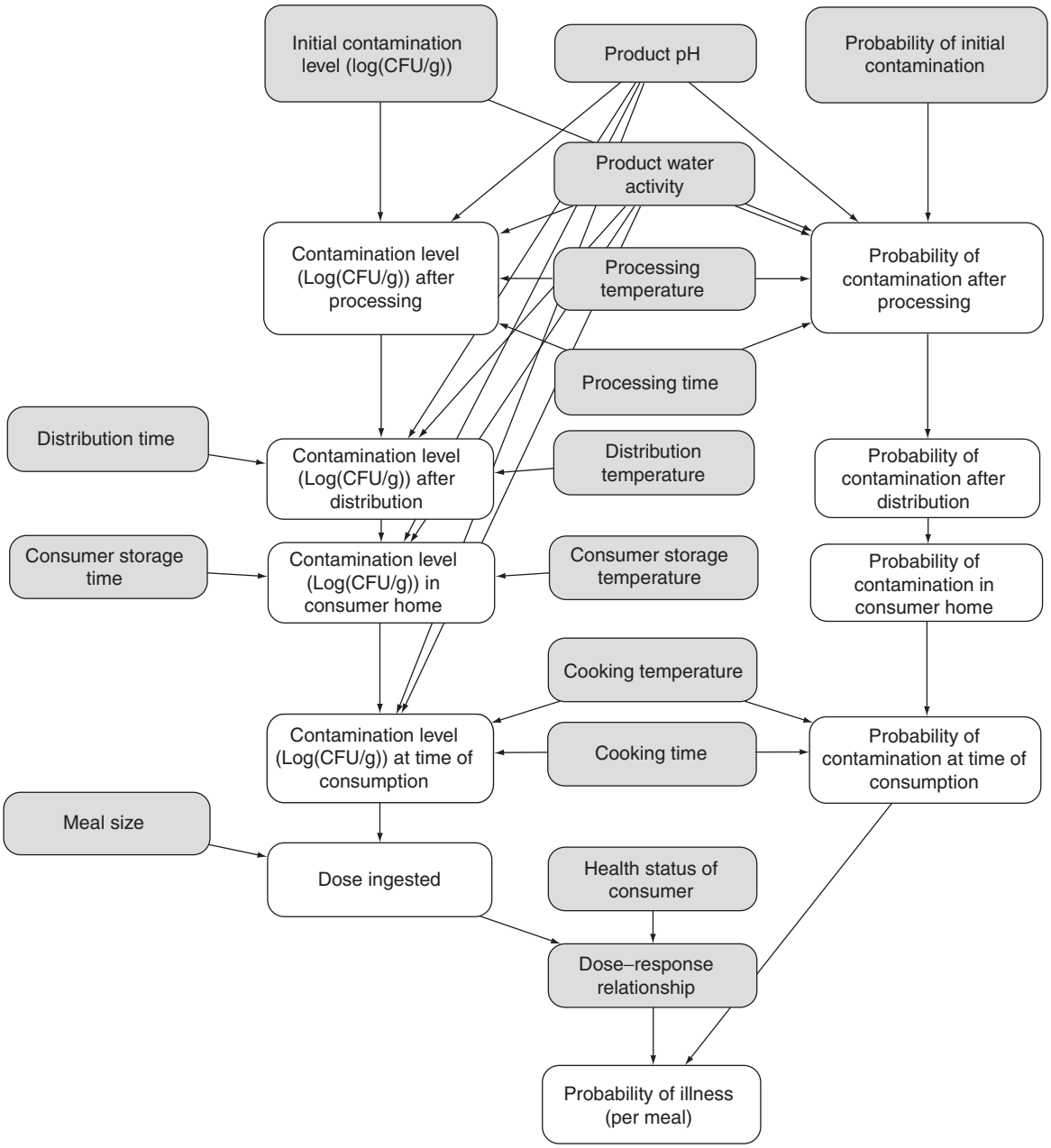

Figure 2 An illustration of a simple 'conceptual model' for assessing risk of microbial foodborne illness from a factory-to-fork food supply chain. The model shown is also described as an 'influence diagram' because arrows in the diagram indicate the influences that various factors have on other variables that affect the final risk to the consumer at the point of consumption. Variables that must be described by data (i.e., obtained by studies or surveys) are shown in shaded boxes whereas variables that are derived from knowledge of other factors, including the final estimate of risk (i.e., probability of illness per meal), are shown in unshaded boxes. In a quantitative risk assessment, the relationships depicted by the arrows are defined and quantified by mathematical equations. The model is a form of 'process risk model' and at each stage in the factory-to-fork process the model calculates both the concentration of pathogen (or toxin) in the food and also the likelihood that the pathogen is present at all in the food.

and yet still provide adequate advice to risk managers. For example, if risk managers determine that the only viable risk management alternatives to manage the risk of pathogen growth in a product are:

1. to reduce the nominal shelf life of the product by 10 days, or
2. to reduce the microbial growth rate in the product by a reduction of pH by 0.5.

A simplified model that estimates the proportional risk reduction arising from either of those alternatives would be sufficient to answer the risk management question (i.e., which alternative would be more effective) without

having to determine the absolute risk to consumers or detail all other risk-affecting factors because they would stay the same. In this case, the risk assessment might devolve to calculation, using predictive microbiology models, of the expected reduction in the extent of growth of the pathogen by either of the alternatives. Conversely, if the purpose of the risk assessment were to identify and rank all possible risk management alternatives, the conceptual model would need to detail the effects of all risk-affecting factors, and their interrelationships, to provide the additional insights needed to support for the decisions of the risk manager.

Methods for Risk Characterization

JEMRA have presented guidelines for the conduct of risk characterization. The recommendations are not prescriptive but recognize that the most appropriate risk characterization approach may depend on the problem being addressed, as illustrated above. Thus 'qualitative' (descriptive) methods may be useful and adequate in some cases, and use terms or rankings like 'higher' or 'lower' to describe the relative risk. This provides limited guidance for decisions, however, so that terms like 'much' higher or 'significantly' higher, etc., may be required. Clearly, however, such terms are subjective and could be interpreted in different ways by different people. In most cases a risk assessment will need to 'quantify' the magnitude of the risk, or magnitude of differences in risk (or relative risk). In essence, risk is a quantitative concept, and mathematical approaches to risk assessment are usually needed. In those cases the conceptual model is expressed as a series of mathematical equations.

In a quantitative risk assessment each of the values that contribute to risk can be defined as a single, representative, value. This is termed a 'deterministic' approach. But such values are rarely constant and the question arises, what is the best representation of the value of each factor? The average value is an obvious choice, but can lead the risk assessment to underestimate or ignore risks. For example, if the average storage temperature and time were used to estimate the potential growth of *Salmonella* in a salad sandwich, it might be concluded that there would never be a problem because any *Salmonella* present would never grow to unacceptable levels. In reality, it is likely that sandwiches are occasionally kept too warm or kept for too long. Such conditions may lead to an unacceptable risk, and need to be included in the risk assessment because they are the scenarios of most interest for identifying risk management needs. An alternative approach is to use the worst-case levels for each factor contributing to the risk, but this will lead to an overestimation of the risk because a situation in which each factor is at the most hazardous level will almost never occur.

In practice, the risk from a particular food and process will be variable, depending on many variable factors. Given this, one approach to characterize the risk would be to calculate the risk for a range of scenarios, with different values for each of the risk-affecting factors. From this, an estimate of the 'range' of the risk, and extreme levels of risk, and some representative measures (e.g., average, mode, etc.) could be determined. The more such scenarios are calculated, the more complete the risk estimate will become. The process of calculations would be tedious, however, unless it could be automated. Stochastic simulation modeling using computer software does this and, for reasons outlined above, currently is often the preferred method for MFSRA though other methods are equally valid, if not preferable, depending on the risk assessment question. An example of the difference between insights obtained from deterministic compared to stochastic approaches is presented in **Box 4**, and discussed further below.

In stochastic simulation modeling each factor in the conceptual model is described by a 'distribution' of possible values, i.e., the full range of values that the variable could take, as well as the probability that any given value in that range will be observed. The software systematically evaluates the model tens or hundreds of thousands of times, each time taking a different combination of values from each of the distributions and calculates the expected outcome according to the conceptual

Box 4 Example of the differences in deterministic and stochastic risk assessment

Stochastic models are usually more informative than deterministic models because most processes leading to foodborne risk are variable, and not readily defined by a single representative value. As an example, **Figure 3** presents a very simple 'farm-to-fork' food safety risk assessment model for an infectious pathogen. The conceptual model is presented as an influence diagram on the left side of the figure. The right hand side of the figure shows the distributions of values of factors that contribute to the risk, namely:

1. the initial contamination level on the food,
2. the potential growth that occurs between farm and consumption,
3. the size of the meal consumed (which affects the dose ingested), and
4. the dose–response relationship or, in the case of the deterministic model, the ID_{50} as a representative value for the average dose required to cause illness.

In the deterministic model, the average value of the distributions was used for calculations, i.e., 1 cell per 50 g (i.e., below the reliable detection limit), 100-fold increase in cell numbers (i.e., 2 logs of growth), 100 g meal, and $ID_{50} = 700\,000$ cells. Using these values, the dose ingested never exceeds a dose that is typically expected to cause illness and no cases of illness are predicted even in 1 million meals consumed.

Conversely, when distributions of values and a dose–response model are used, the expected number of illnesses from 1 million meals is 254. Most risk managers would consider that this level of illness is too high and would implement actions to further reduce the risk to consumers so that an appropriate level of consumer protection was achieved. Note that in this comparison, the average of values contributing to the risk (i.e., the initial contamination level, potential for pathogen growth, and size of the meal) are identical in the two approaches to the risk assessment, but the stochastic approach shows that in some cases an unacceptable risk occurs, whereas the deterministic approach predicts that the risk, 'on average,' is acceptable. Thus, the stochastic approach leads to a very different risk management decision than the deterministic approach.

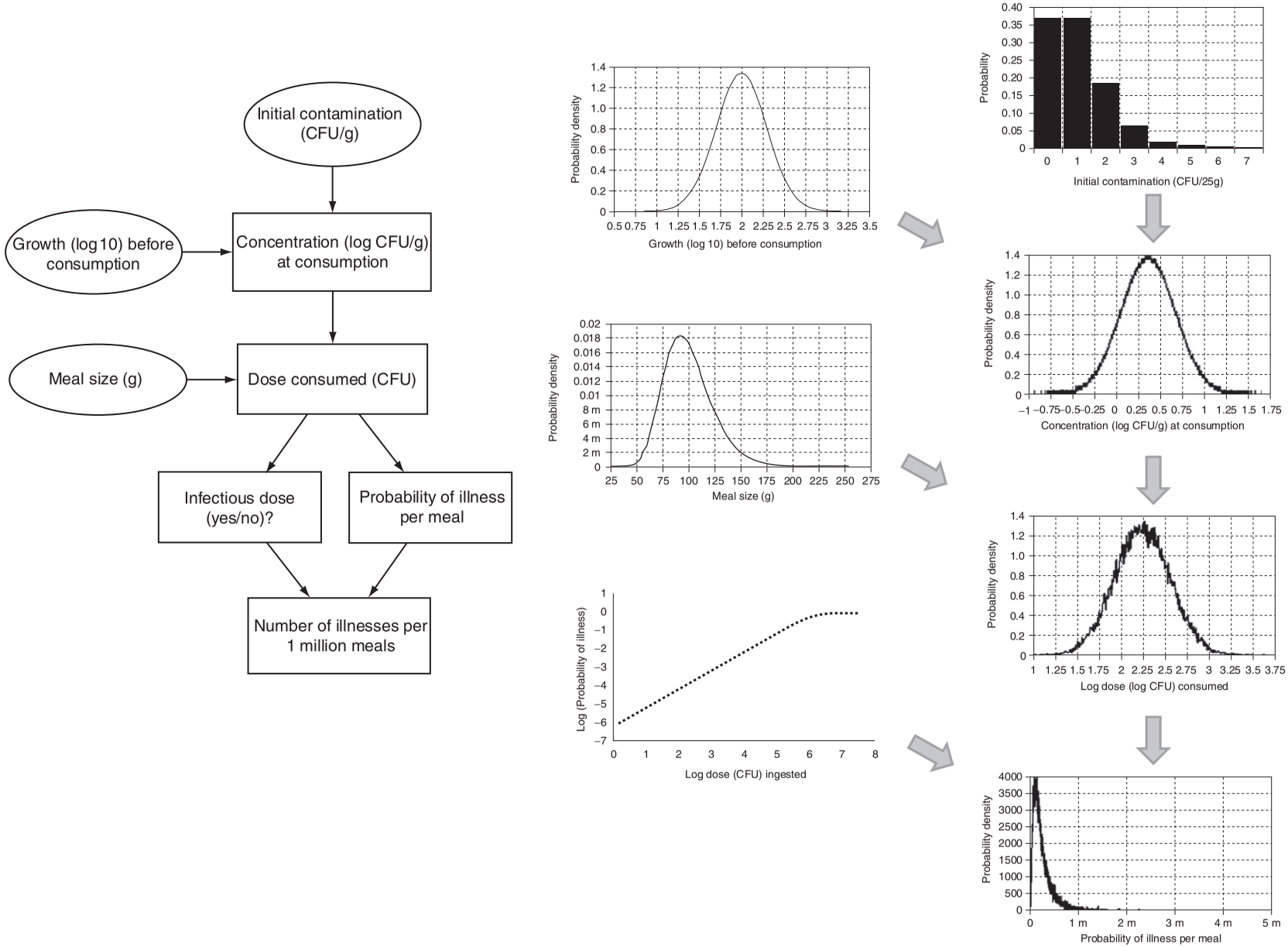

Figure 3 A simple risk assessment model illustrating a stochastic risk estimation process (see text for details).The graphs on the right represent the distributions of values for the variables in the model (oval shapes) and results derived from them (rectangular shapes). When distributions of values are used for calculations, the estimated risk is 254 illnesses per 1 million meals. When the average values of the distributions are used for the estimation of the risk, no illness is predicted.

model. Each calculation is termed an 'iteration' and represents one possible meal scenario, i.e., the risk from one unit of food under a particular set of circumstances. As more iterations are performed a distribution of possible levels of risk is generated, representing more and more possible sets of circumstances for that food and its consumers. In some cases the sets of circumstances will lead to an unacceptable risk to the consumer that would not have been revealed by calculations that considered only typical, or average, sets of conditions.

The four steps of risk assessment are conducted sequentially, i.e., hazard identification must precede the other steps in the risk assessment process because it is a precursor to the risk assessment. Similarly, the risk characterization step is undertaken last because it is the synthesis of the exposure assessment and hazard characterization steps, but those two steps can be conducted in parallel. Sources of more detailed and practical information about the conduct of the four steps of MFSRA are listed in the 'Further Reading' section.

Uncertainty and Variability

Complete and perfect information and knowledge is rarely available for the conduct of risk assessments. As such, a risk assessment provides an imperfect estimate of risk and the significance of the various factors that contribute to it. Risk assessments are used to assist decision making and, accordingly, the level of confidence in the accuracy of the risk estimate, or the range of the risk estimate, needs to be explained to risk managers so that they can balance the risk assessment information against other information and considerations when making risk management decisions.

Uncertainty and variability both affect the confidence one can have in the risk estimate. Uncertainty describes information that is required, but is not available, or that one cannot have much confidence in the data that is available. Variability reflects the fact that in many systems slight differences in conditions affect the outcome of the process, for example, the amount of food that different consumers eat, the health status of an individual consumer, etc. Although uncertainty can be reduced by gathering further data, usually variability cannot. It may be possible to decrease variability by making the model more detailed, for example, specifically modeling individual differences in consumer susceptibility to infection due to their individual health status. This approach is sometimes described as 'disaggregation,' and refers to differentiating average values for larger groups into estimates specific to subcategories within those larger groups. Doing so, however, may make the conceptual model more complex because disaggregation may lead to the need for other related data, specific to the individual cases. For example, if a model discretely considered differences in individual susceptibility to infection by virtue of health status, it might be necessary to also recognize corresponding differences in consumption amount, or meal preparation methods, that might also affect risk to those subgroups, and thus require more data. If that data were not available, the net effect of the decreased variability in dose–response could be to increase uncertainty overall.

Mathematical methods for systematic treatment and evaluation of variability and uncertainty are considered in JEMRA publications, and the published literature. In many MFSRAs variability is estimated by the stochastic simulation modeling process, but it is also important to document sources of uncertainty in the risk assessment (e.g., missing/unavailable data or knowledge, assumptions that were made) and the potential consequences of that uncertainty for the estimate of risk. Such an evaluation can be undertaken by calculating the conceptual model for different assumptions, or different values of uncertain factors, and comparing the new risk estimates with those under the original set of assumptions. If the risk estimate is little affected by the new set of assumptions, it suggests that the missing or uncertain information is not critical to the risk estimate and risk management decision.

Other Methods for Risk Assessment

MFSRA has become almost synonymous with stochastic modeling of the entire farm-to-fork food chain and, for some kinds of risk management decisions, these approaches may be the most appropriate. This perception may, however, have inhibited the uptake of risk assessment principles for food safety management decisions because of concerns about the cost, time, and expertise required because those approaches to MFSRA can be very time- and labor-intensive.

As noted, risk assessment is intended to improve the quality of decision making. JEMRA does not specify methods for risk assessment but rather emphasizes principles. The actual methods used for a risk assessment should be appropriate to answer the specific questions of a risk manager. Accordingly, for some risk management questions (as illustrated earlier), simpler approaches can provide equally rigorous support for decisions, particularly when the decisions are more specific, for example, what is the relative public health burden of salmonellosis compared to listeriosis, which might be addressed by considering the number of cases of each and the typical severity of each infection using the DALY concept. Similarly, epidemiological data and exposure assessment (based on consumption data and predictive microbiology) have been used to rank the relative risk of illness from listeriosis in a wide variety of ready-to-eat foods but without having established a dose–response relationship for listeriosis.

There is also a desire for risk assessment methods that are quicker, for example, for questions that require only a 'first estimate' to determine whether the apparent risk warrants further, more detailed, investigation. Accordingly, risk assessors are attempting to define simpler, less resource demanding, methods that are appropriate to different types of risk assessment questions. To this end, various types of 'risk matrices' have been presented in which users select categories that describe hazard severity, effects of processing, sensitivity of exposed populations, etc., and then use a decision matrix (often a series of tables) to generate a risk estimate. These approaches can suffer from the limitations of qualitative risk assessments because it is difficult to achieve logical consistency when combining subjective categorical/descriptive terms to generate an essentially quantitative risk estimate, or risk ranking. Several simplified MFSRA models have been presented and adopted, however, that lead risk assessors through relevant questions, requiring that the user selects descriptive terms (that are equated to values or ranges of values), and that

then combine the numerical response via a generalized conceptual model. The models are presented as interactive software that, once values are selected or entered, automates the risk calculation. Their effectiveness, however, requires that reliable data or knowledge are available to enable the various questions to be answered correctly.

Summary

The systematic, objective, and science-based approach of risk assessment can support decisions about food safety management at all levels, from individual businesses, to local and national governments, setting international rules for trade in food. At the international level, MFSRA was advocated by the World Trade Organization as a means to harmonize international trade in foods by providing a framework to establish objective regulations. This impetus led to the development of guideline documents for the conduct of MFSRA by the UN's FAO and WHO to provide advice to the CAC and also to assist member nations to develop capacity in MFSRA. Within individual nations MFSRA can be used, among other applications, to prioritize resources to those hazards with the greatest public health burden, to establish risk management approaches relevant and achievable within that nation and culture, etc.

It is widely recommended that MFSRA follow a systematic approach involving four steps that describe (1) the hazard and consequences of its ingestion, (2) the likely exposure of consumers to that hazard in food, (3) the relationship between the amount of the hazard ingested and the probability and severity of illness, and (4) this information is synthesized to generate an estimate of risk (i.e., likelihood, severity, and magnitude of harm to consumers) that can be compared to the risk under other circumstances or proposed management strategies, thus enabling the elucidation of the most effective risk management strategies, or comparison of different risk management systems in different regions, etc.

Although stochastic simulation modeling is generally regarded as the method of choice for risk assessment, there are no internationally agreed methods for MFSRA. This is because different methods will be appropriate to different types of risk management questions, and because time, data, and/or resource limitations may preclude the development of fully quantitative, stochastic, farm-to-fork risk assessment models. Instead, FAO/WHO through the CAC have articulated a series of guidelines that represent 'best practice' in MFSRA. Paramount in those guidelines is the need for transparency of the MFSRA process, in terms of the data and knowledge used, assumptions made, resource or other limitations on the conduct of the risk assessment, and where uncertainty exists. These elements should be documented as part of the risk assessment.

Transparency is needed to gauge the reliability of the risk assessment and estimate derived because risk assessments are used to support decisions concerning food safety management. Those decisions will often have both positive and negative consequences for the food industry and risk managers need to ensure that a net benefit results from the risk management strategies they implement. Risk assessments combine data and knowledge from diverse sources and often require complex mathematical treatments and logic, but they are also often based on imperfect data and knowledge and rely on various simplifying assumptions. Transparency and documentation enable peer review and open evaluation of the risk assessment and its conclusions by those that may be affected by the risk management decisions arising from the MFSRA. The review process also contributes to better and more effective risk management decisions and strategies.

See also: Bacteria: *Mycobacterium avium* ssp. *paratuberculosis*. Foodborne Diseases: Foodborne Diseases and Vulnerable Groups; Overview of Biological Hazards and Foodborne Diseases. Institutions Involved in Food Safety: FAO/WHO Codex Alimentarius Commission (CAC). Public Health Measures: Evaluation of the Efficacy of National Food Control Programs; International Standards and Harmonization of Food Safety Legislation; Modern Approach to Food Safety Management: An Overview. Risk Analysis: Estimating the Burden of Foodborne Disease; Risk Assessment: Principles, Methods, and Applications; Risk Communication: Biological Hazards; Risk Communication; Risk Management: Application to Biological Hazards

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Risk Assessment: Chemical Hazards

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Introduction

Over the past century, the use of natural and synthetic chemicals has largely created our modern world, which has improved the lives of many billions of people. Some of these chemicals have been used by the food industry to improve food safety and quality and reduce spoilage and wastage, thus increase its availability, accessibility, and/or affordability. As with any technology, the use of such chemicals are not without certain risks; therefore these need to be addressed and actively managed. Besides these intentionally added chemicals, there are also chemical contaminants that are inadvertently present in food that may also pose risks to health. These include chemicals that are naturally occurring toxins or that are produced during handling, storage, and processing, including cooking. Chemical contamination of food can also arise from the environment through polluted air, water, and soil. Finally toxic chemicals may be illegally introduced into food to defraud consumers or for other criminal purposes.

With the development of risk analysis principles and procedures in the US, the approach to managing chemical hazards has become more methodical and to some extent, harmonized. In the risk analysis paradigm, the scientific evaluation of a chemical is based on an assessment of the potential risk, consisting of hazard identification, hazard characterization, exposure assessment, and risk characterization. Since 1995, the use of risk assessment for food safety has been required by all member countries of the World Trade Organization (WTO) under the WTO's Agreement on the Application of Sanitary and Phytosanitary Measurers (SPS Agreement). Note that the SPS Agreement explicitly recognizes the standards, guidelines, and other recommendations of the Codex Alimentarius Commission (Codex) as representing the *de facto* international reference for risk assessment and management for food safety. In this regard, Codex has developed definitions and principles for risk analysis that should be consulted to ensure compliance with the SPS Agreement.

In relation to risk assessment, Codex has provided guidance on risk assessment policy and risk assessment as follows:

> *"RISK ASSESSMENT POLICY*
>
> *Determination of risk assessment policy should be included as a specific component of risk management.*
>
> *Risk assessment policy should be established by risk managers in advance of risk assessment, in consultation with risk assessors and all other interested parties. This procedure aims at ensuring that the risk assessment is systematic, complete, unbiased and transparent.*
>
> *The mandate given by risk managers to risk assessors should be as clear as possible.*
>
> *Where necessary, risk managers should ask risk assessors to evaluate the potential changes in risk resulting from different risk management options.*
>
> *RISK ASSESSMENT*
>
> *The scope and purpose of the particular risk assessment being carried out should be clearly stated and in accordance with risk assessment policy. The output form and possible alternative outputs of the risk assessment should be defined.*
>
> *Experts responsible for risk assessment should be selected in a transparent manner on the basis of their expertise, experience, and their independence with regard to the interests involved. The procedures used to select these experts should be documented including a public declaration of any potential conflict of interest. This declaration should also identify and detail their individual expertise, experience and independence. Expert bodies and consultations should ensure effective participation of experts from different parts of the world, including experts from developing countries.*
>
> *Risk assessment should be conducted in accordance with the Statements of Principle Relating to the Role of Food Safety Risk Assessment and should incorporate the four steps of the risk assessment, i.e. hazard identification, hazard characterization, exposure assessment and risk characterization.*
>
> *Risk assessment should be based on all available scientific data. It should use available quantitative information to the greatest extent possible. Risk assessment may also take into account qualitative information.*
>
> *Risk assessment should take into account relevant production, storage and handling practices used throughout the food chain including traditional practices, methods of analysis, sampling and inspection and the prevalence of specific adverse health effects.*
>
> *Risk assessment should seek and incorporate relevant data from different parts of the world, including that from developing countries. These data should particularly include epidemiological surveillance data, analytical and exposure data. Where relevant data are not available from developing countries, the Commission should request that FAO/WHO initiate time-bound studies for this purpose. The conduct of the risk assessment should not be inappropriately delayed pending receipt of these data; however, the risk assessment should be reconsidered when such data are available.*
>
> *Constraints, uncertainties and assumptions having an impact on the risk assessment should be explicitly considered at each step in the risk assessment and documented in a transparent manner. Expression of uncertainty or variability in risk estimates may be qualitative or quantitative, but should be quantified to the extent that is scientifically achievable.*
>
> *Risk assessments should be based on realistic exposure scenarios, with consideration of different situations being defined by risk assessment policy. They should include consideration of susceptible and high-risk population groups. Acute, chronic (including long-term), cumulative and/or combined adverse health effects should be taken into account in carrying out risk assessment, where relevant.*
>
> *The report of the risk assessment should indicate any constraints, uncertainties, assumptions and their impact on the risk assessment. Minority opinions should also be recorded. The responsibility for resolving the impact of uncertainty on the risk management decision lies with the risk manager, not the risk assessors.*

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> *The conclusion of the risk assessment including a risk estimate, if available, should be presented in a readily understandable and useful form to risk managers and made available to other risk assessors and interested parties so that they can review the assessment.''*

On behalf of Codex and member countries of the World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO, the risk assessment for chemicals is carried out mainly by two international expert bodies, namely the Joint WHO/FAO Expert Committee on Food Additives (JECFA), which evaluates food additives, contaminants and veterinary drug residues in food, and the Joint FAO/WHO Meetings on Pesticide Residues (JMPR), which evaluates insecticides, herbicides, fungicides and related chemicals. At times special *ad hoc* expert committees are convened by FAO and WHO to address urgent issues, as was the case with the first evaluation of acrylamide. The methods and procedures used by JECFA and JMPR for evaluating chemicals in food have been recently updated in 2009.

Although there are some similarities, with chemical risk assessment the risk assessment of microbiological hazards has a number of significant differences. It should also be noted that health risks may also be posed by nutritional hazards, such as the inadequate intake of essential micronutrients, including vitamins and minerals. Within Codex, the responsibility for nutritional hazards is under the Codex Committee on Nutrition and Food for Special Dietary Needs. Whereas JECFA reviews the potential risk of excessive consumption of food fortification agents, the establishment of minimum intake levels is handled by other FAO and WHO expert committees.

Risk Assessment and Risk Management Interface

One of the earliest communications between risk assessor and risk managers involves the preparation of the risk profile upon which the risk manager decides whether a full risk assessment is appropriate. The potential toxicity of a chemical is based not only on its chemical structure, but also its likely levels in the diet. Therefore, even a chemical with low toxicity can be harmful if exposure is sufficiently high.

Obviously, a chemical that produces toxic effects shortly after ingestion of the food can be easily identified as a candidate for risk assessment. However, most cases are not so clear. In the early 1900s in the US, for example, the public health risks posed by the widely used, but unregulated food additives benzoic acid and boric acid were hotly debated. At one point, human volunteers were recruited to actually test the toxic potential of these chemicals, although laboratory animals are now used for this purpose.

Today in most countries, food safety legislation requires that any chemical intentionally added to food be shown to be safe through appropriate tests in animals, usually rats, mice, and sometimes dogs. Consequently, risk assessments are systematically carried out in the case of food additives. Premarket risk assessments are also required for pesticides and veterinary drugs. In some countries, chemicals added indirectly to food, such as processing aids and chemicals that can migrate from food contact materials, are also required to undergo risk assessment. Premarket evaluation is also required for new technologies that may introduce a new chemical or increase the exposure to a known toxic chemical. For example, food irradiation was evaluated for 'unique radiolytic products' and genetically modified plants are evaluated for possible immunoactive proteins.

Many of contaminants and toxins would fall under the traditional term 'poisons' because the time between ingestion and onset of adverse effects is short, in most cases in the order of minutes and hours. For example, certain heavy metals, mycotoxins, and biotoxins are easily recognized as potential foodborne hazards. However, certain chemicals cause adverse effects only after many days, months, or even years of exposure. These so-called 'slow poisons' have proved more difficult to identify. Whereas some chemicals are inherently more toxic than others, delayed effects can be difficult to identify because of the nature of the toxic effects in humans are not known. This is particularly difficult for effects with a large baseline of cases, such as cancer. Sometimes chemicals can cause behavioral changes that are subtle and difficult to measure. Some chemicals, such as endocrine disruptors, can cause adverse effects at extremely low levels and for short durations of exposure. Furthermore, effects may not be manifested for decades, such as in the case of diethylstilbestrol. Consequently, these chemicals are currently the subject of scientific and policy debate.

The presence of some toxicants in food has been revealed by new analytical methods, which have steadily become more sensitive over the years. However, the adverse effects of low-level, long-term exposure to many toxic chemicals remain unknown. Each year approximately 1500 new chemicals are brought onto the market, adding to the approximately 70 000 existing ones. The United Nations Environment Program has estimated that production of chemicals is likely to increase at the rate of 15% a year. Few of these chemicals have been rigorously tested and many of them could potentially contaminate food. Consequently, the food safety database for many chemicals of potential concern may be inadequate. If exposure is expected to be significant, governments and/or industry should undertake the necessary studies to support a risk assessment of these chemicals.

Based on preliminary information from risk assessors along with other information in the risk profile, risk managers may decide that the potential health risk warrants the completion of the full risk assessment paradigm. In that case, the risk manager would usually formulate the questions that the risk assessor needs to answer. The risk manager will also provide the risk assessment policy to be followed, such as default assumptions and procedures for addressing uncertainty (see the Codex recommendations above). In many countries, the risk manager also provides the resources to conduct the risk assessment, but a functional separation between the risk manager and risk assessor is necessary to ensure integrity of the process. The next step is hazard identification, which mainly focuses on certain aspects of toxicology.

Hazard Identification

The first step in a risk assessment process is hazard identification. Hazard identification is the process of determining

whether the nature of potential adverse health effects caused by exposure to a chemical and whether adverse health effects are likely to occur in humans. It examines the available scientific data for a given chemical and uses a weight of evidence approach to characterize the link between the adverse health effect and the chemical exposure. Note that exposure to a chemical may generate many different or multiple adverse effects in humans, including various diseases, organ failure, cancers, reproductive defects, developmental deficits, or other effects. These may occur after high level short-term (acute) exposure or low-level long-term (chronic) exposure.

A wide variety of *in vitro* and animal studies are used to support hazard identification, including toxicokinetics, which involve how the body absorbs, distributes, metabolizes, and eliminates specific chemicals and toxicodynamics, which focus on the effects that chemicals have on the human body. Models based on these studies can describe mechanisms by which a chemical may impact human health, thus providing insights into the possible effects of a chemical.

Epidemiological studies of human populations to examine whether there is an association between exposure to a chemical and a human health effect are preferred, but are often not available because accurate exposure information is lacking, especially for diseases with long induction periods. For many of these chemicals, there is a paucity of reliable data to identify the potential health effects and the populations at risk. Results from animal studies can be used to extrapolate observed adverse effects to humans. *In silico* and *in vitro* test are also useful for identifying toxic potential. In some cases, predictive models based on structure–activity relationships can be used to assess potential toxicity for reproductive and developmental toxicity, genotoxicity, and carcinogenicity.

Hazard Characterization

Hazard characterization may include some of the toxicity studies considered under the hazard identification component and in this regard, it should be noted that risk assessment is an iterative process as new data can alter previous conclusions or raise new concerns. The toxicological evaluation of a chemical for regulatory purposes is based on a principle first identified in the sixteenth century by Philippus Paracelsus, a Swiss chemist and physician. He recognized that 'all chemicals are toxic; the dose makes the poison.' For example, even water can cause death if consumed in large enough amounts that cause an electrolyte imbalance. However, a corollary to the Paracelsus Principle might be 'all chemicals are harmless at some low dose,' which is true for most chemicals with the notable exceptions of so-called 'nonthresholdable' toxicants. The concept of a threshold (with appropriate uncertainty factors) below which exposure is 'safe' underlies the risk assessment, or more precisely, the safety assessment of chemicals. Therefore, besides understanding the dose–response characteristics of a chemical, the practical goal of regulatory toxicology is to find the dose of a chemical, which poses no appreciable risk to health over a lifetime of exposure.

To accomplish this, hazard characterization relies on standard toxicity tests performed according to internationally accepted protocols, such as those published by the Organization for Economic Cooperation and Development. Hazard characterization considers the dose levels at which no adverse effects occur in order to establish a dietary exposure from all sources that is acceptable (intentionally used chemicals) or tolerable (contaminants and naturally occurring chemicals). The standard reference value used at the international level to indicate the safe level of intake of an intentionally used chemical is the 'acceptable daily intake' (ADI) which is defined by JECFA as the estimate of the amount of a substance in food and/or in drinking water, expressed on a body weight basis, that can be ingested daily over a lifetime without appreciable health risk to the consumer. Note that because the adverse effects are thresholdable, these safety assessments employ a standard of risk that is virtually zero. The ADI is usually derived from establishing a No Observed Adverse Effect Level in the most sensitive animal species and then apply a 100-fold uncertainty factor (sometimes referred to as a safety factor) to take into account the extrapolation from animals to human and variability among humans.

For contaminants and naturally occurring chemicals, the corresponding reference intake value is the 'provisional tolerable intake (PTI),' which can be expressed on a daily, weekly, or monthly basis. The PTI is referred to as 'provisional' because there are often insufficient data on the consequences of human exposure at low levels, and new data may result in a change to the tolerable level. For contaminants that may accumulate in the body over time, such as lead, cadmium, and mercury, the provisional tolerable weekly intake is used as a reference value in order to minimize the significance of daily variations in intake. For contaminants that do not accumulate in the body, the provisional tolerable daily intake is used. For dioxins and dioxin-like PCBs, the reference is the provisional monthly tolerable intake, which is used to emphasize the long half-lives of these chemicals in the body. These tolerable intakes are the primary health reference limits that apply to chronic low-level exposures.

For 'non thresholdable' toxicants, the lower one-sided confidence limit of the bench mark dose (BMDL) is used as the point of departure for the determination of a margin of exposure (MOE), which is defined as the BMDL divided by the actual or estimated exposure. The bench mark dose (BMD) is determined from the dose–response curve to represent the dose that causes a specific incidence of adverse health effects in test animals. The MOE is not itself a health-based reference value, but can be used by the risk manager in setting priorities and selecting interventions, if necessary. Such dose–response assessments are occasionally used to define the dose associated with a negligible response, such as one case in a million population.

The principles and methods for the risk assessments carried out by JECFA and JMPR for chemicals are described in Environmental Health Criteria 240 prepared by the International Program on Chemical Safety (IPCS) and published by WHO. The use of animal studies for assessing toxicity and their consideration for public health purposes are also described by a number of national agencies. In most cases, a tiered approach is used based on the potential toxicity of the chemical

and the likely levels of exposure of the population. For chemicals of lowest concern, only a battery of genotoxicity studies and short-term toxicity study in the rodent would be required. For chemicals of intermediate concern, genotoxicities study, two subchronic toxicity studies in the rodent and nonrodent species, a developmental/teratogenicity study and metabolism and toxicokinetics study would need to be performed. For chemicals of highest concern, chronic toxicity and carcinogenicity studies in two rodent species, usually the mouse and rat, would need to be performed in addition to those required for chemicals of intermediate concern. Other specific studies may also be required depending on the nature of the chemical and the specific requirements of the national authority, including neurotoxicity, immunotoxicity, and acute toxicity studies.

The ability of certain chemicals to cause disease through a similar mechanism of action has been incorporated into most risk assessments. Metabolites formed in the bodies of humans or in food-producing animals from the parent molecule have to be considered in the risk assessment for both hazard characterization and exposure assessment. For some structurally-related chemicals, a group ADI will be established. In cases where the toxic potentials of related chemicals are different, toxic equivalence factors will be assigned to each isomer. In this regard, the endocrine disruption potential of dioxins and certain polychlorinated biphenyls (PCBs) has been estimated with toxic equivalence factors, such factors have not been assigned to the many other chemicals that also possess such potential, including pesticides, packaging materials, industrial pollutants, and even products commonly found in the home. In addition, the impact of mixtures of chemicals with different toxicities remains largely unknown although some evidence indicates that this may be a concern.

In regard to chemicals present in the diet at very low levels, JECFA has applied another approach mainly for flavorings. The approach, called the threshold of toxicological concern (TTC), recognizes that risk can be predicted based on chemical structure and exposure. For example, if a chemical possess a structure that is not associated with genotoxicity or other nonthresholdable toxicity, there is a high probability of negligible harm if dietary exposure to the chemical is sufficiently low, for example, below 1.5 mg per day. TTC is not intended to replace established risk assessment procedures, but rather to be applied to certain structural classes. The approach might also be useful for evaluating processing aids, packaging migrants, and other chemicals present at low levels in the diet, given the large number of chemicals involved and significant resources that would be required to prepare a complete toxicological profile for each.

Finally, some chemicals need to be assessed for potential acute toxicity. For example, certain pesticides, mycotoxins, veterinary drugs, and metals can give rise to acute health effects after short periods of exposure, usually within 24 hours or less. JMPR routinely considers the need to set an acute reference dose (ARfD) for all pesticides it evaluates. JECFA also includes consideration of acute effects, including the possibility of acute effects in sensitive individuals. JMPR has developed guidance for a single-dose study in experimental animals to enable a more accurate derivation of the ARfD.

Exposure Assessment

For intentionally an added chemical, methods have been developed to predict the likely exposure to populations if the chemical was allowed on the market. In its simplest form, the estimated exposure is the amount of a chemical in a food multiplied by the amount of the food consumed. If the chemical occurs in more than one food, then these exposures need to be added together. If the chemical occurs in multiple media, such as, air, water, and soil, then these exposures need to be included in the exposure assessment, which is referred to as an aggregate assessment. If the chemical involves other chemicals with similar mechanisms of toxicity, for example, organophosphorus pesticides, then the amount of each chemical needs to be taken into account. These assessments are referred to as cumulative and it is possible to have an aggregate, cumulative exposure assessment.

Estimation of exposure to a chemical depends on knowledge of the level of the chemical in a food coupled with knowledge of that food consumed. Most countries use a tiered approach that makes the best use of resources in estimating exposure. For example, if an ADI is high, only a crude exposure estimate is necessary in most cases. However, if a more refined exposure estimate is necessary, additional resources are needed to obtain a more realistic assessment, either through better estimates of concentration levels or consumption amounts. The level of contamination of food can also be influenced by a variety of factors, such as food preparation and storage practices as well as geographical and climatic conditions, agricultural practices, and industrial pollution. The level of contamination of food can be determined from food monitoring data when available, but this data has a number of deficiencies. Different methods of dietary intake modeling combine data on contaminant levels in food with food consumption data to provide estimates of the daily, weekly, or monthly dietary exposure. The models may be either deterministic or probabilistic. In the deterministic model, the mean and high percentile are usually calculated, whereas with the probabilistic model, a more complete picture of the distribution of intakes is calculated to take into account all sections of the population for which food consumption data are available. In some models, distributions of levels of contaminants are also used.

In the case of pesticides and veterinary drug residues, appropriate levels of use are first established to ensure the efficacy of the chemicals and then the safety of those levels is assessed. As a first estimate, a theoretical maximum daily intake (TMDI) is often used to provide a rapid and resource-efficient assessment of exposure, which is compared to the ADI. The assessment assumes that the entire consumed commodity is treated with the chemical and that residues are present at the proposed maximum residue levels (MRLs). Although this first tier is a significant overestimate of exposure, it is often sufficient to resolve any exposure concerns for the chemical under the proposed conditions of use. However, the JMPR routinely applies a more realistic assessment using the median residues instead of the MRLs. Further refinements of the exposure assessment may also be applied, such as processing factors for cleaning, peeling, and cooking. Reducing the number of foods considered for approval may also

be used to reduce exposure to an acceptable level, i.e., the ADI. In the case of veterinary drug residues, the withholding period from the time of administration of the drug to the time of slaughter or collection of eggs or milk for human consumption is used to reduce residues to acceptable levels.

For food additives, various screening methods, like the budget method, have been devised based on the proposed maximum levels permitted in the foods or food categories in which the additive would be used. For some additives, such as flavors, the exposure is based on annual production and assuming that 10% of the population consumes the chemical. If any of these screening methods indicate that the ADI may be exceeded, national exposure assessments are consulted because these offer more precise estimates of consumption and use levels in specific foods.

Another approach is to use total diet surveys to estimate the exposure of selected contaminants that is already present in the food supply. These surveys are a direct measure of the level of dietary exposure to contaminants and other chemicals in food as consumed. When periodically repeated using the same protocol, such data provide useful time trends in exposure, which is very useful for evaluating control measures and anticipating potential problems. WHO considers total diet studies to be the most cost-effective method for generally assuring the safety of the food supply from hazardous chemicals. Many countries share information on the levels of contaminants in their food supply with the WHO GEMS/Food Program as part of internationally coordinated efforts to build national capacities, provide data for international risk assessments, and promote confidence in safety of the global food supply.

In some cases, biomonitoring may be used to measure integrated exposure from all media, including food, air, water, and soil. Biomonitoring is particularly useful for chemicals that bioaccumulate and whose toxicity is related to the body burden of the accumulated chemical. Various tissues may be used for biomonitoring including blood, hair, urine, adipose tissue, and human milk. Such monitoring may measure the chemical or an adduct directly or another substance that serves as a marker of exposure.

In the case of emergencies, the risk assessor will need to use any and all available information to formulate a preliminary risk characterization. To protect public health, the WTO allows the establishment of standards based on partial information, but such measures can only be temporary and are subject to other disciplines, for example, not be discriminatory.

Risk Characterization

Risk characterization brings together the information on the level of exposure to the chemical for various population groups and compares this with health-based reference values for health effects. If the exposure does not exceed the health-based reference value, then it is generally assumed that the chemical does not pose a health concern. For nonthresholdable chemicals, the risk assessment will provide an MOE. This information allows a decision to be made regarding necessary management action, if any. At times, the risk manager may ask the risk assessor to provide additional information related to the risk assessment. For example, the likely health outcomes of various maximum permitted levels of a contaminant in food may be requested by the risk manager to allow balancing of the risk of a chemical with health benefits of food availability. Finally, it should be noted that any risk assessment is based on the scientific evidence available at the time of the assessment and that whenever significant new information warrants, the chemical should be reevaluated.

See also: Foodborne Diseases: Overview of Chemical, Physical, and other Significant Hazards. Nutriential Hazards: Micronutrients: Vitamins and Minerals. Risk Analysis: Risk Assessment: Microbiological Hazards; Risk Management: Application to Chemical Hazards

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Relevant Websites

http://www.who.int/foodsafety/chem/gems/en/
Global Environment Monitoring System/Food Contamination Monitoring and Assessment Programme (GEMS/Food).

http://www.who.int/foodsafety/chem/jecfa/publications/en/index.html
Joint FAO/WHO Expert Committee on Food Additives (JECFA) Publications.

http://www.who.int/foodsafety/chem/jmpr/publications/en/index.html
Joint FAO/WHO Meetings on Pesticide Residues (JMPR) publications.

RISK ANALYSIS

Risk Management: Application to Chemical Hazards

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Glossary

Chemical hazard A chemical in food with the potential to cause an adverse health effect.

European Food Safety Authority (EFSA) The European Union body responsible for risk assessment of and risk communication on risks associated with food and feed, as a basis for European policies, legislation, and measures to manage such risks.

JEFCFA Joint FAO/WHO Expert Committee on Food Additives. An expert body that carries out toxicological evaluations of chemical hazards, including food additives and contaminants and residues of veterinary drugs, in food and proposes specifications for food additives.

JMPR Joint FAO/WHO Meetings on Pesticide Residues. An expert body that carries out toxicological evaluations of pesticide residues in food and proposes maximum residue limits (MRLs) for such residues in foods.

Risk assessment A scientifically based process consisting of the following steps: (1) hazard identification, (2) hazard characterization, (3) exposure assessment, and (4) risk characterization.

Risk assessment policy Documented guidelines on the choice of options and associated judgements for their application at appropriate decision points in the risk assessment such that the scientific integrity of the process is maintained.

Risk estimate The quantitative estimation of risk resulting from risk characterization.

Risk profile The description of the food safety problem and its context.

SPS Agreement The World Trade Organization Agreement on the Application of Sanitary and Phytosanitary Measures.

Introduction

This article describes how the general principles of food safety risk management are applied to chemical hazards in food. Examples are given of different risk management options and their application in dealing with the risks posed by the wide range of chemical hazards that may be present in food.

Chemical Hazards in Food

Many different kinds of chemical hazards may be introduced into food at different points in the food production to consumption continuum. Some hazards are well characterized, single compounds (e.g., basic elements and simple organic molecules), whereas others are complex mixtures of varying composition (e.g., polychlorinated biphenyls (PCBs), polychlorinated dibenzodioxins (dioxins), and many biotoxins). Some are added intentionally to foods (e.g., food additives) or are residues of products used in primary production (e.g., pesticides and veterinary drugs) and others occur naturally in foods or are formed during food processing. Still others are a derived from environmental contamination.

Although bacterial toxins can be regarded as chemical hazards, they are not dealt with here, but instead in the article dealing with the management of biological hazards. Likewise, although genetically modified organisms (GMOs) used as food may contain chemical hazards, they are not dealt with here but in the article on GMOs .

The following are some of the most important types of chemical hazard that may be present in foods.

Environmental Contaminants

Contamination of the environment with persistent organic pollutants, such as PCBs, dioxins, DDT, and several other pesticides and brominated fire retardants, has led to contamination of foods of animal origin, in some cases via contamination of animal feed. Similarly, methylmercury derived from industrial pollution or natural sources accumulates in some predatory fish species. Toxic metals, such as cadmium, lead, and arsenic can be taken up by food plants, for example, cadmium in rice from grown in volcanic soils. Food may also become contaminated with radionuclides as a result of releases from nuclear power plant incidents.

Naturally Occurring Toxins

A wide range of chemical hazards may be present naturally in some foods, for example, cyanogenic glycosides in cassava, caffeine in coffee and tea, solanine in potatoes, hydrazine derivatives in mushrooms, safrole in flavors, and lectins in legumes. In addition, algal toxins present in the aquatic

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environment can accumulate in mussels and other shellfish and give rise to a range of intoxications, such as diarrhetic shellfish poisoning (DSP) and paralytic shellfish poisoning.

Food Allergens and Other Components of Food

Chemical components, especially proteins, of many common foods, for example, eggs, milk, fish, shellfish, nuts, legumes, and cereals, may give rise to allergic reactions in sensitive individuals. In addition to atopic allergy, some food components (e.g., lactose) may give rise to hypersensitivity or intolerance reactions.

Mycotoxins

Growth of certain molds on foods can lead to the formation of mycotoxins, for example, aflatoxins in nuts and maize, ochratoxin A, fumonisins and trichothecenes in grains, and patulin in certain fruits such as apples and pears. The presence of mycotoxins in animal feed can result in contamination of foods of animal origin, for example, aflatoxin M_1 in milk derived from aflatoxin B_1 in feed.

Residues of Pesticides

A wide range of pesticides, including herbicides and fungicides, are used in agriculture and horticulture to protect plants from insects, molds, etc. and to control weeds. This can result in residues in foods of plant origin. Several different pesticides may be used on the same product, resulting in multiple residues in the same food. Pesticide residues present in animal fodder, such as hay, or feed ingredients, such as citrus pulp, may result in the contamination of foods of animal origin, for example, meat and milk.

Residues of Veterinary Drugs, Including Feed Additives

Veterinary drugs are used to prevent or treat diseases in food-producing animals and for growth-promotion and other purposes and residues of such drugs may be present in foods of animal origin. In addition to the risks arising from residues, the use of antimicrobials in animal husbandry may increase the risk of development of antimicrobial-resistant microorganisms.

Intentional Food Additives, Including Flavoring Substances and Enrichment Substances

Certain chemicals are added to foods to improve their quality and shelf-life (e.g., preservatives and antioxidants) or organoleptic properties (e.g., colors, sweeteners, and flavoring substances) or for technological and other reasons (e.g., emulsifiers, acidity regulators, and anticaking agents). Other substances, for example, vitamins and minerals, are added to foods to enhance their nutritional value or to replace nutrients lost during processing.

Substances Formed During Food Processing/Preparation

Industrial food processing and food preparation in catering establishments or at home, for example, heat treatment, such as baking, frying, grilling, and smoking, can result in the formation of chemical hazards in some foods, for example, acrylamide in French fries and bread, polycyclic aromatic hydrocarbons (PAH) in smoked foods, nitrosamines in nitrite-treated meat products, and 3-monochloropropane-1, 2-diol (3-MCPD) in acid-hydrolyzed vegetable protein. In addition, some substances used as processing aids may become part of foodstuffs.

Substances Derived from Materials Coming into Contact with Food

During their production, processing, storage, and preparation foods often come into contact with materials from which chemicals can migrate or be leached. For example, monomers and plasticizers can migrate from plastic packaging materials, tin can contaminate foods stored in tin-plated cans, and lead and cadmium can be leached from ceramic tableware.

Intentionally Added Contaminants/Adulterants

Dishonest food business operators may add chemicals to foods in order to deceive the purchaser as to their identity, quality, or other properties. Examples of such practices in recent years include the addition of melamine to milk to give the impression of higher protein content and the adulteration of spices with the color Sudan Red.

Generic Framework for Food Safety Risk Management

Food safety risk management can be divided into the four steps shown in Figure 1.

Step 1: Preliminary Risk Management Activities

The first step of the risk management process, preliminary risk management activities, comprises the following:

- Identify the food safety issue.
- Develop a risk profile.

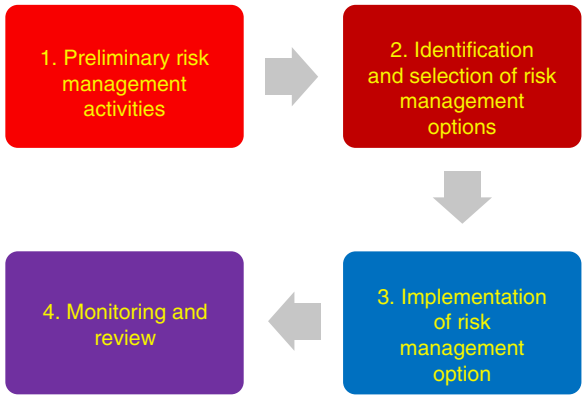

Figure 1 The four steps of food safety risk management.

- Establish broad risk management goals.
- Decide if a (new) risk assessment is needed.
- Rank risks, if necessary.
- Establish a risk assessment policy.

Identify the Food Safety Issue

Identifying and describing the nature and characteristics of food safety issue is an essential first step in its management. In some cases the issue will already have been recognized as a food safety problem and a considerable amount of information about the risks involved is available, whereas in others the hazard may not have been identified earlier and little information on its toxicity or human exposure is available. Food safety problems may be identified in many different ways, for example, by food inspection and monitoring programs (e.g., dioxin contamination of foods of animal origin in Ireland and Germany arising from feed contamination), biological and environmental monitoring, human and animal disease outbreak investigations (e.g., humans affected by methylmercury in Minimata and animals affected by dioxin contamination of feed in Belgium in 1999) and clinical and toxicological studies (e.g., studies on processing contaminants such as acrylamide and 3-MCPD). Industrial incidents, for example, the Chernobyl and Fukushima disasters, may also result in food safety problems from radionuclides. Potential problems may also be identified by academic and scientific experts, the food industry, consumer organizations, and the media. For example, the problems with 2-isopropyl thioxanthone and semicarbazide were first reported by industry.

A brief initial description of the food safety issue provides the basis for developing a risk profile, which in turn generates a context and guide for further action.

Develop a Risk Profile

A risk profile is a description of a food safety problem and its context. Its main purpose is to assist risk managers in assessing if and what further action may be required. Essentially, it is a summary of what is known, and not known, about the food safety issue in question. A risk profile is usually developed by risk assessors in cooperation with others who can provide complementary background information, for example, on production and trade aspects of the foods involved. The contents of a risk profile can vary widely, depending on the amount of information available on the specific issue. However, the following are examples of the types of information that may be included in a risk profile:

- Broad statement of the food safety issue.
- Description of the hazard and food(s) involved.
- Chemical characteristics of the hazard involved.
- Scientific information on possible health risks, including descriptions of potential adverse effects on human health.
- Gaps in and uncertainties in scientific knowledge.
- How and where the hazard enters the food chain.
- Levels of the hazard in relevant foods and intake of these foods by different population groups, providing the basis for estimating exposure.
- Nature of values at risk (human health, economic, ethical, etc.).
- Distribution of risk (who produces, benefits from, and bears the risk).
- Current risk management practices, including any regulatory standards and control measures.
- Information about possible risk management measures.
- Public perceptions of the hazard and possible risks.
- Implications for international agreements, for example, the World Trade Organization (WTO) Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement) and the International Health Regulations 2005.

Examples of risk profiles of caffeine in beverages, ciguatoxins in seafood, and natural toxins in New Zealand crop plants can be found on the website of the New Zealand Food Safety Authority.

Establish Broad Risk Management Goals

Risk management goals are statements of the intended purpose and the end result that risk management is intended to achieve. The goals can be expressed in different ways, for example, to ensure that the intake of a certain chemical hazard by a vulnerable population group does not exceed a defined acceptable daily intake (ADI) or provisional tolerable weekly intake (PTWI) or to achieve a specified reduction in exposure of a population group to a chemical hazard within a certain time period. For example, Germany has set a goal to reduce acrylamide exposure via food by taking an as low as reasonably achievable (ALARA) approach (see Evaluate the Options).

Decide if a (New) Risk Assessment is Necessary

A risk assessment is a scientifically based process to develop an understanding of and characterize the human health risk associated with a particular hazard. It consisting of the following steps: (1) hazard identification, (2) hazard characterization, (3) exposure assessment, and (4) risk characterization. The process is described in more detail in the article on risk assessment of chemicals. A risk assessment can be qualitative (e.g., when data on toxicity and exposure are inadequate) or, preferably, quantitative and should answer specific questions posed by the risk manager on likely risk, including the likelihood and severity of a particular adverse health effect and any population groups that are at particular risk. The degree of uncertainty in the risk estimate should be clearly communicated to the risk manager.

Full risk assessments require considerable scientific resources and are also time-consuming and it is, therefore, necessary for the risk manager, in consultation with risk assessors, to decide whether such a risk assessment is needed or not. In some cases, sufficient information will already be available from risk assessments carried out internationally or nationally. Risk assessments of many chemical hazards in food have already been carried out by expert bodies such as the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) and the Scientific Panels of the European Food Safety Authority (EFSA). These assessments can be accessed via the

FAO, WHO, and EFSA websites (see Further Reading). It is desirable to carry out a risk assessment in cases where the issue is of significant concern to risk managers and other stakeholders, including consumers, and where little scientific data exists and there is considerable uncertainty about the nature or magnitude of the risk. However, it may not always be necessary (or, due to time constraints, possible) to carry out a risk assessment before taking action. For example, when sufficient scientific information on likely risks is already available, where an issue is of minor food safety concern or when rapid action is needed in an emergency. In such cases, the information in the risk profile can be used as a basis for identifying and selecting risk management options. If, in an emergency, preliminary action is taken on the basis of a risk profile, this should be reviewed when further scientific evidence becomes available to enable a risk assessment to be performed.

Rank Risks, if Necessary

In cases where resources for risk assessment are insufficient to meet immediate needs, it may be necessary for the risk managers to prioritize risks for risk assessment. This ranking should be based on a preliminary assessment of the risks by risk assessors, but other factors, such as consumer, political, or trade concerns are often taken into account.

Establish a Risk Assessment Policy

Many of the risk assessments of chemical hazards in food are based on the results of studies carried out on experimental animal receiving relatively high doses of the hazard. When assessing health risks for humans based on such studies, it is often necessary to make certain judgments to bridge gaps in scientific knowledge. This may concern, for example, extrapolating results seen at high doses in cancer studies in animals to low exposure in man and choice of appropriate animal species to test for teratogenic effects. Another issue is the use of default safety factors or uncertainty factors in estimating ADIs for man for chemical hazards from no adverse effect levels determined from animal studies. Many risk assessment bodies follow the practice developed by the JECFA, in which a factor of 10 is used to account for any variation within species (e.g., higher susceptibility in the very young) and an additional factor of 10 in case there is any interspecies variation when extrapolating from experimental animals to man. Additional safety factors may be applied if the underlying scientific data are deficient in some way. Risk assessment policies are usually established by risk managers in consultation with risk assessors well in advance of the risk assessment. These are often incorporated into guidelines or standard operating procedures for conducting risk assessment, such as Environmental Health Criteria 240, which is used by JECFA.

Commission a Risk Assessment

If it is decided to conduct a risk assessment, then the scope of the assessment and the questions to be answered must clearly be defined and the time frame and resources to be allocated agreed. In some cases, where considerable knowledge about the toxicity of a hazard is already available, the risk assessment may be restricted to assessing the risk of a particular health effect, for example, cancer.

Consider the Results of the Risk Assessment in the Context of Risk Evaluation

It is important that risk managers clearly understand the results of the risk assessment and the associated uncertainties. An executive summary is useful for this purpose. For communication with other stakeholders, it is also useful to have a lay summary of the risk assessment.

Step 2: Identification and Selection of Risk Management Options

The second step of the risk management process, identification and selection of risk management options, comprises the following activities:

- Identify risk management options.
- Evaluate the options.
- Select the preferred option or options.

In this second step of the risk management of chemical hazards in food, the risk manager leads the process of identifying and selecting risk management options, taking into account the size and severity of the risk together with the likely effectiveness and costs of the different options for risk reduction and, where relevant, any social, ethical, and political aspects.

Identify Risk Management Options

There are several options available for managing risks associated with chemical hazards in food: in some cases, two or more approaches are used to tackle a problem. The identification of risk management options can be facilitated by close cooperation and good communication between the food authorities, food business operators, technical and academic experts, and other stakeholders.

The modern farm-to-fork approach to food safety requires that good practices (good agricultural practice, good veterinary practice, good hygienic practice, good manufacturing practice, etc.) and be applied along the whole of the food chain in order to minimize the risk of contamination with chemical and other hazards. Food manufacturers should base their internal control systems on hazard analysis and critical control point (HACCP) principles. Traceability, at least the ability to trace feed/foods/ingredients one step forward and one step back in the food chain is important in managing food safety risks, especially when the risks have only been detected in the later parts of the food chain. For instance, in the case of the dioxin incident in Belgium in 1999, lack of traceability was a major constraint in managing the crisis. Later incidents in Ireland and Germany caused by dioxin-contaminated feed also show the importance of traceability of feed and feed ingredients.

The following are some examples of different risk management options and areas in which they can be applied.

- Introduce legislation to require prior approval of a substance/product before it may be used in connection with food production or handling and set conditions for its use. Examples of chemical hazards that are managed in this way are intentional food and feed additives, pesticides, and veterinary drugs. When positive lists of approved substances/products have been established, the use of other products is prohibited. In some cases negative lists of substances/products that may not be used, i.e., are banned, in connection with food production and handling are established.
- Set and enforce maximum levels (MLs) for the substance in various foods and animal feed. Examples of this are setting and enforcing maximum residue limits (MRLs) for residues of pesticides and veterinary drugs, and for MLs for food additives and food contaminants such as heavy metals (lead, cadmium, mercury, etc.), mycotoxins (aflatoxins, ochratoxin A, etc.), persistent organic pollutants (PCBs, dioxins, etc.), and PAH. For operational reasons, food business operators may establish MLs that are below the existing legal limits. These may be used as critical limits in conjunction with HACCP-based internal control systems to ensure the timely correction of processes and to ensure that noncomplying products do not leave the plant.
- Set and enforce maximum limits for migration or leaching of substances from food contact materials. Examples of this approach are setting limits for global migration and migration of specific monomers from plastic food packaging materials and leaching of lead and cadmium from ceramic tableware. Specifications may also be set for metal, for example, stainless steel, equipment coming into contact with food.
- Intensify control and enforcement activities in cases where MLs have already been established and experience shows that these levels are often exceeded.
- Recommend the relevant authorities to take action to reduce or eliminate emissions from sources of environmental pollution with, for example, heavy metals or persistent organochlorine compounds such as PCBs and dioxins.
- Ban food or animal feed that presents an unacceptable or imminent risk. For example, prohibit the sale of foods from a specified geographical area known to be heavily contaminated with a chemical hazard or foods (e.g., certain fungi) known to regularly contain unacceptably high levels of certain chemical toxins.
- Establish specific food labeling requirements so that persons that are allergic or hypersensitive to or intolerant of certain food components can identify and avoid foods that can cause untoward reactions. For example, in some countries there is a requirement to label products containing the sweetener aspartame with information that it is a source of phenylalanine, which is important information for persons suffering from phenylketonuria.
- Introduce legislation requiring food business operators to develop, operate. and document internal control systems based on HACCP principles and to ensure that their staff receive adequate training to prevent contamination of food with chemical hazards.
- Promote voluntary action by food business operators to adopt new production or handling methods, selection of ingredients, etc.
- Issue recommendations, such as codes of practice, on the handling of certain foods in order to keep the levels of chemical hazards at acceptable levels. For example, advice on smoking and grilling of foods to keep down the levels of PAH (see Further Reading).
- Issue dietary advice to consumers, especially vulnerable groups such as pregnant women, to restrict their intake of foods known to regularly contain relatively high levels of certain chemical hazards, for example, to restrict the intake of fish species with elevated levels of methylmercury.
- Develop specific compliance programs to focus on food producers and traders who do not comply with food safety legislation.
- Prosecute food producers and traders who do not comply with food safety legislation, in particular those intentionally adulterating foods with chemical hazards.
- Take no action because it is considered that the risk is negligible or that the current control measures are adequate. A decision to take no action is also a risk management decision and should be documented.

Evaluate the Options

The risk management options should be evaluated against a set of criteria linked to the broad risk management goals established (see Establish Broad Risk Management Goals) and related to public health, economic, social, political, environmental, ethical, and legal issues. The primary goal is to provide an appropriate level of protection of public health (see below) and the options considered should be compared with the status quo in order to consider whether any changes are in fact needed to achieve this goal.

For WTO members (i.e., most countries of the world), it is important to remember that, according to the SPS Agreement, the chosen level of sanitary protection and the measures introduced to protect public health should be as least trade restrictive as necessary to achieve the established goal.

The criteria to be considered in selecting various options include:

- Likelihood that the measure(s) will lead to the achievement of the established public health goals. The risk assessors should be asked to estimate the risk reductions that can be achieved by various options considered.
- The feasibility of implementation, verification, and enforcement of the measures proposed.
- Possibility to evaluate if the public health goal has been achieved.
- Consistency with government policy.
- Consistency with the legal powers of government agency, if legal powers are delegated.
- Costs of compliance for food business operators and other stakeholders.
- Impact on international trade and consistency with the country's international obligations, for example, WTO membership and, in particular, the SPS Agreement.

- Impact on the availability and affordability of the foods concerned and any nutritional implications for the population.
- Acceptability to stakeholders, bearing in mind that some stakeholders may have competing interests.
- Possibility to communicate recommendations, advice, etc. to the population group(s) concerned.
- Any ethical or religious considerations.

One of the most important factors to be considered when evaluating the various risk management options is the human health protection goal. In the SPS Agreement this is referred to as the appropriate level of sanitary protection (ALOP) and it is up to each WTO member to set this level. There are various approaches to setting an ALOP and in selecting the best risk management option(s), including:

- Notional zero risk approach. Hazards are kept at or below levels that correspond to a predetermined negligible or notional zero risk, based on a risk assessment that indicated that such low exposure levels are reasonably certain not to cause harm to human health, even with lifetime exposure. This is the approach used by JECFA in setting ADIs for food additives and veterinary drug residues and PTWIs for chemical contaminants in food. Similarly the JMPR uses this approach in setting ADIs for pesticide residues. The MLs for food additives and contaminants and pesticide and veterinary drug residues adopted by the Codex Alimentarius Commission for various foods are based on JECFA and JMPR evaluations.
- ALARA approach. MLs for hazards are set at the lowest levels technically achievable and economically feasible under the circumstances. These MLs may vary over time and from country to country, due to agro-climatic conditions, technical developments and capabilities and economic feasibility.
- Threshold approach. According to this approach, risks must be kept below a certain specified numerical level that is deemed to represent a finite, but acceptable, risk. This approach may be used for chemical carcinogens, but up to now very few countries have established or applied such levels.
- Benefit–cost approach. Both a risk assessment and a benefit-cost assessment are carried out and the risk managers then weigh the expected risk reduction against the estimated cost of achieving this reduction.
- Comparative risk approach (risk–risk comparison). In some cases, reducing a risk from one hazard using certain measures has to be compared with the risk that may be generated as a consequence of the decision. For example, prohibiting the use of nitrite as a preservative in certain meat products to reduce the formation of carcinogenic nitrosamines may increase the risk of growth of *Clostridium botulinum*. Prohibiting the sale of fish with moderate levels of methylmercury may have nutritional consequences for populations for which fish is an important source of protein and other nutrients.
- Precautionary approach. When information exists to suggest that a hazard in food may pose significant risks to human health, but the scientific data are insufficient for a full risk assessment, interim measures may be adopted to limit the risk while steps are taken to obtain the necessary information to carry out a full risk assessment. This approach (sometimes referred to as the precautionary principle) is included in the basic food legislation in the European Union and some other countries.

Select the Preferred Option(s)

Although final selection of risk management option(s) is the responsibility of the risk manager, it is important to involve other stakeholders, for example, food business operators and consumer representatives, and to document and explain the rationale behind the decision made. The selected option(s) should result in the achievement of the desired level of public health protection at reasonable cost, without causing unwarranted barriers to international trade and, as far as possible, be acceptable to all stakeholders. Where possible, a preventive approach to food safety should be used, i.e., problems should be tackled at source, because this is generally more effective and less costly than other approaches (see Further Reading). Requiring prior approval (including setting conditions for use) of food additives, veterinary drugs and pesticides is an example of this approach.

In managing the risks posed by food additives, pesticides and veterinary drugs, the commonest approach is to establish positive lists of such substances/products and to set conditions for their use and maximum limits for their presence in foodstuffs, such as MRLs for pesticides and veterinary drug residues. WTO members are required by the SPS Agreement to base their national food safety measures on Codex standards, where they exist, although they may set more stringent requirements based on a risk assessment for their population and the appropriate level of protection they have set. Thus most countries base their national legislation on food additives and pesticide and veterinary drug residues on Codex standards.

A number of food safety risks arise due to contamination of animal feed with chemical hazards, such as dioxins and aflatoxins. Management of such risks requires regulation and control of feed ingredients and feed manufacturing processes and recommendations to farmers and other primary producers. Additives added to feed are regulated in most countries.

Managing risks arising from environmental contamination (e.g., methylmercury and PCBs and dioxins in fish) is more complicated. For example, some countries have set maximum limits (e.g., 1 mg kg^{-1}) for the levels of these hazards in fish that can be offered for sale, issued fish consumption recommendations to vulnerable population groups (e.g., pregnant women and young children) and taken measures to reduce or eliminate sources of pollution (e.g., from waste incinerators).

Food safety risks arising from food contact materials can be managed by establishing positive lists of substances that may be used to produce such materials, setting limits for global or specific migration or leaching of chemicals from such materials and regulations or recommendations on the appropriate use of certain materials in contact with specific groups of food, for example, acidic or fatty foods.

Management of risks associated with natural toxins can be achieved by setting limits for their presence in food (e.g., aflatoxins in peanuts and DSP in mussels), advice to consumers

on treatment of some foods before consumption (e.g., treatment of cassava to remove cyanogenic glycosides) or that consumption of certain foods should be restricted or even avoided altogether by vulnerable population groups.

The main method of managing risks from food allergens and other substances causing intolerance or hypersensitivity reactions is through detailed regulations on food labeling, information to affected population groups and voluntary action by food business operators to provide further information on their products in food stores and via the Internet and other channels.

Food safety risks due to substances formed during food processing or commercial or domestic food preparation (e.g., acrylamide and PAHs) can be tackled by regulations or recommendations to food business operators and advice to consumers concerning, for example, grilling and smoking of foods.

Management of risk arising from intentional contamination/adulteration of foods with chemical hazards and gross negligence on the part of food business operators is best achieved by legal action to remove contaminated products from the food supply and prosecution of the offenders. Good coordination with foodborne disease surveillance programs is also essential in detecting such problems as early as possible so that recalls and remedial action can be taken. In this regard, incidents of international public health concern must be reported under the WHO International Health Regulations, which serves to protect the world community in this era of globalized food supply. The European Union has also established the Rapid Alert System for Food and Feed (RASFF) for the rapid communication of information about food and feed safety incidents. Publication of the results of food control and, in particular investigations of malpractices, will often encourage compliance with legal requirements.

Step 3: Implementation of Risk Management Options

The third step in risk management, Implementation of risk management options/measures, consists of the following activities:

- Validate control(s) where necessary.
- Implement controls.
- Verify implementation.

The measures chosen to manage risks arising from chemical hazards must be effectively implemented if the desired risk reduction is to be obtained. Food control authorities and the food and feed business operators have the main responsibility for implementation, but consumers and other stakeholders may also play an important role in some cases.

Validate Controls, Where Necessary

When it has been decided to introduce new or revised official control measures, these should be validated before being introduced. This may be done by the food control authorities or by independent bodies working under contract to the authority. Food business operators should also ensure that their internal control measures are validated.

Implement Controls

Controls may be introduced at various points along the food production to consumption chain, depending on the source of the chemical hazard and an assessment of where controls are likely to be both practical and most effective.

Responsibility for introducing and enforcing legal requirements rests with the food safety authorities. This includes standard setting and inspection and other measures to ensure compliance with existing standards. In some countries inspection of food production establishments and analysis of food samples is carried out by qualified independent bodies working under contract to the food control authorities. Analysis of food samples for control purposes should be carried out by suitably qualified laboratories, preferably accredited for the type of analysis in question. The food safety authorities are also responsible for providing consumers, food business operators, and other stakeholders with information, recommendations, and advice on food safety matters.

Major responsibility for food safety rests with food business operators, who must ensure that they have in place appropriate internal control systems based on HACCP principles, that the control results are documented and that their staff has adequate training in food safety. Furthermore, business operators should have in place systems to ensure traceability, at least one step forward and one step back in the food chain. In other words, they should have records of where they obtained the ingredients, etc., for their products and to whom their products were sold.

Consumers have a responsibility for food safety in the home, including domestic food preparation and storage. Although consumer education programs tend to focus on microbial hazards, consumers can also reduce exposure to certain chemical hazards, such as acrylamide and PAHs, through better food preparation in the home. It is also important that consumers, especially vulnerable population groups such as pregnant women, follow the recommendations of the food safety authorities on restrictions on the intake of certain foods that may contain elevated levels of chemical hazards.

Verify Implementation

The food safety authorities are responsible for verifying that measures that have been introduced in order to manage a food safety problem have been implemented by food business operators. This may be done either by the authorities themselves or by qualified independent bodies working under contract to them.

Step 4: Monitoring and Review

The fourth, and last, step in the risk management process, monitoring and review, comprises the following activities:

- Monitor the outcomes of the control measures.
- Evaluate the results.

- Review controls if the risk management goals are not achieved.

Monitor the Outcomes of Control Measures

When deciding on the measures to be introduced to tackle a food safety problem, it is important to plan how to monitor the outcome of the control measures. Depending on the problem and the desired outcome, this can include, for example, monitoring the levels of pesticide or veterinary drug residues in specific foods before and after introducing the measures, measuring human exposure to chemical hazards (e.g., PCBs, dioxins, lead) or control of food labeling of especial importance for persons who are allergic or hypersensitive to certain food ingredients. For instance, it is through such a monitoring program that the Irish authorities discovered contamination of pork meat with dioxin in 2008. Such monitoring programs are important for biotoxins and radionuclides.

When the control measures have been aimed at producing improvements in food production processes and handling by food business operators, food inspection should monitor and confirm that the desired improvements have been made.

Where the measures introduced comprise mainly information and recommendations to consumers, this should be followed up by surveys to establish if the message has reached the target group and whether it has resulted in the desired changes in food safety behavior.

Many countries periodically carry out total diet studies to assess the exposure of their population to a range of chemical hazards. Such studies can provide assurance that the food supply is safe from such hazards and can identify problems or potential problems based on time-trends. Total diet studies are considered to be an important management tool for addressing the multitude of potentially toxic chemicals that may be present in food.

Evaluate the Results

The results of monitoring after the introduction of measures to reduce human health risks from chemical hazards in food should be evaluated to see if the desired outcome has been achieved. This is the responsibility of the food safety authorities and the results of the evaluation should be made public.

Review Controls if the Risk Management Goals are not Achieved

If, following the evaluation, it is found that the measures introduced have not achieved the established risk management goals, the controls should be reviewed and possibly intensified. If the controls introduced have been found to be ineffective, then the list of risk management options should be reexamined and other or further measures introduced in order to reach the desired food safety outcomes. In some cases, the risk manager may request that the risk assessment be reviewed in order to take into account the latest science and knowledge.

See also: Risk Analysis: Risk Assessment: Chemical Hazards; Risk Communication: Chemical Hazards; Risk Management: Application to Biological Hazards. Safety of Food and Beverages: Safety of Genetically Modified Foods

Further Reading

Codex Code of Practice Concerning Source Directed Measures to Reduce Contamination of Foods with Chemicals. CAC/RCP 49-2001.

Codex Code of Practice for the Prevention and Reduction of Lead Contamination in Foods. CAC/RCP 56-2004.

Codex Code of Practice for the Reduction of Acrylamide in Food. CAC/RCP 67-2009.

Codex Code of Practice for the Reduction of Contamination of Food with Polycyclic Aromatic Hydrocarbons (PAH) from Smoking and Direct Drying Processes. CAC/RCP 68-2009.

Codex documents. The standards, guidelines, codes of practice, and recommendations for chemical hazards in food adopted by the Codex Alimentarius Commission can be accessed via the Codex website: http://www.codexalimentarius.org

Codex Working Principles for Food Safety Application by Governments. CAC/GL 62-2007.

EFSA risk assessments. The reports of the risk assessments carried out by the Scientific Panels of the European Food Safety Authority (EFSA) can be accessed via the EFSA website: http://www.efsa.europa.eu

Food safety risk analysis. A *Guide for National Food Safety Authorities*. FAO Food and Nutrition Paper No. 87. FAO, Rome, 2006.

JECFA and JMPR reports. The reports on toxicological evaluations, etc. of food additives and contaminants and veterinary drug residues carried out by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the toxicological evaluations, etc. carried out by the Joint FAO/WHO Meeting on Pesticide Residues can be accessed via the FAO website (http://www.fao.org) and the WHO website (http://www.who.int).

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Principles and Methods for the Risk Assessment of Chemicals in Food. WHO Environmental Health Criteria 240. Geneva: WHO.

Risk management and food safety. FAO Food and Nutrition Paper No. 65. *Report of a Joint FAO/WHO Expert Consultation*. Rome, Italy, 27–31 January, 1997.

Relevant Websites

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European Commission Directorate General for Health and Consumers (DG SANCO).

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http://www.fao.org
Food and Agriculture Organization of the United Nations (FAO).

http://www.fsai.ie
Food Safety Authority of Ireland (FSAI).

http://www.nzfsa.govt.nz
New Zealand Food Safety Authority (NZFSA).

http://www.food.gov.uk
UK Food Standards Agency (FSA).

http://www.usda.gov
US Department of Agriculture (USDA).

http://www.fda.gov
US Food and Drug Administration (USFDA).

http://www.who.int
World Health Organization (WHO).

http://www.wto.org
World Trade Organization (WTO).

RISK ANALYSIS

Risk Management: Application to Biological Hazards

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Glossary

Food Safety Objective (FSO) The maximum frequency and/or concentration of a hazard in a food at the time of consumption that provides or contributes to the appropriate level of protection (ALOP).

Performance criterion (PC) The effect in frequency and/or concentration of a hazard in a food that must be achieved by the application of one or more control measures to provide or contribute to a performance objective (PO) or an FSO.

Performance objective (PO) The maximum frequency and/or concentration of a hazard in a food at a specified step in the food chain before the time of consumption that provides or contributes to an FSO or ALOP, as applicable.

Risk assessment policy Documented guidelines on the choice of options and associated judgments for their application at appropriate decision points in the risk assessment such that the scientific integrity of the process is maintained.

Risk estimate The quantitative estimation of risk resulting from risk characterization.

Risk management The process, distinct from risk assessment, of weighing policy alternatives, in consultation with all interested parties, considering risk assessment and other factors relevant for the health protection of consumers and for the promotion of fair trade practices, and if needed, selecting appropriate prevention and control options.

Risk manager Governmental food safety organization with the authority to decide on the acceptability of risks and measures needed for their management.

Risk profile The description of the food safety problem and its context.

Introduction

In the late 1990s, the approach to prevention of foodborne illness and management of food safety was thoroughly revisited and drastic changes were brought to the process of decision-making. This paradigm shift was the result of the application of risk analysis to food safety. Risk analysis consists of three elements, namely risk assessment, risk management, and risk communication. Risk management is the core element of the risk analysis process, as risk managers have the responsibility and leading role in managing risks.

For the purpose of this text, the term risk manager refers to a governmental food safety organization with authority to decide on the acceptability of risks and measures needed for their management. Here, the definition does not include all of the individuals who are involved in the implementation phase and activities associated with risk management, such as industry and consumers. However, the risk management process can also be used in by large food companies as a model for decision-making process on food safety matters.

This article presents the principles and procedures for risk management of biological hazards and is based on those recommended internationally by the Codex Alimentarius Commission (see Further Reading section). Risk assessment and communication aspects of risk analysis are addressed in other articles of this encyclopedia.

Background

The need for the risk analysis process emanated from an increasing incidence of foodborne illnesses, such as salmonellosis, campylobacteriosis, enterohemorrhagic *Escherichia coli* infections and listeriosis, in industrialized countries. In addition, epidemics of food- and waterborne diseases in the developing countries, such as cholera and other diarrheal diseases, were becoming more recognized for their adverse impact on health and development. At the same time, the globalization of the world's food supply raised awareness of risk managers that a foodborne disease outbreak in any country had the potential to become an international issue. Even if the contaminated food was not exported, 24/7 media coverage could have a negative effect on consumer confidence. The deteriorating situation raised a large number of questions and called for strengthening of the food safety management system including:

- Reviewing existing management practices, including the efficacy of preventive measures;
- Revising the approach to decision-making based on the risk analysis paradigm, which had already been successfully applied to chemical hazards in food;
- Developing concepts which would make management of food safety (including evaluation) more quantitative; and

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- Clarifying responsibilities and accountability along the food supply chain.

Concomitantly, in 1995 the World Trade Organization (WTO) was established and two agreements came into force that had implications for food safety. Although liberalizing trade in food and animal feed among WTO countries, these agreements were intended to safeguard the right of countries to protect their population against import of unsafe food. The Agreement on the Application of Sanitary and Phytosanitary Measures (SPS) and the Agreement on Technical Barrier to Trade were devised to harmonize safety and other technical aspects (e.g., labeling) of foods entering in international trade. According to the SPS agreement, the work of the Codex Alimentarius Commission (Codex) (i.e., its standards, guidelines, codes of practice, and other recommendations) became the international reference for food safety requirements. Countries that accepted Codex standards were considered to be incompliance with the SPS agreement. However, countries wishing to adopt a stricter standard than Codex were required to provide scientific evidence of the risk that the imported food would present for their population. Consequently, Codex became the benchmark for the national legislation.

Among the various SPS articles, the Article 4 on equivalence states:

> Members shall accept the sanitary or phytosanitary measures of other Members as equivalent, even if these measures differ from their own or from those used by other Members trading in the same product, if the exporting Member objectively demonstrates to the importing Member that its measures achieve the importing Member's appropriate level of sanitary or phytosanitary protection. For this purpose, reasonable access shall be given, upon request, to the importing Member for inspection, testing, and other relevant procedures.

This is of importance to risk management of biological hazards as this has a major impact on the principles of risk management. This article stipulates that an importing country ought to accept the control measures of a trading partner as equivalent, even if these measures differ from their own, if the exporting country can objectively demonstrates that through its control measures it can achieve the same degree of risk reduction, and consequently health protection.

Thus, exporting countries had to prove that their control measures were equivalent to those required by the importing country. To facilitate the process, there was a need to introduce a more quantitative approach to the management of food safety. In regard to microbiological risk management (MRM), new concepts were developed, including appropriate level of protection (ALOP) or tolerable level of risk, as low as reasonably achievable, food safety objective (FSO), and performance objective (PO).

The above developments were the background for a new risk-based approach to management of biological hazards in the food supply chain. Although the risk analysis process did not change the basic control measures per se, it had major consequences such as:

1. Improved the process of decision-making by the provision of a structure for the different governmental activities (legislative, enforcement, education, surveillance, etc.) and increased transparency and objectivity;
2. Allowed for a more evidence-based approach to decision-making considering both scientific facts as well as economic and sociocultural factors;
3. Fostered the development of new concepts (e.g., ALOP, FSO, and PO) to make management of safety more measurable; and
4. Increased the interaction between risk managers, scientists, and stakeholder as well as between stakeholders within the food chain.

Definition and Concept

Strictly, risk management is defined by Codex as the process, distinct from risk assessment, of weighing policy alternatives, in consultation with all interested parties, considering risk assessment and other factors relevant for the health protection of consumers and for the promotion of fair trade practices, and if needed, selecting appropriate prevention and control options. This definition underlines that risk management consists of the evaluation of public health policies. It is a societal process and the risk management decision should be seen as a societal decision as how risks should be managed.

Risk management is not a new concept as historically many measures required to prevent foodborne illnesses were types of risk management. Chlorination of water supply, provision of sanitary services, pasteurization of milk, health surveillance of food handlers, and education of consumers are all examples of risk management measures that has been implemented for a number of years. However, under the modern approach to food safety management, risk managers are encouraged to take their risk management decision in the context of risk analysis process. A set of guiding principles for MRM have also been laid down (Figure 1). As stipulated by the Principle 3, risk manager should follow a more structured approach to ensure that decisions are taken objectively and, as far as possible, based on scientific facts and consideration of socioeconomic factors as well as other risks (e.g., nutritional or chemical risks).

Microbial risk management (MRM) process consists of the so-called preliminary MRM activities, followed by identification, evaluation, and selection of MRM options, implementation of the selected option, and monitoring and review of the impact. What is particularly new in the process is that the decisions are not taken arbitrarily or based on the perception of risk managers, political pressure, or to erect nontariff barriers to trade. They should be based, on the one hand, on a scientific evaluation of risk (risk assessment) and, on the other hand, on weighing different policy alternatives to ensure that both scientific factors as well as feasibility, economic, social, and cultural factors are considered in the decision-making process. Decisions taken during such process will not always lead to an action, as it may be that the level of risk is tolerable or the risk is not a priority at the time. Thus, a risk management decision may be to not take action.

General Principles for Microbiological Risk Management

During the MRM process, the risk manager has the leading role. The main responsibilities consist of setting public health

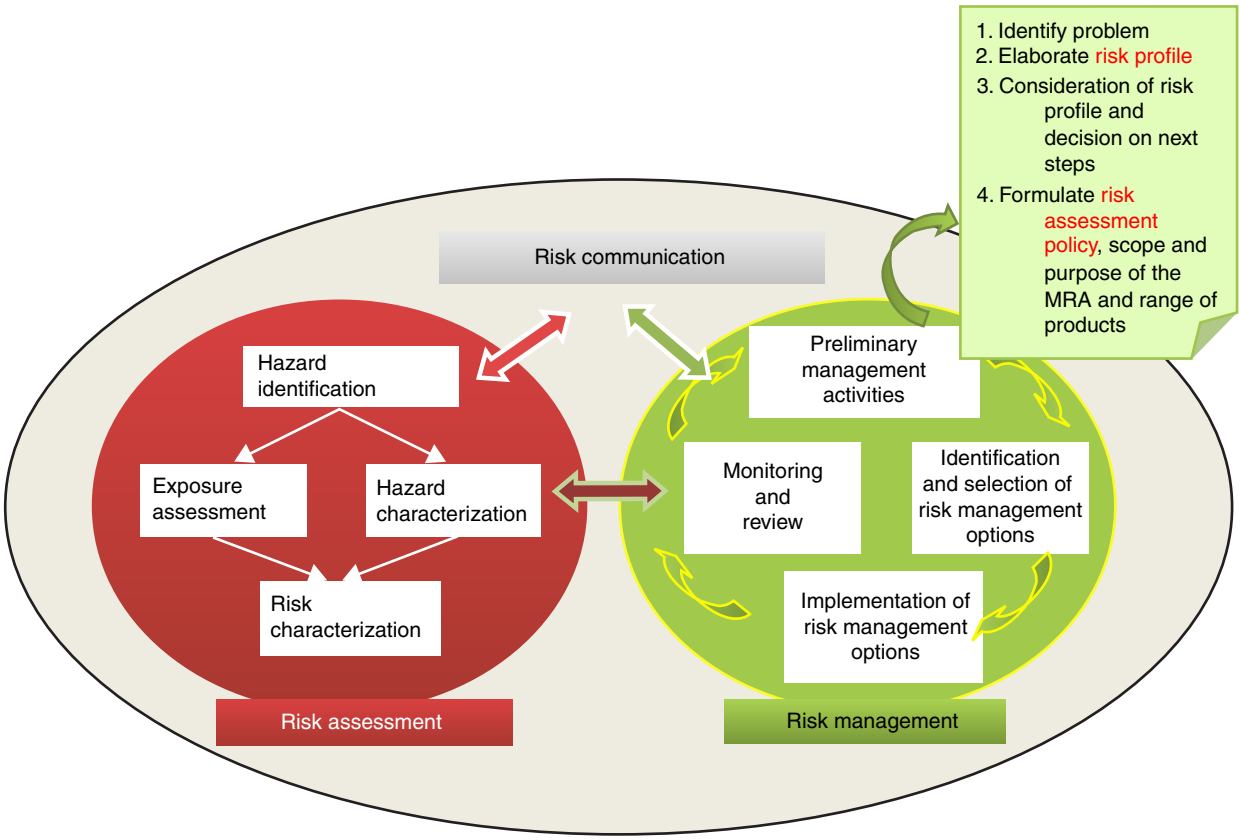

Figure 1 Process of risk analysis, including risk management, assessment, and communication.

goals and priorities, initiating the risk assessment process, and managing the overall risk management process. In MRM, a number of principles need to be observed (see Box 1). These principles result from years of experience in management in food safety and lessons learned from successes as well as failures.

Principle 1: Protection of human health is the primary objective in MRM. The management of biological hazards, particularly when it involves international trade or major economic costs, should recognize that public health is the first priority. For instance, the compulsory pasteurization of milk was initially opposed because of its cost. This principle is to ensure that human health is not compromised for economic reasons.

Principle 2: MRM should take into account the whole food chain. This principle conveys the concept that the entire food supply chain should be considered in the decision-making process. This continuum should cover primary production (including feeds, agricultural practices, and environmental conditions that may lead to the contamination of crops and animals), product design, processing, transport, storage, distribution, marketing, preparation, and consumption. Also, it should include both domestic and imported products to the extent feasible.

The principle ensures that efforts to control foodborne pathogens are implemented, where most feasible and effective, ensuring maximum health protection. For instance, in managing risk of salmonella in poultry, risk managers will have to take the decision as to which measures, or combination of measures, and at which level of the food chain, will be most effective in reducing the risk of illness, e.g. vaccinating flocks, culling infected flocks, irradiating poultry meat, monitoring and recalling contaminated poultry meat, educating consumers to cook poultry thoroughly, or a combination of these,

Box 1 General principles for microbiological risk management (MRM)

Principle 1: Protection of human health is the primary objective in MRM.
Principle 2: MRM should take into account the whole food chain.
Principle 3: MRM should follow a structured approach.
Principle 4: MRM process should be transparent, consistent, and fully documented.
Principle 5: Risk managers should ensure effective consultations with relevant interested parties.
Principle 6: Risk managers should ensure effective interaction with risk assessors.
Principle 7: Risk managers should take account of risks resulting from regional differences in hazards in the food chain and regional differences in available risk management options.
Principle 8: MRM decisions should be subject to monitoring and review and, if necessary, revision.

Principle 3: MRM should follow a structured approach. As mentioned above, this includes preliminary MRM activities, identification and selection of MRM options, implementation of MRM activities, and monitoring and review of the options taken. This principle prevents bias and arbitrary decisions and directs risk managers to follow a consistent process of interaction and consultation with scientists and stakeholders.

Principle 4: MRM process should be transparent, consistent, and fully documented. Transparency and documentation will foster a broader understanding of issues and rationale for decisions among interested parties. Thus, it will minimize conflict among stakeholders.

Principle 5: Risk managers should ensure effective consultations with relevant interested parties. This principle underlines the need for consultation with stakeholders while keeping the independence of the decision-making process. The extent and nature of public consultation will depend on the urgency, complexity, and uncertainties related to the risk and management strategies being considered. Effective and timely consultation with all relevant interested parties provides a sound basis for decision-making to ensure that decisions are feasible, effective, and where applicable, enforceable. Also, it provides an opportunity to consider potential implications and to explore if the considered options are practicable and culturally acceptable by consumers. This is important when they will have to play a role in their application. The acceptability of risk management options depend on the perception of risk and other factors underlying risk communication of biological hazards. For instance, the reduction of the risk of listeriosis by a ban of cheese made from raw milk may not be culturally acceptable in certain European countries. However, education of pregnant women and other vulnerable populations to avoid consuming such products may be more feasible.

Principle 6: Risk managers should ensure effective interaction with risk assessors. This principle is to ensure that the decisions are based on science and where necessary, on a formal risk assessment. This principle recognizes that risk managers and risk assessors should have an iterative dialog so that the risk assessment meets the needs of the risk manager. However, a separation of functions between risk assessment and management helps to prevent conflicts of interest and ensures the integrity of the process.

Principle 7: Risk managers should take account of risks resulting from regional differences in hazards in the food chain and regional differences in available risk management options. This principle recognizes that there may be regional differences in the spread of microbiological hazards, or conditions of production, processing, food handling practices, and food use or consumption patterns; subsequently exposure of consumers may vary. Risk perception may also vary among countries. Thus, risk management options should be adapted to local or regional conditions. For instance, in regions where hepatitis A is highly prevalent and personal hygiene may not be respected, it may be decided to vaccinate professional food handlers against hepatitis A.

Principle 8: MRM decisions should be subject to monitoring and review and, when necessary, revision. Risk management should be a dynamic and an iterative process. Through a process of monitoring and review of verification or newly available data, the efficacy of implemented measures should be examined and evaluated; where necessary risk management decisions should be revised. This principal embodies the concept of continuous improvement, i.e., to strive to continuously reduce risk of illness. A case in point is that the review of the efficacy of routine medical examinations of food handlers working in food service establishments. A World Health Organization expert committee recommended that such practices be discontinued as they only could confirm the health of the food handler on the day of the examination. Instead, it was recommended that food handlers should not directly handle food when they have symptoms of foodborne disease. However, in line with the Principle 7, some countries with high prevalence of typhoid and paratyphoid fevers have decided to continue periodic physical examinations.

Public Health Goals

A prime responsibility of risk managers is to decide on the public health goals. In the context of foodborne diseases, ideally, the public health goal should be prevention of all foodborne diseases, i.e., no cases. However, for most diseases, this is not possible to achieve and a more attainable goal must be decided. In practice, this means that a certain level of risk is tolerable. The concept of ALOP mentioned in the SPS agreement is a type of public health goal and expresses the idea that in managing food safety risk managers should have clear objectives on what they want to achieve, what would be the tolerable target level for the incidence of foodborne diseases, and manage the safety of their food supply so that this is not exceeded. In the SPS agreement, it is defined as ''*The level of protection deemed appropriate by the member establishing a sanitary or phytosanitary measure to protect human, animal or plant life or health within a territory*.'' Sometimes the concept is referred to as 'acceptable or tolerable level of risk.' For instance, an ALOP can be an expression of the yearly incidence of foodborne disease that may be accepted in the population. As an example, it may be decided that the annual incidence of listeriosis should not exceed 2.5 cases per 100 000 of population.

Managing the Risk Management Process

Another responsibility of the risk managers is to manage the risk management process. This consists of the following steps:

Preliminary MRM Activities

See **Figure 2**. These activities consist of:

Identification of a Microbiological Food Safety Issue

Risk managers are responsible for the identification of food safety issues and their communication to risk assessors and stakeholders. The information may reach the risk managers from different sources (e.g., scientists, trading partners, stakeholders, and media). Examples of issues may include:

- Emergence of a new pathogen that is foodborne or suspected to be (e.g., avian influenza or *Mycobacterium paratuberculosis*, the etiological agent of the Johne's disease

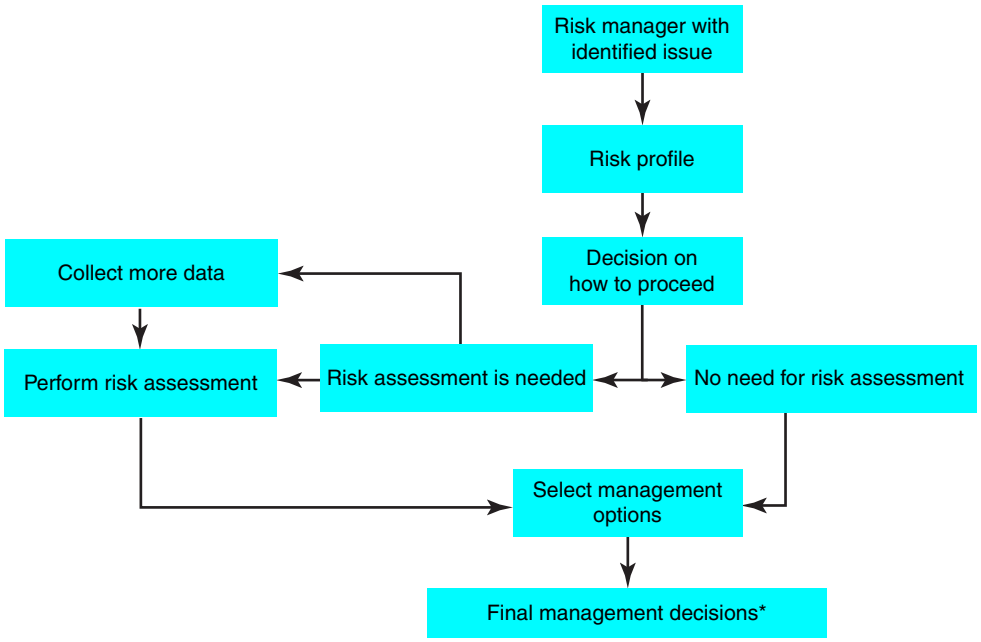

Figure 2 Risk management process. (*Includes the decision that no specific action is required). Adapted from FAO/WHO 2002 Principles and guidelines for incorporating microbiological risk assessment in the development of food safety standards. Guidelines and related texts. Report of a Joint FAO/WHO consultation, Kiel Germany 18–22 March 2002. FAO, Rome, 2002.

in cattle). Although the latter is not yet confirmed as foodborne, a food safety risk management decision was initially required);

- An unexpected outbreak of a serious foodborne illness (e.g., Shiga-like enteroaggregative *E. coli* O104:H4 outbreak in Germany and France in 2011);
- Recurring incidents in industry (e.g., chocolate contaminated with *Salmonella*);
- Frequent food contamination recalls (e.g., beef contaminated with *E. coli* O157 in USA);
- Data on the prevalence and concentration of hazards in the food chain or environment (e.g., surveys of campylobacter in poultry showing a high degree of contamination);
- Trends in human and/or animal disease surveillance data showing increase in a foodborne or zoonotic illnesses;
- Epidemiological or clinical studies, laboratory studies, and scientific, technological, or medical advances; and
- Lack of industry compliance with regulatory requirements.

In emergency situations where there is an imminent public health concern, risk managers may not have time to wait for a full fledged risk management process and may need to take immediate precautionary risk management measures. For instance, in case of an industry-associated incident which may expose large population, even if the source of the outbreak is not yet confirmed or the scientific data on the epidemiology of the pathogen are not fully available, temporary measures such as withdrawal or recall of a product may be justified. However, the provisional and precautionary nature of the information and decision should be communicated to stakeholders and general public and care should be taken to not cause undue panic. Therefore, as part of emergency preparation and crisis prevention, a concise risk profile on various pathogens needs to be compiled beforehand so that it can be readily available to risk managers. As further information becomes available, the decision should be reviewed and, if warranted, revised. Another issue to be addressed is the need for setting priorities and public health goals, or in the context of SPS agreement, comparing the impact of different risk management options in order to demonstrate equivalence of measures.

Risk Profile

If not available, the risk manager will initiate the development of a risk profile. In some cases, these may be available internationally or from another country and can be used as a starting point. A risk profile is a description of a food safety problem and its context in a concise form; it includes the state of knowledge on the subject, potential risk management options, and the food safety policy context that will influence further possible actions. A risk profile provides information on a range of elements that are relevant to the risk management decision. Box 2 provides a list of suggested elements to be included in a risk profile.

The purpose of risk profile is to provide risk managers with an information base that can enhance their understanding of a food safety issue. Through the analysis of this initial data, the risk profile can help managers to decide whether a full microbial risk assessment (MRA) or more limited assessment (e.g., exposure assessment and hazard characterization) is needed. In some cases, the risk profile could provide enough information for the risk manager to make an immediate decision to take precautionary measures. However, the preliminary information may also persuade the risk manager to decide to take no further action.

Box 2 Elements suggested for inclusion of a microbiological risk profile

A risk profile should present, to the extent possible, information on the following:

1. Hazard–food commodity combination of concern:
   - Hazard of concern;
   - Description of the food or food product and/or condition of its use with which problems (foodborne illness and trade restrictions) due to this hazard have been associated; and
   - Occurrence of the hazard in the food chain.
2. Description of the public health problem:
   - Description of the hazard including key attributes that are the focus of its public health impact (e.g., virulence characteristics, thermal resistance, and antimicrobial resistance);
   - Characteristics of the disease, including;
     - Susceptible populations;
     - Annual incidence rate in humans including, if possible, any differences between age and sex;
     - Outcome of exposure;
     - Severity of clinical manifestations (e.g., case-fatality rate and rate of hospitalization);
     - Nature and frequency of long-term complications;
     - Availability and nature of treatment; and
     - Percentage of annual cases attributable to foodborne transmission.
   - Epidemiology of foodborne disease
     - Etiology of foodborne diseases;
     - Characteristics of the foods implicated;
     - Food use and handling that influences transmission of the hazard;
     - Frequency and characteristics of foodborne sporadic cases; and
     - Epidemiological data from outbreak investigations.
   - Regional, seasonal, and ethnic differences in the incidence of foodborne illness due to the hazard.
   - Economic impact or burden of the disease if readily available:
     - Medical and hospital costs;
     - Working days lost due to illness, etc.
3. Food production, processing, distribution, and consumption:
   - Characteristics of the commodity that are involved and that may impact on risk management;
   - Description of the farm to table continuum including factors which may impact the microbiological safety of the commodity (i.e., primary production, processing, transport, storage, and consumer-handling practices);
   - What is currently known about the risk, how it arises with respect to the production, processing, transport, and consumer-handling practices of the commodity, and who it affects;
   - Summary of the extent and effectiveness of current risk management practices including food safety production/processing control measures, educational programs, and public health intervention programs (e.g., vaccines); and
   - Identification of additional risk mitigation strategies that could be used to control the hazard.
4. Other risk profile elements:
   - Extent of international trade of the food commodity;
   - Existence of regional/international trade agreements and how they may affect the public health impact with respect to the specific hazard/commodity combination(s);
   - Public perceptions of the problem and the risk; and
   - Potential public health and economic consequences of establishing Codex MRM guidance document.

Risk Assessment Policy

Another responsibility of risk managers is setting a risk assessment policy. However, it should be established in full collaboration with risk assessors and possibly with other stakeholders. The MRA policy is a document providing guidance to the risk assessors on the scope and range of the risk assessment, for example, sector of the food chain, types of food, products to consider, population to cover, source and type of data to be considered, default decisions for data gaps and uncertainties encountered during the course of the assessment, and how to present the data, particularly the types of assumptions and uncertainties that should be mentioned. The purpose is to protect the scientific integrity of the risk assessment, ensure that risk assessment is systematic, unbiased, and transparent, and to offer guidance for balancing value judgments and policy choices and weighing of adverse health parameters in evaluating risks to human health. The risk assessment policy could be of a generic nature or specific to a planned MRA and should be documented to ensure consistency, clarity, and transparency.

Commissioning a Microbiological Risk Assessment

Figure 2 presents the process of risk management and commissioning MRA. It shows that the process can be complex

and can follow different pathways. MRA does not follow a single model, especially as it is a lengthy and expensive exercise. Therefore, risk managers may decide to commission an MRA only when absolutely needed. The purpose of an MRA is to provide an objective systematic evaluation of relevant scientific data to help risk managers to make an informed and science-based decision. An MRA can also be instrumental in deciding whether public health goals are realistic by estimating the resources that would be needed to achieve them. Furthermore, it can be used to compare the efficacy of management interventions. In relation to the SPS agreement, it can be used to demonstrate that different measures are equally effective and thus equivalent.

In commissioning an MRA, the mandate should be as clear as possible. Risk assessors must inform risk managers of any constraints, data gaps, uncertainties, and assumptions and their impact on the risk assessment. Where there is a disagreement among the risk assessors, the risk managers should be informed of the minority opinions and these differences should be documented. Risk assessors have to also ensure that the result of the MRA is relevant to the questions raised by the risk manager. Through an iterative communication process, risk assessors should further clarify their mandate and fine tune the results to meet the needs of the risk managers. The results of the MRA should be presented by risk assessors in a way that it is comprehensible to risk managers. It can be in different forms such as:

- Risk of illness by consumption of specific product/pathogen combination (e.g., risk of botulism from canned products is 1 in 10^{-12});
- Estimated number of illnesses (e.g., per year in a country) due to consumption of specific food-hazard combination (e.g., listeriosis from smoked fish is 0.5 cases per 100 000 inhabitants);
- Risk estimates for different processing, distribution, and consumer-use scenarios; and
- Efficiency of control measures (e.g., incidence of egg-borne salmonellosis can be reduced by 80% by changing preparation practices).

For the best use of a risk assessment, risk managers should be fully informed of the strengths and limitations (key assumptions, key data gaps, and variability in the data and their influences on the outcomes), especially a pragmatic appreciation of uncertainties associated with an MRA and its outputs.

Identification of MRM Options

Risk management options are control measures that risk managers may consider to manage the microbiological risks and prevent or control foodborne diseases. The identification of management options should be based on the consideration of the ability of the control measures to mitigate the risks to desired level of health protection and on the practical feasibility, acceptability, and consequences of the options. An MRA can help in determining the efficacy of different control measures in achieving the desired objectives.

One category of risk management options is legislation. For managing biological hazards, a number of options are possible, including:

- Introducing a microbiocidal treatment or a temperature control (e.g., refrigeration) to prevent growth of pathogens. For instance, pasteurization of milk is a legal requirement in many countries to prevent milk-borne infections;
- Labeling of products with a safety warning or with instructions for safe handling and preparation of products by consumers;
- Specifying microbiological criteria (MC). For example, following emergence of *Cronobacter sakazakii*, the MC of infant formula was revised;
- Establishing requirements for certification or approval procedures. Such measures can be important when safety is based on hygienic practice during production, processing, or manufacturing (e.g., cheese made from raw milk);
- Requiring import certificates for certain products. Such measures were required at the time of bovine spongiform encephalopathy epidemic;
- Banning sale of certain foodstuffs. For example, many countries ban the sale of raw milk to prevent milk-borne diseases; and
- Inspecting. Many countries require the inspection of slaughtered animals to prevent parasitical diseases.

Another option for managing biological risks is strengthening the food safety assurance system. For example, new measures may be recommended as part of good agriculture practice or in the application of the Hazard Analysis and Critical Control Point (HACCP) system. Following the outbreak of avian influenza, specific measures were recommended by many countries as part of good animal husbandry. Another example is an outbreak of botulism associated with pasteurized carrot juice in 2006 in the USA. As a result, the US Food and Drug Administration (FDA) recommended that firms incorporate validated control measures for *C. botulinum* spores into their HACCP plans. To ensure that growth and toxin production does not occur, the US FDA required that the juice be kept refrigerated in distribution channels and by consumers. Similarly, after outbreaks of *E. coli* O157:H7 associated with unpasteurized apple cider in 1996, the US FDA required the pasteurization of the product.

Another risk management option is the establishment of a FSO for a particular food safety issue. This approach offers flexibility to industry to select appropriate control measures that meet the FSO.

For certain problems, the risk management option of choice is the education of food handlers and consumers in hygienic handing of food. This is perhaps one of the key control measures applicable to most biological hazards, and particularly important for the preparation of food for infants and young children.

In the cases of a food safety emergency, the withdrawal/recall of a food product may be warranted. The effective use of this risk management option often depends on the ability of the producer to trace the distribution of the product. Requirements for traceability, both forward and backward, have now become common for food producers in many countries.

Evaluation and Selection of MRM Options

The primary responsibility for evaluating and selecting appropriate risk management options lies with the risk manager. However, risk assessors and stakeholders play an important role in this process by providing information that permits the evaluation, comparison, and selection of the most suitable options. When evaluating the various options, the risk managers need to consider a number of factors including:

- Efficacy of the measure in reducing risk to the ALOP. For instance, if cold storage of eggs can reduce the risk of egg-borne salmonellosis to a set target;
- Feasibility, economic cost, and culturally acceptability. These need to be assessed from the perspectives of the industry, consumers, and other stakeholders; and
- Any practical issues regarding the implementation of the selected risk management options (e.g., enforceable, verifiable, and additional resource needed).

In selecting among risk management options, it should be remembered that prevention is always better than cure. Moreover, preventive approaches, such as HACCP, have largely replaced test and release system because the level of protection afforded by the latter is no longer considered appropriate.

Implementation of MRM Options

Implementation involves communicating the selected risk management options to stakeholders, if applicable, providing training and education, and assuring that the risk management option is implemented as intended. Implementation may involve different interested parties, including competent authorities, industry, and consumers.

Where a risk management option involves industry, the industry may need time to introduce the measures in their food safety management system and establish performance metrics such as PO or performance criteria (PC), which validate the efficacy of control measures and the contribution to the achieving FSOs or other regulatory requirements.

Where control measures relate to consumers practice, information should be provided to consumers on their responsibilities. It is essential that their education is based on an understanding of their risk perception and socioeconomic conditions and culture.

Monitoring and Review

Implementation of risk management options requires continuous monitoring and surveillance of implementation of MRM options as well as criteria that will be used for evaluation of the impact of MRM measures on the foodborne disease of interest. These can include:

- Surveillance of foodborne diseases in humans or related zoonotic diseases in animals. This can include outbreak data, notification data, epidemiological surveys, or laboratory data on human, animal, and food isolates;
- Monitoring of environmental contamination in food processing establishments;
- Monitoring of contamination of the food supply for a specific pathogen or a food–pathogen combination. Food contamination data collected at different point of the food chain allow a better understanding of where failures occur. Where monitoring data indicate a gap in achieving expected results, consultation with the stakeholders and root cause analysis of the gaps can help in understanding how the implementation of control measures can be improved; and
- Surveying of practices, habits, and behavioral risk factors of food workers and consumers.

As for any management process, the relevance and effectiveness of the selected MRM measures need to be periodically reviewed. The purpose of review is to ensure that expected consumer health protection is achieved. Review of MRM activities should be seen as an integral part of the MRM process, and criteria for the review should be considered when monitoring plans are made.

Ideally, reviews should take place at a predetermined time but may also be triggered by the availability of relevant new information such as new information on the virulence of a pathogen, prevalence of the disease, occurrence of the pathogen in foods, changes in dietary intake patterns, and sensitivity of subpopulations.

The outcome of a review could be the confirmation of appropriateness of the existing measures or a decision to amend the selected MRM option. Industry and other interested parties (e.g., consumers) can also suggest the review of MRM options. For transparency, the results of the review process and the actions that risk managers are considering to undertake should, where appropriate, be made public and communicated to interested parties.

Microbiological Risk Management Metrics

Although various risk management options are presented in greater depth in other articles, a few words should be said about the risk management metrics for microbiological hazards. Traditionally, food safety management and prevention of foodborne illness involved control measures which, although effective, were generally based on metrics that were not directly related to specific levels of public health protection. Therefore, it was often difficult to evaluate the efficacy of such control measures.

To develop risk-based management approaches that can directly and transparently relate the stringency of control measures to achievement of specific levels of public health protection, there was the need to relate the performance of a control measure, or a series of control measures, to the level of control needed to manage a food safety risk. In other words, there was a need to translate the desired level of health protection to the tolerable level of a hazard in a food to achieve the public health goals.

For this reason, a number of metrics for the MRM were developed (see Table 1). These metrics serve various purposes. As explained above, they translate public health goals (e.g., ALOPs) into values which can help industry to design more precisely their food safety control systems to meet

Table 1 Microbiological risk management metrics

Public health goal (e.g., ALOP)	A public health goal can be an expression of acceptable yearly incidence of foodborne disease or expected reduction in incidence during a given period of time
Food safety objective (FSO)	The maximum frequency and/or concentration of a hazard in a food at the time of consumption that provides or contributes to the appropriate level of protection (ALOP)
Performance objective (PO)	The maximum frequency and/or concentration of a hazard in a food at a specified step in the food chain before the time of consumption that provides or contributes to an FSO or ALOP, as applicable
Performance criteria (PC)	The effect in frequency and/or concentration of a hazard in a food that must be achieved by the application of one or more control measures to provide or contribute to a PO or an FSO.
Process or product criteria (PcC and PdC)	PcC specify conditions of treatment that a food must undergo at a specific step in its manufacturing to reduce the level of a hazard to meet PC. PdC specify chemical and physical characteristic of a food to prevent or limit growth of bacteria
Microbiological criteria (MC)	MC specify the conditions of acceptability of a product or a food lot, based on the absence or presence or number of microorganism per unit(s) of mass, volume, area, or lot

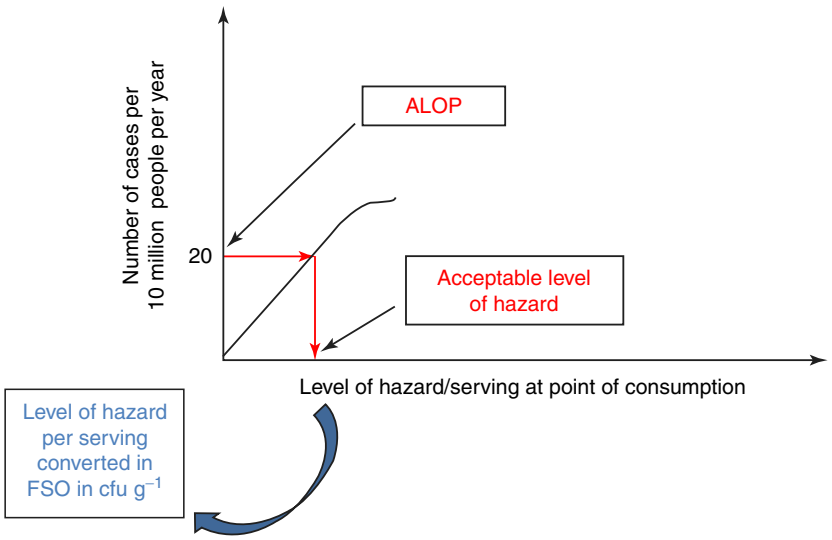

Figure 3 Schematic presentation of relationship of ALOP and FSO.

public health goals. They also provide a conceptual framework that fosters a dialog with all stakeholders on the efficiency and efficacy of control measures. In this way, they can be used to evaluate whether the existing food industry control measures are adequate to support public health goals. In the context of the SPS Agreement, they can be used to demonstrate equivalence of control measures.

To explain these concepts, the term control measures, which is defined by the Codex, should be referred to as the actions and activities that can be used to prevent or eliminate a food safety hazard or reduce it to an acceptable level. The question is then "What is an acceptable level?" At the point of consumption, the acceptable level of hazard is expressed by the term FSO (see Figure 3).

An FSO is defined as the maximum frequency and/or concentration of a hazard in a food at the time of consumption that provides or contributes to the ALOP. For instance, for a given product, it may be required that the level of *Listeria monocytogenes* at the point of consumption should not exceed 100 cfu g^{-1} or *Salmonella* be absent in a ready-to-eat (RTE) product.

As explained above, in establishing an FSO, the acceptable level of a hazard is considered at the moment of consumption. However, this level can also be extrapolated to other points in the food chain. These levels are then referred to as POs. The PO is defined as the maximum frequency and/or concentration of a hazard in a food at a specified step in the food chain before the time of consumption that provides or contributes to an FSO or ALOP, as applicable. Because a PO is conceptually linked to the FSO and ALOP, the impact of various steps in the food chain both before and subsequent to the PO should be considered in setting its value. If the microbial level of a food is likely to change during the steps of the food chain, the POs may be different from each other and from the FSO.

FSO can also be transformed into PC which express the effect of a control measure on the level of a hazard (for instance the effect of a cooking step). A PC is defined as the effect in frequency and/or concentration of a hazard in a food that must be achieved by the application of one or more control measures to provide or contribute to a PO or an FSO.

A PC is most often applied in association with various measures to eliminate the pathogen, such as heat processing and irradiation, or to control their growth, such as use of antimicrobial agents, refrigeration, or lowering water activity. A PC for elimination will specify the reduction of the microbial population that will occur as a result of the treatment (e.g., 12-log reduction in the levels of *Clostridium botulinum* spores). A PC for preventing growth will indicate the maximum acceptable increase in number of pathogens during the application of the control measure (e.g., less than a 1-log increase in *L. monocytogenes* during refrigerated distribution of a RTE food).

The microbial acceptability of a product is described by MC that can be applied as a direct control measure to accept or reject each lot of food or as a means for verifying that a food safety management system, such as HACCP, is functioning as planned. Other control measures may be characterized by a number of process parameters (e.g., time and temperature) or product parameters (e.g., water activity and pH). In turn, these need to be specifically designed to ensure that the set PC is achieved. These conditions are referred to as process criterion (PcC) and product criterion (PdC), respectively.

A PcC specifies the conditions of treatment that a food must undergo at a specific step in its manufacture to achieve a desired level of control of a microbiological hazard. For example, to achieve a PC of 12D reduction in the number of *Clostridium botulinum* spores (so-called 'bot cook') for canned meat products, the PcC is typically designed to be 2.5 min at 121 °C, or parameters that allow the same degree of reduction. However, the PdC specifies a chemical or physical characteristic of a food (e.g., pH and water activity) that, if met, contributes to the food safety of the product.

In general, PCs are established by individual food companies. However, a PC may also be set by national governments for a specific control measure such as the case with low-acid canned food. The advantage of a government PC is that all food businesses are applying the same safety standards regardless of their size and resources. It also facilitates the validation of control measures in industry.

Conclusion

In conclusion, the modern approach to food safety management calls for a more risk based, quantifiable, and transparent approach to food safety and increased accountability of regulatory authorities and stakeholders.

To ensure that management of the safety of the food supply meet the set public health goals, the control measures at different steps of the food chain, from farm-to-fork, should be designed such that the overall reduction or increase in the level of hazards does not exceed the FSOs at the point of consumption.

Although the modern approach to risk management of biological hazards present multiple benefits for the society, a key question remains its implementation as it will require incalculable amount of resources and expertise. Most developing countries and small, or less developed, business may not be able to follow this trend. Nevertheless, this should not undermine the importance and validity of the guidance and its application where feasible.

See also: Public Health Measures: Evaluation of the Efficacy of National Food Control Programs; Food Inspections and Enforcement Systems; Fundamentals of Food Legislation; Health Education, Information, and Risk Communication; Modern Approach to Food Safety Management: An Overview; Monitoring of Contaminants; Surveillance of Foodborne Diseases. Risk Analysis: Food Safety Training and Health Education: Principles and Methods; Risk Assessment: Chemical Hazards; Risk Assessment: Microbiological Hazards; Risk Communication: Biological Hazards; Risk Communication: Chemical Hazards; Risk Communication: Diet, Nutrition, and Health; Risk Communication: Novel Foods and Novel Technologies; Risk Communication; Risk Management: Application to Chemical Hazards

Further Reading

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Relevant Websites

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Centers for Disease Control and Prevention: Microbial Risk Assessment Guidelines.

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http://www.fda.gov/Food/FoodScienceResearch/RiskSafetyAssessment/
FDA Risk and Safety Assessment.

http://www.who.int/foodsafety/micro/jemra/en/
Joint FAO/WHO Expert Meetings on Microbiological Risk Assessment.

http://www.fsis.usda.gov/science/Microbial_Risk_Assessment_Guideline/index.asp
USDA Food Safety and Inspection Service: Microbial Risk Assessment Guideline Pathogenic Microorganisms with Focus on Food And Water.

RISK ANALYSIS

Risk Communication

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Glossary

Attitude A hypothetical construct that represents an individual's degree of like or dislike for an item.
Nanotechnology Focuses with structures sized between 1 and 100 nanometers in at least one dimension, and development of materials or devices within that size range.
Risk assessment Risk assessment is the determination of quantitative or qualitative value of risk related to a concrete situation and a recognized threat (or hazard) from genetically modified (GM) food. GM foods are foods derived from genetically modified organisms.
Risk communication The exchange of information about health or environmental risks among risk assessors and managers, the general public, and other interested stakeholders and end-users.
Risk management A process to identify, assess, manage, and control potential events or situations.
Risk perception The judgment, underpinned by psychological determinants, that people make about the characteristics and severity of a risk.
Stakeholder Any person or group affected by an event or activity, for example the occurrence of a food risk.

The Historical Background of Risk Communication and Food Safety

Risk communication has been a focus of academic study for the past 35 years. Initially, research in this area aimed to translate risk assessments into messages for the general public, in order to align their views with expert opinion, and to facilitate understanding on the part of nonexperts. However, today risk communication forms an integral part of the practice of risk analysis as defined by FAO/WHO. Food risk analysis comprises three components: risk assessment, risk management, and risk communication. Risk communication is now established as an interactive exchange of risk information among stakeholders. Stakeholders include the food industry, farmers and food producers, food risk assessors, communicators and managers, and other regulators, representatives of civic society (e.g., consumer and environmental organizations, and other nongovernmental bodies), and, perhaps most importantly, food consumers. It is risk communication which will be the focus of discussion in this article.

A series of food safety crises has occurred over recent decades. These include, for example, bovine spongiform encephalopathy (BSE), dioxin contamination in different food chains, the debate about the effects of acrylamide on human health, the US *Salmonella* peanut contamination incident, cases of deliberate fraud, such as the melamine contamination of milk in China, and accidental contamination of food at different stages of the food chain, such as the case of Sudan red colorant. These incidents have resulted in a widespread public concern about food safety, emerging food risks, and adequacy of risk management measures. One consequence has been the decline in consumer confidence in regulatory measures and activities associated with consumer protection in the agrifood sector, as well as strengthened regulations and their enforcement (e.g., traceability).

Unsuccessful and successful examples of food risk communication can be identified. Unsuccessful communication efforts typically fail to take into account the concerns and perceptions of the public. For example, in the case of genetically modified (GM) foods, European consumers were concerned that the resulting products were unnatural, and had potential for long-term bioactive consequences. Consumer control over consumption was not possible as products were untraced and unlabeled. Communication was based on the premise that the novel foods were 'substantially equivalent' to the existing ones. Not only did risk communication not address consumer concerns, but also conveyed the unintended message that regulators and industry had dismissed consumer concerns as irrelevant and potentially trivial, increasing societal distrust in the motives of communicators. In contrast, communication about the potential dioxin contamination of the milk supply following the 2001 foot and mouth crisis was effective. Potential contamination resulted from the close proximity of animal cremation sites to milk-producing areas. The UK Food Standards Agency communication was open about the potential uncertainties (signaling honesty) and it also contained information about what was being done to reduce these uncertainties in scientific terms. In contrast, both these types of information had been omitted in communication from the UK Ministry of Agriculture, Fisheries, and Food (MAFF) about BSE a few years earlier, which contributed to public outrage and an international crisis.

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In addition, as a consequence of the increasing globalization of the food supply, food safety problems may have impacts across broader geographical regions than before in terms of human and animal health, or environmental protection. It is at this broad level that the issues of risk assessment, management, and communication must be addressed. As part of this, it is important to understand cross-cultural differences and similarities in consumer risk perceptions, and how these influence consumer behaviors. This information is essential to understanding how these influence best practice in risk communication targeting the needs of consumers in different socio-political contexts.

One aim of risk communication is to influence potentially risky end-user behaviors. Another aim is to enhance transparency and inform consumer choice through the provision of relevant risk information. As part of this, it is important to understand the risk perceptions of the individual, who is the target of the communication, as this will influence what information is perceived to be relevant to the individual, as well as provide the basis for further interactive exchange of opinions and ideas.

Risk Perception and Communication

Research focused on understanding risk perception identified different psychological dimensions of risk between lay and experts, which explained why societal reactions to risk differed from those of experts. This research demonstrated that factors that are not included in technical risk estimates may nevertheless influence people's perception of risk. The extent to which a risk is perceived to be unnatural, potentially catastrophic, or to which an individual perceives exposure to be involuntary influences risk perceptions. These psychological dimensions are excellent predictors of people's responses to potential risks associated with hazards across different hazard domains, including that of food hazards.

Various methodologies have been used to study peoples risk perceptions. These may be both qualitative and quantitative. For example, focus groups (qualitative planned discussions that facilitate detailed analysis of an issue within groups of individuals) or semi-structured interviews may be used as a 'stand alone method,' or provide the foundation for the development of quantitative surveys, for example, those sampling the opinion of representative samples of a population of interest. Although survey based methods for identifying risk perceptions is amenable to comparison across different demographic segments of the population, or in time and space, the use of fixed questions results in the method being inflexible, and unable to incorporate new concerns and issues into comparisons. More interactive methods such as interactive workshops or consensus conferences (classified as deliberative public engagement or public participation) facilitate discussion between members of the public and experts in the area of food risk. The advantage is that an in-depth insight into public concerns and values about food risk can be identified, at the expense of gaining an understanding of the priorities and preferences of the overall population, as only small and unrepresentative groups of the public can be included in such workshops. Expert stakeholder views are frequently elicited using several rounds of a survey (the 'Delphi method'). Delphi methodology provides feedback from the initial rounds of expert responses (e.g., through statistical aggregation), which allows some degree of interaction between experts, even if they are dispersed geographically, or if expertise from a broad range of disciplines, where participants are unlikely to be members of the same scientific or policy networks, is required.

Consumer trust in different actors and institutions responsible for guaranteeing and controlling food safety, as well as trust in the information provided by different information sources that communicate about food safety or food-related risks, is an important determinant of consumer food risk perceptions, and reactions to risk communication. In addition, trust in food chain stakeholders may influence consumer evaluations of the efficacy of food risk management practices.

Individual differences in consumer responses to food hazards, and communication about the associated risks, have also been a focus of empirical investigation. Risk perceptions, food safety related behaviors, consumer responses to food safety incidents, and consumer use of food risk information may be dependent on consumer characteristics such as an individual's tendency to anxiety, their tendency to engage in habitual food choices, or their perceptions that their own health is amenable to influence by their own behaviors.

One important barrier to effective communication about food safety issues, which may vary across individuals, is that of optimistic bias which refers to an individual's judgment that negative events are less likely to happen to the person making the judgment in comparison to the risks experienced by an average member of society or other people in general. The importance of optimistic bias in developing an effective risk communication strategy will be discussed in the context of domestic food hygiene practices in the next section.

Behavioral Change through Effective Risk Communication

Many health-related behaviors, such as home preparation of foods, may determine whether an individual is at high or low risk from a particular hazard. In such cases, risk communication efforts may be specifically aimed at behavioral change of the consumers. In the example of food safety, domestic food hygiene practices are of particular concern.

The Case of Improving Domestic Food Handling Practices

Foodborne illness remains an issue of concern in terms of potentially negative consequences for public health. The overall aim of food safety objectives is to reduce the number of cases of foodborne illnesses, and hence reduce the consequent burden on public health. Although legislative changes may improve food safety up to the point of retail of food products, it is far more difficult to legislate for risky consumer behavior. Inappropriate storage, food preparation, and cross-contamination by end-users may result in illness, despite foods being safe at the point of sale, necessitating implementation of appropriate and effective information interventions. Improved public health is dependent on food preparation practices by the consumer (as well as other stakeholders in the food chain)

implying that effective ways have to be developed to communicate about the risks of food safety and inappropriate food-handling practices with the consumer.

Domestic food preparation tends to be lifestyle-related risk, in particular involving frequently repeated behaviors, where individuals think they have a lot of control over the risks associated with their behavior. Perceived personal control over the risk diminishes perceptions of risk. As a lifestyle risk, domestic food preparation represents an example where optimistic bias may be influential in acting as a barrier to effective risk communication. Optimistic bias has been observed for a range of food-related hazards, but tends to be more pronounced for that which can be described as 'lifestyle' related as opposed to 'technological' in origin. Influenced by optimistic bias, consumers may perceive that the risk information in risk messages is directed toward more vulnerable and less knowledgeable members of society.

People exhibiting optimistic bias may not take precautions to reduce their risk from a particular hazard. Risk communication interventions have been developed which have succeeded in reducing optimistic bias, including increasing perceived accountability associated with individual's risk judgment. This can be achieved through providing information about actual risk-taking behaviors or through making people compare themselves with an individual similar to themselves or an identified individual similar to the receiver of the risk information. Targeting information to those individuals most at risk may optimize use of available resources. An important first step is to differentiate or segment consumers who are most at risk, as a consequence of both attitudinal factors and their vulnerability to the risks.

Developing effective risk communication interventions (e.g., targeted risk communication or information campaigns focused on the needs of those consumers most at risk), is contingent on understanding what constitutes safe behavior as well as consumer beliefs about what constitutes safe behavior. Understanding social psychological factors which determine behavior is likely important if people are to make changes in domestic food preparation practices in order to minimize the risks of foodborne illness. Furthermore, although people may actually possess the information needed to reduce food risks associated with food preparation, they may not use this knowledge at the moment they need it most. For example, most consumers know that cross-contamination should be avoided, and heating should be adequate, if food safety problems are to be reduced, but they may only be aware of this information when prompted. This knowledge is not always applied when consumers are actually preparing food. Differences in the likelihood of conducting safe domestic hygiene practices across the population suggest that stratified risk communication strategies represent an important element in targeting risk communication to those most at risk, designing both message content and using information delivery systems so that vulnerable individuals will utilize the information. Various factors have been shown to increase consumers' receptivity to food safety risk communications focused on domestic hygiene practices. The first involves adding relevant emotional content to food safety messages, (e.g., imagery designed to elicit the emotion disgust). This increased consumer awareness and motivation to behave in such a way is to reduce the problem of food poisoning. Another approach to increase safe behavior, involves introducing a risk message into recipes used as part of food preparation practices. This not only reminded consumers of the relevance of the food safety message included in the recipe, but also activated their existing knowledge about other aspects of food safety related behaviors that consumers already possessed, but were not putting into practice. Research of this type demonstrates that consumers possess more knowledge about safe food preparation than they use, and breaking behavioral habits and activating this knowledge at the moment the behaviors are actually conducted may represent a useful approach to developing risk communication strategies to reduce the incidence of food safety problems.

Communication about Food Technologies

Besides those risks that can be controlled by consumers through their own behaviors, some consumer concerns may focus on production processes of food technologies, which are not under the consumers' control. These types of risks require a different type of communication as it is not end-user food handling, but end-user food choice that determines the risk. This type of communication is, for example, suited for communication about new food technologies. Communication about such risks should deal with a number of factors that may influence risk perception including dealing with uncertainty in risks and trust in relation to risk management structures.

Communicating Uncertainty Associated With Food Risk

There is increasing societal and political pressure directed toward greater transparency in risk management practices. For this reason, the uncertainties associated with technical risk assessments, on which risk management decisions are founded, need to be explicitly communicated to all end-users and stakeholders, including the public. Public response to communication of uncertainty may also need to be taken into account by decision-makers.

Historically, it has been assumed by experts that the public is unable to conceptualize the scientific uncertainties associated with technical risk estimates, and that providing the public with this information will have a negative impact on public risk perceptions and related attitudes. However, the effects of different types of uncertainty on lay people's understanding of technical risk estimates have been investigated. A minority of individuals experience difficulties with the concept of uncertainty *per se*. For others, discussion of uncertainty appears to signal more honestly on the part of the communicators, although may, at the same time, result in reduced competence ratings attributed to the communicator or risk manager. From a risk communication perspective, it is also interesting to note that graphical presentations of uncertainty to lay people seem to result in higher comprehensibility ratings, but lower trustworthiness ratings.

Different types of uncertainty have been found to be associated with different levels of concern on the part of the general public. These are related to perceptions of lack of knowledge on the part of relevant stakeholders (e.g., lack of

scientific information regarding a specific risk, or conflicting scientific information or opinion), scientific uncertainty about the potential impact or extent of a particular hazard should it occur, and the perceived need by consumers for further research to be conducted in order to reduce the uncertainty. Perceptions that regulatory institutions are withholding uncertainty information from the public tend to have a negative impact on public trust in those institutions. The public is more accepting of uncertainty resulting from inadequacies in scientific process (e.g., associated with risk estimates) *per se* compared to uncertainty associated with the failure of institutions to reduce scientific uncertainty through conducting appropriate empirical investigations. Individuals appear to have difficulties in interpreting low probabilities and tend not to seek out probabilistic information under conditions of uncertainty. Other heuristics (or 'decision-rules') may exert influence on lay interpretations of uncertainty information, although it is not known how different individuals, or indeed people with different levels of expertise, are influenced by these heuristics. Example of heuristics relevant to the discussion of uncertainty includes the availability heuristic, where the people find it is easier to recall a past event, the greater they estimate the probability of the event occurring in the future, and representativeness, where the likelihood of an event is estimated according to the similarity to the class of events of which it is seen as an example. Developing effective communication about uncertainty is important if it is to be presented in the public domain as part of the risk analysis process. For example, there is evidence that public preferences for information about proactive risk management increases as uncertainty about a particular risk also increases, although this effect may be prone to cross-cultural variation.

Communicating about Risk Management Strategies

Consumer trust in different actors and institutions responsible for guaranteeing and controlling food safety is as important as trust in the information provided by these different institutions. In particular, trust may act as a proxy for extensive consumer scrutiny of food risk messages or communications. For example, although many people do not want to receive detailed information about the risks and benefits of food technologies, they may accept these same food technologies if they perceive that they are able to trust those institutions with responsibility for consumer and environmental protection. From this, it is arguable that risk communication should not only focus on food-related risks, but also provide information about risk management activities in an accessible form, which can then be made available to consumers. The question arises as to what constitutes optimal risk management practices from a consumer perspective. Results from qualitative and quantitative research have provided insight into which underlying factors have the potential to influence consumer evaluations of risk management practices. The research demonstrated that initiation of preventative risk management activities by risk regulators, strict enforcement of, and communication about, relevant regulations, transparency associated with regulatory activities, and information provision about the application of control systems and their performance were shown to be essential indicators for consumer perceptions of high-quality food risk management. Country-specific factors included skepticism regarding the practice of risk assessment and management. One conclusion is that communication with the public about how risks are managed is an important part of effective risk communication. Making such information available also enhances perceptions that food risk governance is transparent.

Communicating about the Risks (and Benefits) of Food Technologies

Research into consumer attitudes to emerging food technologies, such as GM foods, food irradiation, or highly technological food-processing practices has demonstrated that consumer acceptance of these technologies and their applications is driven not only by perceptions of potential personal benefits and health effects, but also by concerns and beliefs such as ethical and moral considerations, and values such as concern about the integrity of nature. For example, the (European) public perception that institutions and industries were pushing the introduction of GM foods in order to protect their own vested interests, rather than to provide societal benefits, did little to alleviate societal concerns. In the future, emerging technologies applied to food production (e.g., nanotechnology), may give rise to other public concerns. This may be exacerbated by increased complexities and uncertainties regarding both risks and benefits associated with food-production processes and food products. In response to public concerns, institutions may adopt a precautionary approach in terms of regulation, although greater regulatory attention may be paid to the trade-off between risks and benefits associated with these novel foods.

From this, it seems likely that consumer responses are contingent on perceptions of both risk and benefit associated with specific applications. An example is provided by Schenk *et al*. (2008), who examined the trade-offs consumers make between perceived risks and benefits regarding genetic modification applied to mitigating allergies (e.g., applied to the development of hypoallergenic foods, or birch trees with hypoallergenic pollen). Allergic patients perceived greater benefits associated with the birch application compared to nonpatients, an effect which correlated with increased perceived allergy severity. However, no differences were found between patients and nonpatients for the hypoallergenic food applications, possibly because the severity of the allergic response experienced by apple allergy sufferers is rather low. Thus it appears that the personal relevance of a particular benefit associated with a technology application will influence whether a particular consumer perceives the application of a technology to be acceptable.

The problems associated with consumer acceptance of GM foods in Europe will not be discussed in depth in the present article. However, a summary of the risk communication issues associated with GM foods is relevant at this juncture. Consumer values such as concern about the integrity of nature, and trust in the regulatory system, were an important part of societal and consumer acceptance. Communication efforts (from international institutions, national governments, and the industry) focused on the concept of 'substantial equivalence,' in other words that GM foods were in no way 'substantially different' in terms of food safety to foods that consumers were already consuming, and which had been

produced using conventional production techniques. As a consequence, developing communication about substantial equivalence did not address consumer concerns. In addition, control over consumption of GM foods was important to European consumers, necessitating the labeling of GM foods (as an additional form of communication) and implementation of effective traceability systems, as well as communication about how both the risks of genetic modification were managed and controlled.

Once established, consumer attitudes to technologies appear difficult to change, and consumers are unlikely to attend to further risk communication about a specific issue. For example, it has been shown that providing additional information about food-production technologies associated with established attitudes has little impact on these attitudes, independent of whether the information is about potential benefits or benefits and risks. Negative attitudes become slightly less negative, and positive attitudes become slightly less positive, although the actual extent of the change may be rather small.

In the case of nanotechnology, however, there is evidence to suggest that the provision of balanced risk–benefit information has a differential effect on different consumers. In this case, consumer attitudes to the technology still remain inchoate. Some consumers do not change their attitude at all (i.e., they remain indifferent or ambivalent to the use of nanotechnology in food production). These consumers are likely to be convinced either by the benefit they perceive to be associated with specific products, or by the views of opinion leaders with whom they perceive to share values, or whom they trust. A large minority appear to differentially process, and react to the risk information, and a smaller minority process the benefit information, developing more positive attitudes toward nanotechnology and its agrifood-related applications.

It is likely that many consumers are less interested in information about the risks and benefits of novel agrifood technologies, but will accept these technologies if they trust those perceived to be responsible for consumer health and environmental protection. To some extent this will be contingent on the extent to which these institutional and organizational stakeholders are perceived to be both honest (telling the truth) about risks and benefits, other salient issues associated with emerging technologies applied in the agrifood sector, as well as presenting accurate information which addresses risks, benefits, and uncertainties. In the case of nanotechnology, much of the debate about risk communication relates also to whether appropriate risk assessments have been conducted. In addition, trust might be developed through communication about proactive risk management strategies adopted by regulators and industry to ensure that the products of emerging technologies are safe.

Future Research Needs

Although there is a need to develop effective risk–benefit communication with consumers, historically, communication with consumers about food issues associated with health and environmental impact has focused almost exclusively on food risks, whereas health benefits have been communicated separately, as nutritional information. Other areas relevant to consumer food choices (e.g., innovations in food-production technologies) may also involve consumers trading off perceived benefits (e.g., improvements to consumer health, or more sustainable production) against perceived risks, for example, uncertain long-term effects associated with production processes, or ethical concerns about the integrity of nature negatively impacted by the production process. In reality, consumer food consumption decisions frequently involve weighing risks against benefits. Indeed, integrated assessment of risk and benefit is increasingly forming an integral part of the assessment phase of risk analysis.

It is increasingly recognized that benefit assessment should be considered in the risk analyses process. Risk--benefit assessment again is a science-based process, and can be divided into analogous steps: problem formulation, risk–benefit identification, dose–response relationships, exposure assessment, and risk–benefit characterization. Risk–benefit management involves the same process of consideration of societal interests in the issue as dose risk management. Similarly risk–benefit communication has proven to be more complex than simply delivering a health message. At the present time, it is not understood how to effectively communicate to end-users about the combination of both inherent food risks and benefits associated with a particular food product. This will be especially challenging when a specific hazard may differentially harm or benefit subgroups of the population (e.g., pregnant women or the immune-compromised, or those suffering from specific illnesses that can be prevented or would benefit from specific nutritional interventions). In addition, quantitative approaches to risk–benefit assessments may provide information on the associated uncertainty and variability, which can be particularly difficult to communicate. There is an increasing interest in the provision of personalized information, which contemporary communication strategies assume is more effective than those focused on the population more generally. All these types of information (risk–benefit communication, and its impact on consumer decision-making, differential information needs and preferences of specific population groups, discussion of uncertainty associated with both risks and benefits, and development of personalized information) have great potential for better informing consumer choices and food governance activities, but require very careful design and evaluation to avoid the risk of provoking unwarranted alarm, or ill-informed consumer decision-making. An important part of this is investigating what information consumers actually want, and how they may respond to it. At the present time, there is little theoretically underpinned and empirically tested information available on which to develop a pan-European risk–benefit communication strategy associated with foods. Scientific understanding regarding how consumers perceive risks and benefits associated with foods is urgently required, together with further understanding of the cognitive processes underlying risk–benefit communication, and associated decision-making regarding food choices. This knowledge will form the basis of an effective pan-European risk–benefit communication strategy associated with nutritional and technological food issues.

The need to conduct research in this area is particularly timely, given recent and ongoing advances within the natural sciences, for example, regarding the development of integrated risk–benefit assessment measures associated with food consumption, and their consideration by food authorities and innovations in the agrifood sector such as those associated with genetic modification of food production animals and innovations associated with emerging technologies, such as nanotechnology.

See also: Institutions Involved in Food Safety: FAO/WHO Codex Alimentarius Commission (CAC). Public Health Measures: Food Inspections and Enforcement Systems; Health Education, Information, and Risk Communication; Modern Approach to Food Safety Management: An Overview; Risk Governance. Risk Analysis: Food Safety Training and Health Education: Principles and Methods; Risk Communication: Biological Hazards; Risk Communication: Diet, Nutrition, and Health; Risk Communication: Novel Foods and Novel Technologies

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Risk Communication: Chemical Hazards

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Glossary

Knowledge deficit model A concept addressing lay–expert discrepancy in the perception of risk; in this view the discrepancy exists because lay people lack the experts' knowledge and understanding of the issues; the main objective of risk communication is to educate the public in terms of aligning its views to those of the experts.

Latency Concealed or dormant risks. Latency refers to those risks where the harm emerges a considerable time after exposure.

Qualitative risk factors Factors that describe properties of risks (e.g., naturally occurring or human-induced) or risky situations (e.g., degree of ability of self-control) by which people judge risks, beyond the two classic components of risk analysis (i.e., level of probability and extent of possible harm).

Risk communication The interactive exchange of information and opinions throughout the risk analysis process concerning risk, risk-related factors, and risk perceptions, among risk assessors, risk managers, consumers, industry, the academic community, and other interested parties, including the explanation of the risk assessment findings and the basis of risk management decisions (Codex definition).

Risk perception The outcome of the processing, assimilation, and evaluation of personal experiences or information about risk by individuals or groups in society.

Synopsis This contribution highlights insights gained from research in the areas of risk communication and risk perception showing both the relevance and challenge of communication about chemical food hazards. It points out the importance of perceived unnaturalness, involuntariness, delayed effects, and lack of control for judging chemical risks as high compared to natural, voluntary, and controllable risks. Effective risk communication requires addressing these qualitative characteristics. The particular role of public trust of authorities in this context is emphasized. This exposition is preceded by a clarification of the risk communication concept in the field of food safety and followed by an outline of research needs.

Chemical Contamination in the Context of Food Risk Communication

The World Health Organization (WHO) identifies the contamination of food by chemical hazards as a worldwide public health concern and a leading cause of trade problems internationally. Chemical hazards may result from chemicals that were added to food intentionally or from food contaminants that are present in food inadvertently. Residues of pesticides, veterinary medicines, and other agrochemicals, as well as food additives such as coloring and preservatives can lead to adverse health effects if the chemicals are not applied to food in an appropriate manner. One way in which hazardous chemicals can accidentally enter the food chain refers to indirect absorption via environmental pollution of the air, water, and soil, for instance by toxic metals, dioxins, or polychlorinated biphenyles (PCBs). Another possible source of food contamination is naturally occurring toxicants such as mycotoxins. These chemical hazards may arise at various points of the food chain. Contamination may also occur through chemicals used in food contact materials, from which they migrate into the food.

Although exposure to heavy metals such as lead has decreased in developed countries, this problem is still high on the public health agenda of developing countries. Heavy metals and other inorganic substances pose a constant threat to low income population in developing countries with respect to water and food safety. Despite strict regulatory standards and practices chemical food hazards remain a major concern of stakeholders and the public at large also in the industrialized countries. Public health concerns that have evolved here in the recent past include concerns about risks for foods contaminated by dioxins and dioxin-like PCBs (in wild and farmed fish and shellfish, for instance), increased levels of heavy metals in seafood, effects of cumulative exposure (to different pesticide residues in foods, for instance), and impacts on particularly vulnerable parts of the population such as fetuses, pregnant women, children, and the elderly (in relation to exposure to chemicals used in food contact materials, for instance). Although these are public health concerns widely shared by all industrialized countries, controversies arise over the concentrations that are deemed dangerous to human health or the environment (dose–effect relationships) and on the nature of what constitutes a food chemical hazard.

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One illustration of different hazard concepts can be found in the so-called transatlantic beef dispute. On the contrary to the US and Canada, which consider meat produced from cattle treated with certain growth-promoting hormones as safe for consumption, the competent authorities in the European Union (EU) regard it as a risk to human health. A long-running dispute between Europe and the US, and Canada on the import of such meat has been the result of these different views.

Over the past 15 years food safety and public health authorities in the Western industrialized world have increasingly come to recognize that there is a need for purposeful and targeted communication about these and other food safety-related hazards. Although communication of food safety issues is still a developing field of practice, there are a growing number of national and international agencies that offer documentation and advice on the functions and forms of food risk communication and its role within their institutions and for other actors in the food safety field (e.g., WHO, the Codex Alimentarius Commission (CAC), the Organization for Economic Co-operation and Development (OECD), the US National Research Council, the European Food Safety Authority (EFSA), and many of the European national food safety authorities). They can build on a growing body of literature of academic research on the subject of communication on risk in general and on food risks in particular. The repercussions of the series of food-related scares and controversies that afflicted Europe between the late 1980s and the late 1990s (besides mad cow disease, most notably dioxin contamination, beef hormones, and genetically modified organisms) have promoted the expansion of food risk communication research. This expansion occurs in line with the development of the overall risk communication area, which builds greatly on risk perception research.

In this contribution, we will highlight some major insights offered by this overall body of knowledge, which indicate both the relevance and challenge of communication about chemical food risks. We will point out that people tend to worry more about unnatural (i.e., human-induced) and externally imposed risks, that their concerns are often multidimensional, and that the role of trust in risk management is pronounced under circumstances typical of many chemical food hazards. Finally, we will point to some major current research themes deserving further investigation. As the field of food risk communication research is currently dominated by scholars from the Western World concentrating on the situation in Europe, we will use mainly the European situation when we draw on examples.

The Concept of Risk Communication in Food Safety

Food risk communication is widely seen as an integral component of the entire food safety regulation process. The risk analysis framework as outlined by the WHO and the Food and Agriculture Organization (FAO) is comprises three interrelated. But functionally separated components, risk assessment, risk management, and risk communication. Nowadays this framework underlies and informs food safety regulation at the EU-level (and at national level in many European countries) as well as in the US and also at the global level in the form of standard-setting activities of the CAC, a joint WHO and Food and Agricultural Organization standard-setting body. The CAC defines risk communication as the 'interactive exchange of information and opinions throughout the risk analysis process concerning risk, risk-related factors, and risk perceptions, among risk assessors, risk managers, consumers, industry, the academic community, and other interested parties, including the explanation of the risk assessment findings and the basis of risk management decisions.' In this view, food risk communication includes exchanges between those who are central to the risk analysis process (i.e., the risk professionals responsible for framing, assessing, evaluating, and managing risks) and exchanges between these and other actors outside the immediate risk analysis process (mainly experts in food-related research, stakeholders in the food supply chain, and consumers). It has been suggested to refer to the former communicative exchanges as internal risk communication and the latter as external risk communication. By including risk-related factors and risk perceptions as subjects of communication the CAC defines food risk communication as not being strictly about risk. Instead it acknowledges that there is a need to communicate factors other than the technical estimates of a risk, i.e., the probability of an adverse effect and the severity and magnitude of that effect.

Risk perception research has been shown that understanding and communicating risk is influenced by a number of additional factors, such as whether the risk is voluntary or involuntary. Food risk understanding and communication has shown to be also influenced by the particular concerns that groups and individuals might link with a certain food hazard. These are not necessarily restricted to immediate impacts on human health or the environment but might include issues of perceived equity violations, symbolic associations, and attributions with the respective ingredient, or concerns that relate chemical risk with other negative evaluations such as 'big business.'

Attributes of Chemical Hazards that Increase Concern

Many chemical food hazards have attributes or are associated with attributes that risk perception research has identified as factors likely to increase the perception of risk. One of these factors is unnaturalness. From risk perception studies highlighting the importance of both quantitative and qualitative risk factors it is well known that natural hazards are seen as more acceptable than human-induced hazards. Anything that is seen as natural tends to be considered familiar and therefore less fearful and dangerous. Anything that is seen as chemical or artificial tends to be regarded as foreign, unnatural, and thus more threatening and dangerous. Therefore, everything chemistry does to our food tends to be considered as particularly risky. Although this dualist perspective does not withstand scientific examination, it is an expression of the longing of many people in highly industrialized countries for a contemplative refuge from society, technology, industry, and capitalism, and nature has come to represent such a refuge.

Another important qualitative characteristic of most chemical food hazards is involuntariness. People tend to

worry more about risks caused by external factors, i.e., imposed from the outside. This applies particularly in those cases in which the hazard-causing substances are used intentionally in the production of consumer goods, and this is the case for instance with potentially hazardous agrochemicals and food additives.

As shown by the 2010 EU-wide public opinion survey commissioned by the EFSA, European consumers persist in being concerned primarily about chemical contamination in food. Unnaturalness and involuntariness are attributes of the chemical hazards that consumers are most concerned about and likely to be among the top concern-producing factors (besides context-dependent factors such as the level of trust and distrust in the actors of the food supply chain and food safety authorities, social exclusion from risk governance processes, or social amplification effects caused by the media or nongovernmental organizations). This assumption takes into account that in Europe (as opposed to the US, for instance) it is a widely held belief that food should be produced in the most natural way possible and the criterion of naturalness enjoys great popularity.

Research in the areas of risk perception and risk communication links the two risk attributes with a basic difference between expert and public food risk perception. Although food safety experts judge microbiological hazards to be the main risk to human health from food, the public is much more concerned about chemical hazards with residues from pesticides and veterinary drugs ranking especially high. In contrast, acrylamide considered as a natural hazard in relation to food and at least partly under one's personal control has not developed into a major public concern in Europe (acrylamide in food is a natural byproduct of exposing in particular starch to high temperature, which is not related to alien ingredients or additives in food). When public authorities or food producers assess the need and the required design for communication to stakeholder organizations and consumers about chemical food hazards, it is essential that both the major quantitative and qualitative risk attributes and the particular role that these risk attributes might play in a specific social and cultural context are taken into account and explicitly addressed.

Multidimensionality of People's Risk Concerns

Research looking at the psychology of risk perception and the social factors governing eating behavior has further shown that in many cases people link various concerns with food-related hazards. Often the fear of immediate or long-term adverse health effects is not the only or the decisive component in the position that people take towards potentially hazardous aspects of food production. For instance, concerns about the environment and animal welfare have been found to be important in people's judgment about the use of pesticides and not only worries about health risks. Concerns about animal welfare form also part of the skepticism or opposition of people towards what appears to them as an unreserved use of veterinary medicines in animal husbandry. Risk perception studies have further found that people do not always clearly articulate worries about largely invisible hazards such as food additives and chemical residues. Instead, such worries were sometimes expressed more indirectly through indication of a general uneasiness about food quality and general reservations about contemporary social trends such as a progressive industrialization of food production. This unease with industrialization of food processing is often looked at as a threat to the traditional or nutritional value of foods.

The multidimensionality of people's risk perception has immediate implications for risk communication. In many instances, a narrow focus on human health will not suffice. The possibility for effective risk communication significantly increases when risk messages address the specific concerns that individuals, social groups, and different cultures might link with specific food hazards. If the use of growth-promoting hormones in meat production in Europe is opposed partly (or by some consumers even mainly) on the grounds of skepticism towards an ever increasing industrialization of animal husbandry, information campaigns simply explaining or discussing the safety of the meat for human consumption are highly unlikely to be effective; campaigners and addressees would talk at cross-purposes. The effectiveness of risk communication to a large part rests on the knowledge of what matters to the targeted audience and also on the willingness of the risk managers to incorporate the revealed concerns of affected and interested public groups into the design of risk management policies.

The Pronounced Role of Trust in Chemical Risk Management

A prominent topic in risk communication research is trust in the ability of food producers or public food safety authorities to maintain adequate levels of food safety. Trust has been found to be particularly important under circumstances where risks are considered as an early indicator of insidious danger and people feel that they have very little personal control over these risks. Many chemical food hazards are perceived in these terms, most notably food additives and pesticide residues. The perception of these hazards is closely linked with the need to find clear causes for seemingly inexplicable effects such as allergies or cancer in children. Knowledge of the possibility of cancers caused by certain food additives or pesticide residues does at least legitimize the suspicion that any cancer can be explained by the consumption of certain foods. For those suffering from cancer (assumed) knowledge about a concrete reason (such as consumption of foods contaminated with carcinogenic agrochemicals) can help to accept the disease because its occurrence provides a meaning to the affected person. This coping strategy is often linked with the tendency of people to focus on the hazard, the potential for harm, and ignore or downplay exposure and dose–response relationships. Empirical research on intuitive toxicology has shown that people tend to underestimate uncertainties and ignore exposure or dose when they are informed about a potential causal relationship between an agent and an effect.

For dealing with risks belonging to the category of early indicators of insidious danger, affected people depend on information provided by third parties. As a rule these risks cannot be perceived with the human senses. Moreover, these

risks are highly complex, i.e., there are usually many years of latency between emission and effect. Research in the fields of risk perception and risk communication has argued that people will be willing to make a balanced judgment between risks and benefits only if they trust the information providing party (regardless if whether this party advocates the food or warns about it). If there is lack of trust, risk–benefit tradeoffs will not be accepted but zero exposure will be demanded. A basic prerequisite for effective communication about chemical hazards perceived as early indicators of insidious dangers is therefore public trust in the performance of those institutions responsible for dealing with these hazards. Trust cannot be produced or manufactured but only earned in terms of performance and targeted communication. To (re-)gain trust implies to be sensitive to public concerns and to organize communication as a two-way process, i.e., from and to the target audience.

In summary it can be stated that risk communication by those responsible for assuring food safety is particularly difficult, if chemical food risks are invisible to the consumer and may cause negative health effects after a long incubation time. These risks are particularly frightening for the consumer: they are associated with involuntariness, delayed effects, inability to be sensed by human organs, lack of control, and unfamiliarity. To address these negative risk characteristics, it may be helpful to point to functional equivalents of these characteristics in a broader societal context. Potential equivalents are the assurance of a democratic decision-making process to counteract the impression of involuntariness and, as a replacement for personal control, the independence and impartiality of operating and regulating agencies. This may produce trust in their capability to monitor food items on the shelves, check composition and durability of goods, and intervene if safety in the risk-producing facility is not managed properly. In addition, familiarity can partially be compensated for by better functional knowledge about the risk and the associated procedures.

Need for Further Research

Recent risk communication research has indicated and largely supported a general trend towards understanding and performing risk communication as a two-way communication process. In this process it is not only the members of the public who are expected to engage in a social learning process, but also the risk assessors and risk managers. Although risk communication implies a stronger role for risk professionals to provide information to the public rather than vice versa, it is increasingly regarded as a mutual learning process. Concerns, perceptions, and experiential knowledge of the targeted audience(s) should guide risk professionals in their selection of topics and subjects. In contrast to the so-called knowledge deficit model, it is not the task of the communicators to decide about what people need to know but to respond to the questions of what people want to know. The question of what are basic requirements and major challenges of moving beyond the education- and persuasion-based approach to risk communication and towards a more discursive approach based on mutual learning deserves further investigation.

This is not a trivial task. Many scholars of risk communication emphasize that there is not a single homogenous public with one lay view of risk that needs to be taken into consideration. Rather there are a number of differentiated publics with varying values and concerns and also different levels of technical expertise and involvement with a given food issue. When communicating about the effects of cumulative exposure to different pesticide residues in foods, for instance, it needs to be recognized that communication demands of stakeholders along the food chain (such as farmers, producers, caterers, and retailers), environmental and consumer organizations, public authorities involved in risk regulation and public health, and the average consumers are very different. More research is required on what are the pros and cons of the various available methods and tools to achieve understanding of this variability (such as focus groups or mental models approaches) and on appropriate ways to incorporate these insights into the design of risk communication (in terms of content and tools, which can be oriented towards information, dialog and/or participation).

One issue that researchers have been dealing with since the field of risk communication was born is how one communicates uncertainty. This is a major research topic in the food risk area too and a communication challenge for food safety authorities with many chemical food hazards. Uncertainties about the possibility of adverse human health effects at low doses of Bisphenol A, for instance, have led to controversy in the scientific community about the safety of the use of the industrial chemical in food contact materials. This has resulted in different risk management decisions taken by national authorities and received much attention in the media and in the general public. Under these circumstances, food safety authorities are faced with the question of whether they should communicate about the uncertainty issue also to the wider public. Empirical research of the recent past has indicated that communicating uncertainty does not in all cases increase public trust of authorities and help consumers make informed choices but may also lead to public distrust and confusion. The nature of the relationship between uncertainty communication and trust-building is in urgent need of further investigation.

A subject of research that has emerged only recently is the composition of communication messages under circumstances where there is both risk and benefit associated with consuming a particular food. A prominent example is consumption of fish. From a nutritional perspective, increased fish consumption is likely to be beneficial for human health by helping to prevent the occurrence of cardiovascular diseases. From a toxicological perspective, however, it might be a risk to health as fish is associated with environmental contaminants such as methyl mercury, PCBs and dioxins. The challenge for risk communicators addressing the wider public here is to select messages that do not induce consumers to fully eliminate fish from their diet (and, what would make it even worse, switch to increased consumption of red meat), instead of including moderate fish consumption (defined in relation to their potential specific vulnerability) in a well balanced diet. Future research in the area of risk communication on chemical hazards should include further investigation of the potential and conditions for effective risk–benefit communication.

See also: Foodborne Diseases: Overview of Emerging Food Technologies. Risk Analysis: Risk Analysis of Hazards in Food: An Overview; Risk Management: Application to Chemical Hazards

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Organisation For Economic Co-Operation And Development: OECD Guidance Document on Risk Communication for Chemical Risk Management.

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WHO: Chemical Risks in Food.

Risk Communication: Biological Hazards

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Glossary

Foodborne disease Any disease of an infectious or toxic nature caused by or thought to be caused by consumption of contaminated food, including drink.
Foodborne pathogens Disease-causing microorganisms that are transmitted to humans via foods.
Food safety Assurance that food will not cause harm to the consumer, when it is prepared and/or eaten according to its intended use.
Hazard A biological, chemical or physical agent in, or condition of, food with the potential to cause an adverse health effect.
Pathogen An organism capable of causing disease.
Risk A function of the probability of an adverse health effect and the severity of that effect, consequential to a hazard(s) in food.
Risk analysis A process of decision-making (usually at government level) for managing food safety, consisting of three components: risk assessment, risk management and risk communication.
Risk assessment A scientifically based process for evaluating risks associated with foodborne hazards, consisting of the following steps: (i) hazard identification, (ii) hazard characterization, (iii) exposure assessment, and (iv) risk characterization.
Risk assessment policy Documented guidelines on the choice of options and associated judgments for their application at appropriate decision points in the risk assessment such that the scientific integrity of the process is maintained.
Risk communication The interactive exchange of information and opinions throughout the risk analysis process concerning risk, risk-related factors and risk perceptions, among risk assessors, risk managers, consumers, industry, the academic community and other interested parties, including the explanation of risk assessment findings and the basis of risk management decisions.
Risk manager A person or an organization (usually government-related) with the authority to decide on the acceptability of risk and, if necessary, measures needed for their management.
Risk profile The description of the food safety problem and its context.
Uncertainty Uncertainty refers to lack of knowledge of a variable, or effect, or lack of certainty that a measured value is correct. Uncertainties include those that might arise in the extrapolation of information obtained from epidemiological, microbiological and laboratory animals, or complete lack of data for some variable that affects consumer risk.
Validation Obtaining evidence that a control measure or combination of control measures, if properly implemented, is capable of controlling the hazard to a specified outcome.
Variability Variability refers to differences in values of the same variable (e.g. the initial level of microbial contamination, the amount of food eaten in a single meal) that are not readily related to some known cause that could also be measured. It also includes biological variation such the difference in virulence that exists in microbiological populations and the variability in susceptibility within the human population and particular subpopulation.

Introduction

Risk communication is defined as 'the exchange of information and opinions concerning risk and risk related factors among risk assessors, risk managers, consumers, and other interested parties.' This definition emphasizes that risk communication is a two-way process and can occur among different sectors of society.

The goals of risk communication are:

1. exchanging information on the knowledge, attitude, values, practices, and perceptions of interested parties concerning risks associated with food and practices,
2. raising awareness and understanding of the specific health issues under consideration during the risk analysis process,
3. providing a sound basis for risk management decisions,
4. communicating the reason for the risk management decisions proposed,
5. contributing to the development and delivery of effective information and education programs, when they are considered as a risk management option (including when industry views that consumers have to control the risks), and
6. fostering public trust and confidence in the food supply.

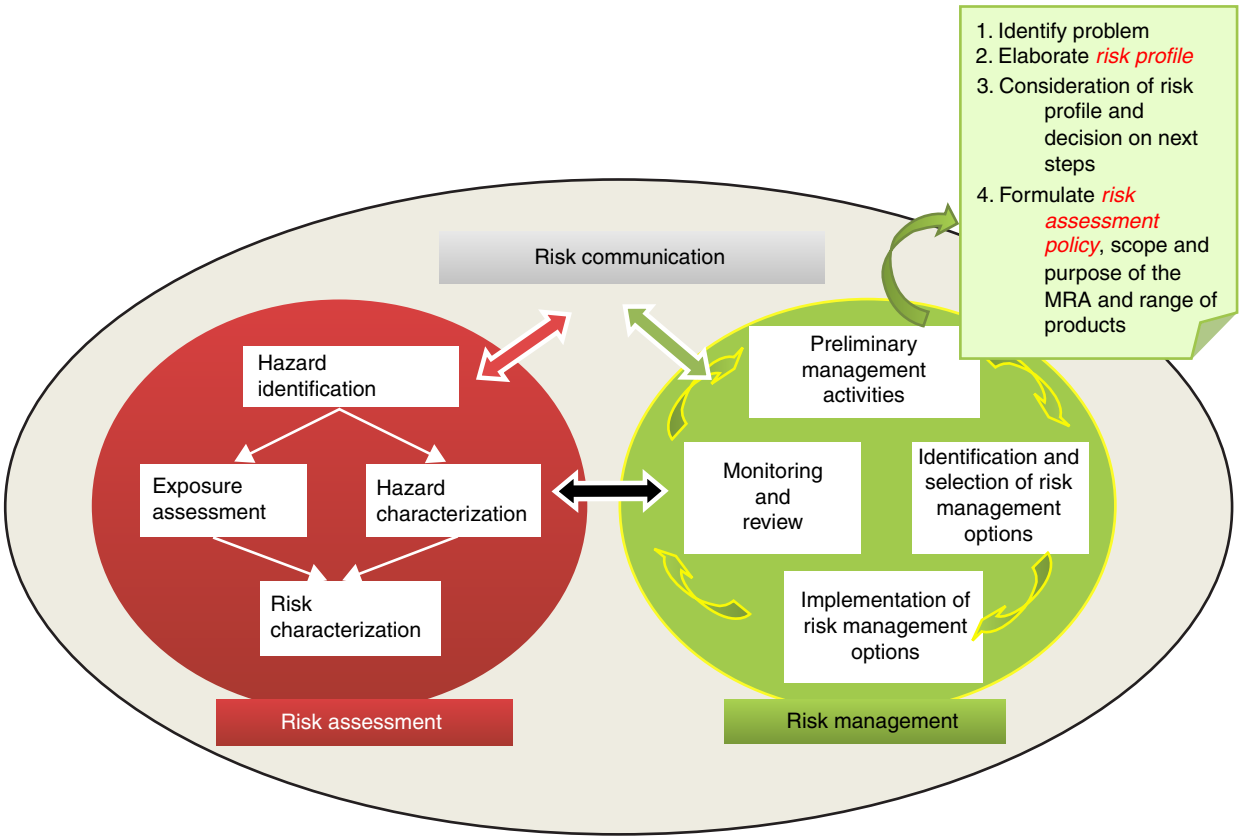

Figure 1 Risk communication in the context of risk analysis process.

In this article, risk communication is examined from two perspectives:

1. Risk communication as part of the risk analysis process.
2. Risk communication as part of health education in food safety (which itself is a risk management measure for prevention of foodborne diseases).

The focus of this article is on risk communication as applied to biological hazards. The principles of risk communication to chemical hazards or food technologies are addressed in other articles of the encyclopedia. The principles of risk communication are similar for all potential hazards in food.

Risk Communication as an Integral Part of Risk Analysis

Since the mid-1980s, the increase in incidence of foodborne diseases and a greater awareness of their health impact, together with a series of food safety incidents and crises causing public outrage, led public health authorities to revisit the process of decision making and the management of food safety at international, national, and local level. The outcome was the advance of the risk analysis process, consisting of risk assessment, risk management, and risk communication, as a model for risk-based food safety management (Figure 1).

Because, there has been an increasing attention to the science of risk communication and the associated concept of risk perception. Risk communication is a dynamic concept, which applies at different levels, for instance between:

1. Scientists involved in risk assessment and risk managers (e.g., regulatory authorities) and *vice versa*.
2. Internationally, between trading partners, i.e., importing and exporting countries.
3. Risk managers and stakeholders involved in the 'farm-to-fork' food chain; such food producers, processors, distributors, retailers, consumers, and others involved in assuring food safety and protecting public health, for example, health professionals.

In the context of risk analysis, be it at national or at international level, risk communication involves processes such as:

1. Understanding the concerns of the stakeholders.
2. Commissioning a risk assessment, and articulating the question that the risk assessment should address and including a risk assessment policy by a risk manager.
3. Communicating the outcome of risk assessment to risk managers at national or international level.
4. Communicating (e.g., explaining or justifying) risk management decisions to stakeholders.

Risk Communication Between Risk Managers and Risks Assessors

Risk managers have a leading role in driving the risk analysis process. As part of the risk communication process, they may:

1. require that the risk assessors advise on issues such as the 'impact' of foodborne disease, their relative importance (e.g., the public health burden they impose), and develop a risk profile for a preliminary appreciation of risks, or
2. commission a full-fledged risk assessment exercise to advise on risk management options. These may, for instance, involve:
    1. the efficacy of various control measures,
    2. points in the food chain where controls can be most effective (e.g., comparing efficacy of vaccination of poultry at the farm, cold storage of eggs in retail stores, or cooking of the eggs at the consumer level for preventing eggborne salmonellosis),
    3. evaluate the acceptability of risk as compared to public health goals, for example, risk of illness by consumption of specific product/pathogen combinations,
    4. risks associated with different processing, distribution, and consumer use scenarios, etc.

In commissioning a risk assessment, the interaction between risks managers and risk assessors starts with commissioning a risk profile, and in the eventuality of a full risk assessment, with preparing and communicating a document referred to as risk assessment policy. This is a document, initiated by the risk manager, specifies the context and objectives of the risk assessors' work. The preparation of the risk assessment policy should take place jointly with risk assessors, and is, of itself, a risk communication exercise. In the risk assessment policy, the risk managers will communicate to risk assessors:

1. the purpose and the scope of the risk assessment, i.e., what should be included and what should be disregarded, such as the segment of the food chain to examine, target population (general population or, e.g., a focus on pregnant women, the elderly, or a minority ethnic group), the data that could be relevant as evidence (outbreak data, sporadic cases, consumption patterns, industry monitoring data, etc.);
2. the procedure to be followed, the required documentation and the form of the risk estimate or the output to be presented (e.g., an estimate of the prevalence of illness, an estimate of the annual rate, or an estimate of the rate of human illness and severity per eating occurrence);
3. interpretative aspects, for instance, guidance on acceptable assumptions, how variability and uncertainties need to be addressed, the acceptable level of protection as a basis for acceptance or rejection of a risk, uncertainty in the risk estimate, transparency in the constraints that impact on risk assessment such as costs, resource, or time.

Risk communication between risk assessors and risk managers is a dynamic process during which iterative exchange of questions and information occurs, and the risk management questions are refined until the risk assessment exercise is focused to a point that risk assessment addresses the issue for which the risk managers need additional advice in an optimal manner.

The communication between risk assessors and risk managers includes the documentation of the risk assessment to ensure transparency and consistency as a reference for the future. Such documents can be important in case a risk reassessment is needed by other experts or when new information becomes available, or if the risk assessment is to be applied to different scenarios or to changed situations.

However, as stated above, a risk analysis process does not always start with a microbiological risk assessment. A starting point is the preparation of a risk profile, and this may in some cases suffice for decision making. At times, the risk profile may indicate the lack of data, in that case the risk managers may need to interact with scientists for the collection of additional data to enable a risk assessment to proceed.

Risk Communication Between Risk Managers and Stakeholders

As for communicating with risk assessors, risk managers should hold a two-way communication with stakeholders to consider the feasibility, the financial or cultural constraints, or preferences of stakeholders in the risk management options. Understanding these, as well as understanding the stakeholders' perceptions of risks, is important for effective communication of risks and risk management options. Even if the risk management option is a decision to accept the status quo and not implement further measures or reinforce the existing measures, it would be important to explain to the stakeholders the reason behind these decisions.

Risk management measures may be targeted to industry, for example, setting microbiological criteria, requiring specific processing such as pasteurization of the product, labeling and instructions for consumers, an inspection or certification process, development of a code of practice, training of food-handlers, or risk communication and health education of consumers and perhaps explaining to consumers the benefits of accepting responsibility for some management of the risk. In the latter case, the communication with consumers can range from simple information to specific educational programs (e.g., school health education, education of pregnant or breast-feeding mothers) or health education campaigns. **Box 1** shows the type of information that could be included in a risk communication message, as needed.

Risk Communication Between Risk Assessors and Stakeholders

As scientists, risk assessors also have a responsibility to alert and advice risk managers, and/or the stakeholders, on emerging pathogens, or conditions leading to potential increase in risk of illness, for example, contamination of environment where food is produced, change in practice or food habits, or to an increase in trend of foodborne illnesses. Such communication to risk managers will, as explained above, initiate the risk analysis process. However, scientists need to be particularly careful in risk communication to the general public

Box 1 Content of risk communication. Depending on what is to be communicated, and to whom, risk communication messages may contain the information described in this box

The nature of the risks

1. The characteristics and importance of the hazard of concern;
2. The magnitude and severity of the risk;
3. The urgency of the situation;
4. The trend;
5. The probability of exposure;
6. The distribution of exposure;
7. The amount of exposure that constitutes a significant risk;
8. The nature and size of the population at risk;
9. Who is at greatest risk.

The nature of the benefits

1. The actual or expected benefits (magnitude, importance) associated with each risk.
2. Who benefits and in what ways.

How the risk was assessed

1. The method used for risk assessment;
2. The assumptions on which estimates are based;
3. The effect of changes in estimates on risk management decisions;
4. Uncertainties in the risk assessment;
5. The weaknesses of, or inaccuracies in, the available data;
6. The sensitivity of estimates to change in assumptions.

Risk management options

1. The actions taken to control or manage the risk;
2. The actions individual's may take to reduce personal risk;
3. The justification for choosing a specific risk management option;
4. The effectiveness of a specific option;
5. The benefits of a specific option;
6. The cost of managing the risk, and who pays for it;
7. The risks that remain after a risk management option is implemented.

Source: Adapted from Food and Agriculture Organization of the United Nations (1999) The application of risk communication to food standards and food safety matters. *The Report of a Joint FAO/WHO Expert Consultation*, Rome. 2–6 February 1998. Rome: Food and Agriculture Organization of the United Nations.

in order not to generate panic among the population or to wear out their trust because, in times of crisis, the attention of the general public will be essential for managing risks. Therefore, as far as possible, it is important to coordinate any communication to the general public with the risk managers, for example, the public health authorities.

Risk Communications Between Stakeholders

Risk communication can also take place between the stakeholders of the food chain and this is reflected in the definition of food safety, which is 'Assurance that food will not cause harm to the consumer when it is prepared and/or eaten according to its intended use.' Therefore, in trading foods, businesses are liable to inform their customers and/or consumers about the conditions for the use or handling of their products, and declare or warn of any counterindication. With regard to allergic reactions to food ingredients or food additives used, this is usually done through labeling as required by the legislation of the country where the product is marketed.

With regard to biological risks, food businesses will have to provide clear instructions for conditions of transport, storage, cooking, etc. While doing so, it is important that the information be provided in a format that is visible and clearly understood by the customer/consumers. Also, businesses should not make any assumption about the knowledge of the consumers and should try to understand the possible perception of consumers and/or their habits as well as the feasibility of the recommended measures. In other words, businesses should communicate in a way that not only are they understood, but also are not misunderstood. Therefore, for measures expected to be taken by customers that are critical for food safety, the coverage, and clarity of the communication should be validated by a representative group of consumers (focus group). There have been several outbreaks where misunderstanding of the conditions of transport, or use of products, have led to outbreaks of foodborne diseases. For example, in 2006, four cases of botulism linked to refrigerated carrot juice occurred in the US and two cases occurred in Canada. The implicated product was pasteurized carrot juice contaminated with spores of *Clostridium botulinum*. The carrot juice was distributed under refrigeration and was labeled with statements 'Keep Chilled,' 'Keep Refrigerated.' Nevertheless, it is believed that the juice involved in these outbreaks may have been left unrefrigerated for an extended period, either during distribution or while being held by consumers, allowing *C. botulinum* spores to grow and produce toxin.

In another outbreak in 2009, cookie dough contaminated with *Escherichia coli* O157 was eaten raw. The label indicated that the product contained raw ingredients. However, consumers did not realize that this was a safety instruction, as the statement was presented as a general information, and not as an instruction or a warning. Seventy people from 29 States in the US fell ill. These cases illustrate the importance of communication with the stakeholders and of validation of the information for clarity. This is particularly important when consumer actions are considered as a critical control point for controlling the hazard in question.

Risk Communication Between Trading Partners Internationally

Communication on risks between importing and exporting countries also occur occasionally. A case in point was the epidemic of cholera in 1993 in Peru. The epidemic led to food export restrictions causing major economic losses for Peru. In this context, World Health Organization (WHO) issued a policy on cholera and international trade in food that eased the situation. Similarly, in the crisis of bovine spongiform encephalopathy (BSE), the communication of WHO in relation to the safety of bovine products paved the way for managing the risk at national and international levels, and reassuring consumers on the safety of products on the market, in particular milk.

Other trade disputes between the European Union and the US involving risk communication have concerned cheese made with raw milk and meat produced with growth hormones.

Understanding Risk Perception as Basis for Risk Communication

Whether communication targets stakeholders of the chain with the purpose of explaining a risk management decision, or for providing guidance on what they will need to do, as mentioned above, understanding the intended audience's perception of the risk is essential. Experience has shown that acceptance of risks varies according to the factors influencing the perception of risk. This depends on a number of factors (these are also discussed in other articles).

Voluntary Versus Imposed

Voluntary risks, i.e., those that are experienced knowingly and by choice, are tolerated more readily than those that are imposed. For instance, in many European countries, there is the cultural habit of eating 'steak tartare' (raw minced beef), although this practice can expose consumers to a wide range of pathogens such as *Salmonella* spp. or *E. coli* O157. Some consumers also prefer raw milk, although it has been repeatedly demonstrated that raw milk can be the source of many illnesses. In Asian countries, consumption of raw freshwater fish is common, even though these are frequently the source of trematode infections. The risks associated with such foods are accepted because they are voluntary, while the same persons may be outraged with industrially produced foods that will expose them to the same level of risk. With regard to their own practices and food choices, consumers are often overoptimistic that nothing will happen, or even in the eventuality of an event, that the illness will be benign and self-limiting, an inconvenience they often qualify as 'simple diarrhea.'

Risk That are Under Individual's Control

Similarly, where risks are under individual's control, they are accepted more readily than when consumers need to rely on governments and/or industry for their protection. A case in point is the risk associated with poultry contaminated with *Salmonella* or *Campylobacter* versus risks of avian influenza (AI) and BSE. In the former, consumers can manage the risk themselves by cooking, whereas in the latter case they perceive the risk as greater or unacceptable due to their lack of control. In the AI and BSE epidemics, sales of products were significantly affected.

Risks That Seem Fair

Risks that seem fair, i.e., where the benefits of accepting the risk are fairly distributed between producers and consumers.' In such conditions, risks are better accepted than when the private sector would be the main or sole beneficiary. For instance, one of the reasons that food irradiation is not accepted by consumers, in spite of the record of proven safety, is because of the perception that the technology is introduced to cover up the poor hygienic practice of producers.

Natural Risks

Natural risks seem more acceptable to consumers than man-made risks. Although scientific evidence shows that where the food control system is well developed and implemented, the risks from food additives and residues of agrochemicals are comparatively lower than foodborne pathogens consumers perceive the contrary. Exposure to microbes is generally seen as a natural risk and as part of life.

Exotic risks Seem Greater Than Familiar Risks

Similar to the section Natural Risks, as people frequently recover from foodborne diarrhea, they are more tolerant of risks associated with the causative agent. In some regions of the world, where infectious diarrhea is a common health problem, it is even perceived as normal part of life. Consequently, such consumers may be more relaxed with their own malpractices, whereas, at the same time, they will be more concerned about an unknown or less familiar illness, even if it is of lower risk, such as AI.

Dread Factor

The second BSE crisis in the year 2000 demonstrated the importance of the outrage factor in the acceptance of risk. Even though with the measures taken by food control authorities, the risk of variant Creutzfeldt–Jakob disease (vCJD) was not particularly high, the fact that new cases of BSE were due to failures in control measures and that the consequences of an eventual vCJD infection were particularly dreadful (i.e., incurable, lingering, and terminal illness), the situation led to outrage and panic among consumers who lost trust in the safety of meat and meat products.

The Authority and Credibility of the Source of Information

Experience and surveys have shown that the trust of the general public in the information they receive depends on the credibility of the source. Generally, public health institutions and professionals, for example, physicians, are the most trusted source of information and consumers may more readily accept a risk when the communication is made by this professional group (because they are perceived to have no vested interest).

Risk Communication in the Context of Health Education

Health education is one of the essential public health functions and, in preventing foodborne illnesses, it is one of the most important risk management measures. In educating the general public and motivating them to adopt healthy practices, more economic constraints, public health authorities must also consider the perception of risk and the social and cultural factors that can influence consumer behavior and their acceptance of risk. Thus, in this context of health education, risk communication and understanding risk perception are also fundamentally important.

Similar to other risk management decisions, health education should be based on two types of considerations:

1. Scientific and technical information on factors leading to foodborne illness, and

Box 2 Causes of diarrhea as perceived in different cultures

1. Foods that are fatty, not cooled adequately or heavy.
2. Imbalance of heat and cold associated with food.
3. Exposure to draughts or seasonal changes.
4. Poor quality of breast milk.
5. Physical factors such as a fall or poor care of a child.
6. Supernatural causes, including possession, sorcery, or the evil eye.
7. Pollution or exposure to inauspicious contact with ritually impure persons or things.
8. Moral misbehavior, including the deeds of the sick persons or a sick child's parents.
9. Natural consequences of development milestones, especially teething, crawling, and walking.
10. Infection which may be associated with hygiene and sanitation (but which may be thought due to pollution).

Source: Adapted from Weiss, MG (1988) Cultural methods of diarrheal illness: Conceptual framework and review. *Social Science and Medicine* 27: 5–16.

2. Sociocultural and economic factors, including risk perception, which may impede the change of behavior or adoption of good practices.

For instance, an educational message on safe foodhandling will have little impact if people do not even believe that foodborne pathogens are the source of diarrhea (Box 2), or that diarrhea can have serious sequaele. Therefore in health education activities, it is important to understand the perception of risks of the population which is often a reason for their practices.

Therefore, in any health education activity, before advising any good practice, it is important to explain the scientific and technical reasons for the recommended measure and their importance. As explained in the article on health education in this encyclopedia, the recommended measures should also be adapted to the cultural and economic conditions of the society, and where necessary, policies and services be provided to ensure the feasibility of the measures. As an example, when promoting breast-feeding, it would be important to explain to parents the risk associated with infant formula, for example, *Cronobacter sakazakii* (formerly known as *Enterobacter sakazakii*), and that these products are not sterile and surviving pathogens may grow if the product is left at room temperature. Newborn babies are particularly sensitive as their immune system is not fully developed. Products may also be recontaminated during preparation by water or the environment, and feeding infants with infant formula will deprive them of the physiological and immune protective functions of breast milk. Naturally, breast-feeding would be feasible only if maternity leave or other type of facilities are provided to mothers.

Unfortunately, misperceptions are also common in the medical and public health community and many public health workers ignore the role of food in the transmission of infectious diarrhea and its chronic sequaele, or judge the extent of the problem by statistics on reported outbreaks, while most cases of illness occur sporadically and are not reported. Therefore, in any strategy for effective communication of risks, education of health professionals on the job and/or education of medical and public health students should be the starting point.

Conclusion

Risk communication is an integral part of food safety management. Understanding the science of risk communication, in particular, factors influencing risk perception, is important for effective management of food safety.

Although the regulatory authorities have the key role in communicating risks and control measures to industry and consumers, public health professionals remain the most trusted source of information, thus the most competent authority to advise the general public on preventive measures. Education of the public health professionals in risk communication and health education is the first stepping stone in the prevention of foodborne illnesses.

See also: Public Health Measures: Health Education, Information, and Risk Communication; Modern Approach to Food Safety Management: An Overview. Risk Analysis: Risk Analysis of Hazards in Food: An Overview; Risk Assessment: Microbiological Hazards; Risk Communication: Chemical Hazards; Risk Communication: Novel Foods and Novel Technologies; Risk Communication

Further Reading

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World Health Organization - Food Safety.

Risk Communication: Novel Foods and Novel Technologies

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Glossary

Risk It is usually defined as the magnitude of a potential harm multiplied by the probability of that harm actually occurring.

Risk management The activities and strategies undertaken to maintain risks within the limits of what is judged to be acceptable for that risk. It is built upon a reliable scientific assessment of the risk and an appropriate standard of risk acceptability (safety).

Safe A level of risk that is deemed to be acceptable by some standard. The question of safety always involves the question of to whom the risk is acceptable, and by what criteria that party judges it so.

Uncertainty The situation created when firm scientific conclusions cannot be drawn because of insufficient or conflicting data, disagreements among scientists on the interpretation of data and other potential sources of misjudgment.

Distinctive Challenges Presented by Novel Technologies and Food

Risk communication around new technologies presents unique challenges because of the special concerns they raise in the public mind. This is especially true in the case of food. The concerns that are raised by food risks stem from the very intimate relationship all people have with food. It is one of only a few truly intimate interactions with our environment, in which we take into our bodies parts of that environment, they become part of us. Sexual activity is the other most typical way in which we do this. So, eating, like sex, raises distinctive concerns about the security of one's person. We are no more protective of our health and welfare than when we ingest food into our bodies.

Food and water are the source of the basis of life. They are the fundamental symbols of nourishment and nurture. Those foods that are the 'staples' of diet in a culture – bread, milk, rice, beans, and maize – usually carry added symbolic significance and, also added concern. When that which is the essential source of life turns into a perceived threat to life or health, it is not surprising that this should trigger much greater aversion to risks than might be tolerated from other sources.

The introduction of a new or unfamiliar technology also triggers concerns and sensitivities that need to be given special consideration in risk communication. Risk perception studies uniformly find that people are more accepting of risks that are familiar and present long term in their environment than of risks perceived as new and unfamiliar. There should be nothing surprising in this fact; that which is a regular part of our lives we accept as the normal conditions of life, and even if they are relatively risky, they are acceptable.

We also learn, or think we have learned, how to control our lives in the face of the familiar risks. The sense of control of the risks one faces is another highly important factor at work in the acceptability of risk for most people. This explains the widely observed fact that people are accepting very high levels of risk if they feel they exercise some measure of control over them. It is one reason why people generally accept the risks of driving automobiles, skiing, and extreme sports (relatively high risk), whereas at the same time may be unwilling to fly in commercial airplanes or eat foods grown with pesticides (relatively lower risk).

Studies also show that people generally are much more accepting of the risks they consider inherent in nature itself than they are of risks created by human activity. They trust 'God' or 'Mother Nature' more than their fellow humans. Thus, the risks posed by foods, food ingredients, or health products thought of as 'natural' do not raise nearly the level of concern as those considered artificial, technologized, or otherwise 'unnatural.' Even though the concept of 'natural' is ambiguous and contested, it plays a significant role in the perception of acceptable risk. There is a close link between the 'familiar' and 'natural' – those things with which we are entirely familiar are more likely to be seen as natural.

New foods and technologies often present new and previously unknown or unexpected risks. For example, a new food or food production process can be the vehicle for new diseases to appear (e.g., bovine spongiform encephalopathy (BSE) and variant Creutzfeldt–Jakob disease (vCJD)) or for old diseases to suddenly emerge as threats to human health on much larger scales (e.g., *Listeria* in processed meats, *Escherichia coli* in vegetables, etc.). When the media reports crises in food safety, public trust in the management of the food system is seriously eroded and notoriously difficult to reestablish. Major food crises and technological failures produce long memories. Competent risk communication practices cannot prevent these reactions to risk

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management failures, but it is an essential tool in the restoration of public confidence in food and technology after these failures occur.

The Importance of Understanding Risk Perception

Risk perceptions like these are often judged to be irrational by risk experts, because they appear to defy the scientific assessment of the actual risks. It is often clear from the scientific point of view that relatively higher risks (e.g., the microorganism risk in organic vegetables) are of less concern to many people than relatively lower risks (e.g., risk of pesticide residue in nonorganic vegetables). This is irrational if the only reasonable consideration in the acceptability of a risk is its magnitude (the probability and severity of the potential harm).

But, as nearly every account of risk acceptability acknowledges, the concept of 'safety' is best understood as 'the level of risk that is acceptable.' This being so, the question becomes one of determining the criteria people use for deciding that different types and levels of risk are acceptable or unacceptable. Different people, and groups of people, will understandably make different judgments about what is acceptable to them, depending upon how risk averse or risk-accepting they are in general, and where they stand with respect to the risks and benefits. There is no one objective standard of safety that can be determined by scientific or other means, for the simple reason that safety is a subjective judgment, relative to the values and interests of those parties who face the risk (or stand to benefit from it).

The factors that have been shown to be most salient for people in their evaluations of risk acceptability are the following:

- The magnitude of the risk and benefits.
- Distribution of the risks and benefits (who gets the benefits and who bears the risk?).
- Familiarity/unfamiliarity of risk (including levels of uncertainties in the science).
- Ability to control/manage the risk.
- 'Dread' factors associated with risks (e.g., dreaded diseases).
- Risk threatens children or other vulnerable populations.
- Catastrophe scenario attached to risk (loss of control and of potential remediation/recovery).
- Voluntary/nonvoluntary (imposed) risk.
- Trustworthiness of the risk manager.

Only the very first factor on this list involves empirical criteria measurable by scientific means. All of the others are primarily value judgments of various kinds, including personal preferences, ethical standards (e.g., 'I have a right to choose the risks I take,' 'You do not have the right to harm me without my consent,' etc.), and deep-seated psychological tendencies within human beings. As value judgments, they illustrate clearly the fact that the question of what constitutes a 'safe' level of risk in food or technology is very complex. It is not just a matter of knowing the magnitude of the risk, because even a very low risk can be unacceptable if it is imposed on persons without any consent and benefit to them, if it is uncertain, and if there is little ability to control it. However, a very significant risk might nevertheless be acceptable if voluntary, controllable, familiar, and highly beneficial to the one taking the risk.

The question of safety cannot be answered except by looking carefully at the actual judgments people (especially those who are the potential bearers of the risk) make about what is acceptable to them. There are no widely agreed criteria, ethical, philosophical, or psychological, for deciding which of these value judgments are 'rational,' 'irrational,' or which should form the basis for a risk management decision.

Basics of Risk Communication

Science is the essential basis for the assessment and management of risk. The most important aspect of risk management of novel foods and technologies is the scientifically reliable assessment of the risks they pose. Judgments about the safety of these foods and technologies that are based on significant underestimates or overestimates of the actual risk are flawed, and where such misunderstandings occur it is appropriate for risk communicators to try to correct them.

Often, however, risk communicators assume that educating the public about the correct scientific assessment of the risk is the primary, or even the sole, objective of risk communication. When this assumption is conjoined with the further assumption that the public's view of the acceptability of a risk can be changed if people can be properly educated about the science, it produces a view of risk communication that is rarely successful in practice, and it often has precisely the opposite effect by undermining public trust in the risk communicators and managers they represent.

This view of risk communication is that its primary objective is to persuade the public to accept the safety views of the risk assessment and management experts, and that the most effective means to achieving this goal is to address the lack of scientific understanding, or 'knowledge deficit,' or the public. Recent research has demonstrated that the 'knowledge deficit' view of risk communication is fraught with serious problems. Attempting to fill in the gaps in the public's scientific understanding of food and technology risks has less impact on its attitudes toward the safety of these things than is commonly supposed. The reason for this is not difficult to see, if one understands the factors, outlined above, that are most important in shaping people's view of acceptable risk, most of which are not matters of scientific understanding of risk magnitudes. Many studies have shown that risk experts generally view safety issues quite differently from lay people. Thus, when they try to persuade the public of their own (nonscientific) views, they move well beyond their own scientific expertise.

The fundamental aim of risk communication is to provide the public and consumers with the most accurate and reliable information they need to judge the acceptability of a risk according to their own standards. To do this the risk communicator needs to respond accurately and sensitively to the safety concerns of stakeholders and the public, helping them to put these concerns together with the most accurate

understanding of current science. Success in this task is the most likely way to build public understanding of, and confidence in, the safety of food and novel technologies. It creates public confidence that the risk managers are competent, truthful, and serious about public safety because they are implementing responsible management strategies.

The fundamental tasks of risk communication include the following:

- Providing the most accurate account of current scientific knowledge. This includes being clear about what is known (by general scientific consensus), and also about what is unknown or controversial within the scientific community.
- Engaging all stakeholders in the risk in an open discussion of perceptions of the risk and standards by which it is judged acceptable or nonacceptable.
- Demonstrating that public concerns and risk perceptions about risk acceptability are understood and are being taken seriously.
- Demonstrating that risk management strategies are appropriate for the type and level of risk.
- Demonstrating that risk management strategies are competent – that they meet or exceed standards and best practices of other respected parties.

These points illustrate graphically that risk communication can never substitute for good risk management, because it is most effective when it is about competent risk management. If the approach to risk management is appropriate and defensible, the risk communication is easier. It is simply being clear about what is being done to protect the public from risks they find unacceptable.

Common Errors of Risk Communication

Identifying the factors that lead to successful risk communication is a complex and uncertain task. There is no guarantee of success. It is much easier to identify the mistakes that often lead to its failure. These mistakes are often made by risk communicators, especially in crisis situations.

'Zero-Risk' Messages

There is a widespread view among scientists and risk experts that lay people generally expect food and technology to be risk-free. This is part of a more general assumption that lay people do not understand the concept of safety as 'acceptable risk' rather than 'zero risk.' This assumption is far from valid in the case of many lay people, and certainly in the case of the more informed portion of the public that involves itself in the public debates about risk and safety. Often, risk experts confuse public resistance to what are viewed as increases in the risks imposed upon it by new practices as a demand for 'zero-risk' in these practices. These are not the same thing.

It is neither surprising nor unreasonable in any way that most people are resistant to increases in the risk burdens they share. Consequently, not only do risk communication claims that a new food or technology is 'risk-free' fail to convince (they are not plausible), but they also serve as traps in the long run that can be devastating to an industry or practice. All it takes to disprove a 'zero-risk' claim is one instance of the potential harm occurring, and when this happens, public trust in the risk managers and communicators is seriously undermined and very difficult to restore. When trust is undermined, risks, however small, will be viewed with much greater suspicion.

Reluctance to Admit Scientific Unknowns and Uncertainties

Another common error in risk communication is the failure to be entirely transparent about the science underlying the assessment of risk. Risk assessment is an inexact science at the best of times, as every risk assessor knows. It is often full of uncertainties and disagreements among scientists themselves. However, because of the high demand in many sectors of society for 'science based' decision making, many scientists, when speaking in public, are reluctant to admit the uncertainties in the science they are willing to admit among themselves. This is, ironically, a very 'unscientific' stance, because it misrepresents the real nature of science to the public. Scientists themselves often create high expectations in the public of the accuracy and objectivity of science, and when scientists turn out to be wrong, or change their view in light of new evidence, the credibility of science itself is undermined in the public eye.

A common form of this risk communication error is the reliance on what is essentially lack of any serious scientific data to support claims that a food product or technology does not pose any threats to human or environmental health. This is known widely as the fallacy of confusing 'absence of evidence (of risk)' with 'evidence of absence (of risk).' A good risk communicator carefully respects this distinction, being ready to admit that where there is little scientific evidence regarding risks posed by a food or technology, this should be admitted. The best risk communication message in this situation is transparency about the state of the scientific understanding, and clarity about what precautions are being taken with respect to human health and the environment in light of the unknowns.

Invoking Standards of Safety that are not Sensitive to Public Concerns

Risk experts tend to rely on standards of acceptable risk that are unpersuasive to the lay person, and appeal to them is usually unpersuasive. The preferred safety standards for risk experts are risk–benefit standards and comparative risk standards. In the first case, the argument made is that the benefits derived from the novel food or technology outweighs the risks, and should therefore be acceptable. In the second case, the argument is that, in comparison with other risks most people already take, the one associated with this food or technology is lower or no greater.

Neither of these arguments has proven to be very effective in risk communication. There are some very easily understandable reasons why this is so. In the first case, pointing out that certain risks are outweighed by the benefits often ignores

the vitally important question of how the risks and benefits are distributed. Often the benefits of a particular novel food or production process accrue to the producers rather than the consumers. They may legitimately feel that they are being exposed to risks in order that others enjoy the benefits – and this is not likely to be persuasive.

In the second case, comparative risk arguments are also notoriously ineffective. This too, is understandable: People often reason, quite legitimately, that the fact that one risk is no greater than another they already take, does not account for the fact that the new risk is being added to the one they already take. Most people are reluctant to add additional risks to what they perceive as an already heavy risk burden. In addition, the comparative risk argument serves to remind people that they are already exposed to risks they did not realize were there.

Assuming that the Primary Objective of Risk Communication is Communicating the Scientific Evidence of Low Risk

The reasons for the ineffectiveness of this strategy should be evident from the previous discussion of the factors that inform attitudes to acceptable risk. Although information about the best scientific assessment of the risk is crucial to risk communication, it is rarely definitive. The question in the mind of the audience is not only that of how great the risk is but also what aspects of the risk make it acceptable or unacceptable, in addition to its magnitude. Emphasizing that the risk assessors and communicator believe the risk to be low, whereas failing to address the other aspects of acceptable risk, simply make them both appear to be insensitive to public safety concerns.

How does this Apply to Novel Foods?

When new food products, food processes, and new foodborne risks are introduced into the market, it is not difficult, given what we know about risk perception, to predict what concerns will need to be addressed in effective risk communication. Recent examples of these concerns include food products from genetically modified organisms (GMOs, plants and animals), cloning of animals, the BSE crisis in beef and the associated vCJD in humans, the use of the pesticide Alar in apples, and the debate about irradiation of food for sanitation. In all these examples we see the following concerns raised in the public mind:

- This is something entirely new in my experience. I do not know whether to trust it. I do not know how to deal with it. It does not seem 'natural.' Should we be 'tampering' with our food in this way?
- What is the state of the science which assures us that the food product is safe? Has sufficient research been done? How much uncertainty is there in the science? Why do some scientists say there are serious risks? Can the science be trusted?
- Who benefits most from this new food product or process, and who is most exposed to the potential risks? Why should I accept the risk if others (e.g., the producers) are the primary beneficiaries? Why should I voluntarily accept this risk anyway?
- If there are risks in this product, is there anything I can do to identify them? There does not seem to be anything I can do (e.g., food preparation) to control my exposure to the risk.
- I do not trust the people who are producing these potential risks (e.g., the scientists and industries). I remember that they assured us that other products were 'proven safe' (e.g., drugs that were later removed from the market because of serious side effects). Why should I trust them to manage this risk?

The debate about GM foods involved each one of the concerns above. The technology of gene splicing was new and difficult to understand by lay people. It appeared to be a significant way of bypassing the familiar processes of 'nature,' which, being familiar, are largely trusted. This raised the questions about the reliability of the science in assessing the potential health risks to humans (e.g., allergenic foods) and environmental risks (e.g., outcrossing of pesticide-resistance into weeds, biodiversity), and trust in the science–industrial complex that profited from the development of the technology.

A commonly acknowledged factor in consumer apprehension about GMOs was that the first generation of these products modified the organisms to serve producer interests (e.g., lower production costs and easier transportation) rather than consumer interests (e.g., better food quality and lower prices). Thus, the question naturally presented itself, 'Why should I accept any level of risk in this technology, if I do not receive any real benefit?' Because several of the companies behind the major GMOs introduced to the market were companies associated with chemical pesticide production (and issues of chemical pesticide residues in food were very much in the news), there were serious issues of public trust in the producers of GM food.

In the early days of the serious public debate that swirled around GM food technology, especially in those countries in Europe and Africa that were forced to adopt restrictive legislation about their production and importation, the communication from the industry, government, and other promoters of the technology largely failed to address these concerns about the acceptability of the risks. Instead, they focused on the scientific assessment of the risk, arguing that the science had not identified any serious risks about which the public should be concerned. This argument was of limited effectiveness, and for obvious reasons, given the nature of public concern, which was focused on other issues. Failure to take these concerns seriously led to weak risk communication about GM foods.

Conclusion: What does the Public Need to Know?

Good risk communicators know that they have succeeded in their task if their intended audience is left with a sufficient understanding of the relevant issues surrounding the risks of a new food or technology to make reasonable judgments about the acceptability of these risks (which they are entitled to

make according to their own values) and about the appropriateness and effectiveness of the risk management strategies in place with respect to these technologies. The keys to this success can be summarized as follows:

- The best and latest scientific information has been communicated in ways that the public can understand sufficiently to make informed judgments;
- The nature and extent of the uncertainties and unknowns in this science have been communicated honestly and transparently, as well as the steps that are being taken to fill in the gaps in scientific knowledge;
- The risk managers and communicators have engaged in the necessary consultation with the various stakeholders in the issue to have developed an understanding and appreciation of the factors that influence their attitudes toward the acceptability of the risk; and
- The communicator is able to address the public sensitivities with respect to this risk, and make a convincing case that these are being addressed responsibly by appropriate risk management strategies.

To the extent that these tasks can be carried out, public confidence and trust in the safety of novel foods and technologies can be sustained.

See also: Foodborne Diseases: Overview of Emerging Food Technologies. Hazards of Food Contact Material: Nanotechnologies and Nanomaterials. Prions and Agents of TSEs: Creutzfeldt–Jakob Disease. Public Health Measures: Assessment of Novel Foods and Ingredients. Risk Analysis: Risk Communication: Biological Hazards; Risk Communication: Chemical Hazards; Risk Communication: Diet, Nutrition, and Health; Risk Communication

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Risk Communication: Diet, Nutrition, and Health

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Glossary

Attentional bias The tendency of individuals to direct attention selectively toward specific information in one's environment, resulting in less attention to other relevant information when making decisions.
Attitude Enduring systems of beliefs that represent individuals' affective feelings of like or dislike of an object.
Credence attributes Attributes of a product that cannot be verified by consumers or can be verified only after a long time period.
Food labeling A panel attached to a package of food that gives a variety of information about the nutritional value and safety of a food item.
Health halo effect One positively perceived feature of a food (such as claim or logo) extends to the broader product evaluation, leading to an overestimation of the overall healthfulness of the food.
Health locus of control A theoretical construct developed to assess individuals' beliefs regarding personal control over one's health. A person may believe that internal forces have control over health or that health primarily is determined by outside forces, such as physicians and chance.
Nutrition education A set of educational strategies to encourage specific dietary choices and behaviors in order to improve health and reduce the risk of diet-related diseases.
Optimistic bias The tendency of 'unrealistic optimism' in which an individual judges that negative events are less likely to happen to the person making the judgment compared to the risks experienced by an average member of society or comparative individual within that society.
Self-efficacy The extent to which a person believes in his or her ability to overcome the difficulties inherent in performing a specific task in a particular situation.
Self-regulation Individual's capacity to change behavior to the attainment of personal goals.

Introduction

Good and safe nutrition are closely connected with good health. Consumers have various concerns about the influence of food on their health, ranging from food safety issues (e.g., additives and foodborne illnesses) and concerns related to longer term health risks because of unhealthy food choices (e.g., overweight, micronutrient deficiency, chronic heart diseases, and cancer). As a consequence, the provision of information about diet, health, and nutrition is increasing. Information is rapidly spread by the (mass) media, which includes social media. But it also includes communication developed to reach consumers at the exact place where they make their food decisions, for example, through food labels, at the supermarket or in catering outlets.

Communication regarding diet, health, and nutrition is about the provision of knowledge and skills so that consumers are better able to select and consume healthy and safe foods. Various barriers to effective communication about diet, health, and nutrition can be identified. Before people can process information they need to be aware of it, either because they are exposed to it or actively search for it. This requires a certain degree of motivation. Even if consumers process this information, it may not subsequently lead to changes in behavior. Hence, communicators in the area of nutrition, diet, and health deal with a variety of issues, ranging from reaching the right target population to making sure that the message is understood and implemented in daily life. In this chapter some relevant consumer behavior issues facing the communication of risk in the field of nutrition and diet will be examined.

Consumer Awareness and Motivation to Process Information

Information about diet, health, and nutrition can only influence consumers if they are exposed to it. Exposure can be intentional when consumers search for information relevant to their goal or problem. Accidental exposure occurs when consumers unexpectedly encounter the information. Attention is influenced by both environmental factors, and those related to the consumer, such as motivational and time constraints. The location of information on a food package, or the novelty of the information presented, potentially influence the likelihood that a message becomes salient and captures a consumer's attention.

Nutrition education has gradually shifted to include food retailers. As a consequence, consumers are confronted with an

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extremely rich information environment with competing choices being made available. Consumers are often too rushed to intentionally search for all types of information in retail environments such as supermarkets which are noisy and provide many other types of information. People are more likely to look for information when they have a specific health problem (e.g., trying to lose weight or food allergies). Owing to cognitive limitations in the extent to which multiple messages can be simultaneously processed, consumer attention is focused on what matters the most to them, and so diet, health, and nutrition information potentially competes with information about price, brand, convenience, and taste.

Research has suggested that health is reported by consumers to be a key choice criterion when making food choices. However, even when consumers are aware of health information associated with particular foods, they are not necessarily motivated to spend energy and time to process this information and adapt their behavior if needed. In persuading individuals to perform or refrain from certain behaviors, the emphasis has typically been on developing uniform education materials for the general population. Although these materials may provide accurate and understandable information, they may not be processed by individuals and hence be less likely to motivate behavior changes. Information processing is influenced by the level at which an individual is involved in its content, and considers the information to be personally relevant. For example, 'optimistic bias' (or 'unrealistic optimism') is where an individual judges that negative events are less likely to happen to the person compared to the risks to which an average member of society or comparative individual within that society is exposed. Optimistic bias has been observed for a range of food related hazards, but tends to be more evident for 'lifestyle' hazards (e.g., alcohol and fat intake) compared to hazards of 'technological' origin (e.g., pesticides and genetic modification). For these lifestyle risks, consumers' optimistic bias may stem from the 'illusion of control' whereby consumers are confident that they are able to prevent the risk from occurring, or the 'illusion of knowledge' where they perceive they know more about the risk than others. Optimistic bias may hinder educational efforts to motivate consumers to change risky behaviors, because they perceive that information is directed towards more vulnerable and less knowledgeable members of society.

Attentional biases influence what kind of information people are likely to focus on, and – in particular – what kind of information they tend to neglect. In the research field of persuasion, some approaches to improving compliance with 'good' behaviors have been identified. For example, people naturally like to behave in line with others. The likelihood that people will adopt a good behavior can be increased by providing them with the information that other people also show similar behavior. If the aim of information provision is to persuade people to eat, for example, more fruits and vegetables, it would be helpful to stress the number of people who are already doing so or to supply a testimonial from someone who eats fruits and vegetables. It also suggests that, if one wants to persuade people not to do something, for example, eating too much saturated fat, one should not focus on the number of people who are engaging in such bad behavior. In this case, the message that people will remember is that others are also acting badly (i.e., eating too much saturated fat) and thus it is normal to do the same. Research shows that providing people with normative information (i.e., information about how others are behaving) is more effective when the bond between the reference group and receiver of the message is closer. For instance, a message like 'many people living in Amsterdam eat sufficient amounts of fruits' should be more persuasive for an inhabitant of Amsterdam than the simple message 'many people eat sufficient amounts of fruits these days.' Some studies have even shown that strong affiliation with the reference group is needed for normative information to be persuasive.

People also differ in the degree to which they believe they possess control over their own personal health. This so-called 'health locus of control' theory categorizes people into 'internals' if they believe that they themselves are in control of their health and 'externals' if they believe that their fate is controlled by external forces such as powerful others or chance. 'Internals' have been shown to be more likely to carry out health-promoting behaviors, such as exercise, and to take responsibility for their own actions. As such, the theory is closely related to the construct of self-efficacy which reflects a person's belief in his or her ability to overcome the difficulties inherent in performing a specific task in a particular situation. Self-efficacy has been shown to be a powerful predictor of many health behaviors. For example, people tend to pursue tasks in which they feel competent and are confident of accomplishing and avoid challenging tasks which involve complex behavior changes (e.g., eat less fat). Messages that boost self-efficacy focus on reasons and skills that make people believe they will be able to carry out the behaviors successfully. Research has shown that messages consistent with the locus of control of individuals are more effective than general messages.

Potential Misinterpretations of Communication about Risks, Health, and Nutrition

Once consumers are exposed to information, whether accidently, or because they deliberately searched for it, the interpretation and comprehension process begins. Comprehension is a precondition of correct interpretation of information. Various studies have shown that individual differences, such as food safety and nutrition knowledge, and health status influence the interpretation of related information. In general, consumers, and some researchers and policy makers, feel that health and nutrition information is often conflicting and confusing. To prevent confusion, it is often stressed that information should be simple without adding too many details. When asked for their opinion, for example, in group discussions or personal interviews, consumers tend to express a preference for simple and understandable information. However, the more information is summarized, for example, in a single logo front-of-pack, the more it is lost, which may make misinterpretations more likely to occur. Information about food often produces inferences (beliefs that are not based on information directly presented to an individual). Consumers are heavily influenced by existing knowledge that is activated during comprehension. Consumers can make

interferences from small amounts of information. For example, some consumers might infer that a food is healthy because the advertisement emphasizes the naturalness of the product. Research on the effect of positive descriptive food names on food showed that these descriptions might create a 'halo effect' such that the food consumed becomes more attractive and liked.

Closely related to this is that healthy and safe eating messages typically stress only one particular risky or beneficial aspect of foods. People tend to rely strongly on one piece of information when making decisions, the 'anchoring bias.' Moreover, faced with information overload, individuals tend to just categorize foods as good or bad and do not evaluate a food or message about the food in the context of the total diet. This is also called 'dichotomous thinking'; the oversimplification of information in either bad or good. Consequently, messages that aim to change undesirable behaviors may be not well received if consumers think that they have to give up their favorite 'good' foods. Restricting the amount of information provided, and presenting a balanced view of risks and benefits, may reduce this tendency.

Food decisions of consumers are based on both the expected benefits and risks. When people are concerned, they typically desire more information about the object of their concern and want to be taken seriously. Risk perceptions of consumers have been found to increase when a potential food risk is perceived to be unnatural, potentially catastrophic, or unfamiliar. Understanding these psychological drivers of risk perceptions are of key importance. For example, research has shown that food additives were rated as more harmful when their names were difficult rather than easy to pronounce, indicating unfamiliarity. The trade-off between risks and benefits involves weighing the benefits of a food (e.g., the health benefits of consuming fatty fish in relation to heart diseases) with the safety risks (e.g., contamination of fish with heavy metals). Perceived risk and benefit seem to be inversely correlated. In case the benefits clearly outweigh the risks associated with a food, risks become more acceptable. This implies that when something is perceived as highly risky, it is correspondingly only acceptable when there are high benefits. This suggests that certain trade-offs between risks and benefits are being made by consumers. The information processing of consumers in terms of perceived benefits is to a large extent the result of cognitive processes. For risky information, consumers process that type of information in a more heuristic way which refers to people's fast and intuitive responses to dangers.

Consumer Trust in Information

Consumer responses to communication are influenced by more factors than the information itself, such as the trust in the source of information. This concept of trust has been extensively studied in relation to consumer perceptions of the risks and benefits associated with the consumption of different foods (for example, in relation to the efficacy of regulatory assessment and governance practices) and information about the risks and benefits of consuming different foods. For example, when consumers judge information to be overcomplicated and difficult to comprehend, they will use their perceptions of the motives of the information source in providing the information to make decisions about the value of the information. They may also adopt the attitudes of groups with whom they perceive that they share values, rather than process the information directly.

To evaluate the healthiness and nutritional value of specific food products, consumers have to resort to the use of credence attributes. Credence attributes are those product characteristics that cannot be verified by the consumers or can be verified only after a long time period. Consequently, consumers have to believe that these attributes deliver without proof at the moment of purchase or consumption. Hence, consumers have to rely on their trust in food manufacturers or regulators. Generally it has been observed that trust in scientific governance has increased across Europe in recent years. Industry sponsored initiatives (such as front-of-pack nutrition labeling) may or may not be subject to regulation, depending on local or regional legislative frameworks in force. For example, in Europe, nutritional labeling must be 'evidence-based,' whereas in South East Asia no such legislation has been enacted. In a global society this may result in different messages being delivered to consumers which vary across regions, causing further consumer confusion about dietary choices. From an industry perspective, such labeling may be used voluntarily to indicate the industry is responsive to societal concerns about food and nutrition.

Changing Consumer Behavior in a Desired Direction

Even under circumstances when people process nutrition information correctly and are motivated to change their diet accordingly, they still often fail to do so. Choices regarding the purchase and consumption of food are made on a daily basis and most of the time is performed in the same context. This repetition in behavior and context means that eating behavior is largely habitual and consequently difficult to change. Habits can be understood as learned sequences of acts that have been reinforced by rewarding experiences in the past and that are triggered by the environment to produce behavior, largely outside of people's conscious awareness. This automaticity means that educational interventions attempting to change behavior by fostering deliberate intentions towards healthy eating are often ineffective. Instead of promoting healthy eating intentions by means of informational campaigns, habit theory suggests that interventions may be more effective when they target environmental cues that trigger automatic behavior. Habits may be easier to break when there is a change in context. Therefore, attempts to change or create eating habits may be more effective when they coincide with a change in someone's situation, such as starting with a new job or moving to a new neighborhood.

Another, related, reason why people often fail to implement their good intentions has to do with their limited self-regulation capacity, or in more popular terms 'willpower.' To enact a healthy food choice when tempted with unhealthy, but hedonically attractive, alternatives requires a high level of self-regulation. People need to be able to resist the reward of short-term gratification over the long-term health benefits associated

with healthy food choices. In particular, in moments of low self-control, for instance when one is tired or hungry, self-regulation often fails. Education interventions that include self-regulation techniques have shown to be more effective than interventions that are based on information provision only. These interventions often include a component that encourages people to make specific plans as to when and how to implement the desired behavior. These so-called implementation intentions have shown helpful in enacting positive intentions.

A major question in research and policy making is whether communicating about diet, health, and nutrition will actually lead to healthier and safer food choices and less nutrition-related diseases. To date, little empirical and consistent evidence exists to support that education improves eating habits. Research on actual impact on diet and health is complex and time consuming. Traditional consumer research approaches based on self-reports are valuable but limited as consumers tend to give socially desirable answers (and besides may not be aware of the factors that underlie their habitual food choices.

Concluding Remarks

Educational campaigns and health interventions are widely used to promote healthy and safe eating practices. Despite these initiatives, consumers do not always notice and act on information provided, particularly when they are overwhelmed with high levels of information. Hence it is important to target messages according to different consumer needs and preferences, for example by referring to other people's action with which the target group closely identifies. Careful segmentation of different population groups may lead to more readily engagement in health-promoting behaviors.

Easy accessible and understandable information is needed to enable consumer to make informed choices about risks and benefits. To increase the likelihood of acceptance, information is increasingly provided at the place where it is most needed. For example, food preparation information should reach the kitchen by putting clear instruction labels on particular foods. Nutrition information 'front-of-pack' or on shelf tags in retail support consumers make in-store decisions.

Information that is not personally relevant and generic is less likely to attract attention and influence attitudes or behavior. Messages that are closely tailored to individuals' interests are more likely to be internalized. Over the previous decade, there has been growing interest in the role of the internet, including social media. Developments in communication technology have enabled efficient delivery of information to large number of individuals. The increased availability of the internet and new social media tools facilitate interactive communication that can help inform the public and create a dialog between communicators and consumers. In particular, one characteristic of social media is that consumers themselves play an important role in sharing and 'cocreating' information. At the same time these additional communication channels are a relatively unfamiliar domain leading to new challenges for researchers, risk managers, and communicators and may create groups of excluded individuals who do not have access to these new media.

In the current food environment, consumers are regularly confronted with relatively cheap, tasty, and unhealthy foods. This is supposed to be a strong contributor to the increase in nutrition-related diseases such as obesity. As a consequence, environmental interventions are highly needed as a complementary strategy to improve healthy and safe eating habits among consumers. In addition, for effective communication to be developed, it is important to understand the factors that influence consumer trust in information about diet, health, and nutrition.

See also: Risk Analysis: Risk Communication

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Food Safety Training and Health Education: Principles and Methods

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Glossary

Food safety competence The ability to perform a food preparation task safely and to the required standard (this may be legislative, in-house or external).
Food safety culture The aggregation of the prevailing, relatively constant, learned, shared attitudes, values, and beliefs contributing to the hygiene behaviors used in a particular food-handling environment. It provides staff with a common sense of food safety purpose.
Food safety management Coordinated activities to direct or control food safety. The attainment of business food safety goals in an effective and efficient way through planning, staffing, organizing, directing, and controlling organizational resources.
Food safety training One way to deliver/achieve learning, a planned process to modify knowledge, attitudes, skill, or behavior relating to food safety through a learning experience. In work, to develop the abilities of an individual to work effectively and efficiently.
Formal training Successful completion of a widely recognized, independent food safety training program.
Health education Range of definitions; a simple one is: a communication activity designed to enhance positive health or diminish ill health in individuals or groups. Considered in a broader work and social framework than training – not necessarily in a limited field of activity.
Informal training Usually undertaken at work, can be theoretical or more usually practically based on the specific needs a company has, for a food handler to produce food safely.
Learning Acquisition and understanding of new skills, knowledge, or attitudes (with respect to food safety). Ability to do something the person was previously unable to do. A relatively permanent change in behavior as a result of tuition, practice, or experience.
Management systems All the documented procedures, practices, and operating procedures which influence food safety.
Operational food safety performance The collective food safety practices used within an organization.

Introduction

The World Health Organization (WHO) at its 126th Executive Board meeting in January 2010 highlighted concerns over food safety and described it as "a continuing public health concern" that required a global solution. Whether actual cases of foodborne disease are increasing or whether there are just more being reported is uncertain, but what is clear is that the number of cases and the associated levels of morbidity and mortality are far too high. The global nature of food safety relates to the world trade and movement in food and raw materials, the mobility of labor from one country to another with the possible risk of infected food handlers moving from countries where some intestinal pathogens may be endemic, coupled with the fact that pathogens spread easily and do not respect international barriers.

The responsibility for food safety involves governments, industry, and consumers working together. Governments should undertake surveillance to establish the level of food contamination and the patterns, risk factors, and organisms involved in foodborne disease at a local level. They should draft and then enforce relevant food safety legislation, but this should be linked to good communication and support for industry. The food industry should construct and use appropriate management systems which need to be implemented within the context of a positive food safety culture. Consumers themselves must also play their part although many recent studies indicate nonimplementation of known food safety practices within the domestic kitchen.

It has been said that in spite of millions of words being written about food safety, coupled with the expenditure of large sums of money, levels of foodborne disease have not decreased significantly. There may be a number of explanations for this including the evolving nature of food pathogens. Many of the organisms causing problems today have been described as emerging pathogens, having been identified as problematic over the past 40 years. *Campylobacter*, identified as causing only foodborne disease in the early 1980s, is thought by WHO to be the most common cause of bacterial food related illness with possibly more than 300 million cases a year. However, even this is likely to be far fewer than the burden of illness caused by norovirus which

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maybe one of the world's most infectious organisms with many cases spread by food handlers. However, focusing only on the microbial nature of foodborne disease is never going to reduce its burden to an acceptable level. Another major contributory factor as, or even more important than pathogen emergence, is the role played by food handlers. The controls necessary to manage foodborne disease are not new, but their full implementation by food handlers is a stubborn problem. Concerted efforts to reduce the burden of foodborne disease must, therefore, understand and address issues of food handler compliance with food safety practices.

Understanding Food Safety Behavior

Research over the past 20 years has attempted to quantify, understand, and improve food handler behavior in both the home and the food industry. Although food handlers in industry are also likely to prepare food in the home (although clearly the reverse may not always be true), a key difference is that practicing good food hygiene is a legal requirement in industry, and desirable, but only optional, in the home. Other differences include the facilities available to practice hygiene and food handler knowledge about safety practices. Many countries have legislation requiring food handlers to be informed about food safety and to be trained. However, strategies to inform and educate consumers about food safety are far more variable or even nonexistent. This may in part contribute to the large number of cases of foodborne illness thought to be acquired in the home.

The need to understand the human aspects of food handling with the involvement of behavioral scientists, has been recognized. In one recent study of food handlers' beliefs, 62% of food handlers admitted to sometimes not carrying out all known food safety behaviors on every appropriate occasion with 4% admitting that they often did not. Lack of time was the most quoted reason for failure to implement relevant practices. In food service operations, this may be due to the need for food handlers to be hygienic when a large number of customers are waiting to be served. This need to serve to order, as opposed to supply stock, which is more common in food manufacturing, can lead to greater food handler noncompliance. Possible factors influencing human food safety behavior are summarized in Figure 1. However, it must be realized that food handlers, especially consumers, are not a homogeneous group, they are individuals and will not behave in the same way or necessarily in a way food safety experts might expect.

In attempting to interpret, study, or even predict behavior it may be helpful to use existing theoretical behavioral models. The simplest of these is the knowledge, attitudes, practice (KAP) model, and this has been used as the basis for much hygiene training. This relies on the provision of information (knowledge) to modify a food handler's attitudes, which would in turn change behavior. The approach takes no cognizance of the conditions, culture, and environment in which the food handler may be working.

More sophisticated and successful models try to consider many more of the factors which can influence behavior. Social cognition models start from the assumption that an individual's behavior is best understood in terms of his or her perceptions of their social environment. Two of the most widely used models are the theory of planned behavior and the theory of reasoned action (TRA). These theories aim to measure behavioral intentions, recognizing that certain uncontrollable factors can inhibit implementation and thus prediction of actual behavior, for example, an individual intends to wash their hands, but arrives at the sink to find there

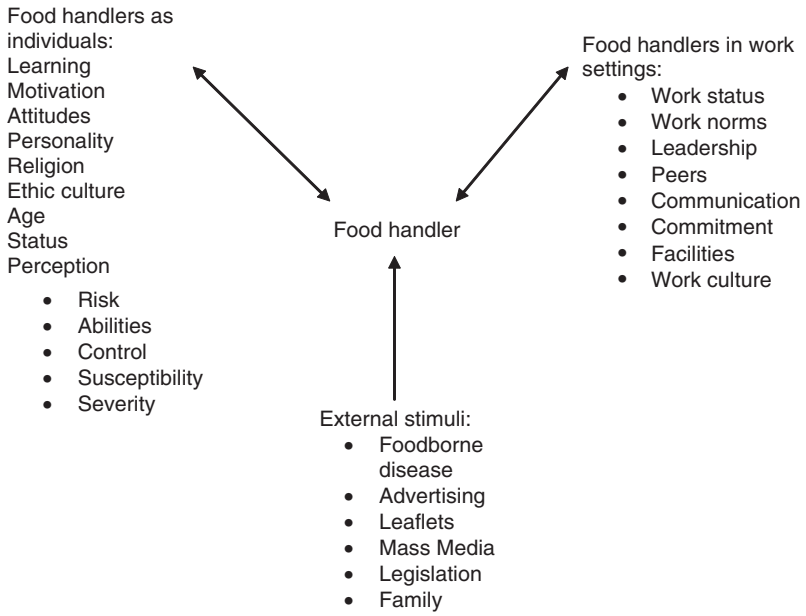

Figure 1 Factors influencing food handlers behavior. Reproduced from Blackburn CW and McClure PJ (eds.) (2009) *Foodborne Pathogens: Hazards Risk Analysis and Control*, 2nd edn. Boca Raton: WP/CRC Publishing.

is no soap. These models include the element of subjective norm, which considers the perceived beliefs of others and the individual's desire to comply with those beliefs.

One model that has been applied to food hygiene behavior is the health belief model (HBM). However, this model has been criticized as being more a catalogue of variables rather than a model. The health action model combines elements of the HBM with the TRA. Other models exist and, although they vary in structure, they can provide a basis for understanding and studying food hygiene behavior and a list of target areas for interventions to focus on. Considering these at the time of hygiene training could help to make the training more effective.

Another model that has attracted interest for use in consumer health education is the transtheoretical model developed by Prochaska and Diclemente. This model is considered to be suitable for social marketing applications and suggests that behavioral change is not usually instantaneous but is part of a series of five stages of change comprising precontemplation, contemplation, preparation, action, and maintenance. Use of this model for audience segmentation at an appropriate stage of change has been proven successful for a variety of health-related behavioral initiatives. What is clear is that food handlers (commercial or consumers) need to know about food safety practices and the underlying reasons for their implementation in order to prepare food safely. This usually requires knowledge transfer of some description which is usually by formal or informal learning/supervision or education.

Training and Commercial Food Handlers

In an attempt to improve food safety practices, legislation in a number of countries requires that all food handlers are supervised, instructed or trained in food hygiene matters commensurate with their work activities. This has in part resulted, in the UK, in an estimated 5 million food handlers receiving formal or certificated training over the past 10 years. This may only represent a fraction of all food handlers and there is evidence that businesses are still not complying fully with training requirements. Some countries are keen to encourage standardized food hygiene training. However, there is debate concerning the most appropriate type and nature of the training and whether introducing competence-based training is more, or less appropriate, than that delivered to meet the needs of various external awarding bodies. The latter are often private organizations and maybe international in nature. Endorsement of their courses may be seen as giving commercial advantage or preference to one organization or one country. However, some countries are less concerned with examinations and assessing the training and are more concerned with proof of attendance on training courses. Certificated formal training is preferred by auditors and inspectors because they can easily assess the level and content of the learning. Employers often prefer this approach because in some ways it is easier and allows them to display hygiene certificates and present a positive image. There is some evidence that employees prefer more specific internal, bespoke on-the-job training or supervision, believing it is more likely to lead to competence than more formal classroom-based training. Buddying can be used as part of on the job training/supervision, especially if based on a training-needs analysis.

Food service establishments are the main location for reported outbreaks of foodborne disease. It has also been implied that many outbreaks of foodborne disease result from faulty food-handling practices with one study suggesting that food handlers' malpractices contributed to 97% of foodborne illness in food service establishments and the home. This may be due to a lack of knowledge or nonimplementation of known food safety procedures. Effective training requires planned and systematic efforts to modify or develop knowledge, skills, and attitudes through learning experiences to achieve an effective performance in an activity or range of activities. Applied to food safety, this means people will receive, know, and understand food safety advice and be motivated to practice it. This is not just about sending people on a training course and hoping they will behave hygienically afterwards. To achieve behavioral change, people need to be motivated and be provided with the resources, including time, to act hygienically. The potential importance of training has been recognized over a number of years and recommended in many influential reports. Effective training offers, potentially, the best way to reduce cases of foodborne disease, yet it is often not well documented, managed, or supported, is taken for granted, and it is often seen by some enforcement officers and management as an end in itself rather than a means to an end.

Food handlers constitute a constantly changing and dynamic workforce, and some businesses may be reluctant to train staff fearing that they might leave and work for the competitors. Evidence of successful training can be seen in the form of hygiene certificates displayed in many food service establishments and small retailers, where they may be perceived to have a marketing potential and provide reassurance to customers. However, there is uncertainty regarding who should be targeted and the efficacy of current food hygiene training in changing handling practices and reducing foodborne disease. Many training initiatives have targeted the ordinary food handler rather than supervisors/managers, although there is evidence that businesses with better trained managers (who set standards and devise procedures) may sell food of better microbiological quality and are more likely to perform better in hygiene inspections. In a number of countries, training the supervisor or person in charge is seen as the most appropriate strategy. Training in food safety should involve all levels, not just food handlers and supervisors but also senior/top managers. They may need slightly different information with strategies designed to promote positive attitudes and culture as opposed to knowledge of specific practices. It is important for managers and supervisors to show food safety leadership. Statistics, although difficult to interpret, have not shown significant reductions in notified cases of foodborne disease as a result of all the training that has taken place. It is however possible that if the training had not been delivered, the number of cases could be even higher.

Catering or food service locations may be responsible for up to 70% of general outbreaks and it may be more difficult to ensure training is effective, i.e., behavioral change may be more difficult to achieve in this sector than in larger food manufacturers. Particular problems with ensuring training is effective in food service are summarized in Table 1.

Table 1 Characteristics of the food service industry that leads to training difficulties

Characteristics of food service industry	*Problem*
Industry dominated by small businesses	Small businesses have less time, flexibility, and resources for training combined with fewer/no opportunities for in-house courses. Need to conform to wishes of training providers
High turnover of staff	Requires new people to be constantly trained, time, and cost implications
Large number of part-time workers	Greater numbers of people with limited time available to be trained
Low staff pay	Poor morale, little incentive to implement training
Low staff status/poor career structure	Poor morale, little incentive to implement training
Staff language problems and/or low educational standards	Food service often employs larger number of overseas workers or ethnic minorities who may have poor language skills. Some who do speak a language well may be unable to read or write. This can make it difficult to find appropriate food safety training aids and can cause embarrassment for people being trained
Poor access to food safety information	No technical departments, little back up and support and many small bodies do not even belong to trade or professional bodies. Owners, of small catering business may have no hygiene or business training, with little or no knowledge and appreciation of food safety
Nature of business	Food manufacturers work to stock and there is more time available to be hygienic, food service works to order and this creates lack of time to practice hygiene when demand is heavy and customers are waiting. This creates attitudinal ambivalence, although hygiene may be considered important workers feel it is more important to serve customers quickly. Large volumes of food are handled within short periods of time – uneven work patterns. This makes it more difficult to practice food hygiene consistently
Premises design and construction	The ability to be hygienic cannot be divorced from the environment in which the food is handled. Food service premises, may be in nonpurpose built, poorly designed, and constructed premises – or even no premises (street vending). Premises maybe cramped and inadequate – greater opportunity for cross contamination

It is now well recognized that the KAP approach to training is incomplete and greater use of other social cognition models is advocated. These argue that behavior is the outcome of an interaction between cognitive processes and environmental events, both of which can be affected by the overall training need and the views of interested parties. A model for effective transfer of training (**Figure 2**) and the role and influence of interested parties is presented. What people learn and whether they learn is influenced by training design and delivery, i.e., the course structure, information contained, physical learning environment, and quality of training. In addition, it is also influenced by their own internal motivation to learn and how this may be influenced by others, and their desire to comply with the wishes of others. The outcome from this is the individual's intention to behave hygienically, which can be again influenced by both the physical and social environment, the availability of the necessary facilities to practice hygiene, the operating procedures and the management systems they have to comply with, and whether there is a prevailing business culture to be hygienic. The latter are very important in determining whether the acquired learning is, or not, implemented. The sum of the individual food handler's practices gives rise to the collective operational food safety performance, which is an integration of the food safety management systems and food safety culture.

The likely effectiveness of training is a dynamic between the employer and the trainee. Employers should not automatically assume training will be implemented. The role of the food industry is not just about providing training, but it has very important functions in helping to create the desire for learning within their employees as well as providing the right culture/climate for this learning to be transferred into practice. This consists of identifying and maintaining behavioral food safety norms and standards, i.e., the level of hygiene expected. This is related to rewards for compliance and sanctions for noncompliance, as well as the provision of appropriate and adequate facilities to allow its implementation. Employers, and in turn employees, may display attitudinal ambivalence. Alternatively, employers may display a positive general attitude to hygiene and food safety, but they may be more negative toward the implementation of specific hygiene practices and be more concerned about making money. One of the greatest enemies of a positive food safety culture maybe an opposing economic culture where saving money is considered more important. For example, although good hygiene may be valued, managers may feel specific practices are too time consuming or inconvenient. In turn, food handlers themselves may perceive this desire to serve customers quickly or save money.

Another dilemma that may face industry concerns the type of training and who will deliver it. Options included work-based training, usually delivered in-house, or a more formal certificated training which can be delivered in-house or contracted out to one of the many training providers/consultants available. One of the concerns experienced by industry in the past, particularly with respect to externally accredited courses, has been their generic nature. This has often resulted in food handlers from manufacturing being trained with caterers or butchers and following the same general syllabus. The lack of specificity of this type of approach has been cited as one of the reasons why training can fail to change behavior. As a consequence, some countries now offer sector-specific syllabus and exams. In practice, the different sections may or may not be taught together. All trainers should provide relevant examples showing application of knowledge to specific sector work practices, although this may be difficult with large mixed

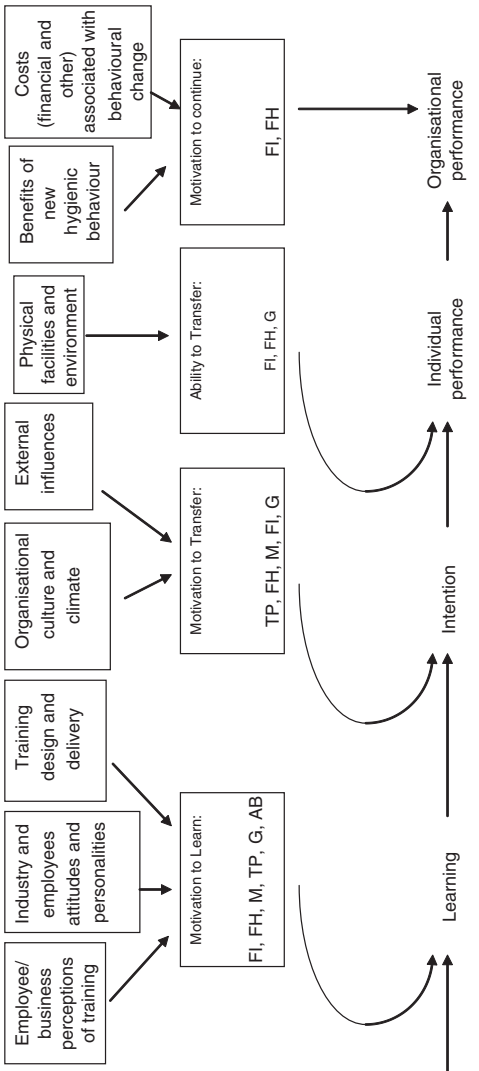

Figure 2 A model for training and behavioral change (see also **Table 2**).

classes. Some awarding bodies/training providers have attempted to provide bespoke courses for larger companies who can provide sufficient student numbers. Tables 2 and 3 provide a self-assessment checklist for employers to try to ensure maximum benefit from the training their employees receive.

A frequently neglected component of training is the requirement for refresher/remedial or update training. Most training focuses on the critical need for food handlers when they start work. Yet for longer-term compliance especially in the light of new information, refresher/remedial training is essential.

Health Education

Given that zero risk with regard to food safety is unlikely, governments and the agrifood industry have a responsibility for communicating information on risk and risk management to consumers. However, until recently, educating the consumer about food safety has been largely ignored. Consumers have an important role to play in handling food safely, yet increasingly hygiene and cooking skills are no longer taught in schools. Parents may not teach their children hygiene skills. Consumer food hygiene education and risk communication are important elements of the work of governments or their agencies, for example, the Food Standards Agency (FSA) in the UK. It is vital to ensure transparency and, where possible, consumer's participation in decision making. It is important to inform and advise them about risks in a way which makes it clear that they have the ability to manage or control those risks.

This latter aspect could be of concern to specific sectors of the food industry. If there is a problem with presence of pathogens in raw foods, for example, *Campylobacter* in poultry, the risk needs to be communicated to consumers in a way which both alerts them to the problem and informs them of how they can reduce/manage the risk. This is known as the reassurance/arousal paradox. Failure by a sector of industry to inform consumers about risk associated with a food could lead to an increase in consumer outrage. What is clear is that risk is not just about science but is heavily influenced by psychology, and experts and the public perceive risk in very different ways.

In general terms, risk communication should enable the recipients to understand the nature of the problem, help or support decision making and be unbiased. There is debate over the precise role of the government in this process – should this be restricted merely to provision of information and raising hygiene awareness or should it extend to modifying or influencing behavior? A detailed study of risk communication is beyond the scope of this article, however, it is worthwhile considering some of the pitfalls/barriers in effective risk communication for raising general consumer awareness of food safety.

Consumers acquire food safety information from a variety of sources including the home, school, and others, for example, the media including television, printed materials, and food safety campaigns. The role of the mass media for informing consumers about food safety risks is of critical importance, and the media have a responsibility to ensure that advice they provide is accurate and adequate. Although media coverage of food safety issues in recent years has heightened consumer awareness of microbiological safety, it has also increased consumer concern and confusion. The media have been responsible for sensationalizing so-called food scares whereby consumers' initial anxieties regarding the issue are commonly amplified. For example, in response to the *Salmonella* in eggs scare in 1980s/90s, consumers' risk perceptions rose to a level far greater than the actual risk. Consumers need to receive correct and consistent food safety information not only to prevent panic instigated by careless communications, but also to promote the safe food-handling behaviors in a manner that is accurate and credible.

Channels and sources generally used in public food safety communication include a variety of formats such as television, radio, posters, leaflets, newspapers, cookery books, magazines, and reminder aids. Although limited research has been conducted to evaluate the effectiveness of different intervention types, it has been reported that the potential effectiveness of different media elements, despite having common characteristics, does vary considerably. Research in the UK evaluating consumer perceptions of different sources of food safety information has shown that Environmental Health Departments and the Food Standards Agency were perceived to be the most trusted and credible organizations providing food safety information. The most preferred source of food safety information was identified as food packaging, followed by advice from a medical doctor.

Parents may not have the correct information to give to their children and there is evidence that consumers' (parents) knowledge about food safety maybe inadequate. Schools are acknowledged as important places for influencing health-related behaviors – including hygiene practices. Hygiene teaching in schools has suffered, certainly in the UK, as a result of a packed school curriculum. Recently, food safety material specially designed for schools has been produced in a number of countries and its usage could help to initiate good practice at an early age. Elements of the mass media, especially TV, offer a powerful means of influencing behavior; unfortunately they do not always project the correct message and TV cookery programs, with highly respected or admired chefs acting as a role model, can illustrate bad practices. One such show in the US even caused an outbreak of foodborne disease. Furthermore, there are suggestions that the media present conflicting advice causing confusion in consumers. In spite of this in the USA, the majority of consumers depend on the media as their primary information source. Initiatives designed to inform the consumer need to use a wide variety of strategies. Traditionally, food safety health education has excessively depended on the use of leaflets, although this is changing.

Educating the Consumer

Publicity concerning foodborne disease in the UK in the late 1980s led to the widespread distribution of standardized knowledge-based leaflets with food safety advice. Although acquisition of knowledge must precede behavioral change,

Table 2 A model for training and behavioral change: factors influencing effective training

Model component	*Interested party*	*Influence*
All stages	All parties	Business survival depends on safe food and consumers want to eat safe food. The food industry has a legal obligation to produce safe food, with government and society accepting their responsibilities. Industry has to contribute to providing the required knowledge for food safety and stimulating the desire to learn and transfer this knowledge into practice
Food safety knowledge	All parties	Accurate information on risk-based food safety practices and efficacy of control measures needs to be available. However, there is still some confusion over how some key practices should be best implemented, for example, defrosting, cooling, handwashing. Needs to cover theory and practice and promote understanding
Learning/motivation to learn	Food handlers	Individual desire for continued learning and acquiring knowledge, desire to succeed and do well, organizational commitment. Language and educational ability. Recognition of key role in food safety, and the ability and need to do things properly. Recognition of specific learning needs, response to feedback at work. Peer values. Perceived importance. Customer expectations
	Industry (management)	Recognition of importance of food safety. Job specific training. Attitudes of managers and supervisors. Assessment of specific hazards and risk and food safety management systems. Promotion and incentive schemes. Access to training. Provision of a training culture
	Training providers	Quality of delivery and training materials, role and enthusiasm of individual trainer. Quality of course materials, visual aids and learning environment . Flexibility of delivery and breadth of provision. Involvement of trainees in the learning process. Extent of precourse preparation
	Media	Adverse publicity about businesses/foodborne disease. Communication of risk. Demands for higher standards. Awareness of food borne illnesses
	Government	Stick and carrot approach to training: legislation, coupled with provision of support materials and finance for training initiatives
Intention/motivation to transfer	Food handlers	Positive reactions to training/enjoyment of learning process. Rewards, for example, enhanced status or salary for succeeding in training followed by its implementation
	Food industry	Food safety and organizational culture and climate: Appropriate cultural norms: Expression of what is desirable and acceptable. Influence of peers, managers, and supervisors, clear statement of hygienic values. Appropriate management systems to be available and in use. Customer expectations
	Training providers	Quality of course delivery. Emphasis given to the need to implement practices rather than just knowledge and rote learning and passing tests. Identification of likely barriers to hygiene implementation coupled with provision of coping strategies during course delivery
	Media	External cues toward hygiene implementation, for example, publications concerning named businesses and foodborne disease outbreaks
	Government	Overarching legislation. Communication that poor hygiene unacceptable, provision for closure of unhygienic operations and other sanctions
Individual performance/ability to transfer	Food handlers	Time to be hygienic, adequate knowledge in sufficient detail concerning theory and practices, i.e., why it needs doing and how it is to be done. Support and approbation from supervisors and peers
	Industry	Provision of all necessary facilities and equipment to practice/implement all food safety measures, for example, cleaning equipment, handwashing facilities, monitoring, design of work environment, etc.
	Government	Environmental health officers (EHOs) ensure premises of appropriate standard

(*Continued*)

Table 2 Continued

Model component	*Interested party*	*Influence*
Organizational performance/ motivation to continue	Food handler	Benefits of greater hygiene (moral and ethical values) greater consumer safety, better status and/or pay, greater job security, and satisfaction. Evaluation and eradication of personal negative aspects, for example, time, possible drying of skin from additional handwashing. Evaluation of peer values, positive feeling of doing things properly, self-satisfaction from understanding what to do and why it is necessary
	Food industry	Profitability, evaluation of cost of implementing hygiene, review, and recognition of failure costs if foodborne disease occurs (also no fines, bad publicity, compensation). Feedback from public and/or EHOs (compliance with legislation). Job description and appraisal, feedback from employees. Reduction in food waste (through better management and control), cleaner premises, integration of training, and hygiene into business management

Table 3 Employer's self-assessment checklist for effective training

Employer activity before the course	*Yes*	*No*
1 Inform the trainer of the type of food business you run and the role of the trainee		
2 Inform the training provider of any language or learning difficulties the trainee might have		
3 Check the qualifications/credentials/background/reputation of the training provider and the individual trainer		
4 Check that you have chosen the best course for your trainee. Get advice from your environmental health officer (EHO), or other food safety professional		
5 Check that the training sessions are not too long		
6 Ensure that training is not scheduled directly after work shifts		
7 Check that your trainees know how to get to the training venue. Consider the option of training at your premises		
8 Tell your trainee about the course content before training		
9 Explain how the course will be assessed. Show them sample questions		
10 Tell your trainee that he/she is expected to make an effort to learn on the course		
11 Ensure your trainee understands that training is important and is a necessity in your business		
12 Tell the supervisors about the training arrangements and encourage them to support the trainees during and after training		
13 Provide your trainee with consistent good examples of hygiene performance		
14 Devise a reward scheme for successful completion and implementation of training – based on increased status or pay		
15 Ensure all facilities are in place for hygiene implementation		
16 Ensure you provide sufficient time for trainees to attend and do any coursework.		
17 Ensure flexibility in time provision and job cover		
Employer activity during the course		
1 Encourage the supervisor to debrief the trainee		
2 Tell the training provider if the trainee has experienced any problems		
3 Recognize the course is maybe more theoretical than practical. Trainees will need to practise the hygienic behaviors at work.		
Employer activity after the course		
1 Debrief your trainee after training		
2 Collect his response to the training on an evaluation form		
3 Praise/reward your trainee for success on the course		
4 If they fail the test, mentor the trainee and encourage them the achieve success in the future		
5 Get the supervisor to check training effectiveness – hygiene knowledge and behavior		
6 Provide safety notices, and other visual aids, in the workplace to reinforce training		
7 Provide written work procedures with performance standards		
8 Check that facilities for hygiene performance are still adequate		
9 Check that work schedules (especially during peak demand) allow time for hygienic behavior		
10 Get the supervisor to provide encouragement and praise for demonstration of hygiene practices		
11 Give newly trained staff opportunities to perform trained tasks on the job		
12 Give extra responsibility to those who demonstrate competence and who motivate others		
13 Criticize and investigate hygiene failures. Learn from them, especially why. Implement corrective actions		
14 If hygiene performance is unsatisfactory, retrain on the job		
15 Carry out frequent refresher training sessions and maintain training records		
16 Encourage staff to discuss hygiene issues and make suggestions for improvements		

Table 4 The 4 Ps of marketing – marketing mix

Product	What is being offered must be acceptable and as tangible, accessible and attractive as possible. The changed behavior must not be excessively expensive, time consuming, totally impracticable, painful, etc.
Price	Decisions to act are a result of consideration of costs and benefits. Ways to minimize the cost as well as the benefits need to be addressed in strategies for behavioral change
Place	The means to implement new behavior must be readily available and convenient, for example, availability of soap for handwashing
Promotion	The message needs to be advertised in the most appropriate way. This could include leaflets/TV/cook books/magazines or individual advice, for example, health visitors

Source: Reproduced from Blackburn CW and McClure PJ (eds.) (2009) *Foodborne Pathogens: Hazards Risk Analysis and Control*, 2nd edn. Boca Raton: WP/CRC publishing.

and knowledge of the consequences of unsafe food-handling practices can enhance consumer motivation to change behavior, findings from other areas of health education suggest that although leaflets can play a role in raising awareness and supporting communication initiatives, they generally do not result in behavioral change. Leaflets can provide people with the information enabling them to change, if they are motivated and want to change. In the UK, the FSA, the Food and Drink Federation (FDF), and the Food Safety Promotion Board (FSPB) (Ireland) are the largest providers of consumer food safety information. The UK campaign was based on increasing awareness and understanding of 'The 4 Cs' (cleanliness, cooking, chilling, and cross-contamination). In the USA, a National Food Safety Initiative was set up to provide targeted information for consumers. The Fight BAC! Campaign was a product of the Partnership for Food Safety Education, which is a unique public–private partnership of government and consumer groups dedicated to increasing awareness of food safety and reducing the incidence of foodborne illness. The Campaign is based on four food safety messages ('Clean' – wash hands and surfaces often, 'Separate' – do not cross-contaminate, 'Chill' – refrigerate properly, and 'Cook' – cook to proper temperatures), and 'BAC!', a big, green bacterium character, has served as the focal point to the campaign. A recent addition to the Fight BAC! initiative has been the introduction of 'Thermy,' a cartoon thermometer. Such a character has been used to support the Fight BAC! message of 'Cook,' based on studies that have indicated there is significant risk of foodborne illness when color is used to judge if a food has been cooked to a safe temperature. Intervention materials have not only been targeted at specific food safety behaviors but also aimed at specific groups of consumers, and they have included a wide range of media formats, some of which have been interactive.

To improve food safety at an international level, The World Health Organization has developed a food safety initiative entitled 'The Five Keys to Safer Food', to teach safe food-handling practices. This initiative has been developed from the 'Ten Golden Rules for Safe Food Preparation' in the early 1990s but is simpler and generally more applicable to food handlers, especially those in high-risk groups and their caregivers. Implementation of this initiative has been undertaken by WHO regional offices, and interventions have been translated into more than 40 languages.

In addition to national educational initiatives, a number of countries undertake research initiatives for collecting information concerning consumer food safety. However, they mostly rely on self-report of behavior. A few studies have focused on actual behavior, and these have indicated that consumers often do not know or understand hygiene advice but more importantly, they often may not implement known hygiene practices. Other behavioral and attitudinal studies have identified that consumers may believe they implement adequate safe food-handling behaviors, but their perception of what is adequate has been frequently observed to be inadequate.

It has been suggested that the future of hygiene promotion should be based on the analysis of the specific needs of the target audience. A contemporary approach to structured behavioral change for health education initiatives has been the application of social marketing to a variety of public health-related disciplines including consumer food safety. Social marketing is a social change strategy that uses traditional marketing techniques from the commercial sector. The approach focuses on voluntary behavioral change to benefit the individual and society, rather than coercing consumers to adopt healthy behaviors. Use of the social marketing approach facilitates the development of a consumer-orientated strategy whereby the needs and wants of targeted consumers are actively sought and acted on in program planning, management, and evaluation. This approach requires a careful evaluation of consumer behavior and the identification of target risk groups using market segmentation. A precise message is identified and the target audience's response to, and beliefs about, the message is assessed as well as the likelihood that they will change behavior. Results from these preparatory stages are then used to plan the initiative based on marketing principles (involving the 4 Ps or marketing mix, see **Table 4**). After implementation, the initiative is evaluated, preferably based on assessment of actual behavior.

This type of approach, which has been used in both the UK and the USA (e.g., for certain cheeses eaten by the Spanish speaking community in California), is more comprehensive than an ordinary health educational approach. The latter is generally more concerned with imparting and receiving messages and with people learning facts. Social marketers argue that learning is only important if it results in the desired behavioral change. Although there are differences in marketing commercial products compared to improved safety in food-handling, not least in terms of budgets available, there are potential benefits in adopting the approach. The social marketing approach was successful in one UK campaign, but change was only achieved in the short term and concerted efforts need to consider a longer time scale. Relatively small improvements/changes in behavior could significantly reduce the costs and consequences of foodborne disease.

See also: Food Safety Assurance Systems: Labeling and Information for Consumers; Personal Hygiene and Employee Health. Institutions Involved in Food Safety: FAO/WHO Codex Alimentarius Commission (CAC). Public Health Measures: Challenges of Developing Countries in Management of Food Safety; Evaluation of the Efficacy of National Food Control Programs; Food Inspections and Enforcement Systems; Health Education, Information, and Risk Communication; Modern Approach to Food Safety Management: An Overview. Risk Analysis: Risk Communication.

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International Association for Food Protection (IAFP).

http://www.usda.gov
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ANALYTICAL METHODS

Contents

Overview of Methods of Analysis for Chemical Hazards

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Glossary

Analyte Residue or contaminant being targeted for analysis.
Analytical quality assurance Measures taken in the laboratory to demonstrate the validity of analytical data.
Cleanup Laboratory procedure involving concentration and purification of analyte.
Chromatography Analytical technique used for separation of analytes in gas phase (gas chromatography) or liquid phase (high-performance liquid chromatography).
Data systems Computerized means of processing outputs from analytical instruments.
Digestion Heating a food sample with strong acids to remove organic material(s).
Mass spectrometry (MS) Instrumental technique used to identify analytes by fragmentation to form unique fingerprints known as mass spectra.
Method validation The process undertaken in the laboratory to establish a set of performance parameters for any analytical method.

Introduction

Agricultural and food products are extensively tested in the laboratory to ensure they meet compositional requirements, that they are authentic, free of adulteration, and comply with regulatory limits for residues and contaminants. Additionally, processed foods need to be analyzed so they can be labeled for nutritional purposes, checked for levels of food additives, and finally any packaging system used to provide protection during transport and storage must be checked so that it does not lead to trace levels of contamination. Sophisticated techniques have been developed to facilitate this food testing ranging from field-tests used for rapid screening to complex multitarget instrumental analysis to be used in the analytical food laboratories.

Analytical chemistry has made tremendous advances over the last 10–15 years with dramatic improvements in methods of analysis exemplified by significantly higher sensitivity and vastly improved specificity of detection. The improved sensitivity has meant that detection of residues and contaminants in foods at parts per billion (ppb) levels (ng g^{-1}) is now carried out routinely. Improved specificity means that complex food matrices can be analyzed, with a high degree of confidence in the correct identification and accurate quantification of the targeted residue or contaminant. With increased specificity, the time spent on sample extraction and cleanup has been reduced and many multimethods have been developed enabling screening for a large number of analytes in a single analytical run.

Despite these significant developments in trace analysis, one should not underestimate the degree of difficulty in testing foods for residues and contaminants. First, foods are complex and diverse in composition making it difficult to develop a single method to cover all food types. Second, the limits for permitted levels of residues and contaminants in foods that are required by regulations in some instances are exceedingly low. This means that high concentration factors are required and sample preparation of necessity means removing as much of the background co-extractives from food as possible, before instrumental analysis.

A trend that has been apparent in recent years has been an increased emphasis on ensuring the quality of analytical data. It is now generally accepted that to demonstrate reliability of data, food laboratories should be accredited to the International Organization for Standardization's (ISO) Standard 17025, should only use validated methods of analysis, and should prove analytical competence through participation in proficiency testing.

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Sampling

It is widely acknowledged, but frequently forgotten, that any analytical result is only as good as the sample that has been taken and submitted to the laboratory. The sample submitted must be representative of the batch or lot from which it was taken. It is well established that for various reasons, residues and contaminants are rarely evenly distributed throughout a large batch of material. This lack of homogeneity is extreme in the case of mycotoxins, where fungal infection is a random process and individual nut kernels or individual grains can be highly contaminated, whereas the bulk of the material might be essentially free of contamination. This same variability has been found with pesticides, where uneven spraying can result in some individual fruit or vegetables having higher residue levels, as a result of their location within an orchard or agrochemical treated field.

Generally sample sizes of only 50–100 g are required for analysis in the laboratory, but for that sample to be representative a significantly larger sample must be initially taken, thoroughly homogenized, and then a subsample taken for laboratory testing. Protocols known as 'Sampling Plans' have been developed that must be followed, and these dictate the total sample weight comprising a minimum number of subsamples and the points from which samples should be taken from throughout a food consignment.

Sample Preparation and Homogenization

When a sample is received by the laboratory, it is necessary that it undergoes appropriate preparation before subsampling for analysis. The type of preparation will depend on the specific matrix. Liquid foods like oils require only mixing, whereas dry foods like cereals can be blended to a fine powder in a hammer mill. Some products such as oily nuts can be blended to a paste, but in other instances (e.g., dried figs) it may be necessary to add water (or another solvent) to form a slurry during blending. If any of the target analytes are volatile, it may be necessary to blend the sample at low temperatures to avoid losses and this is known as cryogenic milling. This procedure is frequently used for sample preparation for the analysis of volatile pesticides in fruit and vegetables.

Screening Methods (Field Tests)

In some situations, it is highly desirable to test raw materials for residues or contaminants before transport, from the point of production to the factory for subsequent food processing. For example, it is desirable to test milk at the farm for veterinary drug residues or aflatoxin M_1 before mixing milk from a number of sources in a tanker. It is also desirable to test individual animals at the point of slaughter for illegal substances. In these instances, rapid field tests which do not require sophisticated laboratory facilities are employed. Also, many raw materials are produced in developing countries, which do not have access to sophisticated laboratory facilities, but nevertheless need to ensure that products are free of contaminants before export.

Screening Methods

Screening for Single Compounds

There are a wide range of different screening assays targeted at measuring individual analytes. These 'test kits' are simple and 'easy to use' and can be semiquantitative or can be developed to give 'YES' or 'NO' answer above or below a predetermined regulatory limit. These kits are invariably based around antibodies and the format can range from classical enzyme linked immunosorbant assay (ELISA) style through to dipsticks, test cards, and lateral flow devices. The majority of this type of test kits have been developed for screening for individual mycotoxins (aflatoxin B_1, ochratoxin A, deoxynivalenol, and zearalenone) and for individual veterinary drug residues (e.g., chloramphenicol). Results are read visually or using simple reading devices which can be battery operated and suitable for field use.

Multianalyte Screening

Some screening tests are widely used to indicate the presence of classes of compounds rather than individual analytes. Microbial inhibition assays such as Premi® Test (R-Biopharm AG, Darmstadt, Germany) or Delvotest® (DSM Food Specialities, Delft, The Netherlands) can be used to test for the presence of classes of antimicrobial substances in various matrices such as fresh meat, fish, eggs, honey, urine, and feed. The CALUX® assay (BDS Biodetection Systems b.v., Amsterdam, The Netherlands) is a dioxin/PCB screening test categorized as a reporter-gene assay. It can measure levels of dioxins and polychlorinated biphenyls (PCBs) faster and is less expensive than traditional instrumental methods, and gives total concentrations of the regulated biologically active dioxins/furans and PCBs.

Portable Instruments

There is considerable interest in miniaturization of laboratory instruments to develop portable hand-held devices that can be used in the field or food factory. This is still an area of development, but already handheld Raman and Fourier transform infra-red (FTIR) spectrometers have been designed for rapid and accurate identification of unknown chemicals directly in the field. Handheld X-ray fluorescence (XRF) analyzers have been purpose-built for measuring levels of metals anywhere and anytime with accurate results available in seconds, rather than the hours or days it can take for traditional laboratory testing. The feasibility of using portable devices for testing has been clearly demonstrated, but improvements in sensitivity and handling diverse food types remain as challenges.

Instrumental Methods

Sample Extraction

Although some new approaches are being developed to directly analyze foods, in general an extraction step is normally

required. This involves blending the foodstuff with a suitable solvent, then filtering or centrifuging to separate solid material from the blended extraction mixture. Although various innovative approaches to improve extraction such as use of supercritical fluids (SFE), microwave-assisted extraction, accelerated solvent extraction (ASE), or cloud point extraction have been developed, these have never really superseded classical solvent extraction, which remains the most widely used approach.

Sample Ashing Techniques (for Metals)

Sample preparation techniques for the analysis of metals of food safety concern such as mercury, cadmium, and lead involve removal of the organic matrix and dissolution of the metals of interest in acid solution. The destruction of organic matter can be achieved by dry ashing in a muffle furnace at 450–500 °C, but this risks loss of several elements by volatilization. Wet ashing is preferred by digestion of the organic material, for example, in *aqua regia* (three parts nitric and one part hydrochloric acid) by heating in an open or closed vessel (digestion bomb). In the past 10 years, microwave techniques have largely replaced conventional heating in most laboratories. Here the sample is digested in a polytetrafluoroethylene bomb and the process can be completed much more quickly than with conventional digestion/ashing techniques.

Sample Cleanup

Sample cleanup involves purification of the initial crude extract to remove background co-extractives, and at the same time selectively concentrating the target analytes(s) in the extract. The efficiency of the extraction and cleanup steps is measured as 'recovery' which should generally be better than 70% for an analytical method to be acceptable.

Solid-Phase Extraction

Traditionally, small glass columns packed with silica gel or another adsorbent were used for sample cleanup. However, these have now been largely replaced with plastic prepacked cartridges, which are commercially available containing a wide choice of different solid phase materials. Careful choice of a suitable solid-phase extraction (SPE) cartridge and development of appropriate conditions for loading the sample extract and eluting the target analyte(s) remains at the heart of most analytical methods. Reverse phase C-18 SPE cartridges are probably the most widely used, with anion exchange (SAX) and cation exchange (CAX) columns being selected for more polar analytes.

Quick, Easy, Cheap, Effective, Robust, Safe Method for Pesticides

One of the biggest developments in sample cleanup for pesticides in fruits and vegetables has been the introduction of the 'Quick, Easy, Cheap, Effective, Robust, Safe' (QuEChERS) method. This dispersive SPE method involves weighing the food sample into a tube, shaking with acetonitrile, adding magnesium sulfate and sodium chloride, followed by internal standards before centrifugation. An aliquot is taken and further purified with sorbent, centrifuged again, and diluted with acetic acid and 'analyte protectants' before direct instrumental analysis. This simple procedure can be carried out rapidly in a single tube and has been demonstrated to give good recoveries for a wide range of pesticide residues.

Immunoaffinity Column Cleanup

Probably the most effective cleanup technique is based on the use of antibodies which have been raised against the target analyte. The antibodies are linked to a gel matrix and contained in a small plastic column known as an immunoaffinity column (IAC). The crude sample extract or even liquid foods themselves (such as milk) are passed directly through the column, and the analyte is selectively extracted and becomes tightly bound to the antibody. The column can be washed to completely remove any potential interferences, and finally the analyte–antibody bond is broken and the target analyte is eluted in solution. These columns have found widespread acceptance as the definitive cleanup method for analyzing mycotoxins. Immunoaffinity columns are commercially available for individual mycotoxins or combinations of two or three mycotoxins which commonly co-occur. Immunoaffinity columns have also been employed for the cleanup of samples for the analysis of veterinary drugs, pesticides, marine biotoxins, process and environmental contaminants as well as vitamins.

Size-Exclusion Chromatography

Size-exclusion chromatography (also known as gel-permeation chromatography) is a cleanup technique that is useful as it can facilitate collection of a fraction from the sample below a predetermined molecular weight. This technique is not widely used for sample cleanup, although for analysis of oils and fats it is very useful for removal of the bulk of the lipids which is difficult with alternative cleanup methods.

TurboFlow Technology

A recent innovative development in sample preparation employs a combination of size exclusion and SPE in a reusable column operating under 'turbulent flow' conditions. This technology (ThermoFisher Scientific, Franklin, USA) is used in an automated mode, which is directly coupled to the detection system, and provides a very cost-effective approach for routine analysis of large numbers of sample.

Separation Techniques

Despite extensive sample cleanup, it is normal in the final stage of trace analysis to carry out chromatographic separation by either gas chromatography (GC) or liquid chromatography (LC). Lower molecular weight analytes that are sufficiently volatile or can be derivatized to increase volatility are generally analyzed by GC, whereas for nonvolatile analytes LC is the chosen approach.

Gas Chromatography

Gas chromatography involves injecting the sample extract (0.2–10 μl) via a heated injection system onto a capillary column with an internal diameter of typically 0.25 mm and a

length of typically 25–50 m. The column is coated internally with a thin film (e.g., 0.1–0.25 μm) of nonvolatile liquid (stationary phase) and gas is passed through the column at a flowrate of typically 1–2 ml min^{-1}. Separation of a mixture injected into the column takes place by partition between the gas and liquid phases, with the more soluble compounds being retained and the less soluble passing more quickly through the column and eluting first. The column can be operated at a fixed temperature (isothermal run), but more normally the temperature is raised from a starting temperature to such a point as all injected compounds have been eluted from the GC column (temperature programming). A detector (see Sections Nonspecific Detection – Gas Chromatography and Mass spectrometry – Gas Chromatography Mass Spectrometry, Liquid Chromatography Mass Spectrometry, and Inductively Coupled Plasma Mass Spectrometry) is connected to the column outlet so that compounds, which have been separated, can be monitored one by one as they elute. Capillary GC has a high separating power with a wide choice of the column stationary phases enabling the GC analysis to be tailored to engineer the desired separation of analyte from other compounds.

Liquid Chromatography

Liquid chromatography involves injecting an aliquot of the sample extract (typically 10–50 μl) onto an LC column with an internal diameter of 3–5 mm (columns ranging in internal diameter from 0.050 to 4.6 mm or even larger are used for performing large-scale preparative chromatography) and a length of typically 50–250 mm. The column is packed with solid support (3–5 μm particle size) coated with a film of stationary phase. A liquid solvent (mobile phase) is passed through the column at high pressure at a flowrate of typically 0.5–2 ml min^{-1}. Separation of any mixture injected into the column takes place by partition between the liquid phase on the high-performance LC (HPLC) column and the mobile phase, with the more soluble compounds being retained and the less soluble passing more quickly through the column and eluting first. The column is held at a fixed temperature. A detector (see Sections Nonspecific Detection – Gas Chromatography and Mass spectrometry – Gas Chromatography Mass Spectrometry, Liquid Chromatography Mass Spectrometry, and Inductively Coupled Plasma Mass Spectrometry) is connected to the column outlet so that compounds, which have been separated, can be monitored one by one as they elute. The stationary phase is generally made up of hydrophobic alkyl chains ($-CH_2-CH_2-CH_2-CH_3$) that interact with the analyte. Such reverse-phase C-18 LC columns are the most widely employed, although there are a wide range of columns which can be used depending on the desired separation.

Detection and Measurement Systems

Nonspecific Detection – Gas Chromatography

The most widely used detector in GC is the flame ionization detector (FID) which gives a mass response depending on the carbon content of the eluting compound. This is a very robust detector, but is nonspecific in that it responds to all compounds and lacks sensitivity. Other detectors are available with varying degrees of specificity such as the nitrogen/phosphorus, flame photometric, and electron capture detectors, which respond to specific elements such as nitrogen, phosphorus, sulfur, and halogens in the target analyte. However, for additional specificity most analysis for contaminants and residues would normally nowadays be conducted by GC mass spectrometry (GC/MS).

Nonspecific Detection – Liquid Chromatography

In LC, the UV detector is a general purpose detector equivalent to the FID. The diode array detector is similar to UV detector but can operate at a number of different wavelengths thereby providing increased specificity. The LC fluorescence detector offers both high sensitivity and good specificity, but its use is limited to a relatively small number of contaminants that have native fluorescence. The fluorescence detector is the detector of choice for routine analysis of mycotoxins such as aflatoxins, and can also be used for the analysis of polycyclic aromatic hydrocarbons (PAHs).

Atomic Spectrometry for Metals

There are a variety of different instrumental techniques which can be employed to analyze an acid solution containing dissolved elements. The most commonly used technique is known as atomic absorption spectrometry (AAS) in which a flame is used as an atomizer into which the liquid sample is aspirated. There are other variations used to atomize the sample, such as graphite furnace atomization (GFAAS) or electrothermal atomic absorption spectrometry (ETAAS). Analyte species are introduced into a thermally or electrically energetic medium such as a flame, arc, or plasma where molecular bonds are broken. Once the analyte species are converted into free atoms and atomic ions, their determination may be carried out by atomic absorption or atomic emission.

Mass spectrometry – Gas Chromatography Mass Spectrometry, Liquid Chromatography Mass Spectrometry, and Inductively Coupled Plasma Mass Spectrometry

In trace analysis, for the determination of low levels of residues and contaminants, the preferred identification and detection system is undoubtedly the mass spectrometer. The mass spectrometer can be directly coupled to either a GC (GC/MS) or an LC (LC/MS) system. In both cases, the analyte(s) can potentially be identified from its full scan spectrum or the instrument can be used as a sophisticated detector in a mode known as selected ion monitoring (SIM). When several ions are monitored simultaneously in SIM, this offers a reasonably high degree of specificity. This can however be further enhanced by using what is known as tandem mass spectrometry (MS/MS). In this mode of operation, the analyte is initially fragmented in the source of the mass spectrometer to form a characteristic pattern of ions. One or more of these fragment ions are then selected and subjected to conditions to induce further fragmentation (known as collision-activated decomposition). The process whereby these decomposition ions are subsequently monitored is known as multiple reaction monitoring (MRM).

Most MS involve measurement of the nominal mass of the ions (low resolution) which takes no account of the small

differences in mass due to different isotopic abundances of the constituent elements. High resolution MS takes account of these differences enabling accurate mass measurement to be undertaken providing an even greater degree of specificity from the instrument. Typically, dioxin analysis needs to be carried out using high resolution GC/MS in order to separate congeners of the same nominal mass which cannot be separated chromatographically. A resolution of 10 000–15 000 in GC/MS is usually adequate for dioxin analysis. A more recent development has been in LC/MS where time of flight (TOF) and Orbitrap instrumental configurations have become commercially available. LC-TOF/MS instruments offer approximately 20 000 resolution, whereas Orbitrap instruments can offer resolution up to 100 000. Both instruments (Thermo-Fisher Scientific, Bremen, Germany) are still relatively expensive and are still the province of research rather than routine food control laboratories.

A mass spectrometer can also be used for metal analysis by combining a high-temperature inductively coupled plasma (ICP) source with a mass spectrometer (ICP-MS). The ICP source converts the atoms of the elements in the sample to ions. These ions are then separated and detected by the mass spectrometer. Inductively coupled plasma-mass spectrometry has many advantages over other elemental analysis techniques such as AAS and optical emission spectrometry with detection limits for most elements equal to or better than those obtained by GFAAS and its ability to obtain isotopic information. In addition, ICP-MS is a fast, multielemental technique. It is however important to acknowledge that several elements including S, Se, B, Si, P, Br, I, K, and Ca have high detection limits via ICP-MS. In the case of I and Br, this is due to the fact that very few positive ions are formed in the ICP for these elements.

Data Systems and Data Analysis

Modern instruments are normally computer controlled, and this computerization covers optimization of instrumental parameters, control of auto-samplers (for running unattended) through to capture, and handling of data. The simplest of systems such as an LC with a UV detector may only require electronic recording of a chromatogram and integrating one or more peak areas. By comparing the area of the analyte peak with that of a standard, the concentration of the analyte can be estimated. More complex analysis might involve screening of 200 or more pesticides in a sample extract by LC/MS/MS. For each retention time, for each pesticide, two or more ion ratios need to be checked and compared with standards, and MRM areas of ions need to be measured. The more sophisticated the detection system, for example, high-resolution LC/MS, the more complex the data sets that are generated, and the more processing required to check the data quality and to compute the results into concentrations.

Chemometrics

In some situations, the complex datasets generated from instrumental analysis are used to characterize food samples, as being part of a certain population rather than making a simple analytical determination. Statistical techniques known as chemometrics are used to examine a dataset of known samples and to select those parameters which 'characterize' samples in terms of their 'sameness,' and those parameters that can be used to distinguish samples in terms of their 'differences.' These statistical techniques recognize that no two food samples within the same population are identical, but by understanding the natural variability within a population it is possible to estimate a probability that two samples in fact come from different populations. These techniques might be used to distinguish geographical origin of honey or distinguish organic from conventional produce or distinguish wild from farmed fish. In all these three examples for the same food product, no single parameter can distinguish the different types, but multiple variables processed with chemometrics can distinguish one from another.

Laboratory Information Management Systems

Running a modern food testing laboratory is a complex management challenge, as large numbers of samples are received daily, each may require different measurements, there are complex calculations, and reports and certificates must be generated. Quality assurance checks need to be undertaken and recorded for accreditation purposes and all data need to be archived. A Laboratory Information Management System (LIMS) is a customized database that serves multiple needs for sample and data handling. A LIMS typically is used to track samples throughout the laboratory from receipt to storage and archiving (bar-coding is effective). LIMS is usually set up to process the huge amounts of electronic data that are generated by modern instruments and to make suitable calculations of concentrations of residues and contaminants. The LIMS can even be used to automatically generate a certificate using a template for specific customers. Although there can be a high initial set-up cost to customize LIMS, the business case is not difficult to make in terms of cost savings to the testing laboratory.

Method Validation

Once any method of analysis has been developed to determine a food additive, residue, or contaminant, it is necessary to establish how well that method actually performs in practice. This process, whereby the performance characteristics of the method are established, is known as method validation. Method validation can be carried out in a single laboratory (the method originator's laboratory) or preferably should be tested among a number of laboratories.

In-House Method Validation

In-house or single-laboratory method validation is the most basic validation that must be carried out to establish the method performance and is the minimum required for accreditation purposes. Spiked and naturally contaminated samples are employed to establish the limit of detection (LOD), limit of quantification (LOQ), recovery, and between-day repeatability.

Inter-Laboratory Method Validation

Although a method may appear to perform well in the hands of the originator and the performance characteristics from in-house validation may be acceptable, in practice it is frequently the case that some steps in the method may be badly described or the method itself may be instrument specific and thereby difficult to reproduce elsewhere. For this reason, the recommended way to demonstrate that a method has widespread acceptability is to carry out a full inter-laboratory method validation (sometimes known as a collaborative study). In such a study, a group of a minimum of 12 laboratories is requested to scrupulously follow the proposed method and is required to analyze coded blind duplicate samples (both naturally contaminated and spiked at different levels). The results from individual participants are usually kept confidential by the study organizer, although the study results are normally published if acceptable performance characteristics are obtained. A minimum of eight sets of acceptable results are necessary after outliers have been removed during statistical analysis of the validation data.

A number of international bodies such as AOAC International and European Committee for Standardization publish validated methods as their own Official Methods or Standards, respectively. In such cases, the validation data have to be submitted to the organization, where it is closely scrutinized to ensure compliance with the AOAC/International Union of Pure and Applied Chemistry/ISO harmonized protocol, and to decide if the performance of the method itself justifies acceptance. In a number of countries, only prescribed official methods can be used for food-control purposes. In the EU, a criteria-based approach is used. This approach means that the minimum performance characteristics of the enforcement method are published in a Directive, and food control laboratories have a 'free choice' of methods provided the chosen method performs as well or better than the published standards.

Method Performance Characteristics

Method performance characteristics form an objective basis by which methods can be selected to ensure they are 'fit for purpose,' and can be used to compare the relative merits of different approaches. These parameters, however, do not provide any insights into costs, speed, or robustness of methods which have to be separately assessed.

Limit of Detection

The LOD is the smallest signal from the analyte that can be reliably seen against the background noise from the instrumental system. There are various ways of establishing the LOD based on different statistical measures, but the simplest and most commonly used approach is to estimate the amount of analyte which can give a signal three times the average background noise.

Limit of Quantification

The LOQ is the smallest signal from the analyte that can be reliably measured against the background noise from the instrumental system. There are various ways of measuring the LOQ based on different statistical measures, but the simplest and most commonly used is a signal 10 times the average background noise.

Recovery

The method recovery is the amount of analyte measured as a percentage of the amount present in the initial food sample. Usually spiking of blank food samples is carried out with known amounts of analyte(s), and the sample is taken through the complete analytical procedure and then the amount in the final extract is measured. Losses of analyte(s) can occur throughout the analytical procedure, for example, incomplete extraction or during cleanup. In general, acceptable methods should have recoveries from 70 to 110% and this range for recoveries is used as the target range in criteria-based approach.

Accuracy

The method accuracy is the closeness of the measured value to the true value. Although spiking gives some indications of accuracy, spiked samples may behave differently from naturally contaminated ones. Therefore, wherever possible accuracy is measured by comparing 'given values' for the analyte(s) with one's own 'determined values' for the test material. The given values for the test material might be those obtained by a number of expert laboratories in the case of a certified reference material, or consensus value in the case of surplus proficiency test material. In either case, one would expect to obtain measured values within the 95% confidence interval for the prescribed values of the test material.

Method Precision (RSD_r and RSD_R)

The method precision is expressed as both repeatability (r) and reproducibility (R). The repeatability is the within-laboratory precision, which can be determined from a single-laboratory validation and reflects variation when the method is performed in the same laboratory by the same analyst using the same equipment. The reproducibility (R) is the between-laboratory precision which can only be determined from a full inter-laboratory validation and reflects the variation when the method is performed by different analysts in different laboratories using a range of different equipments. Both r and R are frequently reported as percentage relative standard deviations (RSDs). The RSD_r is invariably less than the RSD_R and both are dependant on the amount of analyte being determined. In general, the lower the amount of analyte being determined the higher the method precision. Typical requirements for an analyte which is regulated at levels between 1 and 10 ng g^{-1} (ppb) would be that the RSD_r should be better than 20% and the RSD_R should be better than 30% with a recovery from 70to 110%.

Conclusions

Food analysis has made tremendous advances in the past 10–15 years and we can now measure more and more chemicals in

foods at incredibly low levels. Not only has sensitivity improved, but there has also been a focus on analytical quality, and there is a far greater confidence that analytical results are more meaningful than was the case some years ago. For the future the focus will probably not be on achieving lower limits of detection, but on improving the speed of analysis and reducing costs of analytical measurements.

See also: Environmental Contaminants: Dioxins, Furans and Dioxin-like Polychlorinated Biphenyls; Environmental Estrogens – Hazard Characterization; Nitrate and Nitrite; Perchlorate. Food Additives: Antioxidants; Colorants; Flavors and Flavor Enhancers; Food Additives – General; Natural Preservatives; Preservatives; Sweeteners. Food Safety Assurance Systems: Quality Assurance and Good Laboratory Practice. Foodborne Diseases: Overview of Chemical, Physical, and Other Significant Hazards. Hazards of Food Contact Material: Bisphenol A and Endocrine Disruption; Food Packaging Contaminants; Nanotechnologies and Nanomaterials; Phthalates. Mycotoxins: Aflatoxins; Deoxynivalenol and Other Trichothecenes; Fumonisins; Mycotoxins – General; Ochratoxin A; Patulin; Zearalenone. Natural Toxicants: Alkaloids; Mushrooms and Toadstools; Naturally Occuring Toxins of Plant Origin; Tetrodotoxin. Pesticide Residues: Conazoles; Dithiocarbamates; Herbicides; Inorganic and Other Metal-Containing Compounds; Organochlorines; Organophosphates and Carbamates; Pyrethroids. Processing Contaminants: Acrylamide; Advanced Glycation End Products (AGEs); Benzene; Biogenic Amines; Chloropropanols and Related Esters; Furan; Hydroxymethylfurfural; *N*-Nitrosamines; Polycyclic Aromatic Hydrocarbons (PAHs). Risk Analysis: Risk Management: Application to Chemical Hazards. Toxic Metals: Arsenic; Cadmium; Lead; Mercury; Trace Metals – Chromium, Nickel, Copper, and Aluminum. Veterinary Drugs Residues: Anabolics; Anthelmintics; Antibacterials; Coccidiostats; Control of Helminths; Ectoparasiticides; Veterinary Drugs – General

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Transmissible Spongiform Encephalopathy Diagnosis

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Glossary

Bioassay A scientific test in which the effect of a substance on a living organism is investigated. Bioassays are commonly used to measure the relative strength of a pharmaceutical substance or the toxic effects of environmental pollutant in an animal species. Bioassays in animals are the 'gold standard method' for estimation of the concentration of prions causing transmissible spongiform encephalopathies (TSEs).

Enzyme-linked immunosorbent assay (ELISA) A biochemical test by which antigen (e.g., prion protein (PrP)) can be detected and measured by reaction with an antibody directed to the antigen. Often it is also possible to detect antibodies against the pathogen, produced by the host, however, for prions this is not possible as the prion does not seem to be recognized by the host as foreign.

Histology Histological examination of thin sections of formalin fixed and paraffin embedded tissue has revealed the presence of pathological changes (in particular vacuolation also known as spongiform changes) in the brain tissue. The preserved tissue is often stained, usually with haematoxylin and eosin (H&E), before examination by light microscopy.

Immunohistochemistry Immunohistochemistry builds on the preparation of sections of fixed tissue as described for histopathology, by identifying deposits of PrP with the application of antibodies to PrP followed by secondary antibodies allowing the visualization of staining patterns that are indicative of infection. By using a range of antibodies that target different PrP epitopes as well as different pretreatments of the tissue, this approach can offer a degree of discrimination between strains of prion.

Western immunoblot/effect of glycosylation Western immunoblotting is an analytical technique for the study of proteins, which, after appropriate extraction and preparation of the protein to be examined is subjected to electrophoresis through a gel. This enables molecules to be separated according to their rate of migration through the gel, and these are subsequently detected by the application of antibodies to the protein (here PrP), usually after transfer of the protein from the gel to a membrane that will permit such treatment. Sugar molecules are normally found attached to the PrP, but when studied by the use of biochemical tests, such as immunoblotting, the protein is normally cleaved by incubation with proteases. When the ensuing mix is visualized after electrophoresis through gels, the digested protein becomes segregated by molecular weight in the gel, and visualization by combination with antibodies to PrP usually highlights three distinct bands. The high molecular weight band has two sugar molecules attached (diglycosylated), the middle band has one (monoglycosylated), whereas the lowest has none (unglycosylated). The position of this lowest band, relative to control material from known isolates, is increasingly used to discriminate between isolates, and may potentially represent strain differences. The di- and mono-glycosylated bands are also used to discriminate between isolates, but result in more complex interpretations that are particularly influenced by differences in methodologies between laboratories.

Introduction

The appearance of bovine spongiform encephalopathy (BSE) in the middle of the 1980s immediately prompted the (unpleasant) question regarding the distribution and frequency of the disease in individual countries, Europe, or other parts of the world. This led to the development of various diagnostic tests. Initially the disease was discovered because of its clinical symptoms, yet it became clear that more accurate and more rapid diagnostic methods would be needed in order to control the spreading of the disease as well as to limit the exposure of humans to BSE contaminated meat. However, diagnosis was also a political issue as the detection of BSE cases in a country often led to severe trade restrictions by other, presumably BSE-free countries. This also led to the specification that BSE tests should be 100% specific and sensitive, something which is technically hardly achievable. With progressively better diagnostic methods and extended surveillance, it became clear that BSE was more widespread than originally admitted by the veterinary authorities and that carcasses from BSE-infected animals would enter the human food chain. These findings lead to a culmination in November 1999 with the introduction of the "over 30 months"-rule, that is, animals over 30 months had to be tested with an European Union (EU) approved rapid

BSE test before entering the food chain. As this mandatory testing lead to a strong surge of BSE cases, testing was not questioned for the next 6–8 years. In 2008, 15 EU member states requested a relaxation of the BSE monitoring program based on a favorable risk assessment by the European Food Safety Authority (EFSA) and subsequently these countries received permission to raise the age limit for healthy slaughtered cattle from 30 to 48 months. In 2009, the age limit was raised to 72 months and in 2013 testing of healthy slaughtered cattle was stopped and the EU BSE monitoring program was revised to testing only at risk sub-populations of cattle. In hindsight it is clear that diagnostic tests have been accentuating the BSE crisis but in the end also provided the data to re-assure consumers and to point to residual sources of infection.

Clinical Diagnosis

Historically, transmissible spongiform encephalopathies (TSEs) were diagnosed clinically following examination of suspicious animals with abnormal neurological behavior and the disease was confirmed postmortem by histopathological examination of brain tissue (passive surveillance). With the introduction of routine monitoring programs using rapid screening tests in healthy slaughtered cattle (active surveillance) in the EU and other countries, BSE could be diagnosed also in apparently healthy cattle in the preclinical stage. Classical BSE affects adult cattle and manifests from as early as 20 months to 22 years of age with a peak incidence in animals aged 4–6 years. Like in all TSEs, the clinical picture of BSE is characterized by a slowly progressive course with early signs consisting of subtle behavioral changes, hyperreactivity, and incoordination. As the disease progresses, frequent signs include apprehension, hypersensitivity to touch and sound, abnormalities of posture, and gait involving low head carriage, arched back, abducted, stiff, straight and straddled hind limbs, hind limb ataxia, swaying, trotting, hypermetria, and falling as often shown in videos (http://www.defra.gov.uk/vla/science/sci_tse_rl_video.htm). Further signs described include overreaction to tactile, visual or auditory stimuli, startle response to new objects or small gaps in the ground, excessive licking of the nose or tremors of the head. Individually many of these signs may not be diagnostic, however a combination of clinical signs becomes highly accurate. Frequently observed extraneurological signs in the clinical phase include deterioration in body condition accompanied by weight loss and reduced milk production. The clinical course usually extends over weeks or months and can be as short as 2 weeks or as long as a year or more.

Bioassay

Animal bioassays have been used extensively in TSE research to determine the amount of infectivity and as a gold standard for diagnostic testing. However, bioassays are severely limited in their widespread use by the length of time it takes to obtain results (e.g., cattle material into cattle takes at least 3 years) and for the often used rodent models (hamsters, mice) there is a species barrier effect apparently reducing the amount of infectivity in a given sample. Yet animal bioassays remain the only method to measure directly the infectious agent and are, therefore, the most sensitive assay available for the detection of TSE agents. Although ruminants were used early on to assay for infectivity in sheep and goats, bioassays for research purposes improved dramatically with the introduction of rodent-adapted scrapie strains and their titration by intracranial inoculation of mice and hamsters. A further breakthrough in the detection of TSE agents by bioassay was achieved by the genetic engineering of PrP-deficient (knockout), mutant and transgenic mice. In particular, transgenic mice expressing high levels of heterologous PrP^{C} (e.g., human or bovine) on an otherwise PrP knockout background have been very valuable as a diagnostic tool for the detection of human or bovine prions. Transgenic mice expressing high levels of bovine PrP are approximately 10 times more sensitive than cattle and more than 1000 times as sensitive as wild-type mice to infection with BSE prions.

Histology

With the uncertainties surrounding the etiologic agent of TSEs, a definite diagnosis cannot be made by isolation of the infectious agent from tissues of an infected animal. Therefore, routine laboratory diagnosis relied originally on the identification of pathognomonic markers of the disease by histopathological examination of brain tissues. The most prominent pathological feature is a widespread vacuolation of neuronal cells giving brain tissue a sponge-like appearance, hence the name spongiform encephalopathy. Astrocytic gliosis is also regularly seen although it is more consistently seen in scrapie than in BSE. The extent of astrocytic gliosis in brain tissue can be assessed by immunohistochemical staining using antibodies to the astrocytic marker protein glial fibrillary acidic protein (GFAP). The third morphological feature, amyloid plaques, albeit not consistently seen in all TSEs, are composed of abnormal PrP (PrP^{Sc}) and exhibit the typical fluorescence birefringence of amyloid after staining with the dye Congo Red. During the BSE epidemic in the UK, investigations on brain lesions of cattle with BSE led to the identification of the medulla oblongata (at the level of the obex) as the most suitable diagnostic target site for histopathological examination and this method was used as reference diagnostic tool for the confirmation of a clinical suspect case of TSE.

Immunohistochemistry

With the identification of the disease-specific form of the PrP that accumulates in the brain of animals infected with TSE agents, antibodies to PrP were exploited to perform immunochemical staining of formalin-fixed, paraffin-embedded tissue sections. Studies on field cases of classical BSE and experimentally infected BSE comparing the lesion profile determined by histopathology and PrP^{Sc} deposits by immunohistochemistry, confirmed that the obex region of the medulla oblongata is the primary target site for the TSE agent in the brain. Because of its high sensitivity and specificity, immunohistochemistry for PrP^{Sc} has replaced histopathology as the

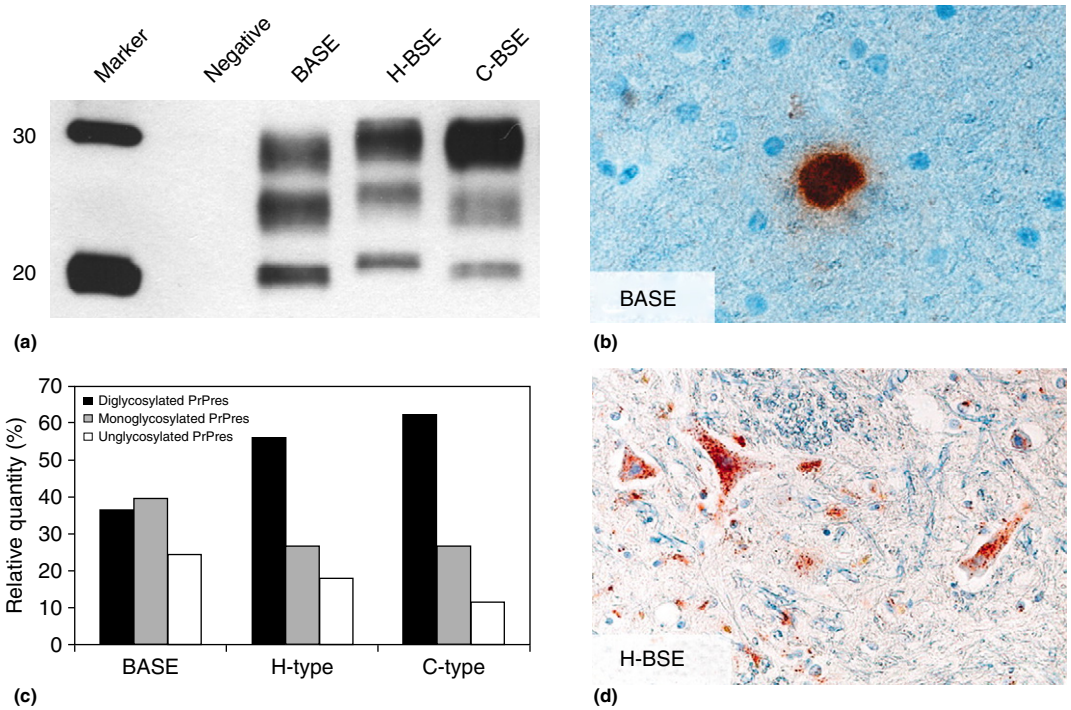

Figure 1 Discrimination of different types of bovine spongiform encephalopathy (BSE) by Western blot and immunohistochemistry. (a) Western blot showing the glycopattern of the two atypical BSE strains, BASE and H-BSE and the classical BSE (C-BSE) strain. In C-BSE cases the diglycosylated PrP^{Sc} band is the most prominent band and is often referred to as PrP27–30 because of its apparent molecular weight ranging from 27–30 kDa. BSE-negative sample served as control. Molecular masses of a protein marker are indicated on the left in KDa. (b) Quantitation of the relative intensities of the three PrP^{Sc} bands reveals that the diglycosylated band in the BASE strain is less prominent than in the H-BSE and C-BSE cases. (c) Immunohistochemistry showing amyloidotic PrP^{Sc} plaques stained with antibodies to PrP as a characteristic feature found in brains of BASE-affected cattle. (d) PrP^{Sc} deposition pattern in H-BSE (zebu [*Bos indicus*], olivary nuclei) resembles that in C-BSE. Reproduced from Seuberlich *et al.* (2010) A typical transmissible spongiform encephalopathies in ruminants: A challenge for disease surveillance and control. *Journal of Veterinary Diagnostic Investigation* 22: 823–842, with permission from Sage.

reference diagnostic method for TSEs and thus was adopted by regulatory authorities and reference laboratories as the standard method for confirmation of a suspect TSE case (Figure 1(b)). In addition, the PrP^{Sc} distribution pattern in various brain regions has become an important tool for the characterization of TSE strains.

Western Immunoblotting

Diagnosis of TSEs by Western immunoblotting is based on the presence of protease resistant PrP (PrP^{Sc}) which is pathognomonic for TSEs. In an unaffected animal PrP^{C} with an apparent molecular weight of 33–35 kDa is protease sensitive and completely digested following incubation with proteinase K. In TSE affected organisms PrP^{Sc} is partially protease resistant and digestion with proteinase K leads to the removal of the *N*-terminus leaving a protease resistant core fragment with an apparent molecular weight of 27–30 kDa (PrP27–30). Sample collection and homogenization is one crucial part of the diagnostic tests and influences the diagnostic sensitivity and specificity. Both forms of PrP are associated with membranous cellular elements and thus detergents are required during tissue homogenization to allow efficient solubilization of PrP. In addition, conditions for protease digestion need to be optimized in order to allow complete digestion of PrP^{C} but only removing the *N*-terminus of PrP^{Sc} thereby leaving intact the protease-resistant core, PrP27–30, for the subsequent detection by anti-PrP antibodies. A piece of tissue from the brain stem (obex region) is homogenized and treated with proteinase K. The proteins in the sample are resolved by denaturing polyacrylamide gel electrophoresis and transferred to a membrane. The protease resistant fragments of PrP are detected on the membrane using a PrP-specific antibody directed to a sequence in the protease-resistant core and visualized using an enzyme-coupled secondary antibody with the corresponding substrate detection system. The presence of a PrP-immunoreactive signal with the additional two criteria of a reduced molecular weight (due to digestion of the *N*-terminus of PrP^{Sc}) and a typical three-band pattern representing unglycosylated, monoglycosylated, and diglycosylated PrP^{Sc} forms result in a TSE-positive diagnosis. The main advantage of the Western immunoblotting technology compared to Enzyme-linked immunosorbent assay is its high specificity due to the three independent criteria used for diagnosis. The Western immunoblotting technology has been validated and approved by the International Office for Epizooties (OIE) as a method to confirm postmortem the diagnosis of suspect or clinical cases including confirmation of a positive result in a rapid screening test. Furthermore, the Western blot is the only

diagnostic laboratory method suited for the discrimination of different TSE strains either using different antibodies that recognize strain specific epitopes on PrP or by comparing the relative intensities of the three protein bands on the blots representing the unglycosylated, mono-, and diglycosylated form of PrP (Figure 1(a)).

ELISA

Several ELISA have been developed and commercialized as rapid screening tests for TSEs. Most of them rely on a tissue homogenization procedure followed by protease digestion to eliminate PrP^{C} from the sample and prepare the analyte PrP27–30 for detection by anti-PrP antibodies. Some ELISA tests make use of the different conformational properties of PrP^{C} and PrP^{Sc} and use PrP^{Sc}-specific binding reagents that allow capture of full-length PrP^{Sc} without proteolytic degradation of PrP^{C}. Detection occurs by sandwich ELISA using two monoclonal antibodies, one of them enzyme labeled. The digested homogenate is either directly incubated with the capture antibody immobilized on the microtitre plate, or alternatively incubated with the detection antibody and the mixture is then transferred to the microtitre plate that is coated with the capture antibody. Detection is colorimetric or based on chemiluminescence and results are analysed using validated cut-off settings. The main advantage of ELISA tests is simplicity, ease of use, and capability for full automation which allows high throughput of samples. The disadvantage of ELISA tests is the occasional false positives due to incomplete digestion of PrP^{C} or cross reactivities of the antibodies with other protein in the brain extract.

Lateral Flow Immunochromatography

Lateral flow immunochromatographic (LFI) assays or strip tests are essentially immunoassays adapted to operate along a single axis to suit the test strip format. The basic technology that underlies LFI assay was first described in 1960s, but it was not until 1988 that the first commercial application as home pregnancy tests was launched. Similar to ELISA tests, strip tests employ a tissue homogenate subjected to treatment with proteinase K followed by incubation with the conjugate consisting of a colored latex bead-labeled anti-PrP antibody in a microtitre format. The strip is inserted into the sample-conjugate reaction mixture and the sample then continues to migrate across the membrane until it reaches the capture zone where the immunocomplexes consisting of PrP27–30 bound to antibody residing on the latex bead are captured by the immobilized second monoclonal anti-PrP antibody sprayed as a line perpendicular to the long axis of a strip producing a colored line on the membrane. The sample then migrates further along the strip until it reaches the control zone, where excess conjugate will bind and produce a second colored line on the membrane. This control line indicates that the sample has migrated across the membrane as intended. Two clear lines on the membrane represent a positive result. A single line in the control zone is a negative result. The reading and result interpretation can be done either visually or automatically allowing a quantitative analysis using a flat-bed scanner, and dedicated software. To allow for high throughput testing, individual strips are arranged in a comb-shaped cartridge holding eight strips. The main advantage of LFI assays is their speed and ease of use with only minimal equipment needed which makes them superior to conventional ELISA tests.

Rapid BSE Tests as Screening Tools

Together with other measures to control and eradicate BSE , postmortem testing of healthy cattle over 30 months of age was introduced in Europe in 2001 and later also in Japan for all cattle over 21 months of age. Although testing for BSE and subsequent removal of infected animals from the food chain was important to regain consumer confidence, rapid BSE tests could only eliminate a proportion of all infected animals even though that part represents the animals containing the highest amount of infectivity. Experimental BSE infection studies have shown that all postmortem diagnostic tests can detect a BSE infected animal up to 6 months before onset of clinical signs when substantial levels of infectivity have already accumulated in nervous tissues.

The introduction of active BSE surveillance required the implementation of routine sampling procedures in the abattoir, the set-up of a logistics chain to send the samples to accredited laboratories, and the development and validation of rapid BSE screening tests for diagnosis using fresh brain tissue as sample matrix. The sampling of brain tissue in the slaughterhouse involves the removal of the medulla oblongata from the skull of slaughtered animals through the foramen magnum. This procedure is described in the latest edition of the 'Manual of standards for diagnostic tests and vaccines' of the OIE. The sample is transported to the laboratory and a subsample of the obex region is used for diagnostic analysis. Testing is performed by accredited laboratories and the test must be performed exactly as described in the manufacturer's instructions of use. If the result from the rapid screening test is negative, the animal carcass can be released from the abattoir. A positive or inconclusive result in the initial screening test needs to be confirmed by the National or European Reference Laboratory (EURL) using one of the approved confirmatory tests. If the initial positive test result is confirmed, the carcass is not to be used for human consumption and needs to be destroyed in accordance with the respective regulations.

The routine laboratory testing with rapid BSE tests in the EU is controlled and overseen by the EURL which organizes regular testing of proficiency sample panels for comparison of performance between testing laboratories. In addition, the test manufacturers have to comply with defined quality management requirements and each kit lot is undergoing a European batch release procedure which is unique in the field of veterinary diagnostics.

Validation of Rapid BSE Screening Tests

Since 2001, when compulsory active surveillance of healthy cattle over 30 months of age was introduced in the EU, rapid postmortem tests for BSE have been evaluated and approved

Table 1 EU approved rapid postmortem tests for BSE (Regulation (EC) 999/2001)

	Laboratory evaluation			*Field trial*				
	Sensitivity in % (95% CI in %)	*Specificity in % (95% CI in %)*	*Detection limit*	*Healthy slaughtered*			*Poor quality*	
				Sensitivity in % (95% CI in %)	*Specificity in % (95% CI in %)*	*IR*	*Specificity in % (95% CI in %)*	*IR*
Prionics® Check WESTERN	100 (99)	100 (99.7)	1:100[a]	nd	nd	nd	nd	nd
Bio-Rad TeSeE	100 (99)	100 (99.7)	≥1:200	nd	nd	nd	nd	nd
Enfer TSE kit	100 (99)	100 (99.7)	1:30	nd	nd	nd	nd	nd
Prionics® Check LIA	100 (93.8)[b]	100 (98)	≥1:200	100 (98.7)	100 (99.97)	0.86	100 (98.5)	
IDEXX HerdChek® BSE Test	100 (94)	100 (98)	≥1:200	100 (98.5)	99.99 (99.95)[c]	0.39	100 (98.5)	0
Prionics® Check PrioSTRIP	100 (94)	100 (98)	1:100	100 (98.5)	100 (99.97)	0.37	99.6 (98.2)	1.34
Roboscreen Beta Prion BSE Test	100 (94)	100 (98)	≥1:200	100 (98.5)	100 (99.97)	0.67	100 (98.5)	0
Roche PrionScreen	100 (94)	99.3 (96.2)[d]	1:100	100 (98.5)	100 (99.97)	0.2	100 (98.5)	27
Enfer TSE kit v2	100 (94)	100 (98)	≥1:200	100 (98.5)	100 (99.97)	5.81	100 (98.5)	0

[a]Detection limit in the 1999 EU evaluation was 1:10; the EU evaluation in 2002 showed a detection limit between 1:81 and 1:243.

[b]One sample which was initially a false negative was found positive after retesting of additional tissue and must be considered as a sample with low and/or heterogeneous PrP^{Sc}.

[c]Four samples were classified initial reactive; three of them were negative after retesting and one retested positive.

[d]One negative samples out of three initial reactives was positive in retesting.

95% CI in %, 95% confidence interval in %; IR, initial reactive rate in ‰; nd, not done.

by the EU authorities. A new rapid BSE test is considered fit for purpose if the performance of the test meets the requirements defined in the 'Protocol for evaluation of new rapid BSE post mortem tests' published under the auspices of the EFSA. The protocol in principle states that each new test submitted by a manufacturer to the EU under a call for expression of interest will be subjected to a two stage evaluation procedure consisting of a laboratory evaluation and a field trial. In this evaluation a new test should perform not inferior to an already approved rapid BSE test. The whole evaluation exercise is supervised by the Institute of Reference Materials and Methods .

The hallmark of any diagnostic test is its diagnostic sensitivity (ability to identify infected reference samples as positive) and specificity (ability to identify uninfected reference samples as negative). This will determine the usefulness of a test in the field, i.e., how many true and false positives it will produce. The diagnostic sensitivity and specificity of BSE tests were evaluated by the EU commission using defined numbers of positive and negative samples from animals previously diagnosed for TSE with a reference method such as histopathology or immunohistochemistry. All rapid BSE tests approved for BSE surveillance have a diagnostic sensitivity of 100% and a diagnostic specificity of 99.3% or 100% (Table 1).

In addition, the evaluation protocol included an assessment of the detection limit using serial dilutions of a BSE-positive brain homogenate diluted in negative brain homogenates. Determination of the detection limit for the analyte PrP^{Sc} should give an indication on the test's capability to detect preclinical cases of BSE. In this evaluation exercise the lowest detection limits of the rapid tests ranged from a dilution of 1:30 up to at least 1:200 (Table 1). However, the analytical sensitivity may or may not correlate with the diagnostic sensitivity depending on the appearance and concentration of the analyte during the course of the disease. For this reason, slight variations in analytical sensitivity do not necessarily reflect differences in diagnostic sensitivity.

The rapid BSE tests were further evaluated in a field trial in order to determine their performance under normal routine conditions. This enabled a thorough assessment of parameters like robustness, applicability in different laboratories, expected rate of false initial reactives under high throughput field conditions, and necessary cut-off adjustment before market introduction. All tests showed a diagnostic specificity of 99.9% – 100% on tissue samples from healthy slaughtered cattle that were tested negative with an approved reference test. Similarly, all evaluated rapid tests showed a diagnostic sensitivity of 100% using a set of true positive samples that were tested positive using a reference test. In addition the tests were assessed for their performance on poor quality samples. This is important because field samples are often in a deteriorated state due to poor storage conditions of carcass samples over long periods of time. Such samples show extended autolysis and a robust diagnostic test has to give accurate results under such conditions. In this evaluation, one test had a specificity of 99.6% and all other tests showed a specificity of 100%. Table 1 shows an overview of the rapid BSE tests that are approved for BSE surveillance within the EU and are listed in Annex X Chapter C of the TSE Regulation (EC 999/2001).

Field Applications of Rapid BSE Tests

Between 2001 and 2010, approximately 10 million rapid BSE tests were performed in the EU per year and approximately 1 million per year in Japan within the compulsory rapid testing

of risk animals and slaughtered cattle. Routine statutory testing algorithm in the EU requires that each screening test-positive sample is retested by either a confirmatory test or a second screening test in order to confirm or reject the initial positive result. As confirmatory test either immunohistochemistry or an ultrasensitive Western blot procedure combined with enrichment of PrP^{Sc} by a centrifugation protocol are employed. Large scale field performance of three rapid BSE tests – Western blot, ELISA, and LFI – for the BSE surveillance program in Italy on more than 2.8 million samples from slaughtered animals resulted in the identification of 81 positive BSE cases. Both the Western immunoblotting test as well as the LFI test gave no false-positive results whereas the ELISA showed a false-positive rate of 0.94 per 100 000 negative samples tested. In the same study the rate of false-positives in fallen stock samples was recorded. In 200 000 fallen stock samples tested, the Western immunoblotting test showed no false-positive results while ELISA and LFI gave false-positives rates of 84.3 and 8.48 per 100 000 negative samples tested, respectively. Although all tests showed a similar high diagnostic sensitivity and specificity in the EU validation, ELISA and LFI based rapid tests tend to show increased rates of false positive results when used in routine testing compared to Western blot technology.

Diagnosis of Atypical BSE Cases

The introduction of mandatory active surveillance in the EU using rapid BSE tests allowed more systematic investigations into the circulating BSE disease phenotypes. Strain typing analysis of spongiform lesions and PrP^{Sc} distribution patterns in the brains of several hundreds of British BSE cases indicated that the epidemic was sustained by a single or major strain of TSE agent which was distinct from the many different strains of scrapie agent. These findings were confirmed by strain typing studies in mice. Nevertheless, given the magnitude of the epidemic in Britain, it seemed likely that a minor strain could have escaped recognition and that new strains of BSE would surface once the epidemic would decline further.

In 2004, the first atypical BSE strains exhibiting novel molecular and pathological phenotypes were identified in Italy, France, and Japan. These strains were detected among routinely diagnosed BSE cases in the active surveillance using rapid BSE tests. All atypical BSE cases were found in apparently healthy cattle with no clinical signs suggestive of BSE. Whereas the cases in Italy and France were found in old cattle, the Japanese case was the first BSE case confirmed in an animal less than 2 years old. Characterization of the atypical BSE cases was performed by strain typing using Western blot analysis of the protease-treated PrP^{Sc}. Classically, TSE strains have been characterized through inoculation of brain homogenates into a series of mouse lines. Distinct strains are then differentiated based on incubation time and lesion profile within the same mouse line. These are the most important criteria for differentiation of strains. More recently and with the availability of immunochemical diagnostic methods, a more detailed analysis was made possible based on glycosylation patterns of protease-treated PrP^{Sc}. On Western blots, proteinase K-treated PrP^{Sc} is resolved into three bands that correspond to the di-, mono-, and unglycosylated PrP. These bands can exhibit different mobility patterns, presumably reflecting different conformations of PrP^{Sc} associated with different prion strains. All classical BSE cases (C-BSE) that were analyzed showed the same PrP glycopattern with the highest intensity for the diglycosylated form of PrP (**Figure 1(b)**). The French animals presented with a higher apparent molecular mass of the unglycosylated PrP (**Figure 1(a)**) hence the cases were later designated H-BSE with 'H' referring to the higher molecular mass. The Italian cases showed a PrP^{Sc} type with predominance and a lower apparent molecular mass of the unglycosylated PrP which led to the designation L-BSE with 'L' for lower molecular mass. Further investigations on the pathology of the Italian atypical BSE cases by immunohistochemistry revealed a different distribution of PrP^{Sc} deposits and PrP-positive amyloid plaques, a feature which has not been described before for classical BSE (**Figure 1(c)**). These findings prompted the authors to name this disease BASE, for bovine amyloidotic spongiform encephalopathy. Worldwide, some 50 atypical cases of BSE have been described with all of them being diagnosed in animals between 8 and 20 years of age. In contrast most C-BSE cases fall into an age range of 5 – 6 years. Clearly, atypical BSE represents a different disease entity than C-BSE and this led to the notion that atypical BSE could arise spontaneously similar as sporadic prion diseases in humans. This is in agreement with the low prevalence of atypical BSE of 1 case per 3 million adult cattle. From a diagnostic perspective, all atypical BSE cases were identified in routine surveillance by approved rapid BSE tests, suggesting that these tests are capable to detect all forms of BSE although they were not evaluated for atypical BSE.

Conclusion

The history of BSE control and eradication is merely 20 years old but the changes that were brought about by the measures implemented to protect the global food supply and public health are unique among newly emerging zoonotic disease in livestock animals. Together with the ban on feeding ruminant derived meat and bone meal to ruminants and the removal of specified risk materials, monitoring programs using rapid BSE tests played an important role in the management of risks posed by the BSE epidemic. Although none of the currently used diagnostic test methods is able to detect the disease in a living animal or at an early stage of infection, routine active surveillance for BSE allowed removal of a significant proportion of infected cattle and therefore the majority of infectivity from the food chain.

Before being applied as routine tests for consumer protection, rapid BSE tests underwent rigorous validation by the competent authorities and, once marketed, were required to undergo quality assurance and quality control measures to an extent that is so far unseen in the field of veterinary diagnostics.

From a scientific perspective, the introduction of rapid BSE tests has led to the identification of atypical cases of BSE which seem to represent sporadic forms of BSE that were most likely present in the cattle population before the BSE epidemic

started in the UK. Although transmission studies in transgenic mice have shown that atypical BSE replicates at least as efficiently as C-BSE, it remains to be determined whether infectivity distribution in different tissues is also similar between the two disease entities. These uncertainties underscore the fact that continued surveillance and monitoring of BSE is crucial to minimize potential risks from atypical BSE cases and maintain consumer protection at a high level.

See also: Prions and Agents of TSEs: Creutzfeldt–Jakob Disease

Further Reading

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CHARACTERISTICS OF FOODBORNE HAZARD AND DISEASES

Contents

Pathogenesis and Virulence

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Glossary

Bovine spongiform encephalopathy A progressive, fatal neurodegenerative condition in cattle, also known as mad cow disease, caused by infection with a prion.
Cytokines Proteins synthesized and secreted by inflammatory leukocytes and some nonleukocytic cells that act as intercellular mediators and affect the immune response.
Guillain–Barré Syndrome A temporary immune-mediated neuropathy that may occur days or weeks after a viral infection, during the recovery stage, and presents with progressive muscle weakness and paralysis.
Integrins Heterodimeric transmembrane glycoproteins consisting of noncovalently linked α- and β-subunits, which mediate cell–cell or cell–matrix interactions and function in signal transduction.
Lysosome A membrane-enclosed low-pH intracellular organelle that contains hydrolytic enzymes, where components destined for breakdown are digested.
Microvilli Actin-rich microscopic cellular projections that are found on many types of epithelia and increase the cellular surface area.
Phagosome An intracellular vesicle that forms after the plasma membrane fuses around particles engulfed by phagocytosis, such as cells, tissue debris, or microorganisms.
Variant Creutzfeldt–Jakob disease A rare, progressive, fatal neurodegenerative condition in humans, causally linked to a prion that also caused BSE, characterized by the spongy degeneration of the brain.

Introduction

Foodborne illnesses represent a global medical and public health concern with an economic and social burden that is difficult to quantitate. One in four individuals in the US and approximately one in five individuals in England develop acute gastroenteric infections annually. For $>80\%$ of the foodborne infections, the causes remain unknown. Foodborne pathogens include bacteria, viruses, protozoa, microbial toxins, or chemicals that employ different pathogenesis strategies at various stages during their interaction with the host. These strategies include resistance to acidic conditions, disruption of the intestinal epithelial barrier, survival in vacuoles, escape from phagocytosis, inhibition of protein synthesis, actin-based motility, or manipulation of the immune response. In certain instances, studies of the host–pathogen interaction enabled the discovery and characterization of new cellular components with physiological roles. For example, research on *Listeria monocytogenes* ActA facilitated the discovery of the Arp2/3 family of eukaryotic actin-nucleating proteins. Another foodborne pathogen, *Campylobacter jejuni*, the main cause of bacterial diarrhea in European Union countries, was causally linked to the Guillain–Barré syndrome, a peripheral neuropathy that represents the most frequent cause of acute neuromuscular paralysis, and exemplified the first instance when molecular mimicry explained the pathogenesis of this condition. Insights into the virulence factors of foodborne pathogens will be

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instrumental in designing and implementing prophylactic and therapeutic strategies to benefit global public health, and should be the focus of expanding research endeavors in the coming decades.

Resistance to Acidic Conditions

A key requirement, for many pathogens that colonize the gastrointestinal tract and cause disease, is their ability to survive acidic conditions. At pH levels between 1.5 and 2.5, the gastric acid is one of the harshest and most inhospitable environments in the human body, but several pathogens are able to survive these conditions and cause disease. The terms acid tolerance, acid resistance, and acid habituation have been used to describe the ability of certain microorganisms to survive acidic environments.

Certain pathogens survive in the gastric environment better than others. For example, studies on human volunteers revealed that 50–100 *Shigellae* are sufficient to cause dysentery in healthy adults, as compared to 10^8 organisms that are required in the case of other pathogens, such as *Vibrio cholerae*. At least in part, this is explained by the better ability of certain pathogens to survive the acidic conditions of the stomach.

Five acid response pathways that were described in *Escherichia coli*, AR1–AR5, protect bacteria in log or stationary phase growth conditions. AR1 operates in stationary phase cells growing under moderately acidic conditions, at pH ~ 5.5, requires the stationary phase alternative transcription factor RpoS and the cyclic adenosine monophosphate (cAMP) regulatory protein CRP, and is repressed by glucose. AR2–AR4 involve amino acid decarboxylase-based pathways – glutamate, arginine, and lysine decarboxylases for AR2, 3, and 4, respectively. AR2, the glutamate decarboxylase system, and the one that was most extensively studied, represents the major pathway allowing bacterial survival under extreme acidic conditions, such as at pH 2.5. In this system, two decarboxylases, GadA and GadB, use a cytoplasmic proton to convert glutamate to GABA and carbon dioxide, and an inner membrane antiporter, GadC, removes GABA from the cell in exchange for more glutamate. *Escherichia coli* acid resistance depends on the growth conditions and is present only during growth in rich media. In minimal media with glucose, the pathogen is not resistant to acid stress. AR5, an ornithine–putrescine antiporter encoded by the *potE* gene, was less extensively studied.

Salmonella has acid tolerance response mechanisms that protect under log or stationary phase conditions. Shifting the bacteria to extremely acidic pH requires a previous exposure to moderate acidity, but the pathogen can survive at pH 3 for several hours. One difference between *E. coli* and *Salmonella* is that in complex media, stationary phase *E. coli* is more resistant than *Salmonella* to low pH, and may survive at pH 2 for several hours. *Salmonella enterica* serovar Typhimurium has an arginine decarboxylase, AR3, which is induced under anoxic conditions, and a lysine decarboxylase, AR4. As opposed to the *E. coli* AR4 system, where *cadC*, which activates transcription of the *cadBA* operon that converts lysine into cadaverine and CO_2, is constitutively expressed, *cadC* in *S. enterica* AR4 is induced by low pH and lysine. *Salmonella* has two additional acid stress response pathways. One of them is induced in exponentially growing cells that undergo a transition from moderate to low pH (pH < 3) and involves > 60 acid shock proteins. The other system, which operates in stationary phase cells, involves 48 genes, of which only five overlap with the genes required during the log phase acid stress response.

Helicobacter pylori, a gram-negative microaerophilic pathogen linked to gastritis, and certain strains to gastric cancer, has a particularly strong ability to survive the acidic gastric environment due to urease, one of its most prominent proteins that is essential for colonizing the mucosa. Urease helps the pathogen hydrolyze urea into carbon dioxide and ammonia. Ammonia is converted to ammonium, which reacts with the carbon dioxide and forms bicarbonate, neutralizing the gastric acidity. *In vitro*, *H. pylori* can survive at pH 1 for several hours in the presence of urea. The urease operon has seven genes. *UreA* and *ureB* have structural roles, and *ureE–ureH* are required for inserting Ni^{2+} into the apoenzyme. *UreI* encodes a six-transmembrane-domain integral membrane protein and functions as a proton-gated channel that is activated at acidic pH, when it increases urea transport into the cytoplasm.

Listeria monocytogenes, a gram-positive facultative anaerobe, utilizes a glutamate decarboxylase system similar to the one in *E. coli*. The *gadA* and *gadB* genes encode two glutamate decarboxylases, and *gadC* encodes a glutamate-γ-aminobutyrate antiporter. In addition, a two-component signal transduction system, encoded by *lisRK*, is important for stationary-phase acid tolerance and *in vivo* virulence, and an F_0F_1 ATPase removes protons from the cytoplasm. This multisubunit complex has a cytoplasmic domain, F_1, with a catalytic role, and an integral membrane domain, F_0, which functions in proton translocation. Its role is to generate ATP aerobically when protons enter the cell, and hydrolyze ATP anaerobically, generating the proton motive force, when protons move out of the cell, an activity that can increase the intracellular pH when it becomes acidified.

Type III Secretion Systems

Many gram-negative bacteria have molecular injection devices known as type III secretion systems, which transport bacterial effector proteins across bacterial and host membranes into the host cells, where they perform specific virulence-related functions. Because the two cells establish direct contact, this process is also referred to as contact-dependent secretion.

Type III secretion was first described in 1994 in *Yersinia*, and over 20 systems were characterized to date in animal, plant, and insect pathogens. It enables pathogens that are extracellular or reside in phagosomes to inject bacterial proteins across the membranes, into the cytosol, where they hijack the eukaryotic cellular machinery, induce cytoskeletal changes, and/or initiate signal transduction pathways. The type III secretion apparatus, often referred to as a 'molecular syringe' or injectisome, represents a complex macromolecular machinery that spans the inner bacterial membrane, the periplasmic space, the peptidoglycan layer, the outer bacterial membrane, the extracellular

space, and the host cell membrane. Although type III secretion systems coexist in many pathogens with additional virulence factors, they represent a major virulence determinant. A simplified view depicts the injectisome as a basal body built of two sets of rings positioned within the inner and outer bacterial membranes, a needle-like structure extending 20–50 nm from the bacterial cell surface, through which the proteins travel, and the translocon, which is the pore formed by hydrophobic proteins inserted into the eukaryotic membrane. The injectisome is assembled in three steps. The basal body is formed first. This is followed by the assembly of the needle structure, from a single protein that is synthesized in the cytoplasm, secreted to the cell surface, and polymerized via its *C*-terminal amino acids. The 2–2.5-nm internal diameter of the needle allows effector proteins to travel in an unfolded state. Straight and hollow needle-like structures were visualized by electron microscopy in several pathogens, including *Salmonella*, *Shigella*, and *Yersinia*. In a third step, proteins that form the translocon, the pore-forming complex inserted into the host cell membrane, are secreted and localize to the target membrane. The components of type III secretion systems are tightly regulated at several levels.

Microorganisms that use different pathogenesis strategies and cause clinically distinct diseases often rely on very similar type III secretion systems as one of their major virulence mechanisms. For pathogens from the *Yersinia* genus, type III secretion is key to virulence. Before contacting a eukaryotic cell, *Yersinia* cells develop, on their surface, syringe-like structures made of ~25 proteins that form the Ysc injectisome. On encountering professional phagocytic cells, the pathogen upregulates a group of proteins known as *Yersinia* outer proteins (Yops) which orchestrate type III secretion. Two secreted proteins, YopB and YopD, are the translocators that become inserted into the host cell plasma membrane, and form a multimeric 500–700-kDa integral membrane complex. This process also requires a chaperone, LcrH in *Yersinia pseudotuberculosis* and SycD in *Yersinia enterocolitica*, and the pores allow the transport of effector proteins into the cytosol. Inside the eukaryotic cell, Yops perform specific functions. YopH, YopJ, YopE, YopT, YpkA, and YopM modulate the dynamics of the cytoskeleton and prevent the pathogen from being phagocytized. YopH, one of the most powerful tyrosine phosphatases known, dephosphorylates proteins from the focal adhesion complex, some of which are involved in adhesion during phagocytosis, and inhibits integrin signaling and phagocytosis. *Yersinia pseudotuberculosis* YopJ (YopP in *Y. enterocolitica*) is a cysteine protease that inhibits mitogen-activated protein kinase (MAPK) and nuclear factor kappa B (NF-κB) signaling pathways, promoting apoptosis in macrophages and suppressing the inflammatory response to the pathogen. In addition, YopJ/YopP is a promiscuous deubiquitinating enzyme that removes ubiquitin groups from key cellular proteins, such as IκB-α, negatively regulating signaling pathways. YopE, a Rho GTPase activating protein (GAP), inactivates Rho GTPases and causes the depolymerization of actin microfilaments, which enhances the ability of the pathogen to resist phagocytosis and inhibits the inflammatory response in the host. YopT, a cysteine protease, cleaves RhoA, Rac, and Cdc42 *N*-terminally from a prenylated cysteine, causing their inactivation by releasing them from the membrane, and subsequently disrupting the actin cytoskeleton. *Yersinia pseudotuberculosis* YpkA (YopO in *Y. enterocolitica*) inhibits nucleotide exchange by the Rac1 and RhoA GTPases, and disrupts the actin cytoskeleton. YopM, unique among the Yops due to its ability to traffic to the eukaryotic cell nucleus, and important for virulence *in vivo*, binds the ribosomal 6 kinase (RSK) family of proteins, a group of serine/threonine kinases involved in cell growth, motility, and survival. YopM binding to RSK causes sustained RSK activation by inhibiting dephosphorylation of the active, phosphorylated form of the protein, revealing a new strategy for pathogenesis, in which a bacterial virulence factor hyperphosphorylates and overactivates RSK family kinases.

Salmonellae provide an interesting example by being the only species identified to date that uses two different type III secretion systems encoded on different pathogenicity islands. After colonizing the small intestine, *Salmonella* invades nonphagocytic epithelial cells and gains access to the lymphatic tissue, a process mediated by a type III secretion system encoded on the SPI1 *Salmonella* pathogenicity island. SPI1, a ~40-kb chromosomal region located at centisome 63 on the chromosome, is the best characterized SPI from the five that exist in this bacterium. The virulence of SPI1 mutants is attenuated in animal models when the pathogen is administered orally, but not systemically, indicating that this pathogenicity island, thought to have been acquired earlier during evolution, might have had roles limited to intestinal colonization, and additional virulence factors, acquired later, enabled the pathogen to cause systemic disease. SPI1 is most important for crossing the intestinal barrier and for establishing intestinal inflammation. In addition to factors required to infect intestinal epithelial cells, *Salmonella* also encodes virulence factors needed for systemic infection. The second type III secretion system, encoded by the SPI2 pathogenicity island at centisome 31 on the chromosome, contains virulence factors needed for the systemic phase of the infection and for survival in macrophages. However, this division is not very sharp, as certain SPI1 components mediate intracellular effects, and certain SPI2 components are important during intestinal inflammation. SPI2 has been more difficult to study *in vitro*, particularly because genes from this pathogenicity island are expressed only after the pathogen enters host cells. Genes encoded in SPI3 and SPI4 are also required for the systemic stage of the disease, and SPI5 is required for inflammation and chloride secretion that are part of the enteric disease.

The *Shigella* type III secretion system represents a key virulence determinant encoded on a large, ~220-kb plasmid. *Shigella* uses membranous epithelial cells (M cells) to cross the intestinal epithelial layer, and invades epithelial cells through their basolateral surface. During entry, type III secretion allows the pathogen to form pores, made of the IpaB and IpaC proteins, two major invasins, in the cytoplasmic membrane of the target cells, and inject effector proteins into the cytosol. Additionally, the *C*-terminus of IpaC recruits and activates the Src tyrosine kinase, inducing actin polymerization. Enninga *et al.* visualized *Shigella* type III secretion in real time, and revealed that the process is activated on contact with the host cell. Approximately 50% of the IpaB and IpaC effectors were released within 24 s after contact. The *Shigella* type III

secretion system, known as Mxi–Spa, involves ~50 proteins, ~25 of which are effectors. Among these, three classes of effectors were described. Certain proteins are produced independently of type III secretion, others are positively controlled by the type III secretion system, and a third class contains proteins that are weakly expressed when type III secretion is not active, but their expression further increases on its activation.

An interesting example reveals that proteins encoded in the type III secretion system enable certain pathogens to initiate cytoskeletal changes in the host even before entering the cell. Enterohemorrhagic and enteropathogenic *E. coli* (EHEC and EPEC), major causes of diarrhea, subvert the actin cytoskeleton even before entering the host cell. EHEC and EPEC cause attaching and effacing (A/E) lesions, intestinal epithelial changes characterized by the loss of microvilli, the adherence of bacterial cells to the host cell membrane, and the formation of actin pedestals, which are raised actin-filament rich structures located under the bacterial attachment sites. Both pathogens harbor a ~35-kb chromosomal pathogenicity island called the locus of enterocyte effacement (LEE), which encodes proteins that are part of a type III secretion system and is sufficient to render a nonpathogenic *E. coli* strain capable of inducing A/E lesions, indicating that it contains all the genetic determinants required for this process. This region has a much lower G + C content than the rest of the *E. coli* chromosome (~38% vs. ~51%), suggesting that it was acquired from a foreign source. In the bacterial chromosome, LEE is located at the site that encodes selenocysteine transfer ribonucleic acid (tRNA), and this is also the location where several other pathogenicity islands, encoding other virulence genes, are inserted, including the ones from uropathogenic *E. coli*.

One of the proteins involved in A/E, translocated intimin receptor (Tir), formerly known as Hp90 (EspE in EPEC) is injected by the pathogen into the host cells to function as the receptor for intimin, a bacterial outer membrane protein. Tir is secreted in an unphosphorylated form, and becomes phosphorylated in the host, after insertion into the target cell membrane. Its *N*- and *C*-termini are in the host cell cytoplasm, where they interact with other molecules and participate in signaling, and an extracellular loop contains the intimin-binding domain. So far, this is the only system where the bacterium injects its own receptor into the host cell cytoplasm. Each Tir molecule has an extracellular antiparallel α-helix joined by a loop. Two α-helices from a Tir dimer establish a four-helix bundle that contacts two intimin molecules via the loops that join them. The Tir–intimin interaction leads to the intimate attachment of the bacterium to the eukaryotic cell and enhances the translocation of additional effector proteins. A distinct feature of Tir-based actin signaling in EPEC and EHEC is that the two pathogens use highly similar gene products, and generate morphologically similar pedestals, but employ different mechanisms to trigger actin polymerization. Kenny *et al.* demonstrated for the first time that EHEC O157:H7 Tir does not support the formation of actin pedestals when expressed in EPEC, and showed that actin signaling was defective, revealing that distinct signaling mechanisms operate in these two pathogens.

Flagellar Motility

For certain enteric pathogens, flagellar motility plays an essential role in the adaptation to various niches, and often represents an important virulence factor during gastrointestinal colonization. The flagellar motor, one of the most common and most studied motility structures in bacteria, is one of their largest molecular machines. Most studies that unveiled the genetics and biochemistry of the bacterial flagella were conducted in *E. coli* and *Salmonella typhimurium*. The flagellum consists of three elements: a basal body embedded in the cell wall, which anchors the flagellum to the bacterial cell envelope and contains the motor that powers flagellar movement; a flexible hook that smoothly transmits the torque generated by the motor to the filament; and a helical filament, 5–10 μm in length and 20 nm in diameter, containing thousands of copies of proteins called flagellins. More than 50 genes are required to encode the products involved in synthesizing the flagellar apparatus, in a complex and highly ordered process. These include structural proteins and proteins that fulfill nonstructural roles, such as secretion, assembly, and regulation. The basal body is synthesized first, followed by the hook and the filament. The flagella contain their own type III protein export machinery, and flagellar proteins travel through diffusion, in a partially or totally unfolded state, through a 2-nm-wide internal channel along the length of the flagellum, to be incorporated into the growing filament.

Helicobacter pylori and *C. jejuni* are two of the pathogens that rely on flagellar locomotion during the infection process. *Helicobacter pylori*, a gram-negative bacterium colonizing half of the world's population, is a major cause of chronic inflammation of the gastric mucosa, peptic ulcer, and gastric cancer, and in 1994 was classified by the World Health Organization as a class I carcinogen. *Campylobacter jejuni* is a leading cause of diarrheal disease worldwide. The two organisms reside within the mucus layer of the gastric and intestinal epithelium, respectively.

The involvement of flagellar motility in virulence was extensively studied for *H. pylori*, a noninvasive, highly motile pathogen that is propelled through a bundle of 4–7 flagella important for its chemotaxis toward urea. By using urease, another virulence factor, to hydrolyze urea and generate ammonia, the pathogen elevates the local pH and lowers the viscosity of the gastric mucus, facilitating its movement. The flagella are covered by a sheath, continuous with the bacterial outer membrane which is thought to protect it against the harsh gastric environment. The pathogen maintains its motility even under high viscosity conditions, when the motility of other bacteria is inhibited.

An interesting strategy, used by several microorganisms, is phase variation, the process of reversibly alternating between the expression of different flagellar antigens. In *C. jejuni*, both *flgS* and *flgR*, which encode a two-component regulatory system required for the expression of several flagellar genes, are targets of phase variation. Homo- or heteropolymeric nucleotide tracts in the two genes are involved in this process, and this represents, to date, the only known example when both regulators of a two-component system undergo phase variation. A unique mechanism for phase variation was described in *H. pylori*. *FliP*, the gene encoding a flagellar body protein,

contains a homopolymeric cytosine tract. As a result of slipped-strand mispairing, after the addition or deletion of a single nucleotide, *fliP* can be reversibly inactivated by the frameshift mutation that prematurely introduces a stop codon. This turns off flagellar assembly and the pathogen loses its motility.

In most *S. enterica* serovars, flagellar expression is biphasic. Only one of two flagellar antigens, FljB or FliC, can be expressed at any given time. This alternative expression is controlled by a site-specific deoxyribonucleic acid (DNA) recombinase that inverts a 996 base-pair chromosomal region, called the H segment, containing the promoter for the operon encoding *fljB* and *fljA*, which is a negative regulator of *fliC*. The significance of flagellar phase variation for *Salmonella* pathogenesis is not yet clear. Even though flagella are not essential for *Salmonella* virulence in a mouse model, they are important in other processes related to pathogenesis, such as in initiating a proinflammatory reaction.

In *L. monocytogenes*, flagellar motility, important for colonizing environmental surfaces and the host, is temperature dependent, and it is controlled by the reciprocal activities of the MogR repressor and the GmaR anti-repressor. MogR is constitutively expressed at all temperatures. The GmaR anti-repressor undergoes temperature-dependent conformational changes. Below 37 °C, GmaR binds and antagonizes MogR. At 37 °C, the GmaR–MogR complex is destabilized and releases MogR, which binds the promoters of flagellar motility genes, repressing their transcription and that of *gmaR*.

The energy for flagellar motility does not come from ATP, but from an ion motor source that converts electrochemical energy into mechanical energy. Although *H. pylori* and *E. coli* flagella are powered by a proton motive force, certain *Vibrio* species, such as *Vibrio parahaemolyticus*, which posses a dual flagellar system, use a sodium motive force for the single sheathed polar flagellum that propels the bacterium in liquid medium (swimming), and a proton motive force for the numerous unsheathed peritrichal flagella that allows translocation in viscous environments or over surfaces (swarming). The two types of flagella, powered by different energy sources, operate within the same bacterial cell, do not share structural components, and are regulated by a single CheY response regulator species, making this organism an interesting model to dissect the interface between flagellar biology and pathogenesis.

Manipulation of the Intestinal Barrier

Pathogens use several strategies to breach the intestinal barrier. Certain microorganisms, such as EHEC, attach to the intestinal epithelium but remain mostly extracellular during the infection. Other pathogens are intracellular, and developed two mechanisms to invade nonphagocytic cells, invasive zippering and triggering. In invasive zippering, a strategy used by *Listeria* and *Yersinia* species, bacterial molecules that mimic host cell ligands bind and activate host cell receptors, and the ligand–receptor interaction initiates a signaling cascade that reorganizes the actin cytoskeleton. After close apposition between the host and the bacterial cell, host cell membrane extensions form like a zipper around the bacterium, engulfing it. In invasive zippering, these cytoskeletal changes are not so dramatic, and usually disappear minutes after bacterial entry. In invasive triggering, used by *Salmonella* and *Shigella* species, bacteria bypass the need to bind cellular receptors and, instead, use type III secretion systems that deliver bacterial effectors into the host cell, where they regulate the actin cytoskeleton. In invasive triggering, cytoskeletal changes are much more extensive.

Intestinal epithelial cells have an apical and a basolateral membrane, each characterized by very distinct compositions and specific roles in maintaining epithelial integrity. Epithelial cells are attached to each other by three structures: tight junctions, adherens junctions, and desmosomes, and many pathogens developed strategies to target one or several of these components.

Disrupting the Mucus Layer

Certain pathogens produce virulence factors that disrupt the protective mucus layer, which forms a physical barrier between the intestinal tract content and the underlying gastrointestinal epithelium that it protects. MUC2 mucin, the major structural component of the mucus gel, is a high-molecular weight glycoprotein produced by the goblet cells in the colon and small intestine. Individual MUC2 subunits assemble into large polymers. Assembly starts in the endoplasmic reticulum, where MUC2 dimers are initially formed via the *C*-termini of individual molecules. After *O*-glycosylation in the Golgi apparatus, multimerization occurs late in the secretory pathway via disulfide bond-mediated trimerization of the *N*-termini. The extensive *O*-glycosylation prevents proteolytic cleavage by pancreatic digestive proteases. One example of a pathogen that disrupts the mucus layer is the protozoan parasite *Entamoeba histolytica*, the etiologic agent of human amebiasis, which is annually responsible for 50 million infections and 100 000 deaths worldwide and represents one of the most frequent causes of death from parasitic infections. Amebiasis is second only to malaria as the cause of mortality due to a protozoan parasite. In the early stages of the infection, *E. histolytica* uses cysteine proteases that depolymerize and disrupt the intestinal mucus gel by targeting poorly glycosylated, cysteine-rich regions in the *C*-terminus of MUC2. Additionally, the parasite releases glycosidases into the intestinal environment, degrading mucin oligosaccharides.

Attachment to the Apical Pole of Epithelial Cells

Colonization of the host is one of the most important early steps toward establishing an infection. One of the strategies employed by many bacteria is the use of adherence factors. Pili or fimbriae, one type of adherence factors that pathogens use, are 5–7-nm diameter, rigid, rod-shaped structures that help attachment to the host. As a result of their key roles in pathogenesis, pili also emerge as attractive vaccine candidates. Enterotoxigenic *E. coli*, the most important cause of traveler's diarrhea, and a leading cause of diarrhea in developing countries, produces several types of pili. Of these, the CS1 pili were the most extensively studied and the most commonly associated with the disease. Only four genes, which encode structural and assembly proteins, are required to produce CS1 pili. The CooA protein represents the major pilin subunit that

forms the body of the pilus. CooD, a protein localized to the tip of the pilus and required for adherence, is thought to initiate assembly, CooC is an outer membrane protein involved in transport, and CooB is a periplasmic chaperone-like protein that stabilizes the other three proteins.

In the first stage of the host–pathogen interaction, EPEC, an important cause of watery diarrhea, adheres to the apical pole of the small intestine epithelial cells by using bundle-forming pili (BFP). These rope-like intertwined filaments bundle together several microorganisms to form microcolonies, and mediate their interaction with *N*-acetyllactosamine glycan receptors on the host cell surface, two events collectively referred to as localized adherence. BFPs are encoded on the EPEC adherence factor plasmid, and their expression requires a cluster of 14 genes that are designated *bfpA–bfpL*, *bfpP*, and *bfpU*. *BfpA* encodes prebundlin, which is processed to bundlin, the major structural subunit of the pili, by two enzymes, BfpP, which cleaves the signal sequence, and DsbA, which catalyzes the formation of a disulfide bond in the periplasmic *C*-terminal region of the protein. Certain BFP mutants are impaired in their ability to cause disease, as shown in human volunteer studies. EHEC does not have BFP and infects the intestinal tract through the colon. BFPs can be extended and retracted by the bacterium. Both the formation of the pili and their capacity to retract, represent important virulence factors, and BfpD and BfpF are involved in the extension and retraction, respectively. The retraction, which closes the gap between the bacterial and the intestinal cells, ensures intimate adherence between the two, and promotes the translocation of bacterial effectors into the eukaryotic cell, contributing to the disruption of tight junctions and to the formation of actin-rich pedestals.

Counteracting Intestinal Epithelium Turnover

One of the innate defense strategies against pathogens is the rapid self-renewal, every 4–5 days, of the intestinal epithelial cells. Kim *et al*. recently described a new virulence tactic used by intestinal pathogens. *Shigella flexneri* OspE, a type III effector protein injected into cells, interacts with integrin-linked kinase, and the complex stabilizes integrin-containing adhesion sites, increasing the adherence of intestinal cells to the basal membrane, and decreasing their detachment. This strategy prevents the turnover of the infected intestinal epithelial cells. Iwai *et al*. provided *in vitro* and *in vivo* evidence that IpaB, a *Shigella* effector, after being injected into intestinal epithelial cells, binds Mad2L2, an inhibitor of the anaphase-promoting complex/cyclosome, and modulates the cell cycle progression of intestinal epithelial cells, slowing down the renewal of infected cells and allowing the pathogen to persist longer.

Disruption of Tight Junctions

Vibrio cholerae adheres to the brush border of the intestinal epithelium and secretes its main virulence factor, the heat-labile cholera enterotoxin, encoded by the *ctxAB* genes located on CTXϕ, the 6.9-kb filamentous bacteriophage integrated into chromosome I. Cholera enterotoxin is an AB5-type toxin. Its five identical B subunits form a homopentamer through interactions between β-sheets from adjacent monomers, and bind to intestinal cell receptors. The A subunit is proteolytically cleaved into two fragments, CT_{A1} and CT_{A2}, which remain linked by a disulfide bond. CT_{A1} harbors the enzymatic activity, and CT_{A2} inserts CT_{A1} into the B-subunit homopentamer. The interaction between CT_{A2} and the B-subunit homopentamer is noncovalent.

The receptor for the cholera toxin B subunit is the GM_1 ganglioside. The toxin reaches the endoplasmic reticulum and the cytoplasm via retrograde transport. Its intracellular target is adenylate cyclase, the enzyme that converts ATP into cAMP, one of the most important intracellular secondary messengers. The CT_{A1} fragment catalyzes the transfer of the adenosine diphosphate (ADP)-ribose moiety from nicotinamide adenine dinucleotide (NAD) to an arginine residue on the α-subunit of the G_S protein that activates adenylate cyclase, and increases intracellular cAMP levels in intestinal epithelial cells. This leads to protein phosphorylation and ion transport changes, including increased Cl^- secretion. The increased Cl^- secretion in crypt cells and the decreased coupled Na^+–Cl^- absorption in the villus cells cause the net movement of electrolytes into the lumen, with subsequent water loss and diarrhea. An interesting aspect about cholera toxin is that the GM_1 ganglioside, its cellular receptor, resides on the apical side of the intestinal epithelial cells, but the effector, adenylate cyclase, is on the basolateral side, and several models were proposed to explain how the signal is transduced between these two distinct cellular locations.

Vibrio cholerae harbors several additional toxins. A cytotoxin, hemagglutinin/protease, the first protease noticed in pathogenic vibrios, is a metalloprotease that degrades the extracellular domain of occludin and reorganizes ZO-1 from tight junctions, perturbing the barrier function of the intestinal epithelium. A virulence factor discovered in 1991, a $\sim$45-kDa outer membrane protein encoded by the cholera toxin bacteriophage CTXϕ and known as zonula occludens toxin (Zot), reversibly opens tight junctions. The biologically active domain responsible for its enterotoxic activity resides in a $\sim$12-kDa *C*-terminal fragment, which is cleaved off and secreted into the intestinal environment. After binding its receptor, Zot is internalized and activates phospholipase C, generating inositol trisphosphate and diacylglycerol. Protein kinase Cα, which is activated either directly by diacylglycerol, or indirectly through the release of Ca^{2+} via inositol trisphosphate, phosphorylates cellular proteins. One of the effects is G-actin polymerization into F-actin filaments, with the rearrangement of actin filaments. This leads to the displacement of other proteins, including ZO-1, from the tight junctions, and to the disassembly of the tight junctions, causing increased intestinal permeability.

Clostridium perfringens enterotoxin, a 35-kDa single-chain polypeptide with a *C*-terminal receptor-binding domain and an *N*-terminal toxicity domain, binds claudin, a tetratransmembrane component of the tight junctions that has two extracellular loops. Claudin is, so far, the only receptor identified for this toxin. From more than 24 proteins in the claudin family, only certain members are receptors for the *C. perfringens* enterotoxin. The second loop represents the toxin recognition site and, within this loop, the *C*-terminal 12 amino acids shape sensitivity to the toxin through an interaction that appears to be electrostatic. Several pathogenic *Shigella* serotypes are also able to remove claudin-1, down-regulate ZO-1,

and dephosphorylate occludin, disrupting tight junctions. One of the virulence strategies of *Giardia lamblia* involves disrupting ZO-1 from tight junctions in a myosin light chain kinase- and caspase-3-dependent manner, leading to increased epithelial permeability.

Another pathogen that manipulates tight junctions is rotavirus, a leading cause of severe diarrhea in infants and young children, which annually claims ~500 000 deaths worldwide. Rotavirus particles are made of three concentric protein layers. The inner layer surrounds the 11 segments of its double-stranded RNA, and is formed of three proteins: VP2, the main structural protein, and VP1 and VP3, which are embedded in this layer and are crucial for RNA replication. The middle layer protein is VP6. The outer layer has two major proteins, VP7 (protease cleaved or P protein), which forms the smooth surface of the virus, and VP4 (glycoprotein or G protein), which forms the spike-like projections. VP4, the main protein involved in the virus–host interaction, is cleaved by trypsin into two peptides, VP5 and VP8. VP8 displaces ZO-1, claudin-3, and occludin from the tight junctions, disrupting them in a reversible, time- and dose-dependent manner.

Disruption of Adherens Junctions

Listeria has developed a sophisticated array of virulence factors. The two most extensively characterized bacterial surface-associated proteins, internalins InlA and InlB, are important for invading host cells. Internalins have an *N*-terminus made of tandem repeats organized into a solenoid shape. Expression of InlA in noninvasive *Listeria* species makes the bacteria become invasive *in vitro*, and the protein appears to be important in human fetoplacental listeriosis. InlA is an 80-kDa protein that engages the most distal extracellular domain of E-cadherin, the adherens junction cell–cell adhesion molecule that, through its *N*-terminus, binds E-cadherin molecules from neighboring cells in a Ca^{2+}-dependent interaction. The three-dimensional structure of the InlA *N*-terminal domain bound to the *N*-terminal part of E-cadherin reveals that the InlA LRR domain wraps around the E-cadherin *N*-terminal domain in a geometry that is very different from the one established between E-cadherin molecules. This interaction is very specific and occurs only for human, but not for mouse E-cadherin, despite an 85% amino acid identity between the two proteins. The specificity is conferred by a single amino acid, at position 16 of E-cadherin, which is proline in the human protein and glutamic acid in the mouse and rat proteins. The replacement of glutamic acid with proline in the mouse E-cadherin confers it with the ability to bind InlA. The second bacterial surface protein, InlB, is a 67-kDa invasin that binds Met, a protein tyrosine kinase that is activated by the binding of hepatocyte growth factor (HGF). X-ray crystallography revealed that InlB does not compete with HGF for the same binding site on Met. The binding of InlA and InlB to their receptors triggers intracellular signaling cascades that result in cytoskeletal rearrangements and actin polymerization.

Using M Cells to Invade the Host

Membranous epithelial cells (M cells) from the intestinal mucosa are cells of epithelial origin that, in addition to their role in capturing antigens and presenting them to the immune system from the underlying lymphoid tissue, are exploited as an entry route by pathogens that are unable to enter epithelial cells or do so inefficiently. M cells have poorly organized brush borders, short and irregular microvilli, a thin layer of glycocalyx, high endocytotic activity, and few lysosomes, and these characteristics are exploited by several pathogens as they gain access to the intestinal epithelium. The hallmark of M cells is the presence, on their basolateral membrane, of invaginations that form pockets and provide a docking site for intraepithelial lymphocytes, greatly reducing the distance that antigens have to travel. This is instrumental in accomplishing one of their major functions, the uptake and transport of antigens from the intestinal lumen to the mucosal immune system.

Some pathogens preferentially, and others exclusively use M cells when invading the host. *Salmonella* crosses the intestinal epithelium through M cells, enterocytes, or dendritic cells, but M cells represent its major route of entry. On entry, the pathogen causes extensive ruffling, which results from apical membrane changes and the redistribution of actin filaments. Virulence factors encoded in the SPI1 pathogenicity island are important during epithelial cell invasion, and also appear to be important for M-cell invasion, but SPI1 mutants, although still capable of invading M cells, do not destroy the follicle-associated epithelium. Inside macrophages, the pathogen survives due to the second type III secretion system, encoded by SPI2.

After reaching the ileum, *Y. enterocolitica* and *Y. pseudotuberculosis* use invasin, a chromosomally encoded outer membrane protein, to bind $\beta 1$ integrins expressed on the apical side of the M cells. The pathogens not only exploit M cells to gain access to the host, but they can also use epithelial cells. *Yersinia* provides an example of structural mimicry at the molecular level, as invasin mimics the $\alpha 5\beta 1$ integrin-binding surface of fibronectin to manipulate the host signal transduction pathways during internalization. Despite tertiary fold and molecular surface differences between invasin and fibronectin, their integrin-contacting residues align almost perfectly, unveiling a strong similarity in the functional features. However, invasin binds integrin with a much higher affinity than fibronectin, with a two-order magnitude difference between the dissociation constants of the two complexes (K_d invasin $= 5 \times 10^{-9}$ M, K_d fibronectin $= 8 \times 10^{-7}$ M).

From the four *Shigella* species that cause human disease, *Shigella sonnei* and *S. flexneri* are most common in the US and developed countries, whereas *Shigella dysenteriae* and *Shigella boydii* are more frequent in developing countries. *Shigella flexneri*, the most intensively studied member of the species, invades M cells that overlie solitary lymphoid nodules in the colorectal mucosa. The pathogen crosses the cells in an intact endocytic vacuole and is delivered to the intraepithelial pockets of the M cells, where it is engulfed by macrophages and dendritic cells, but survives degradation by lysing the phagosomal membrane and by inducing apoptosis. Subsequently, *Shigella* enters epithelial cells through their basolateral surface and is surrounded initially by a membrane vacuole. After disrupting the vacuole, the bacterium replicates in the cytoplasm and induces actin polymerization at one of its poles, forming a 'comet tail' that depends on IcsA (VirG) and *N*-Wiscott–Aldrich syndrome protein (*N*-WASP) to help

it move intracellularly. Subsequently, the pathogen forms membrane-bound protrusions, most of them localized to focal adhesion sites, which fuse with the plasma membrane of the adjacent cell, forming pathogen-containing vesicles. In the new cell, the bacterium is transiently found in these double membrane-bound vacuoles, and it is released into the cytoplasm after disrupting their walls. This strategy helps the pathogen gain access to the cytoplasm of new cells without being exposed to the extracellular environment.

Survival within Vacuoles

Intracellular replication of the *Salmonellae* occurs in a membrane-bound compartment known as *Salmonella*-containing vacuole (SCV), a highly dynamic structure that the pathogen actively remodels to make it permissive for its growth. After internalization, bacteria are enclosed in these vacuoles and prevent their fusion with lysosomes, where they could be degraded, establishing a niche supportive of their replication. At the same time, growth within a vacuole presents the pathogen with several challenges, such as obtaining nutrients or escaping the vacuole to replicate and infect other cells.

Several bacterial proteins maintain the SCVs, which undergo early (<30-min postinfection), intermediate (30 min–5-h postinfection), and late (>5-h postinfection) stages of development during their life cycle. One of the early events in vacuole development is membrane remodeling. SopB, a protein encoded in the SPI1 pathogenicity island and translocated into the cell by type III secretion, is important for this process. SopB is a phosphoinositide phosphatase that hydrolyzes phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$) to generate phosphatidylinositol 3-phosphate, and also decreases phosphatidylserine (PS) levels in the SCV membrane. By decreasing the levels of the negatively charged $PI(4,5)P_2$ and PS, SopB regulates the surface charge of SCVs and alters the recruitment of the Rabs, small GTPase proteins that regulate membrane trafficking. Preventing the recruitment of Rab23 and Rab35, which are important for phagosome maturation and the fusion between phagosomes and lysosomes, inhibits the SCV fusion with lysosomes, changing their fate.

As they mature, SCVs sequentially incorporate and lose endosomal components and move by dynein-dependent transport from their internalization sites toward a perinuclear position, adjacent to the microtubule-organizing center. Actin-based nonmuscle myosin II and dynein-microtubule-dependent movement, along with several SPI1- and SPI2-encoded effectors, appear to be important for this process.

SpiC, an effector protein secreted by the SPI1-encoded type III secretion system, interferes with vesicular trafficking and prevents SCV fusion with endosomes and lysosomes. SpiC binds and inactivates Hook3, a mammalian protein that links the Golgi complex to microtubules and is implicated in cellular trafficking. Additional SPI2-encoded gene products protect against nicotinamide adenine dinucleotide phosphate-dependent killing in macrophages, interfere with inducible nitric oxide synthase localization to the vacuole, and delay apoptosis, helping the intravacuolar replication of the pathogen.

During the late stages of vacuole development, membrane tubules form on their cytoplasmic surface and extend along microtubules toward the cell periphery. These structures, first described in 1993 by García-del Portillo *et al*. were called Sifs, for *Salmonella*-induced filaments. Among the best-studied proteins acting at this stage and required for Sif formation is SifA, an effector encoded in the *Salmonella* SPI2 pathogenicity island that impacts virulence by several mechanisms. One of these is through the maintenance of the *Salmonella*-containing vacuolar membrane. Bacteria harboring a $sifA^-$ mutation are not surrounded by a vacuolar membrane, replicate inefficiently in the cytoplasm, and exhibit decreased virulence in a mouse model. SifA was the first example of a bacterial protein that is responsible for maintaining a membrane-bound vacuole permissive for replication. The SifA *C*-terminal domain resembles a guanine nucleotide exchange factor and binds the guanosine diphosphate (GDP)-bound form of RhoA. This is thought to activate RhoA, which binds and activates SseJ, modifying the SCV membrane phospholipid composition. The *N*-terminal domain of SifA binds SifA and kinesin-interacting protein (SKIP), and the complex binds and activates kinesin-1, linking SCV to the intracellular tubular network. A *Salmonella* SPI1 effector, SptP, harbors a *C*-terminal domain with tyrosine phosphatase activity and dephosphorylates valosin-containing protein/p97, a mammalian protein involved in membrane fusion, and in this manner facilitates pathogen replication inside SCVs.

This tubular network is much more complex than initially envisioned, and involves several additional components, including sorting nexin tubules, *Salmonella*-induced secretory carrier membrane protein 3 tubules, and LAMP1 negative tubules. The complex tubular network involved in SCV maturation was mostly studied *in vitro*, and was not visualized yet *in vivo*, most likely because of technical limitations. This tubular network appears to be important for pathogen survival, replication, and virulence, as mutants are attenuated for pathogenicity in animal models.

Escape from the Phagosome

The ability of some pathogens to avoid phagocytic killing represents an important virulence strategy, and different pathogens developed different approaches to achieve this. Although *Salmonella* employs a strategy to survive inside macrophages by inhibiting the phagosome–lysosome fusion, and is able to replicate inside modified phagosomes, *Yersinia* developed an antiphagocytosis strategy by using the YopH protein tyrosine phosphatase, and *Shigella*, after entering macrophages, can disrupt the phagosome and subsequently multiply in the cytoplasm and kill macrophages by apoptosis within 2 h. A well-characterized system to escape the phagosome is the one used by *L. monocytogenes*. After entering the cell by receptor-mediated endocytosis, *Listeria* is found in primary phagocytic vacuoles, also known as phagosomes, for approximately 30 min. Normally, the phagosome undergoes a process of maturation, in which after gradual acidification it fuses with the lysosome that contains digestive enzymes, to form a phagolysosome where the pathogen is destroyed. *Listeria* is able to prevent this by escaping into the cytosol, where it replicates,

and can subsequently infect other cells. A pore-forming cytolysin, listeriolysin O (LLO), encoded by the *hly* gene, together with two type C phospholipases, phosphatidyl inositol-specific phospholipase C (PI-PLC), encoded by *plcA*, and phosphatidyl choline-specific phospholipase C (PC-PLC), encoded by *plcB*, are important in orchestrating the escape from the phagosomes. In 1990, it was shown that LLO is sufficient to allow the pathogen to escape from the phagosome. LLO, a member of the cholesterol-dependent cytolysins, binds membrane cholesterol as a monomer and establishes large multimers composed of up to 50 subunits. The oligomers extend transmembrane β-hairpins that penetrate the membrane and form 200–300-Å diameter pores, allowing macromolecules to leak from the affected cells. *Listeria* is the only known pathogen to secrete this type of toxin inside the cell, an aspect that opens significant challenges about how it is regulated to prevent cellular damage. LLO is controlled at several levels, including gene transcription and protein synthesis, activity, and degradation. LLO has an optimum activity under acidic conditions, at $pH < 6$, and it unfolds and aggregates at neutral pH. Its transcription is largely controlled by positive regulatory factor A (PrfA), which is a key virulence factor that represents a 'master switch,' as it orchestrates the transition between the saprophytic and the virulent, intracellular form of this pathogen. PrfA positively controls the expression of many virulence genes, including that of its own. A PrfA deletion mutant is 100 000 less virulent in a mouse model. PrfA is a 27-kDa symmetrical homodimer with an *N*-terminal β-barrel similar to the one found in cyclic nucleotide-binding domains that is connected, via a hinge, to a *C*-terminal helix-turn-helix motif. This *C*-terminal domain binds a 14 base-pair palindromic nucleotide sequence centered at position -41.5 in target promoters, known as the PrfA box. Ten genes that are directly regulated by PrfA form the core PrfA regulon, and include *hly*, *plcA*, *plcB*, *actA*, *inlA*, *inlB*, *inlC*, *mpl*, *hpt*, and *prfA*. At least 145 additional genes were predicted, based on microarray experiments and proteomics approaches, to be regulated by PrfA.

An interesting feature of PrfA is its posttranscriptional thermoregulation. *Listeria monocytogenes* key virulence genes are maximally expressed at 37 °C and are almost silent at 30 °C. At temperatures ≤ 30 °C, the *prfA* 5′-untranslated region forms a stable inhibitory secondary structure that masks the ribosome binding site and prevents access of the Shine–Dalgarno sequence to the ribosome. Under these circumstances, PrfA is not translated and virulence genes are not expressed. At 37 °C, which is the temperature in the human host, the leader transcript cannot form this secondary structure, PrfA is translated, and virulence genes are expressed.

LLO has a PEST-like sequence implicated in virulence and localization to the phagosome. Mutants lacking this sequence accumulate in the cytosol and eventually cause cell death. This indicates that, as an additional strategy, the pathogen might have developed a eukaryotic protein degradation signal to achieve a balance between vacuolar escape and avoiding damage to the host cell.

After replicating in the cytosol, the pathogen moves by using the actin cytoskeleton of the eukaryotic host, and when encountering the plasma membrane, it forms an invagination into the neighboring cell, where it is subsequently found in a double-membrane vacuole, also known as the secondary vacuole. In secondary vacuoles, as opposed to the primary vacuoles, the cytosolic face of the inner membrane is close to the bacterium. The pathogen escapes again, by using LLO and the two phospholipids, to initiate a new infection cycle. Compared to wild-type bacteria, which are free in the cytoplasm, *hly* mutants do not produce LLO and are unable to escape from vacuoles.

A similar pore-forming toxin, perfringolysin, is a nonessential virulence factor produced by *C. perfringens*, one of the most frequent causes of food poisoning in developed countries. This pathogen is a medical and public health concern for several reasons, including its very short generation time of ~ 10 min under optimal growth conditions, the wide distribution of its spores in nature and in the gastrointestinal tract of humans and animals, and its ability to produce > 15 toxins. One of these, perfringolysin O (PFO), oligomerizes and creates large pores in cholesterol-containing membranes. X-ray crystallography revealed that PFO is an elongated protein, organized into four domains, with a $\sim 40\%$ β-sheet content. The monomeric toxin is water soluble. A tryptophan-rich loop near its *C*-terminus recognizes the cholesterol-containing membrane and anchors the toxin to the membrane. Conformational changes in this domain are transmitted through the molecule to a more distant domain, which loses many of its contacts with the rest of the molecule, as it undergoes a conformational change in which six α-helices transition to form two transmembrane β-hairpins that span the membrane. Monomers initially oligomerize on the membrane surface into a prepore intermediate, which subsequently forms large homooligomeric complexes that create pores. This profound conformational change, along with the fact that each PFO monomer penetrates the membrane with two amphipathic β-hairpins that completely span the membrane bilayer, defines a new paradigm for the membrane insertion of a cytolytic toxin.

Actin-Based Movement and Actin Remodeling

Several pathogens developed strategies to manipulate the host actin cytoskeleton and promote their own motility during infection. This represents an important virulence strategy and, at the same time, provides an attractive therapeutic target. Pathogens that show actin-dependent movement were organized into two groups. Although some pathogens, such as *Listeria*, mimic nucleation-promoting factors, others, such as *Shigella* and EHEC, recruit nucleation-promoting factors to the bacterial cell surface.

After escape from the vacuole, on being released into the cytosol, *Listeria* uses the host cytoskeletal actin to move intra- and intercellularly. *Listeria monocytogenes* actin-assembly-inducing protein A (ActA) polymerizes actin and provides the force allowing the pathogen to move within the cell. *Listeria ivanovii*, primarily an animal pathogen, uses a related protein, IactA. Cameron *et al*. revealed that ActA is necessary and sufficient to move coated polystyrene beads in cytoplasmic extracts. The protein mimics the actin nucleation-promoting activity of eukaryotic host cell proteins from the WASP and WASP-family verprolin-homologous protein (WAVE) families. The WASP/WAVE family includes actin nucleation-promoting factors that activate the Arp2/3 complex, a highly conserved seven-member protein complex that in addition to Arp2 and

Arp3 contains five other proteins: p16, p20, p21, p34, and p40. This complex binds preexisting actin filaments and nucleates new filaments, and is found particularly in regions with increased actin dynamics.

The ActA protein has three distinct functional domains. ActA-*N*, its *N*-terminal region that is essential for motility, binds monomeric actin (G-actin) and together with other host proteins forms an Arp2/3-containing complex that nucleates actin, enhancing the formation of branched filaments. This basic region is similar to a *C*-terminal region from WASP family proteins. The 146-KKRRK-150 sequence within this region is important for intracellular motility, and mutation of the arginine residues abolishes motility. The central domain contains four consensus proline-rich motifs, and is important for shaping the velocity of movement. *Listeria monocytogenes* mutants lacking these central proline-rich domains produce actin tails that are shorter and move more slowly as compared to the wild-type strains. Each proline repeat is thought to contribute $\sim 2.5\ \mu m\ min^{-1}$ to the rate of the movement. This central domain recruits the focal adhesion protein vasodilator-stimulated phosphoprotein (VASP), which contains a proline-rich region that binds the G-actin-binding protein profillin and stimulates actin polymerization and actin tail formation. The ActA *C*-terminal hydrophobic domain is a membrane anchor that noncovalently links the protein to the cell wall. An interesting feature that distinguishes ActA from the eukaryotic proteins that it mimics is the absence of regions interacting with regulatory proteins. This ensures that pathogen-induced actin nucleation occurs constitutively and is not influenced by host regulatory factors. ActA played a fundamental role in discovering and understanding actin nucleation and dynamics by Arp2/3 in eukaryotes, and *L. monocytogenes* provides one example when studies on bacterial pathogenesis unveiled key physiological cellular processes in eukaryotes.

In contrast, *Shigella* uses IcsA, an outer membrane protein previously known as VirG, which does not share sequence homology with *Listeria* ActA and activates Arp2/3 via a different pathway. Although *Listeria* ActA mimics WASP/WAVE family proteins, *Shigella* IcsA binds directly, through its *N*-terminal glycine-rich repeats, to the actin nucleation-promoting factor *N*-WASP, recruiting it to the bacterial surface, and one of the prevailing models proposes that IcsA, as part of a ternary IcsA–*N*-WASP–Arp2/3 complex, mimics Cdc42-induced *N*-WASP activation, explaining also why *Shigella* actin-based motility is independent of the Rho-family GTPases.

Yarbrough *et al*. reported for the first time that *Vibrio* outer protein S (VopS), a *V. parahaemolyticus* type III secreted effector that causes cell rounding, covalently modifies a conserved threonine residue in the Rho, Rac, and Cdc42 Rho-family GTPases with adenosine 5′-monophosphate. This modification, known as AMPylation, prevents the interaction of these proteins with downstream effectors and actin assembly.

Other pathogens can subvert the actin cytoskeleton even without entering the cell. As previously mentioned, the A/E lesions caused by EHEC and EPEC are accompanied by actin accumulation in the host cell cytoplasm, directly beneath the adherent bacteria, forming pedestal-like structures that sometimes extend as much as 10 μm and anchor the pathogen to the host cell. The loss of microvilli on the intestinal epithelial cells during this process is thought to be a key factor leading to the infection-associated diarrhea. A/E is the result of enterocyte brush border degeneration and represents a unique ability of the pathogen to subvert the host cell actin cytoskeleton from the outside.

On the Tir–intimin interaction in EHEC (EspE–intimin interaction in EPEC), the Tir cytoplasmic domains interact with other host cell molecules and participate in signaling events. Despite similarities in initiating signaling, and despite highly similar gene products and morphologically similar pedestals, EHEC and EPEC use different mechanisms to trigger actin polymerization. One of the major differences between the Tir cytoplasmic domains in the two pathogens is phosphorylation of tyrosine 474 in the cytoplasmic tail of EPEC, but not EHEC. This allows the phosphotyrosine-dependent binding of Nck, a host adapter protein that recruits *N*-WASP and additional host proteins and initiates signaling cascades culminating with actin assembly and filament formation. This strategy is very similar to the one encountered in vaccinia virus, where a protein, A36R, becomes phosphorylated on tyrosine 112, and recruits Nck, indicating that two proteins from these two distinct pathogens employ very similar strategies to manipulate the host cytoskeleton. Both proteins activate WASP and the Arp2/3 complex, and generate actin tails in the vaccinia virus, and pedestals in EPEC and EHEC. In addition, EPEC can activate a less efficient, Nck-independent pathway that uses tyrosine 454, which is less efficiently phosphorylated, to activate *N*-WASP independently of tyrosine 474. Actin pedestal formation in EHEC does not require Nck and occurs through a process that involves additional bacterial proteins and was less extensively characterized. The EHEC Tir molecule lacks the Nck binding site and uses a tyrosine residue from an NPY458 motif within its *C*-terminal domain. This recruits the insulin receptor tyrosine kinase substrate and the insulin receptor substrate protein of 53 kDa, which subsequently recruit the Tir-cytoskeleton coupling protein, also known as $EspF_U$, a protein that has five and a half 47 amino acid repeats. A 17-residue motif within these repeats is sufficient to bind *N*-WASP. The ability of *N*-WASP to activate the Arp2/3 complex is normally regulated by an inhibitory intramolecular interaction between its GTPase binding domain and its *C*-terminal helical region. Cdc42 binding to the GTPase activation domain causes a conformational change that releases the *C*-terminus, enabling its interaction with the actin regulation machinery downstream. $EspF_U$ mimics this autoinhibitory *C*-terminal helix from *N*-WASP and binds the GTPase activation domain, activating the protein by competitively disrupting its autoinhibited state. This subsequently leads to actin polymerization.

Additional pathogens exploit the actin cytoskeleton for various purposes during the infection. At least six *Salmonella* effectors, *Salmonella* invasion protein A (SipA), *Salmonella* invasion protein C (SipC), *Salmonella* outer protein B (SopB), *Salmonella* outer protein E (SopE), *Salmonella* outer protein E2 (SopE2), and *Salmonella* protein tyrosine phosphatase (SptP), manipulate the actin cytoskeleton during invasion. The SipA, also called stringent starvation protein A (SspA), binds G-actin and reduces the critical actin concentration needed for polymerization, protects actin filaments from the

ADP/cofilin-directed depolymerization and from the severing activity of gelsolin, and cooperates with SipC to generate stable F-actin bundles by promoting actin nucleation. These cytoskeletal rearrangements help induce membrane ruffling to envelope and internalize the pathogen. SopE and SopE2 activate Cdc42. In addition, SopE activates the Rac1 Rho GTPase. The Rho family of GTPases, important regulators of the actin cytoskeleton, function as molecular switches that alternate between an active, GTP-bound and an inactive, GDP-bound conformation. During this transition, structural changes occur mainly in two regions, switch I and II, which in the presence of Mg^{2+} form a binding pocket that stabilizes the bound guanine nucleotide. A unique 166-GAGA-169 loop from SopE becomes inserted between Cdc42 switch I and II and induces conformational changes that distort the nucleotide-binding site, promoting nucleotide release. SopE represents the first example of a non-Dbl-like protein that is able to induce the release of a guanine nucleotide in a Rho-family protein. Activated Cdc42 and Rac1 recruit WASP and Scar/WAVE proteins, which activate Arp2/3 and cause actin polymerization and cytoskeletal rearrangements. A virulence factor produced by certain *Salmonella* strains, *Salmonella* plasmid virulence B, contains a *C*-terminal ADP-ribosylating motif and uses NAD as a substrate to covalently attach ADP to arginine 177 of G-actin. The C2 toxin from certain *Clostridium botulinum* strains also ADP-ribosylates the same arginine residue on G-actin. ADP-ribosylated G-actin binds to the barbed (fast growing) end of the F-actin filaments and acts as a capping protein, preventing actin polymerization and filament formation, and shifting the equilibrium toward monomeric G-actin. Another *Salmonella* SPI1 effector, SptP, harbors within its *N*-terminal domain an arginine finger motif 206-GPLRSLM-212, in which the arginine residue is conserved among all Rho GTPases. Through this region, SptP acts as a GAP for Cdc42 and Rac1, reversing the actin cytoskeletal changes caused by SopB, SopE, and SopE2, and restoring the cytoskeletal architecture in the host cell after the infection.

Inhibition of Ribosomes

Shigellae, gram-negative nonsporulating bacilli that belong to the family *Enterobacteriaceae*, cause bacterial dysentery, a disease transmitted by the fecal–oral route and characterized by abdominal cramps and bloody diarrhea with mucus. One of the toxins produced by *S. dysenteriae* serotype 1, and rarely by other *Shigella* species, is the Shiga toxin (Stx). The name comes from Kiyoshi Shiga, the Japanese microbiologist who in 1897 characterized for the first time the bacterial origin of dysentery. The Stx is nearly identical to members of the Stx1 and Stx2 families, produced by certain *E. coli* strains known as Stx-producing *E. coli* (STEC) or verocytotoxin-producing *E. coli*. Shiga toxin and *E. coli* Stx1 differ by only one amino acid in the catalytic A subunit. Stx2, the more potent of the two antigenic forms, is responsible for most instances of fatal human infections. Stx1 and Stx2, despite being approximately 56% identical at the amino acid sequence level, are immunologically distinct and, in addition, they do not target exactly the same cell types and tissues. Some isolates produce one of these toxins, whereas others produce both. Each of the two Shiga-like toxin families consists of multiple variants, and the toxin subtype determines the clinical course and the severity of the infection.

The X-ray crystals of both the *S. dysenteriae* and the *E. coli* toxins reveal an AB_5 structure, in which an ~32-kDa enzymatically active A subunit is noncovalently associated with five identical B subunits, ~7.7 kDa each, organized as a ring. The *C*-terminus of the A subunit is interdigitated into the central pore formed by the B-subunit pentameric ring.

The Shiga toxin cellular receptor is a neutral glycosphingolipid, globotriaosylceramide (Gb3), $\alpha Gal(1-4)\beta Gal(1-4)\beta Glc$-ceramide, also known as CD77, on which the toxin recognizes the terminal α-1,4 digalactose. This receptor also exists on renal endothelial glomerular cells, mesangial cells, and tubular epithelial cells, and cellular differences in its expression pattern are thought to explain the organ-specific pathology associated with the infection. The receptor is not present in the cattle gastrointestinal tract, which explains why cattle, which are a major EHEC reservoir, are asymptomatic. One Stx2 subtype, Stx2e, prefers to bind globotetraosylceramide (Gb4), $\beta GalNAc(1–3)$-$\alpha Gal(1–4)$-$\beta Gal(1–4)$-$\beta 1Glc$-ceramide, in which an additional *N*-terminal *N*-acetylgalactosamine is linked by a $\beta(1–3)$ linkage.

On binding its receptor, the Shiga toxin is internalized by clathrin-mediated endocytosis, in which clathrin-coated pits pinch off the plasma membrane and form sealed vesicles that contain toxin bound to the internal surface. The toxin is taken up into the cell even when clathrin-dependent endocytosis is inhibited, indicating that the clathrin pathway is not an absolute requirement for its uptake. The toxin subsequently undergoes retrograde transport through the secretory pathway to the endoplasmic reticulum. The Shiga toxin was, in fact, the first example of a molecule that is transported from the cell surface to the endoplasmic reticulum through the Golgi apparatus. Early during the endocytic pathway, the A subunit is cleaved at a *C*-terminal protease-sensitive loop, between arginine 251 and methionine 252, by membrane-associated furin, generating an ~27-kDa A1 fragment that is catalytically active and the A2–StxB complex. The A1 fragment remains associated with the complex via a disulfide bond between the A1 and A2 fragments, which is reduced in the endoplasmic reticulum. This releases the A1 fragment, which is translocated to the cytoplasm and performs the molecular damage that includes protein synthesis inhibition. The A1 fragment acts as an *N*-glycosidase, cleaving an adenine base at position 4324 in domain VI of the 60S eukaryotic ribosome 28S ribosomal RNA (rRNA) subunit. This property is shared with the plant toxin ricin. The cleavage prevents the formation of a critical stem-loop structure to which elongation factors should bind, preventing aminoacyl-tRNA binding and elongation, and inhibiting protein synthesis. The toxin was also shown to trigger apoptosis by multiple mechanisms, in several cell types.

The X-ray crystal structure of the Stx1 B pentamer in complex with an analog of its Gb3 trisaccharide receptor, solved at 2.8-Å resolution, was reported in 1998 and revealed that each B-subunit monomer has three distinct trisaccharide moiety binding sites. The 15 binding sites per pentamer account for the high binding affinity between this toxin and its receptor.

Although certain infected individuals remain asymptomatic, others develop the infection, and ~5–8% progress to a life-threatening extraintestinal complication that occurs when the toxin translocates through the intestinal epithelial barrier into the circulation. This complication, known as hemolytic–uremic syndrome (HUS), presents with the hemolytic anemia, thrombocytopenia, and acute renal failure triad, and represents the main cause of acute renal failure in children. HUS may occur even after the pathogen is no longer detectable in the stool. The STEC strains that cause hemorrhagic colitis and HUS are collectively known as EHEC. *Escherichia coli* O157:H7 is the main STEC serotype from >500 that were described in humans, and is most frequently associated with severe disease worldwide, causing an estimated 73 000 foodborne infections annually in the US. Shiga toxin can also be produced by other, non-O157 *E. coli* serotypes.

It is not known why certain individuals develop HUS whereas others do not, but children and the elderly are particularly susceptible. Children <10 years have a 10–15% risk of developing HUS after an *E. coli* O157:H7 infection. The risk of HUS depends on several factors. Some of these factors, such as serotype, inoculum size, and the presence of pathogenicity islands, are pathogen specific. Others, such as age, underlying immune conditions, genetic factors that shape the inflammatory response, and the use of antibiotics and antiemetic medication, are host specific. It was reported that individuals who developed HUS after a Shiga-like toxin-producing *E. coli* infection had higher levels of hydroxylated fatty acid in their red blood cell Gb3, a factor known to affect receptor conformation, and this pointed toward receptor molecular differences that could shape the infection. HUS mortality is currently <5%, but up to 50% of surviving patients may have long-term sequelae, including chronic renal failure and neurological complications.

One of the most important features of the Shiga toxin is its ability to damage vascular endothelial cells. The gastrointestinal tract, the kidneys, and the central nervous system are the most frequent locations affected in patients with HUS. From the intestinal lumen, the toxin is absorbed through the mucosa and enters the circulation; a paracellular pathway was also proposed to exist. Subsequently, it binds receptors on the endothelium and on cells from various organs, causing organ-specific pathology and symptoms. The Gb3 receptor is also present on the renal glomerular endothelial cells, which bind the toxin, resulting in vascular injury, fibrin deposition, thrombus formation, and renal failure. Additional effects of the toxins are cytokine synthesis and release, cytokine-stimulated activation of polymorphonuclear (PMN) leukocytes with the release of oxygen metabolites and endothelial injury, and apoptosis.

A more recently found activity of the Shiga toxin is its ability to stimulate the release of chemokines, cytokines, and adhesion molecules. An apparent paradox is that even though the Shiga toxins inhibit the ribosome and protein synthesis, at similar concentrations they also increase the level of several cytokines. In fact, it was shown that very small amounts of toxin, which have only minor effects on protein synthesis, exert a large impact on gene expression. This was explained by the fact that, at the same time as inhibiting the ribosome, the toxins activate the stress-activated protein kinases and upregulate the inflammatory response.

Another ribosome-inactivating toxin, ricin, is one of the most potent naturally occurring toxins, with as little as 500 μg being sufficient to kill an adult. Ricin is a heterodimeric protein produced by the castor bean plant *Ricinus communis*, made of two polypeptide chains, a 32-kDa A chain and a 36-kDa B chain that are covalently linked by a disulfide bond. The toxin is internalized by endocytosis, which not only occurs by clathrin-coated pits, but was also shown to occur when this pathway is blocked, indicating that other endocytic mechanisms are operational. Experiments with ricin provided the early indications supporting the existence of a clathrin-independent cellular endocytic pathway. After uptake, ricin is transported to the Golgi apparatus and the endoplasmic reticulum, from where the A chain is translocated to the ribosome. The A chain has deadenylase activity and irreversibly depurinates the 28S RNA ribosomal subunit, inhibiting protein synthesis. The B chain is a catalytically inactive lectin that binds β-D-galactopyranoside moieties on glycoprotein and glycolipid receptors with galactose residues, a characteristic that enables it to bind all over the eukaryotic cell surface. The mechanism of action of ricin was first reported in 1987, when investigators revealed that catalytic amounts of ricin modified one nucleotide, adenine A4324, in the 28S rRNA located adjacent to the α-sarcin site in the rat ribosome. A single ricin molecule is able to inactivate more than 1500 ribosomes per minute, a rate that exceeds the ability of the cell to manufacture new ribosomes. This, in addition to other characteristics, such as its stability and its resistance to proteolytic degradation, explains why one or a few molecules are sufficient to cause cell death.

Synaptic Inhibition

Clostridia produce the largest number of toxins among animal and human pathogenic bacteria. *Clostridium botulinum* is a gram-positive, rod-shaped, and spore-forming obligate anaerobe with worldwide distribution. This pathogen causes several types of botulism. Foodborne-, infant-, wound-, and adult intestinal toxemia botulism occur naturally, whereas inhalation- and iatrogenic botulism may result from the deliberate aerosolization and from the therapeutic or cosmetic injection of the toxin, respectively.

All these forms of the disease are potentially fatal and represent medical emergencies. The neurological symptoms of botulism often start with blurred vision and progress with descending bilateral flaccid paralysis, with respiratory and cardiac complications in severe cases. Foodborne botulism has a 5–10% fatality rate and occurs after the consumption of food contaminated with the toxin produced by bacteria growing under anaerobic conditions. This form of the disease is not caused by bacteria or spores themselves, but by the ingestion of preformed botulinum neurotoxin, and it is therefore considered to be an intoxication rather than an infection. The term botulism derived from *botulus*, the Latin word for sausage, and it was used in the eighteenth century central Europe to describe a highly fatal disease presenting with muscle paralysis and breathing difficulties that often occurred after eating blood sausage.

Infant botulism was described in the first year of life, and several studies suggested that the incompletely established intestinal microbial flora, which could compete with the pathogen, facilitates the infection. It classically presents with the clinical triad of bulbar palsy (slow or absent pupil responses), alertness, and the absence of fever. Some of the risk factors for infant botulism are residence in an area with high spore density, decreased intestinal motility, and the consumption of honey.

Botulinum neurotoxins are considered to be the deadliest toxins known to humans, with the median lethal dose of 1 ng kg^{-1} or less. The seven known serologically distinct toxins were designated A–G and differ by approximately 70% in their primary amino acid sequences. Of these, four serotypes, A, B, E, and F, cause human botulism. Botulinum neurotoxins act by irreversibly binding acetylcholine receptors at motor nerve terminations and neuromuscular junctions, inhibiting the release of neurotransmitter at peripheral cholinergic nervous system synapses. Each toxin is synthesized as a ~150-kDa inactive single-chain protein precursor that is cleaved into a ~50-kDa light chain, which is connected by a disulfide bond to a ~100-kDa heavy chain. The heavy chain ensures protein transport across the neuronal plasma membrane into the cytosol, and the light chain represents the active, catalytic moiety.

The toxin, called a 'marvel of protein design,' consists of three modules: an *N*-terminal catalytic domain that is formed by the light chain and a central translocation domain followed by a *C*-terminal receptor-binding domain, both formed by the heavy chain. The light-chain active site is partially occluded in the unreduced holotoxin by a translocation belt, which is a heavy chain loop wrapped around the light chain.

Botulinum neurotoxins bind to the presynaptic surface of motor neurons and are internalized by receptor-mediated endocytosis. In the acidic environment of the endosomes, the heavy chain undergoes a conformational change that helps it insert into the endosomal membrane to form a channel. The light chain is translocated through this channel and reaches the cytosol, where it acts as an endopeptidase. The catalytic center of the light chain contains a conserved zinc-binding HExxH motif, also found in many zinc proteases. Activation of the enzyme occurs when the disulfide bond connecting the heavy- and light chains is cleaved in the reducing environment of the cytosol. Botulinum neurotoxins introduce a single proteolytic cleavage in presynaptic proteins known as soluble NSF attachment protein receptors (SNARES), inhibiting the fusion of synaptic vesicles and the release of neurotransmitter into the synaptic cleft, and blocking neurotransmission. In the B, D, F, and G toxins, the light chain hydrolyzes a specific peptide bond in the synaptic vesicle protein synaptobrevin or vesicle associated membrane protein. In the A, C, and E toxins, a specific peptide bond in the presynaptic membrane protein synaptosome-associated protein of 25 kDa is hydrolyzed. In addition, the type C toxin cleaves a peptide bond in syntaxin, a presynaptic membrane protein.

N- and *O*-Glycosylation

Although relatively little is known about *C. jejuni* pathogenesis, one of the main bacterial causes of human gastroenteritis worldwide, its most important virulence determinants are the flagellum, the adherence to the intestinal mucosa, the ability to invade, and the production of toxins. The pathogen has a polar flagellum made of two *O*-glycosylated structural proteins, FlaA and FlaB, whose biogenesis is controlled by a two-component system comprising the FlgS sensor and the FlgR response regulator. A genetic locus containing ~50 genes is responsible for completing this posttranslational modification. Defects in *O*-linked glycosylation lead to decreased motility, adherence, and invasion. *O*-glycosylation occurs when glycan residues are attached to the hydroxyl oxygen of serine or threonine residues. In *C. jejuni* strain 81–176, each FlaA monomer, the major flagellar protein, has up to 19 serine or threonine residues that can become *O*-glycosylated with pseudaminic acid, a 9-carbon sugar that structurally resembles sialic acid, and with its acetamidino derivatives. As opposed to *N*-glycosylation, *O*-glycosylation is not sequence specific, but appears to be determined mainly by the protein tertiary structure, and exposed residues are mostly the ones that are modified. The residues that undergo *O*-glycosylation are, with one exception, in the flagellar regions in the primary sequence that are predicted to be surface exposed, and this process is necessary for flagellar assembly. The *O*-linked glycans represent up to 10% of the protein mass, making this one of the most extensively modified bacterial proteins described to date. *O*-linked glycosylation is important for flagellar assembly, bacterial colonization, and biofilm formation. In addition, several genes in the flagellar glycosylation locus contain homopolymeric tracts and undergo phase variation, a process that is important for adapting to the changing environmental conditions and for evading the host immune response. An *O*-linked glycosylation system was also described in *C. botulinum* flagellins.

Campylobacter jejuni is the pathogen in which another virulence factor, *N*-glycosylation, was described for the first time. This modification is required for attachment, invasion, and colonization in cell culture and in animal models of infection. Genetic approaches that block *N*-glycosylation render the bacterium significantly less pathogenic, and cause pleiotropic effects that include decreased virulence in human epithelial cell lines and reduced colonization in animal models. Proteins involved in *N*-glycosylation are encoded in a locus with 12 putative genes. During *N*-glycosylation, the oligosaccharyltransferase PglB, the key enzyme in the pathway, transfers en bloc a heptasaccharide to an asparagine residue from a consensus sequence, Asp/Glu-X_1-Asn-X_2-Ser/Thr, where X_1 and X_2 can be any amino acid except proline. More than 65 *C. jejuni* proteins are *N*-glycosylated, and more than 150 proteins are predicted, based on a sequence prediction of the acceptor consensus sequence, to undergo *N*-glycosylation.

Manipulation of the Host Immune Response

Campylobacter jejuni is the most frequent pathogen linked to the Guillain–Barré syndrome, a peripheral neuropathy that develops ~1–3 weeks after *Campylobacter* enteritis in approximately 1 in 1000 individuals. Guillain–Barré syndrome is an autoimmune condition characterized by limb weakness and areflexia, and represents one of the most frequent causes

of flaccid paralysis. At least four subtypes of the syndrome were described. Of these, the two major categories are acute motor axonal neuropathy, in which axons are the primary target and presents with pure motor involvement, and acute inflammatory demyelinating neuropathy, in which myelin is the primary target. *Campylobacter jejuni* infection was associated only with the acute motor axonal neuropathy. Another condition, Miller Fisher syndrome, characterized by ophthalmoplegia, ataxia, and areflexia, is thought to be a variant of the Guillain–Barré syndrome and was also associated with *C. jejuni* infection during the weeks preceding its onset.

Several animal and human studies found that the structural similarity between lipooligosaccharides from the outer membrane of the pathogen and gangliosides, a group of glycosphingolipids anchored to the external leaflet of the lipid bilayer by a ceramide moiety, could generate cross-reacting anti-ganglioside antibodies that are responsible for the autoimmune disease. In 1993, Yuki *et al*. revealed, for the first time, that a *C. jejuni* strain obtained from a patient with acute motor axonal neuropathy with immunoglobulin G against the GM1 ganglioside had the Gal β1-3 GalNAc β1-4 (NeuAc α2-3) Galβ oligosaccharide protruding from the lipooligosaccharide core. This oligosaccharide was identical with the tetrasaccharide present on the GM1 ganglioside. The authors confirmed the existence of molecular mimicry between *C. jejuni* lipopolysaccharide (LPS) and a terminal tetrasaccharide on the GM1 ganglioside, marking the first instance when mimicry between a pathogen isolated from a patient with Guillain–Barré syndrome and the nervous tissue was shown.

The mechanism that explains why certain individuals develop Guillain–Barré syndrome and others develop the Miller Fisher syndrome after *C. jejuni* enteritis was recently elucidated. Cst-II, the bacterial enzyme that transfers sialic acid to the lipooligosaccharide, exhibits a polymorphism. The amino acid at position 51 can be a threonine, in which case the enzyme has α-2,3-sialyltransferase activity and produces lipooligosaccharides that induce antibodies against GM1 and GD1a gangliosides, which exist on motor neurons that control the limbs. If position 51 is an asparagine, the enzyme exhibits both α-2,3- and α-2,8-sialyltransferase activity, and produces lipooligosaccharides that induce antibodies against GQ1b gangliosides, which exist mostly on oculomotor and primary sensory neurons. Thus, this polymorphism appears to determine the clinical course of the autoimmune condition subsequent to *C. jejuni* enteritis.

The current model proposes that during the *C. jejuni* infection, the pathogen produces GM1-like and GD1a-like lipooligosaccharides, which induce anti-GM1 and anti-GD1a antibodies. During the eradication of the infection, the antibodies that form against lipooligosaccharides also bind gangliosides in the nervous system, and initiate autoimmune injury. These findings reveal a new paradigm, in which a bacterial gene polymorphism shapes the clinical presentation of the postinfection autoimmune disease.

Several strategies to subvert the immune response were described for the *Yersinia* Yop proteins, and their ability to interfere with cytokine production and to inhibit phagocytosis is one of these. YopH, one of the most powerful tyrosine phosphatases known, inhibits signaling from the integrin receptor, and dephosphorylates proteins from the focal adhesion complex, some of which are involved in adhesion during phagocytosis, directly blocking phagocytosis. A conserved YopH cysteine residue, C403, is essential for catalytic activity, and a strain in which this residue is mutated can still be phagocytosed but it is not virulent in mice. YopH also inhibits lymphocyte proliferation. YopJ in *Y. pseudotuberculosis* (YopP in *Y. enterocolitica*) reduces tumor necrosis factor-α (TNF-α) and interleukin-8 release and suppresses the inflammatory response by inhibiting IKKβ, a kinase that phosphorylates the NF-κB inhibitor IκB. This inhibits IκB phosphorylation and degradation, preventing NF-κB translocation into the nucleus. Brodsky *et al*. identified a new effector, YopK, without known sequence homology to any protein to date, except proteins from pathogenic *Yersinia* species, and revealed that its intracellular domain interacts with the type III secretion translocon to prevent recognition of the type III secretion proteins by the host innate immune system. In this manner, YopK prevents assembly of the inflammasomes, key molecular complexes that are important for innate immunity and enhance bacterial survival in the host.

An additional strategy to manipulate the immune system exists in *S. flexneri*, which contains two functional copies of the *msbB* gene, one encoded on the chromosome (*msbB1*) and the other one on a virulence plasmid (*msbB2*). The products of these two genes produce a transferase that catalyzes the acyloxy-acylation of myristate at the 3′ position of glucosamine disaccharide on lipid A, a component of the bacterial LPS. This modification is required for the recognition of lipid A in the host. It was recently shown that both genes are required for the maximal acylation of lipid A, and a strain with both genes mutated showed defective TNF-α production in monocytes, decreased intestinal inflammation, and reduced invasion of the intestinal epithelium.

Another group of virulence factors that manipulate the immune system is illustrated by staphylococcal enterotoxins, one of the major causes of food poisoning worldwide, and the most common cause of food poisoning in the US. Staphylococcal enterotoxins are heat-stable toxins, an important characteristic in terms of food safety, and are resistant to inactivation by gastrointestinal proteases, such as pepsin. *Staphylococcus aureus* enterotoxins were first identified in 1959 and more than 20 members are currently known, types A and B being most frequently involved in food poisoning and the most extensively characterized. Less than 1 μg of toxin is sufficient to cause disease. In 1988, Evenson *et al*. reported an outbreak in a US school district where >850 students became ill after drinking 2% chocolate milk. Even though no bacteria were present in the chocolate milk, staphylococcal enterotoxin A was detected. On average, 11 ng of enterotoxin were found in one-half pint cartons. The attack rate was 31.5% among those who drank one carton and 44.4% among those who consumed three or more cartons.

In addition to being gastrointestinal toxins, staphylococcal enterotoxins act as superantigens, and stimulate nonspecific T-cell proliferation. The term 'superantigen' was coined in 1989 in Philippa Marrack's and John Kappler's research group, to describe a group of antigens that can stimulate T cells with very high potency. Unlike conventional antigens, which are processed into peptides in antigen-presenting cells, superantigens are not processed. Instead, they can

simultaneously bind the major histocompatibility complex (MHC) class II molecules on antigen-presenting cells and T-cell receptors on T cells, where they interact with the variable region of the receptor β-chain. The trimolecular complex that forms bypasses the process of antigen presentation, activates more lymphocytes than conventional antigens, and produces large amounts of cytokines. The resulting inflammatory cascade may overwhelm the immune response and lead to fever, shock, tissue and organ damage, and sometimes death. These manifestations, and the uncontrolled T-cell proliferation, sometimes occur at superantigen concentrations <0.1 pg ml^{-1}, and certain superantigens are able to stimulate T cells *in vitro* at 1 fg ml^{-1} (10^{-15} g ml^{-1}). Although most conventional antigens interact with very few naïve lymphocytes, usually activating one in 10^5–10^6 naïve T cells, superantigens interact with a large proportion of the lymphocytes, and may activate as many as one in five T cells. Enterotoxin A is the most potent T-cell stimulator from all staphylococcal enterotoxins.

The X-ray crystal structure of the staphylococcal enterotoxin type A, reported in 1995, reveals a 27-kDa monomer composed of two domains. One domain contains a β-barrel made of two β-sheets, and the second domain contains a β-grasp motif, which is a mixed β-sheet packed against a single α-helix. This β-grasp motif contains the major Zn^{2+} coordination site that is involved in MHC class II binding, and an additional MHC II binding site exists in the first domain. Mutational studies revealed that the two sites cooperate, a process that explains the high binding affinity of the toxin to MHC class II molecules. In both type A and type B enterotoxins, the two unequal domains are separated by a central shallow cavity that represents the T-cell receptor-binding site.

An additional strategy to manipulate the immune response is antigenic variation, and for foodborne pathogens, this is illustrated by intestinal parasites. *Giardia lamblia*, also known as *Giardia duodenalis* or *Giardia intestinalis*, is one of the most frequent human intestinal parasites, and one of the leading causes of parasitic diarrheal disease worldwide. An important strategy used by *Giardia* trophozoites to evade the vertebrate host humoral immune response is antigenic variation. This phenomenon is also observed in other pathogens, such as the ones causing malaria and syphilis. By varying its surface molecules and evading the immune response, the parasite is able to successfully perpetuate long-term infections. Antigenic variation in *Giardia*, unlike in other pathogens, does not occur by gene rearrangements or DNA sequence variation, but through epigenetic mechanisms that involve post-transcriptional silencing mediated by microRNAs. *Giardia* antigenic variation explains the ability of the parasite to cause persistent infection or multiple reinfections. Antigenic variation is mediated by the off/on switching of the *vsp* genes, a family of >190 genes that vary in size between 20 and 200 kDa and encode cysteine-rich surface proteins with conserved *C*-termini and variable *N*-termini that cover the entire surface of the parasite. Variant-specific surface proteins (VSPs) represent the main surface antigen of the parasite that is recognized by the host immune system. The parasite expresses only one VSP at any given time, but it can spontaneously switch to another type. Prucca *et al*. showed that most or all the *vsp* genes are transcribed, and the primary transcripts of all but one gene are subsequently degraded by an RNAi-like mechanism, which explains the expression of specific antigenic variants on the parasite surface. In addition to allowing the parasite to escape the immune system, antigenic variation also appears to facilitate survival in different intestinal environments.

Epigenetic Modifications

The DNA adenine methylase (Dam) system has been increasingly linked to virulence in several bacterial species. Dam methylates the position N6 of adenines in 5′-GATC-3′ sequences, and shapes several processes including DNA replication, chromosome segregation, gene transcription, transposition, and repair. Methylation of the adenine amino group alters DNA curvature and lowers the thermodynamic stability of the DNA, changing DNA–protein interactions. *Salmonella enterica*, *V. cholerae*, and *Y. pseudotuberculosis dam*$^-$ mutants are attenuated for virulence in animal models. In *S. enterica*, the Dam methylation system regulates genes involved in flagellar subunits. *Salmonella dam* mutants have reduced motility, decreased expression of the genes from the SPI1 pathogenicity islands, and in a mouse model, the LD_{50} (median lethal dose) of a *S. typhimurium dam* mutant administered orally was $\sim$10 000-fold higher than for the wild-type strain.

Another epigenetic modification linked to virulence, recently reported by Hamon *et al*. was that early during the infection, before entry into cells, secreted LLO dephosphorylates Ser^{10} on histone H3 and deacetylates histone H4, in a manner that is independent of its ability to form pores. These activities correlated with decreased transcription of key immunity genes in the host, providing a fascinating mechanism by which a bacterial pathogen can manipulate the epigenetic machinery in the host as a novel virulence strategy.

Neurotoxicity Caused by Insoluble Proteinaceous Aggregates

Recent advances in the study of transmissible spongiform encephalopathies challenged existing dogmas and revealed new concepts in disease transmission and pathogenesis. Prions are associated with a group of fatal, progressive neurodegenerative diseases that can be spontaneous, genetic, or infectious in origin. In humans, they include kuru, the sporadic, familial, iatrogenic, and variant forms of the Creutzfeldt–Jakob disease, the Gerstmann–Sträussler–Scheinker disease, and fatal insomnia. In animals, some of the examples of prion diseases are scrapie in sheep, transmissible mink encephalopathy in farmed mink, and bovine spongiform encephalopathy (BSE) in cattle.

One of the early insights into prion diseases came from the study of kuru, a fatal transmissible neurodegenerative condition that was first described among people of the Foré linguistic group in Papua New Guinea and started to be scientifically studied in 1957. The disease was causally linked to endocannibalistic practices, and most frequently occurred in women and children, who were the most likely to consume

the brain during these rituals. Initially, the pathogen was thought to be a slow virus. A challenging finding was that prions could be transmitted and could act as infectious agents in the absence of a nucleic acid genome. In addition, they were unusually resistant to treatments known to inactivate most bacteria and viruses, including formaldehyde, high temperatures, or treatment with UV. Subsequently, the 'protein-only hypothesis' was developed, asserting that nucleic acid is not required for transmission, and that the infectious agent is a proteinaceous particle that can self-propagate by recruiting and converting the normal, noninfectious host cellular prion protein (PrP) into aggregates.

Prion diseases recently reemerged in the public attention with the BSE outbreak that was reported first in the UK in April 1985 and subsequently in several countries in the mid-1980s, reaching its peak in 1992. The supplementation of cow feed with meat-and-bone meal, a high-protein nutritional supplement prepared from the offal of cattle, sheep, pigs, and chickens, was implicated in the outbreak. Even though this food type had been used before, retrospective studies revealed that a change in the organic extraction process, and particularly the abrogation of the organic solvent use, allowed prions, which can be inactivated by lipid solvents, to maintain their infectivity and cause disease. It was reported that, as the use of organic solvent became less profitable, the amounts that were used to prepare the meat-and-bone meals decreased progressively after the late 1970s, leading to meat-and-bone meals with higher lipid content, and in the early 1980s, most plants abandoned the use of organic solvents. This time period coincided with the first reports of BSE in the UK. It is thought that this change in the manufacturing process allowed scrapie from sheep to survive, and it was passed to the cattle through the feed. After the feeding of ruminant-derived proteins to cattle was banned in July 1988 in the UK, and subsequently throughout the EU, the incidence of the disease started to decline after approximately 5 years, a time that corresponded to the approximate BSE incubation period.

Several lines of evidence pointed toward BSE as the cause of a neurodegenerative human disease that became known as variant Creutzfeldt–Jakob Disease (vCJD), first reported in the UK in March 1996, and subsequently in other countries. Both the animal and the human epidemics attracted considerable attention, particularly because of the oral transmission, the causative relationship between the infectious agent involved in the cattle and the human outbreaks, and its resistance to conventional decontamination procedures.

The current view, supported by several lines of experimental evidence, is that the PrP exists in two conformations: a cellular isoform (PrP^{C}) that is properly folded, protease-sensitive, noninfectious, soluble in detergents, and rich in α-helices, and a scrapie-related disease-inducing isoform (PrP^{Sc}) that is aberrantly folded, infectious, protease resistant, insoluble in detergents, and rich in β-sheets. PrP^{Sc} has the propensity to aggregate into amyloid-like fibrils or plaques. PrP^{C} conversion to PrP^{Sc} is poorly understood, and is thought to occur spontaneously or be induced by mutations, but once PrP^{Sc} is formed, it can act as a template to recruit PrP^{C}, and as a catalyst to convert it into more PrP^{Sc}. The cellular synthesis of PrP^{C} replenishes the PrP^{Sc} pool. Prions are capable of self-propagation and nucleation. The initial conversion of a cellular protein to a prion is thought to occur rarely, but once it happens, the prion may bind protein molecules in the healthy configuration and catalyze their conversion.

An interesting observation revolves around a polymorphism described in *PRNP*, the gene on chromosome 20 that encodes the human PrP. Position 129 in this protein can be ATG and encode methionine, or GTG and encode valine. It was observed that homozygosity at this site predominates among individuals with sCJD, and all but one individual with vCJD carried the 129 MM genotype. Heterozygosity at this locus was reported in the recipient of a blood transfusion from a donor who subsequently developed vCJD. The transfusion recipient died 5 years later from a nonneurological condition, and protease-resistant PrP was detected in several tissues. Among Foré women, heterozygosity at the same locus was reported to confer survival advantage, and heterozygous individuals had a much later onset of the disease as compared to homozygous individuals. The association between homozygosity and disease is explained by the ability of homologous protein species to establish protein–protein interactions with greater efficiency, whereas in heterozygous individuals, subtle differences between the protein species could make these interactions more difficult.

An important concept regarding prion diseases is the existence of species barriers: prions isolated from one species are often less infectious when introduced into a different species. For example, a species barrier was shown when it was reported that the hamster protein does not cause disease in mice. This is explained, at least in part, by dissimilar sequences and structures between an infectious prion and the host PrP variant. This species barrier was abrogated when mice were genetically engineered to harbor the hamster *PRNP* gene and express the hamster PrP^{C} protein. Sometimes, the species barrier is unidirectional, for example, although hamster-derived prions did not cause disease in mice even after several years, mouse-derived PrP caused disease in hamsters after $\sim$378 days. Similarly, hamster PrP cannot seed human amyloid, and human PrP does not cause disease in hamsters. Nevertheless, the species-specific substitution of a single amino acid at a critical region was sufficient to change this seeding specificity, and to bypass the species barrier, making the protein acquire the seeding characteristics of the other species. As it is well known by now, vCJD emerged in humans after the BSE epidemic in cattle, showing that the species barrier is not absolute. An interesting model proposes that the species barrier might be shaped by interactions between the PrP and host PrP sequence variants, a concept that opens thought provoking questions and has far-reaching medical and public health implications.

See also: Bacteria: *Campylobacter*; *Clostridium perfringens*; *Helicobacter pylori*; *Listeria monocytogenes*; *Salmonella* Typhi and *Salmonella* Paratyphi; Shiga Toxin-Producing *Escherichia coli* and Other Pathogenic *Escherichia coli*; *Shigella*; *Staphylococcus aureus*; *Vibrio cholerae*. Prions and Agents of TSEs: Bovine Spongiform Encephalopathy in Cattle

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CHARACTERISTICS OF FOODBORNE HAZARD AND DISEASES

Microbial Stress Response

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Glossary

Chaperone A protein which has the role of helping other protein chains fold correctly into a functional form.

Enterobacteriaceae A family of Gammaproteobacteria which are Gram negative, catalase positive, oxidase negative, rod-shaped facultative anaerobes which are bile-salt tolerant and often inhabit the gastrointestinal tract. It includes the genera *Escherichia*, *Salmonella*, *Shigella*, *Yersinia*, *Klebsiella*, *Enterobacter* and *Erwinia*.

Exponential phase Phase of growth curve when cell numbers increase logarithmically; cells are actively growing and doubling their numbers periodically.

Facultative anaerobe A microbe which can grow with or without oxygen present but grows better when oxygen is present. Such organisms can switch their metabolism between oxidative and fermentative states.

Gram-negative bacteria Bacteria that have a thin layer of peptidoglycan in the cell wall and an outer lipopolysaccharide layer. These cells fail to retain the Gram stain.

Gram-positive bacteria Bacteria that have a cell wall with a thick peptidoglycan layer, which helps them to retain the Gram stain.

Humectant A substance that aids the retention of moisture; examples in foods are salt, sugar, glycerol. Their addition causes changes to osmotic pressure and water activity.

Probiotic Live microorganism that, when consumed in sufficient numbers, gives a health benefit to the host.

Sigma factor A variable subunit of RNA polymerase which is often responsible for allowing differential gene expression. Examples: sigmas also known as RpoS, the stationary phase sigma factor; σ^{32}, also known as RpoH, the heat-shock sigma factor.

Stationary phase Phase of a logarithmic growth curve when cell numbers plateau; cells such as *Escherichia coli* change their characteristics though changes in gene expression.

Water activity (a_w) The vapor pressure of a liquid divided by that of pure water at the same temperature. Effectively it measures the amount of available water; a_w for pure water is 1 and this decreases as the solute concentration increases.

Food-Related Stresses

Many foods present a stressful environment for microorganisms. This may occur naturally because of the properties of a food, for example, the acidity in fruits or be part of a food's design with regard to formulation, packaging, and storage conditions. Table 1 shows the factors which control microbial growth in foods: intrinsic factors are those inherent in the food formulation; extrinsic factors are those related to packaging and storage conditions; implicit factors are related to the natural microbial contaminants; and processing factors are those processes which are applied to foods and designed to control microbial growth. For each of these factors there is a varying tolerance amongst microorganisms. For example, the minimum water activity (a_w) for bacterial growth ranges from 0.97 for bacteria like *Pseudomonas* and *Clostridium botulinum* type E to 0.85 for *Staphylococcus aureus* with only specialist halophilic bacteria growing at a_w 0.75 and xerophilic molds down to 0.61. For other factors such as pH and temperature, organisms have an optimum condition under which they will grow and a range either side of this where they grow at a slower rate; an organism will become stressed outside of these extremes but, because of the variation in intrinsic tolerance between different organisms, the levels causing stress will vary. For example, both *Salmonella* and *Campylobacter* have a pH optimum at neutral. However, *Salmonella* will grow over a pH range of >4 and <9.1, while *Campylobacter* is much more acid sensitive with a range of pH >5.9 to <9. These ranges are in turn affected by the other factors present: if another factor in the environment is below optimum, for example, water activity, then this will not only reduce the level of growth of the organism but also may reduce the pH range over which it grows. This is the basis of the 'hurdle effect' which imposes a series of low-level stresses on the organism which together will prevent its growth.

Culture Production

The production of live bacteria for use in food production as starter cultures and as probiotics is a major industry. Where

Table 1 Factors controlling microbial growth in foods

Intrinsic factors
Water activity (a_w): concentration of humectant, for example, NaCl, sucrose, and glycerol
pH
Organic acids: citrate, lactate, and acetate
Eh (oxidative/reductive potential)
Preservatives: for example, sulfite and nitrite
Natural antimicrobial components: for example, allicin, eugenol, and thymol
Nutrient supply
Food structure
Extrinsic factors
Temperature
Time (shelf life)
Gas atmosphere
Relative humidity
Implicit factors
Microflora composition
Microflora levels
Cell state
Spatial distribution
Processing factors
Heat
Freeze–thaw
Irradiation
Biocides

these are provided as dried powders or as frozen cultures the viability of the organisms may be compromised as the processes used in culture production can cause damage to the cells. Optimizing bacterial cellular recovery so that a high level of viable cells is provided is a major concern for the industry and an understanding of stress response in these organisms may be exploited to provide mechanisms by which stress response can be switched on before exposure to the stress, thus providing improved culture viability.

Bacterial Stress Response

Although all organisms have an intrinsic resistance level to different stress, they can show adaptations which allow them to grow outside of their normal range. In bacteria there are a number of adaptive responses which have been demonstrated: some are generic responses which allow adaptation to a range of stresses and some are stress-specific responses.

The Global Response Regulator: *rpoS*

When a population of cells of *Escherichia coli* is exposed to a sublethal stress such as moderate heat treatment (e.g., 56 °C), a proportion of the cells die but some will grow on culture media. The proportion of cells which can be recovered depends on a number of factors including the level of stress imposed, the nature of the recovery conditions, and the phase of growth in which the cells were when the stress was imposed. In particular, stationary phase cells show a greater tolerance to stress than exponentially growing ones. Such a response is seen in other facultative anaerobes such as *Salmonella* and *S. aureus* but not in strictly fermentative organisms such as *Streptococcus mutans*. *Campylobacter jejuni* does not mount this enhanced stress resistance stationary phase phenotype but shows a reduced resistance in stationary phase.

In *E. coli* and other Enterobacteriaceae, the entry into stationary phase of growth causes a series of cellular changes. In particular, cells show increased resistance to oxidative stress, near-ultra violet (UV) irradiation, thermal, acid, and osmotic stress and starvation. The increased expression of a central regulator of stationary phase genes, the sigma factor σ^s or RpoS, is responsible for these changes. Increased levels of the sigma factor lead to upregulation of a large series of genes and operons under its control, many of which create increased stress tolerance. Genes such as *tre*A (*osm*A) and *ots*AB, which are associated with trehalose metabolism, provide increased resistance to thermal and osmotic stress; genes for catalase hydroperoxidase, *kat*G and *kat*E, provide oxidative stress protection against hydrogen peroxide; there are also a series of genes involved in deoxyribonucleic acid (DNA) repair which give increased resistance to oxidative stress and near-UV light. Other changes such as a switch to anaerobiosis and the shortening of cell shape may also have a role in creating increased stress tolerance.

Although originally considered as a gene induced in stationary phase, *rpoS* is now seen as a global response regulon as a range of stress factors cause levels of the sigma factor to increase. These include osmotic stress caused by different humectants, starvation, temperature stress, and acid stress. Redox potential has also been shown to influence RpoS induction leading to increased stress resistance as have concentrations of nitrite >500 ppm, although these levels are above those allowed in the food industry. As such the use of sublethal levels of these stresses in foods and food processing present a particular problem. Exposure to sublethal levels of one stress will switch on resistance in the surviving cells to a whole host of other stresses via RpoS induction and will also induce expression of some virulence genes, for example, *spv* in *Salmonella*. Thus cells surviving one sublethal stress will be primed to survive subsequent different stresses and may be more virulent.

Specific Stress Responses

Acid Stress

Although microorganisms have a pH range over which they naturally grow, many organisms show the ability to adapt to low acidic conditions. The acid tolerance response (ATR) can be defined as the induced resistance to a normally lethal low pH challenge following growth or exposure at moderately low pH. This response has been extensively studied and was founded on the original study of Foster and Hall who showed that when exponential phase *Salmonella* Typhimurium bacteria grown at pH 7.6 were shifted to pH 5.8 for one doubling and then challenged with pH 3.3, they survived this normally lethal challenge. The response has been shown to be influenced by a whole range of factors (such as type of acid, the pH challenge used, and growth phase) and more than one molecular mechanism is in place with exponential phase ATR

and stationary phase ATR being different responses. The acid shock proteins (ASPs) which are induced influence a range of cellular metabolism and repair functions and different subsets of these are controlled by central response regulators including RpoS, OmpR, and Fur, as well as the two-component systems PhoPQ and OmpR/EnvZ, each of which regulate a subset of ASPs. Different ASPs are induced in the exponential and stationary phase responses (60 and 48 ASP, respectively having only 5 in common) and by the nature of the acid used (organic vs. inorganic). However, it must be remembered that other bacteria are also capable of mounting this response, for example, *E. coli* and *Listeria monocytogenes* although the precise cellular mechanisms by which this is achieved vary.

Of the many ATR proteins produced glutamate decarboxylase (*gad*) has been shown to be important for very low pH challenge. This enzyme converts glutamate γ-aminobutyric acid (GABA) and in doing so uses up a proton. GABA is then transported out of the cell by an induced antiporter in exchange for another glutamate. Interestingly, whereas *E. coli* and *Shigella* both contain this enzyme, *Salmonella* does not. Arginine decarboxylase, the product of the *adiA* gene, is a second system which provides resistance when arginine is present in the medium. The enzyme converts arginine to agmatine again after consuming a proton in the reaction.

The importance of acid resistance in food safety is that acid-adapted bacteria may then survive a later more extreme challenge. Hence, *Salmonella* surviving exposure to an intermediate pH in a food may then be more able to survive the acid challenge of the stomach. The rate at which a fermentation takes place could also be crucial as a slow rate of initial acid production could lead to acid adaptation and survival in the final product even which this achieves a presumed lethal pH. Moreover, the acid stress response of exponential phase cells has been shown to provide a cross-protective effect to other stresses such as heat shock, high osmolarity, oxidative stress, and DNA damage (although this does not occur reciprocally). The involvement of global regulars in ATR may be an explanation for this but this does mean that cells surviving acid stress will have increased tolerance to other usually hostile environments and not just low pH. From a more beneficial aspect, the acid stress response in probiotic species like *Bifidobacterium longum* has also been investigated with a view to increasing their ability to survive the stomach acid challenge.

Heat Shock

When cells are exposed to a sublethal increase in temperature or heat shock, a set of proteins called the heat shock proteins (hsps) is expressed. This response is ubiquitous in all cells but the precise nature of the genes induced and their regulation varies. Two examples will be used to illustrate this here: heat shock in the Gram-negative bacterium *E. coli* and in the Gram-positive bacterium *Bacillus subtilis*.

In *E. coli* approximately 20 hsps are temperature responsive showing a large increase in their rate of synthesis by 10- to 20-fold. This increase is regulated at the transcriptional level by sigma factor σ^{32} or *rpo*H; a second sigma factor σ^{E} (σ^{24}) has been shown to be important for the regulation of *rpo*H expression itself at high temperature ($>50\ ^{\circ}C$). However, hsps are not always induced only by heat and other stresses such as ethanol can also switch these on. Many of the functions the hsps affect are basic cellular functions such as DNA replication, ribonucleic acid (RNA) and protein synthesis, and cell division and some *rpo*H- regulated hsps are required at all temperatures. In fact, mutants of *rpoH* are compromised for growth, not growing above 20 °C. Therefore, although upregulated during heat shock, the functions of these proteins appear to be more fundamental to the cell. For example, the hsp GroEL increases from $<1\%$ to 20% of cellular protein when *E. coli* is moved from 30 to 46 °C.

Many hsps under σ^{32} control are chaperones whose function is to ensure correct folding of proteins in the cells. As one of the cellular responses to heat is for proteins to denature, chaperones allow correct refolding of these to take place. Typical of these are chaperone teams DnaK–DnaJ–GrpE and GroEL–GroES complexes which act cooperatively in protein folding and are involved in nascent chain folding as well as refolding of thermally damaged protein species.

Other hsps under σ^{32} control are proteases and these break down damaged proteins and thus regulate protein turnover. Typical of these are Lon and Clp proteases which are produced constitutively but are upregulated on heat shock. Their normal function may be to act to control regulator proteins as well as to degrade abnormal or damaged proteins.

In *B. subtilis* there are three classes of heat shock inducible genes. Class 1 are hsps which include GroESL and DnaK and the operons are controlled by a conserved region in the chromosome, controlling inverted repeat of chaperone expression (CIRCE), which is only induced by heat shock. Class 2 hsps are controlled by the alternative sigma factor σ^{B} which is a general stress sigma factor; class 2 heat shock genes are the largest group comprising >125 genes. These also respond to salt, ethanol, and starvation stress and stationary phase, and so are really general stress-response proteins (gsps). Class 3 hsps are independent of σ^{B} and CIRCE and are proteases including the *Clp* genes and Lon. The cellular control of these three systems is complex at the transcriptional/translational level.

Thus both Gram positives and Gram negatives respond to heat stress by producing chaperones to repair the damaged proteins and proteases to remove those that cannot be repaired. However, their cellular control of these is quite different and under complex regulatory systems.

Recent studies on heat shock in *Salmonella* have compared planktonic and immobilized cells. Immobilized cells may be considered to be more typical of what is found in foods where colonial microenvironments may develop. This study examined transcriptome differences in the response of *S.* Typhimurium to treatment at 45 °C for 30 min and showed that the pattern of expression of 538 genes was different if cells were immobilized or planktonic. In particular, invasion of Hela cells and virulence gene expression were two elements which showed differences in expression with immobilized cells in contrast to planktonic cells, showing an increased upregulation. This study demonstrates that understanding of a bacterium's response to stress is dependent on more than the stress alone and the whole of the environmental conditions under which the response is studied needs to be considered.

Cold Shock

Cold shock is induced when cells suffer a temperature downshift of 13 °C. Unlike heat shock it has a less damaging effect on cellular components and the main attribute is the loss of membrane fluidity, nonstabilization of secondary structures of nucleic acids and proteins and reduced function of ribosomes through the formation of RNA structures that cause these to stall and inhibit translation initiation. Like the response to heat shock, cold shock induces a series of proteins, the cold shock proteins (Csps), which are found widely in many organisms. The major Csp, CspA is sufficiently conserved in sequence that it has been suggested as an alternative taxonomic marker in Gram-positive and Gram-negative bacteria.

In the Gram-negative *E. coli*, two classes of Csps are induced. Class 1 shows a 10-fold or greater induction following cold shock. One of these is the CspA family, a structurally related group of proteins; although there are nine homologous proteins in CspA-I, only CspA, CspB, CspG, and CspI are induced on cold shock and these are nucleic acid chaperones. Of these CspA is expressed at the highest levels and its role is to bind to single-stranded RNA and function as an RNA chaperone to prevent secondary structure formation. CspB and CspG are believed to perform a similar role but may also act as DNA chaperones assisting in DNA replication. Other Csps in class I are CsdA, RbfA, and NusA, all of which are ribosome-associated proteins assisting in ribosome function, and polynucleotide phosphorylase, a ribonuclease which may be involved in mRNA degradation.

Class 2 Csps are all induced to a much lesser extent (<10 fold); three of these are bacterial translation initiation factor IF2, GyrA and H-NS. IF2 is again a ribosome-associated protein involved in translation and is required for efficient ribosome function at low temperatures. IF2 ensures that protein synthesis begins at the correct codon within a messenger RNA by mediating the binding of the initiation tRNA (fMet-tRNA) to the 30 S subunit.

In the Gram-positive *B. subtilis* the block in translation initiation also induces a series of ribosome-associated proteins which are functionally similar to those seen in *E. coli*; however many of these are parts of the standard translation system and are not cold specific.

Loss of membrane fluidity is a major factor which affects the functioning of the cell as transport and secretion systems are impaired. A change to lower temperatures results in a change in lipid composition to ensure that the liquid crystalline nature of the membrane is retained and to prevent the formation of a gel state. Fluidity in part is regulated by the ratio of unsaturated to saturated fatty acids present in membrane lipids. In aerobic bacteria this can occur by direct conversion of unsaturated to saturated states. In *Bacillus* this is through the action of the membrane-bound desaturase enzyme, *des*, the gene for which is cold inducible. In *E. coli* increased activity of the enzyme synthase II converts palmitic acid to cis-vaccenic acid. It is also reported that in *L. monocytogenes* the ratio of anteiso- to iso-branched fatty acids is dramatically changed after temperature downshift.

In *L. monocytogenes* which is a psychrotrophic bacterium (a mesophile which can grow at low temperatures) balanced growth at low temperatures has been called cold acclimation and proteins exhibiting increased synthesis during this period are called Caps. Five of the 12 Csps induced in *Listeria* are also Caps. This includes CspLa, the major Csp protein in *Listeria*. As well as this, an oligopeptide-binding protein, OppA, is required for low-temperature growth.

Osmotic Shock

Osmotic shock in bacteria occurs when they are placed in hypo- or hypertonic conditions and a range of humectants widely used in the food industry can induce this state. Most work has concentrated on the effect of NaCl as this is experienced naturally as well as in artificial conditions such as foods. With the exception of true halophiles, a salt concentration of approximately 1% is optimum for most organisms but some species are naturally halotolerant and will grow at high salt levels, for example, *S. aureus* and *L. monocytogenes* will grow in conditions of up to 10% salt.

In high osmolarity environments the cell needs to prevent water loss to the environment and this is achieved by the accumulation of compatible solutes in the cell. K^+ ion influx is a rapid initial response in *E. coli*, occurring via three different uptake systems. Following this in Gram-negative bacteria, glutamate is synthesized by two enzymes: glutamate dehydrogenase and glutamate synthase and accumulates quickly; this synthesis is dependent on K^+ ion as an initiator for synthesis. Following this, another compatible solute trehalose is synthesized by the *otsAB* operon. OtsA is a trehalose-6-phosphate synthase and OtsB is a trehalose-6-phosphate phosphatase. These genes are under RpoS control as already discussed and *rpoS* is induced in exponential phase by high osmolarity. Gram-positive bacteria show a similar response although proline generally replaces glutamine.

Many compounds found in animals and plants can act as osmoprotectants in bacteria. These include compounds such as betaine, choline, and carnitine. In the presence of these, cells will accumulate derivatives, for example, in *E. coli* choline is converted to betaine aldehyde and then to glycine betaine. The ProU permease in *E. coli* shows a higher affinity for betaine than for proline.

In many of the enteric bacteria, a change in several major outer membrane proteins occurs in high osmolarity. In particular, there is a switch in the expression of OmpC and OmpF. These are porin proteins which form pores in the membrane. The pore size of OmpC is smaller than that of OmpF and in high osmolarity, OmpC porin predominates, reducing the ingress of solutes. *ompR* is one of the regulatory genes for this system, another central regulatory system.

Oxidative Stress

Microorganisms grow under a range of atmospheric conditions from the strict aerobes which have an absolute requirement for oxygen to grow to facultative anaerobes that can grow with or without oxygen to strict anaerobes which are killed by oxygen presence. In respiratory organisms, the generation of oxygen radicals is potentially damaging to DNA, proteins, and lipids. A series of enzymes are responsible for the inactivation of these and these can also protect the organism from applied oxidative stress. Hydrogen peroxide is excreted as a waste product by many heterofermentative lactic

acid bacteria and may also be used in surface sterilization procedures. It can also be formed as a product of superoxide dismutase (SOD) which converts superoxide radicals to hydrogen peroxide and oxygen. Hydrogen peroxide is inactivated by catalase, the enzyme breaking it down into oxygen and water.

In *E. coli* there are two catalase genes, *katG* and *katE*, which are complexly regulated; *katE* expression is solely under *rpoS* regulation and hence its expression correlates with the levels of RpoS in the cell. *katG*, in contrast, is also part of another response regulon under the control of *oxyR*, another global response regulator, which is induced in response to oxidative stress. There is an interdependence of these regulators involved in *katG* expression. OxyR in turn regulates OxyS, a small RNA regulator which negatively regulates the levels of active RpoS in the cell. This is believed to be a fine-tuning mechanism to allow induction of only the oxidative stress genes which are under the control of both regulators (e.g., *katG*) and to avoid the full expression of the RpoS regulon under conditions of oxidative stress. Hence, cellular levels of catalase activity are regulated in a complex manner and different genes may be involved at different stages of growth. Another gene under OxyR control is alkyl hydroperoxide reductase (ahpC and ahpF), the function of which is probably in the detoxification of lipids and other hydroperoxides produced during oxidative stress.

In a number of studies examining the induction of genes involved in the stress response to a variety of stresses, induction of oxidative stress protection systems have been reported even when the organisms were not exposed to oxidative stress. Privalle and Fridovich showed that *E. coli* expresses SOD when exposed to heat stress, and Belkin *et al*. showed that catalase was induced following ethanol challenge. In *S. aureus* Armstrong-Buisseret *et al*. demonstrated that alkyl hydroperoxide reductase is induced when the organism was subjected to osmotic shock. Deficiencies in any of the protective mechanisms against oxidative stress lead to multiple stress sensitivity in bacterial cells, for example, *E. coli* deficient in SOD was reported to be sensitive to heat, as well as O^{2-}.

Dodd *et al*. advanced a theory suggesting that a major cause of damage in sublethally injured cells is intracellularly produced free radicals; this effect was termed the bacterial suicide response. The central tenet of this theory is that, when injured by any applied stress, actively growing cells produce a burst of free radicals which causes oxidative damage to the cell; this contributes to cellular injury and death. This response occurs regardless of the nature of the stress causing the injury and is generic in nature. Exponential phase cells, because of their greater metabolic flux, are more susceptible to the effect than stationary phase ones and hence incur greater damage in response to sublethal injury. The presence of a free radical burst on exposure to sublethal injury has been demonstrated by Aldsworth *et al*. (1998b).

Conclusion

From the work discussed in this article, it can be seen that the induction of resistances following a variety of environmental stresses can also lead to induction of other stress regulons and in particular, some of the central stress regulators like *rpoS* which coordinately induce a whole range of other resistances and indeed virulence genes. There are obvious disadvantages in this for the food industry. The move toward minimal processing to improve the quality of foods may mean that there is a greater risk of this occurring.

See also: Bacteria: *Listeria monocytogenes*; *Salmonella* Non-Typhi; Shiga Toxin-Producing *Escherichia coli* and Other Pathogenic *Escherichia coli*. Characteristics of Foodborne Hazard and Diseases: Sublethally Injured and Viable but Nonculturable Cells. Disciplines Associated with Food Safety: Food Microbiology. Safety of Food and Beverages: Safety of Probiotics and Prebiotics

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CHARACTERISTICS OF FOODBORNE HAZARD AND DISEASES

Sublethally Injured and Viable but Nonculturable Cells

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Glossary

Microcosm Sealed chamber containing water from a natural source into which microorganisms are inoculated for survival studies.

Pre-enrichment An incubation step in a non or mildly-selective medium that allows cells to repair injury and multiply before being subcultured into a more selective medium.

Reactive oxygen-containing species Oxygen-containing compounds, ions, or free radicals that react directly with cell components causing damage or giving rise to reactive products. Examples include hydrogen peroxide, superoxide anion, hydroxyl free radical, and singlet oxygen.

Resuscitation Procedure in which sublethally injured or VBNC cells are incubated under appropriate conditions to allow repair of injury and/or recovery of culturability.

Selective medium A medium containing selective agents that when incubated under appropriate conditions suppresses growth of unwanted organisms, while allowing multiplication of the target group of organisms.

Introduction

Detecting and enumerating viable microorganisms forms an essential part of any strategy for ensuring the microbiological safety and quality of food. However organisms that have survived exposure to food processing treatments may suffer debilitating cellular injury that renders them unable to grow on the selective media used for their detection. Another class of organisms, those in the viable but nonculturable (VBNC) state, are believed to be viable but unable to grow on media that normally support their growth. Under suitable conditions, injured or VBNC cells can resuscitate and regain all their normal properties, including virulence. Understanding the properties of these organisms and how they may be recovered is therefore important in assessing risks to public health.

Properties of Sublethally Injured Cells

Injured cells display a diverse range of altered growth properties. These are summarized in Table 1, together with possible explanations for the altered phenotypes.

An example of the practical implications of sublethal injury when assessing the survival of *Escherichia coli* O157 under conditions simulating those in a fermented sausage is shown in Figure 1. When counts were made on Sorbitol MacConkey Agar (SMAC), it appeared that a 5-log reduction had been achieved by approximately 10 days storage, whereas enumeration on Tryptone Soya Agar (TSA) showed that this underestimated survival by a factor of approximately 10 000 and in fact viable organisms were still present after 30 days.

Resuscitation and Repair of Injury

Repair of injury in pure cultures can be demonstrated by the differential plating method, in which injured cells are incubated in a suitable liquid medium and samples removed at intervals for plating onto selective and nonselective agar media (Figure 2). The nonselective medium allows growth and enumeration of both injured and healthy cells, whereas the selective medium allows growth of uninjured cells only. During the extended lag period, repair takes place and cells regain their ability to grow on the selective medium. Multiplication ensues usually, soon after the repair of injury is complete.

Under optimum conditions, injury caused by chilling, freezing, freeze-drying, γ-radiation, or acid is normally repaired within 4–5 h and often within 1–2 h. Mild heat injury can be repaired in approximately 5 h, but cells with severe heat injury may require much longer times, up to 24 h in extreme cases. The severity of injury varies within injured populations and this is reflected in wide differences in lag times of individual cells. Such heterogeneity becomes relevant when examining samples likely to contain low numbers of pathogens.

Screening for the Presence or Absence of Pathogens

For pathogenic microorganisms that have a low infective dose such as *E. coli* O157, *Campylobacter jejuni*, and *Salmonella enterica* serovars, the presence or absence of the target organism is the main concern and prolonged pre-enrichment can be used, because multiplication after repair will not affect the result and will in fact increase the sensitivity of detection. With *Salmonella*, pre-enrichment in buffered peptone water or lactose broth has been

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Table 1 Symptoms of sublethal injury

Symptom of injury	*Probable or possible cause of injury*
Sensitivity to ingredients of selective media: bile salts, dyes, and hydrophobic antibiotics	Loss of lipopolysaccharide from Gram-negative outer membrane or loss of gram-positive surface protein layer. Loss of transmembrane proton gradient and multidrug efflux pump activity
Sensitivity to lysozyme, bacteriocins, and large hydrophilic antibiotics	Transient physical disruption of Gram-negative outer membrane
Sensitivity to acid, alkali, osmotic stress, and some inorganic ions	Unknown. Possibly impairment of cytoplasmic membrane barrier properties toward cations and protons. Physical disruption of efflux or uptake systems involved in homeostasis. Depletion of cellular adenosine triphosphate or loss of proton motive force required for membrane transport systems
Sensitivity to exogenous oxidative stress	Inactivation of protective enzymes catalase, peroxidase, and superoxide dismutase Depletion of intracellular reductants or inactivation of thiol disulfide redox systems Increased permeability of membrane toward exogenous oxidants
Increased production of intracellular reactive oxygen species	Membrane perturbation leading to disorganization of electron chain components and increased likelihood of univalent reduction of oxygen by intermediates of the chain Imbalance in metabolism leading to increased metabolic production of hydrogen peroxide or superoxide Disruption of iron sulphur complexes, release of iron and production of hydroxyl free radical by Fenton reaction
Altered nutritional requirements for growth (real or apparent)	Enzyme inactivation (e.g., peptidases) Sensitivity to peroxide in rich media or requirement for reductants (minimal medium injury) Metabolic imbalance on nutritional upshift
Restricted temperature range for growth	Unknown. Possibly imbalance between repair and degradative processes
Loss of virulence	Unknown
Extended lag	Need to resynthesize damaged membranes, nucleic acids, ribosomes, etc

Source: Reproduced from Mackey BM (2000) Injured bacteria. In: Lund BM, Baird-Parker AC, and Gould GW (eds.) *The Microbiological Safety and Quality of Food*, pp. 315–341. Maryland: Aspen, with kind permission from Springer Science + Business Media B.V.

in use since the 1970s, but a wide range of other pre-enrichment broths have been approved by official bodies depending on the target organism and type of food being examined. Details of pre-enrichment media, appropriate for specific organisms, can be found in other chapters of this Encyclopedia and from official sources such as the International Organization for Standardization (ISO) or the Bacteriological Analytical Manual (BAM) of the US Food and Drug Administration (FDA).

Recommended pre-enrichment-incubation periods for presence–absence tests are generally around 16–24 h, although much shorter periods have often been advocated; especially with rapid methods employing nucleic acid-based endpoint detection systems. Short resuscitation steps are perfectly adequate for many types of food, but may give false-negative results if severely injured organisms are present. However, prolonged incubation in nonselective medium can lead to overgrowth by competitor organisms that suppress the growth of the target organisms. A compromise arrangement employed with *Campylobacter* spp. is to have a short and nonselective incubation period (e.g., 4 h), followed by the delayed addition of antibiotics, and/or an increase in the incubation temperature. This procedure allowed the detection of *Campylobacter* from bathing beaches, a difficult habitat from which to isolate pathogens. Delayed addition of selective ingredients can be accomplished by the use of timed-release capsules which, though effective, have not found wide use.

Enumeration Using the Most Probable Number Method

Enumeration of injured cells can be achieved by combining liquid resuscitation with the most probable number (MPN) approach. Following serial dilution of the sample, replicate volumes are inoculated from each dilution into separate tubes of nonselective medium to allow resuscitation, and this is followed after a suitable interval by subculture to a selective medium appropriate for the target organism. The number of positive replicates on or in a selective medium at each dilution is recorded and MPNs are estimated by reference to standard tables. The method depends only on the proportion of tubes showing growth, and is thus not affected by the extent of multiplication after repair. An example of this method is the multiple-tube MPN method for the enumeration of *E. coli* in water, in which resuscitation in minerals-modified glutamate medium is followed by incubation in bile salts and brilliant green-containing media. The MPN method is sensitive but labor intensive, and the precision is poor unless the number of replicate tubes per dilution is very large. Microwell plates have sometimes been used for greater convenience rather than tubes or bottles.

Enumeration Using Selective Agar Media

Baird–Parker selective agar for *Staphylococcus aureus* is unusual in that it supports good recovery of cells damaged by drying, freezing, or heating. There are few other examples of non-inhibitory selective media, so a resuscitation step is needed to allow recovery of injured cells before exposure to selective agar. Resuscitation in broth before plating on agar is unsatisfactory because cell multiplication in the broth may occur leading to an overestimate of viable numbers. If resuscitation is allowed to take place in cells immobilized on agar, then enumeration problems arising from multiplication after repair are circumvented. The thin agar layer method consist of pouring a layer of

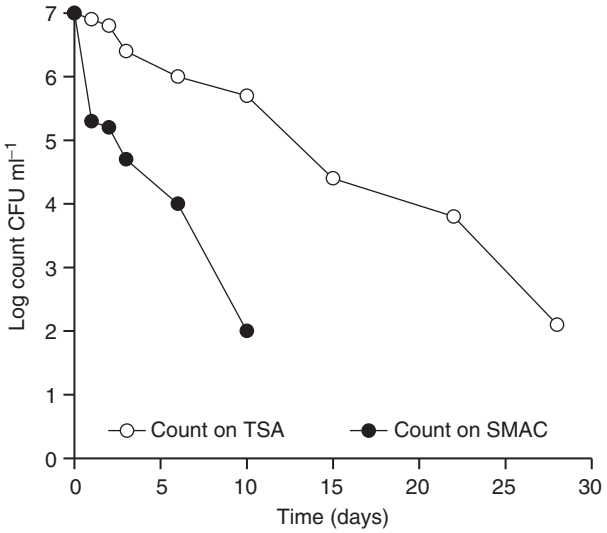

Figure 1 Sensitivity of sublethally injured *E. coli* O157 to selective media. Cells were inoculated into Tryptone Soya Broth containing 13.5% NaCl adjusted to pH 4.9 with lactic acid representing conditions found salami. Samples were removed at intervals for viable counting on TSA or SMAC. Reproduced from McCarthy J, Holbrook R, and Stephens PJ (1998) An improved direct plate method for the enumeration of stressed *Escherichia coli* O157:H7 from food. *Journal of Food Protection* 61: 1093–1097. © International Association for Food Protection, Des Moines, Iowa, USA.

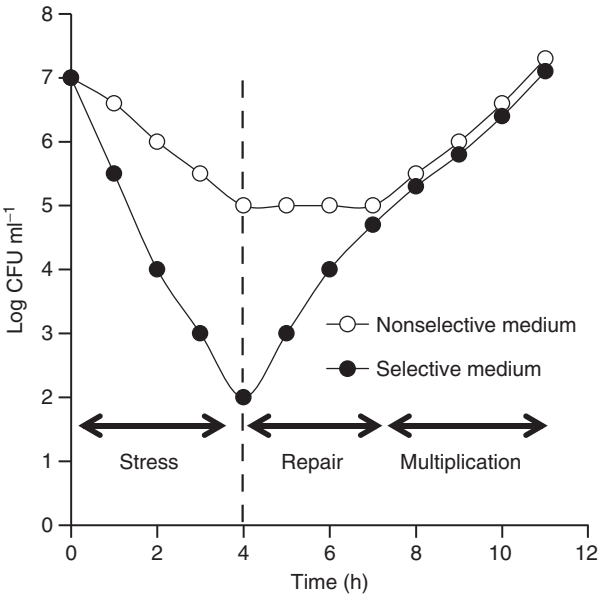

Figure 2 Development of sublethal injury and subsequent repair monitored using the differential plating method.

nonselective agar over a selective medium and allowing this to set immediately before spreading the sample (Figure 3(a)). Resuscitation takes place on the nonselective layer as selective agents diffuse into the upper layer to suppress the growth of competing bacterial species. A method that avoids having to pour agar immediately before spreading the sample consists of spreading samples on acetate or polycarbonate membranes, which are incubated on nonselective medium to allow injury repair before transferring the membranes to selective agar (Figure 3(b)). This method is in routine use for the enumeration of *E. coli* as indicator organisms.

Preventing Death of Injured Cells from Oxidative Stress During Recovery Procedures

Sensitivity to oxidative stress can exacerbate the inhibitory effects of selective media ingredients toward injured cells and can even prevent their growth on supposedly noninhibitory nutrient media. Avoidance of oxidative stress depends on minimizing the metabolic generation of damaging reactive oxygen-containing species (ROS) and/or by removing exogenous oxidants present in recovery media. During normal respiration, small amounts of the damaging superoxide anion or hydrogen peroxide are produced, but in healthy cells these are removed by the action of superoxide dismutase and catalase. The fine balance between the production and removal of ROS can be disturbed either by inactivation of protective enzymes or by an increased metabolic flux of peroxide or superoxide. This appears to happen in injured cells, although the mechanisms are not fully understood (Table 1).

Metabolically-generated oxidative stress can be avoided by allowing injured cells to resuscitate under anaerobic conditions. This has been shown to improve recovery of heat-injured cells of *E. coli* and *Listeria monocytogenes* substantially, sometimes by several orders of magnitude (Figure 4).

Resistance to aerobic incubation is recovered during the lag phase when cells are incubated anaerobically. It is important to note that this dramatic enhancement of the recovery of injured cells during anaerobic incubation in broth restores the ability to grow aerobically on nonselective nutrient media such as TSA. It is therefore highly relevant to laboratory studies with pure cultures designed to establish resistance to heat and other processes used in food preservation. It is also worth noting that aerobic incubation in static liquid medium causes less oxidative stress than incubation on the surface of an agar plate so that MPN counts in broth are much higher than plate counts on agar media of similar composition.

Exogenous oxidative stress can arise from the presence, in recovery media, of hydrogen peroxide, superoxide anion, organic peroxides, and other uncharacterized oxidants. These can be generated in nutritionally rich media by a range of processes including reactions between reducing sugars and phosphate during autoclaving and by subsequent photochemical oxidation of tryptophan, riboflavin, or peptones. Tests using low numbers of injured *Salmonella* cells have shown that the numbers of cells able to recover in commercially available nonselective broth media can vary by orders of magnitude. This was attributed to differences in the inhibitory properties of different peptone ingredients. Exogenous oxidative stress can be minimized by formulating media with noninhibitory peptones and by adding supplements that act as reducing agents or remove exogenous ROS. These include blood, charcoal, catalase, sodium pyruvate, sodium metabisulphite, and ferrous sulphate. For example, a blood-free charcoal cefoperazone deoxycholate (CCD) selective agar commonly used for *Campylobacter* spp.

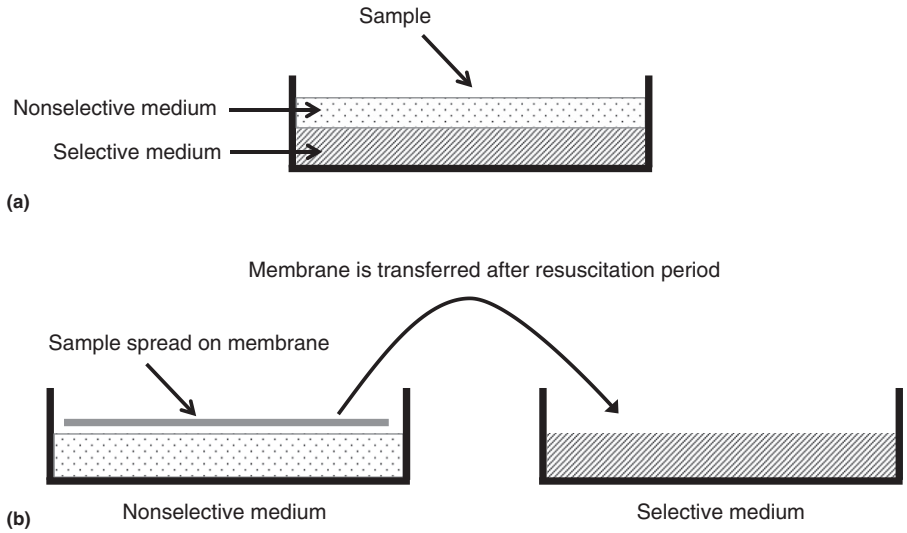

Figure 3 Solid medium repair. (a) Sample is spread on a thin layer of nonselective medium that has been overlaid on selective agar. (b) Sample is spread on membrane filter on nonselective agar. After incubating to allow repair of injury, membrane is transferred to selective agar.

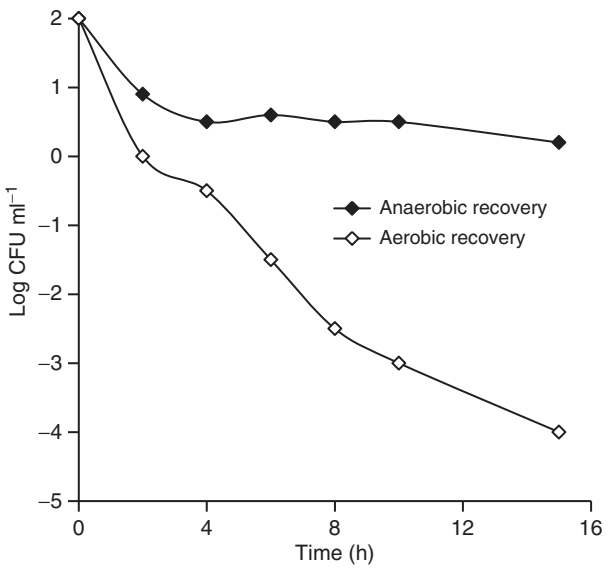

Figure 4 Effect of anaerobic incubation on recovery of heat-injured cells *E. coli* O157. Reproduced from George SM, Richardson LCC, Pol IE, and Peck MW (1998) Effect of oxygen concentration and redox potential on recovery of sublethally heat-damaged cells of *Escherichia coli* O157:H7, *Sal. enteritidis* and *Listeria monocytogenes*. *Journal of Applied Microbiology* 84: 903–909, with permission from Wiley.

contains a mixture of charcoal, sodium pyruvate, and ferrous sulphate, whereas sodium pyruvate is included in buffered *Listeria* enrichment broth and universal pre-enrichment broths specified in the BAM of the FDA.

Strictly anaerobic conditions can be achieved by working entirely within an anaerobic cabinet with pre-reduced media. More convenient methods for achieving anaerobiosis are available including the use of 'Oxyrase,' a proprietary crude membrane preparation from *E. coli* that scavenges oxygen with high affinity. The simple expedient of incorporating the reducing agent cysteine into recovery broth and sparging the headspace with nitrogen allowed the recovery of severely heat-injured *L. monocytogenes*. The use of semisolid media containing cysteine in screw-capped tubes achieved the same end. As a minimum precaution, when estimating viable numbers of stressed cells in pure culture studies, catalase or sodium pyruvate should be incorporated into nutrient nonselective agars, for example, when determining resistance to heat, high pressure acidity, and other preservation processes.

Viable but Nonculturable Cells

In recent years, there has been considerable interest and concern about possible safety problems posed by VBNC cells. These are physically intact and show metabolic activity of various sorts, but fail to multiply on nonselective media that normally support their growth. In many studies, the VBNC state has been induced by incubating cells at low temperature in natural water microcosms, but more acute stresses may induce nonculturability including aerosolization, freezing and thawing, and exposure to weak organic acids and biocides. *Vibrio vulnificus* can be induced to enter the VBNC state by growing in artificial seawater at 20–25 °C and then decreasing the temperature to approximately 4–5 °C. The cells change in shape from rods to cocci and rapidly become nonculturable, but retain metabolic activity.

A number of indicators of cellular integrity and metabolic activity have been used as indicators of viability when studying the VBNC state. One that is widely used is the BacLight Live–Dead kit, which stains cells with a mixture of the fluorescent dyes, SYTO-9 and propidium iodide. SYTO-9 is a membrane-permeant nucleic acid stain, which stains all cells and therefore gives the total cell count, whereas propidium iodide stains only cells with disrupted cytoplasmic membranes, which are scored as dead. Intact cells are counted as viable. Other methods are based on the detection of esterase activity, reduction of tetrazolium salts, membrane potential, induced enzyme synthesis, gene expression, and the presence of short-lived mRNA. The Kogure direct microscope count method depends on the

Table 2 Examples of treatments leading to an increase in culturability *in vitro*

Organism	*Inducer of VBNC state*	*Resuscitation treatment*
Vibrio vulnificus and *Vibrio parahaemolyticus*	Starvation at 5 °C in artificial seawater medium	Temperature upshift to 20–25 °C
Campylobacter jejuni	Anaerobic incubation	Incubation under microaerobic conditions with added spent medium
Salmonella enterica serovar Typhimurium	Starvation in river water 25 °C	Nutrient addition
Sa. enterica serovar Typhimurium	Prolonged incubation in water microcosm. Heating at 53 °C	Incubation in nutrient broth supplemented with Ferrioxamine E, Oxyrase or heat-stable enterobacterial autoinducer (AI)
Sa. enterica serovar Oranienberg	Incubation in 7% NaCl at 37 °C	Resuscitation promoting factor (Rpf)-like protein derived from *Salmonella* Typhimurium; product of *YeaZ* gene possibly a peptidase
Escherichia coli (clinical or food isolates)	Starvation in 0.9% and 4% saline at 4 °C	Minimal medium supplemented with methionine, glutamine, threonine, serine, and asparagine
E. coli O157	Sodium hypochlorite	Conditioned medium, Autoinducer AI-2
Micrococcus luteus, *Rhodococus rhodocrous*, *Mycobacterium tuberculosis*, and *Mycobacterium smegmatis*	Prolonged incubation in stationary phase	Incubation in liquid medium alone or with Rpf with muralytic activity
Staphylococcus aureus	Starvation at seawater at 4 °C	Temperature upshift to 22 °C

ability of cells to elongate in response to nutrients in the presence of an antibiotic that inhibits cell division.

Resuscitation of Viable but Nonculturable Cells

If one accepts the widely held view that a viable microbial cell is one that is capable of multiplying under suitable conditions, then definitive proof that putative VBNC cells are actually alive depends on applying a resuscitation treatment that restores culturability. A wide range of resuscitation treatments have been reported (Table 2).

When VBNC populations of *V. vulnificus* produced by starvation at low temperature are warmed to approximately 20 °C, there is a rapid increase in plate counts accompanied by a transformation of coccoid to vibrioid cell shape. This procedure has been criticized because it does not distinguish between resuscitation and multiplication of a few residual culturable cells present in the population. Nevertheless, some studies with *V. vulnificus* and other organisms have demonstrated resuscitation in samples in which the probability of residual culturable cells was vanishingly low. However resuscitation is often followed or accompanied by cell multiplication, so it is impossible to know by simply following the increase in colony counts and proportion of cells in the original population that were capable of resuscitation. Ambiguity can be avoided by using the MPN approach described in Section 'Enumeration Using the Most Probable Number Method'. Suspensions of bacteria containing VBNC cells are serially diluted in a liquid medium before applying the resuscitation treatment. The MPN after resuscitation can be compared with the plate count to give the proportion of VBNC cells in the population. Unfortunately most reported studies fail to use the MPN approach, so the size of the putative viable population relative to the total number of metabolically active cells is often unknown.

Virulence of Viable but Nonculturable Cells

Virulence of cells in the VBNC state has been tested with *Sal. enterica* serovar Typhimurium, *E. coli*, *L. monocytogenes*, *V. vulnificus*, *Vibrio cholerae*, *Legionella pneumophila*, *Ca. jejuni*, *Helicobacter pylori*, *Aeromonas salmonicida*, *Shigella dysenteriae*, and *Enterococus faecalis*. Infectivity has been measured in a variety of ways including injection into suckling mice, ligated rabbit ileal loops or embryonated eggs, colonization of chicks, and ability to cause symptoms in human volunteers. Unfortunately, the results have been variable and sometimes contradictory. Although several carefully designed studies demonstrated the potential of VBNC cells to cause disease, others found no such effect. This may perhaps be related to differences between strains, protocols, or the method of producing VBNC cells. One study with VBNC cells of *Ca. jejuni* generated by storage at 4 °C in artificial seawater found that cells could be resuscitated by passage though the mouse intestine for considerable time after the loss of culturability, but the capacity for resuscitation diminished progressively and was lost before the loss of metabolic activity measured as ability to reduce the fluorescent tetrazolium salt, 5-cyano-2,3-ditolyl tetrazolium chloride (CTC). Infectivity may thus be retained transiently after cells become unable to grow on agar plates.

Resistance of Viable but Nonculturable Cells

There have been a few reports that VBNC cells are more resistant to physical and chemical stress than culturable cells in accord with their supposed role in survival. *Vibrio parahaemolyticus* VBNC cells were more resistant to heating at 42 °C and 47 °C than exponential phase cells when viability after heating was assessed by the BacLight Live–Dead kit. *V. vulnificus* VBNC cells were similarly found to be somewhat more resistant to high hydrostatic pressure than exponential

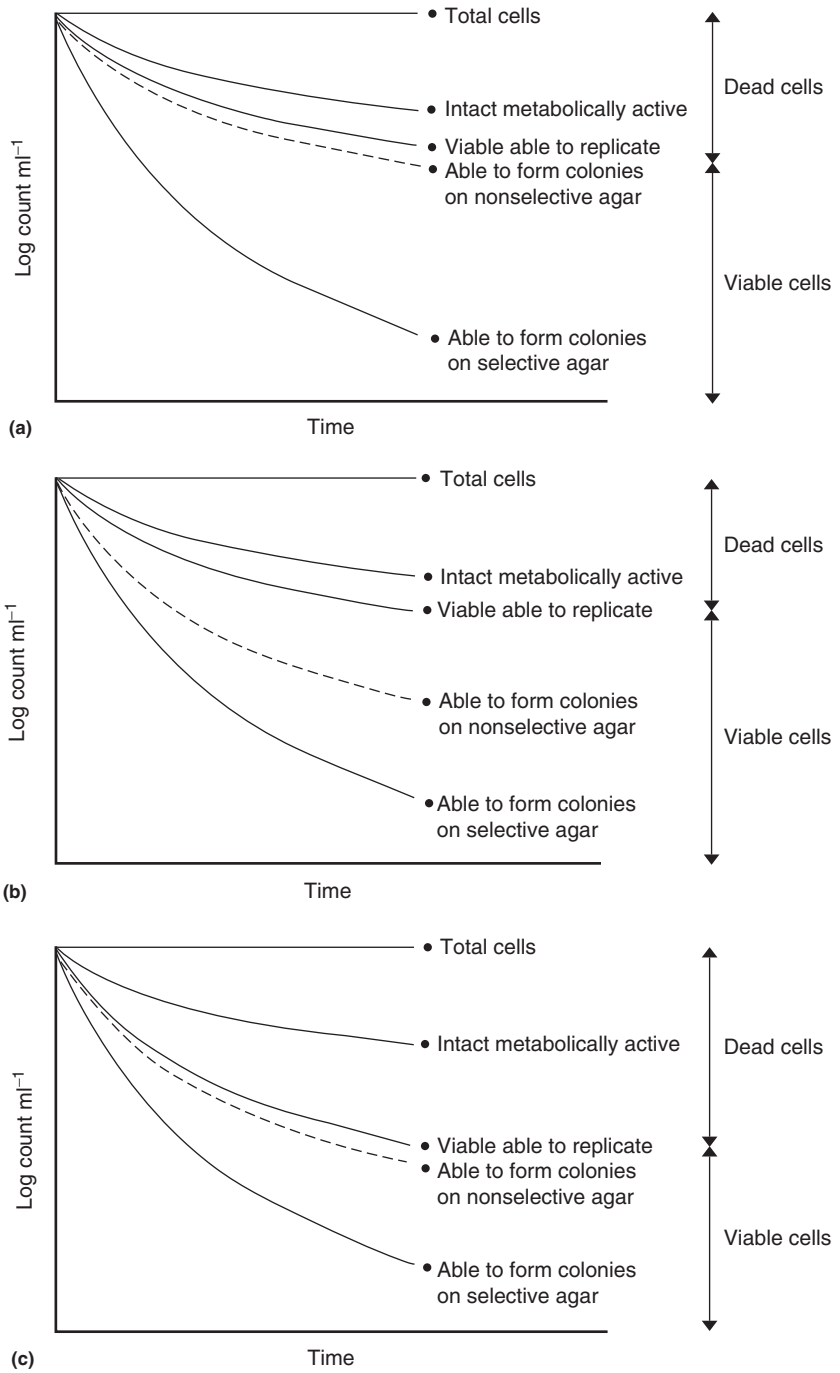

Figure 5 A general scheme for describing development of sublethal injury and entry into VBNC. (a) Loss of culturability occurs at a similar rate to loss of viability. (b) Loss of culturability occurs faster than loss of viability. (c) Loss of viability and culturability occurs much faster than loss of metabolic activity.

phase cells. *Ca. jejuni* cells, however, were more sensitive to a range of disinfectants but more resistant to chlorine. A problem with these and other similar studies is that the number of cells actually able to resuscitate after exposure was not determined. The susceptibility of *V. vulnificus* to sonication can be assessed directly by the microscopy of treated cells. This showed that the resistance of cold-incubated VBNC cells was initially similar to that of growing cells but increased to equal that of starved but culturable cells. Prolonged storage of *Mycobacterium smegmatis* leads to the formation of morphologically distinct ovoid dormant cells that do not form colonies on agar but can be resuscitated in liquid medium. Thermal resistance of starved cells was much higher than that of cells from a 24-h culture. Survival was assessed by colony counts so although VBNC ovoid cells were present in the aged suspension, the survivors must have been derived from

Table 3 Sublethal injury of bacterial spores

Treatment	*Symptom of injury*	*Possible cause or mechanism*
Heat	Better recovery on rich than minimal media	Reduced biosynthetic burden. Probably many different mechanisms
Heat	Recovery dependent on addition of starch or activated charcoal to recovery media	Sensitivity to unspecified inhibitors present in recovery media that are neutralized by additives
Heat	Requirement for non-nutrient germinants (lysozyme, calcium–dipicolinic acid)	Inactivation of germination system
Heat	Sensitivity to lysozyme	Damage to spore coat
Heat and radiation	Restricted temperature range for recovery	Inactivation/activation of germination systems having different temperature optima Damage to spore protoplast membrane leading to lysis on outgrowth at non-optimal temperatures
Heat, ionizing radiation, and oxidizing agents	Increased sensitivity to sodium chloride	Damage to spore protoplast membrane. Damage to metabolic enzymes and inability to generate adenosine triphosphate needed for biosynthesis of osmolytes
Heat	Sensitivity to nitrite (low concentrations)	Inhibition of germination systems
Heat	Increased sensitivity to organic acids	Unknown
Heat and oxidizing agents	Increased sensitivity to surface active compounds and certain antibiotics	Damage to spore protoplast membrane. Inactivation of detoxification mechanisms
Ionizing radiation	Decreased heat resistance	Damage to spore cortex and partial rehydration
Acid	Greatly reduced heat resistance	Loss of cations from spore cortex affecting level of spore hydration
Alkali and/or reducing agents	Sensitivity to iodine chloroform, ethanol, glutaraldehyde, and other disinfectants	Damage to spore coat

Source: Reproduced from Mackey BM (2000) Injured bacteria. In: Lund BM, Baird-Parker AC, and Gould GW (eds.) *The Microbiological Safety and Quality of Food*, pp. 315–341. Maryland: Aspen, with kind permission from Springer Science + Business Media B.V.

culturable cells. Based on the few studies so far available, there is no firm evidence that nonculturable cells are more resistant than cells that have mounted a starvation or stationary-phase stress response. There appear be no studies in which a stress treatment has been applied to a VBNC population, and the number of surviving viable cells has been assessed after exposure by using the MPN resuscitation approach.

Nature of the Viable but Nonculturable Condition

A commonly held view is that the VBNC state is an adaptive survival mechanism analogous to spore formation that results from a genetically regulated developmental process. An alternative view is that it is a transient phase in a degenerative process leading to cell death. Changes reported to be characteristic of entry into the VBNC state include a decrease in metabolic activity, a decrease in cell size and adoption of a more rounded or coccoid cell shape, an increase in cross-linking of cell wall peptidoglycan, and changes in membrane phospholipid composition. It remains difficult to identify changes that are uniquely related to the VBNC condition because somewhat similar changes occur in starved culturable cells.

Although the term VBNC is used as a blanket term, it is now clear that there is more than one cause for nonculturability. Evidence is now accumulating that in *V. vulnificus* and some other organisms, nonculturability is due to sensitivity to oxidative stress rather than being an adaptation for survival. Sensitivity to exogenous oxidants appears to be due to inactivation of catalase following cooling to 5 °C; and the addition of catalase or pyruvate to agar media restores culturability. Conversely, a mutant of *V. vulnificus* that remained culturable when exposed to artificial seawater at 4 °C was found to overexpress glutathione *S*-transferase and was more resistant to hydrogen peroxide. Similarly, mutational inactivation of catalase or superoxide dismutase in *Sta. aureus* made cells hypersensitive to becoming nonculturable in seawater at 4 °C and prevented resuscitation on raising the temperature to 22 °C. The resuscitation promoting effect of ferrioxamine on *E. coli* and *Sal. enterica* serovar Typhimurium may perhaps be related to its ability to chelate iron and prevent the production of superoxide anion via the Fenton reaction. The sensitivity to oxidative stress of this class of VBNC cells is thus identical to that of sublethally injured cells described in **Table 1**. The role of enterobacterial autoinducer in resuscitation remains unclear and organisms responding to this compound may perhaps represent a different class of VBNC cells.

The nonculturable state of *Micrococcus luteus* arises for different reasons. When grown in a minimal medium and maintained for several months in stationary phase *Mi. luteus* enters a state of very low metabolic activity, becomes smaller in size, and loses its ability to grow on agar plates unless first resuscitated in liquid medium. Resuscitation is considerably enhanced by the presence of other live cells, spent culture medium or, more specifically, by resuscitation promoting factor (Rpf) a 17-kDa protein with lysozyme-like activity secreted by active cells. The requirement for Rpf has been interpreted as a mechanism, wherein a few growing cells in the population can communicate to dormant ones that conditions are favorable for growth.

Loss of culturability at low temperature in vibrios can be prevented if the starvation response is first initiated at higher temperatures. Nonculturability may therefore represent a

symptom of debilitation rather than a positive survival response. An interpretation of the VBNC condition is shown in Figure 5. This proposes that culturability on selective or nonselective media, metabolic activity, and viability (ability to replicate under appropriate conditions) can decline at different relative rates during starvation or exposure to other stresses. In Figure 5(a), culturability and viability decline at similar rates during starvation as occurs in cells that have mounted a general stress response. In Figure 5(b), culturability declines much faster than either viability or metabolic activity as occurs in VBNC cells. In Figure 5(c), viability and culturability decline much faster than loss of membrane integrity and metabolic activity as would occur in ultraviolet-irradiated cells. This scheme sees loss of culturability as a transient state before loss of viability during which cells are intact and metabolically active and may retain virulence for a while albeit at a reduced level but require resuscitation before they can grow on agar plates.

Spore Injury

The pioneering studies of Esty and Meyer in the 1920s on the thermal destruction of *Clostridium botulinum* spores and subsequent work by others with *Bacillus* spp. revealed that recovery of heat-treated spores was markedly influenced by the composition of the medium, incubation temperature, and gas atmosphere. It was also discovered that heated spores became more sensitive to sodium chloride and other food preservatives. The spore is a dormant structure and injury is sometimes only detectable during germination or outgrowth. Table 3 gives examples of different manifestations and possible mechanisms of spore injury.

Severely damaged spores may require additives such as blood, charcoal, pea infusion, or starch for maximum recovery. Injured anaerobic spores may be very sensitive to gas atmosphere or redox potential though in some cases the simple expedient of pour plating and incubating anaerobically is adequate. The addition of bicarbonate to Reinforced Clostridial Agar has been shown to increase the recovery of heat-damaged spores of *Cl. botulinum*, *Clostridium sporogenes*, and *Clostridium. histolyticum*. Spore germination times often follow a log-normal or skewed log-normal distribution with some spores having very long lag times. In predictive modeling studies of survival and growth of spores under different conditions, it is advisable to make observations for 3–6 months to allow for delayed spore germination.

Conclusions

Practical resuscitation treatments are often a compromise between recovering all sublethally injured cells and avoiding overgrowth of competitors. With regard to VBNC cells, there is little evidence that nonculturability *per se* confers any enhanced resistance to environmental stress over that of cells that have mounted a general stationary-phase stress response. The dormant condition, however, can readily be understood as a mechanism for enhancing long-term survival and resistance to antibiotics that require active metabolism for their effect. Apart from *Mi. luteus* and related Actinobacteria nonculturability appears to be a consequence of debilitation rather than a survival mechanism.

It is generally accepted that safety is best ensured by the application of hazard analysis and critical control points (HACCP) rather than end-product testing. Provided that critical control points are effective, the presence of injured or VBNC cells in food is unlikely to impact directly on safety during food processing. However, failure to detect injured or VBNC organism may affect other aspects of safety management such as risk assessment, investigations of foodborne illness, and epidemiological studies. It is therefore important to apply recommended resuscitation procedures as appropriate and to continue research into the physiological basis of the not readily culturable state given that there may be several causes requiring different resuscitation measures.

See also: Characteristics of Foodborne Hazard and Diseases: Microbial Stress Response

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CHARACTERISTICS OF FOODBORNE HAZARD AND DISEASES

Drug Resistant Pathogens

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Glossary

Antimicrobials Pharmaceutical compounds that kill microorganisms or slow their growth, and can be classified into antiviral, antibacterial, antifungal, or antiparasitic agents.

Avoparcin A glycopeptide antibiotic that shows cross-resistance with vancomycin and was used for growth promotion in animal farming in several European Union countries before being banned, starting with 1995.

Commensal microorganisms Microorganisms that coexist with the host without causing disease, and sometimes having beneficial effects.

Hemolytic–uremic syndrome A life-threatening medical condition characterized by hemolytic anemia, impaired blood clotting, and kidney failure, that is often preceded by infection with bacterial pathogens, mostly *Escherichia coli* O157:H7.

Horizontal gene transfer Also known as lateral gene transfer, it represents the transfer of genetic material between two microorganisms, in a process that often involves plasmids, and represents the primary cause of antimicrobial resistance.

Methicillin-resistant *Staphylococcus aureus* A pathogen that initially emerged in health care facilities (healthcare-associated methicillin-resistant *Staphylococcus aureus* (HA-MRSA)) but subsequently was increasingly described in the community (community-associated MRSA (CA-MRSA)) and represents a clinical and public health challenge.

Multidrug-resistant pathogens Microorganisms resistant to more than one antimicrobial agent.

Vancomycin A naturally occurring glycopeptide antibiotic made by the soil bacterium *Amycolatopsis orientalis*, used in the prophylaxis and treatment of infections caused by Gram-positive bacteria.

Introduction

In an elegant experiment that administered healthy volunteers a regular diet for 21 days, followed by a sterile diet for 20 days, Corpet documented a drastic decrease in the percentage of ampicillin-, tetracycline-, and streptomycin-resistant lactose-fermenting microorganisms in the stool during the sterile diet period. This finding supported the view that most resistant enterobacterial strains could originate from food sources, and pointed toward the need to allocate enhanced attention to the presence and dynamics of antimicrobial resistance originating in food.

While in the 1950s an average grocery store in the US stored approximately 300 items, by the 1980s this number increased to approximately 25 000 and, in more recent years, it reached 50 000. The growing availability of fresh produce and the increasing use of antimicrobials for many applications other than human medicine, including agriculture, aquaculture, and animal farming, increased the likelihood that certain food products may harbor and transmit antimicrobial-resistant pathogens.

The dynamics of antibiotic-resistant pathogens in food products has two distinct dimensions. One of them involves the transmission of resistant bacterial pathogens from food products to animals or humans; the second one involves the circulation of antimicrobial resistance-conferring genetic determinants, which can be horizontally transferred from resistant to susceptible bacterial strains, sometimes between members of different genera, and often after very short contacts.

Surveillance programs represent one of the most significant initiatives to monitor foodborne pathogens and develop and implement interventions. One of the first national surveillance models, established in 1995 by the Danish Ministry of Food, Agriculture and Fisheries and the Danish Ministry of Health, was the Danish Integrated Antimicrobial Resistance Monitoring and Research Programme. In the US, the Foodborne Diseases Active Surveillance Network (FoodNet) was initiated in July 1995 as a collaboration between the US Centers for Disease Control and Prevention (CDC), the US Department of Agriculture (USDA), the US Food and Drug Administration (FDA), and selected state health departments. It initially included five sites in California, Connecticut, Georgia, Minnesota, and Oregon, and later incorporated Colorado, New Mexico, New York, Maryland, and Tennessee, covering approximately 15% of the US population. This program

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conducts active surveillance and epidemiological studies to characterize the burden and temporal trends of foodborne illnesses, and proposes the development and implementation of initiatives to reduce their incidence. National surveillance of antimicrobial resistance in foodborne bacteria in the US started in 1996, with the establishment of the National Antimicrobial Resistance Monitoring System (NARMS), a collaborative effort of the FDA, CDC, and USDA.

Epidemiology of Major Foodborne Pathogens in Commercial Food

Staphylococcus aureus and MRSA

Staphylococcus aureus, identified in many commercial food products, was involved in several foodborne outbreaks. From an antimicrobial resistance perspective, methicillin-resistant *St. aureus* (MRSA) is the main concern. Recent studies from several countries reported the detection of MRSA in commercial food. In 2007, investigators from the Netherlands found MRSA in 2 (2.5%) of 79 commercial meat samples collected at supermarkets and butcher shops. One isolate, nontypable by *Sma*I digestion, was identical to isolates identified in pigs. In Japan, the first piece of evidence for MRSA in commercial raw chickens came in 2005, when the pathogen was detected in samples collected from retail locations in different prefectures. MRSA was detected in 5 (1.6%) of 318 meat samples collected between November 2007 and March 2009 in La Rioja, Spain. Two isolates were sequence type ST398, a new strain that is nontypable by *Sma*I pulsed-field gel electrophoresis, suggesting animal origin, and they were also resistant to tetracycline, an antibiotic widely employed in the pig industry. Six (3.75%) of the one hundered and sixty *St. aureus* strains collected between 2003 and 2005 from dairy products in Italy harbored *mecA*, the gene that confers methicillin resistance.

In 2009, the first survey on the prevalence and characteristics of MRSA isolated from retail meat in the US, which used samples randomly collected from several supermarket chains in Baton Rouge, Louisiana, reported the presence of MRSA in one beef and five pork samples. More recently, Waters *et al.* revealed that almost half the meat and poultry samples collected in grocery stores from five US cities harbored *St. aureus*, and over half of the isolates exhibited multidrug resistance, defined in the study as resistance to three or more classes of antimicrobials.

In 1995, Kluytmans *et al.* described an MRSA outbreak in the hematology unit of a hospital from the Netherlands, where 21 patients developed clinical disease and five died. Phenotyping and genotyping indicated that food contaminated by a dietary worker, who prepared the food and did not have direct contact with patients, was the most likely cause of septicemia in the first patient, and this marked the first documented instance of foodborne MRSA transmission. Twenty-one patients developed the infection, and five of them died. Jones *et al.* were the first to report gastrointestinal illness caused by community-acquired MRSA, when they showed that strains isolated from stool samples in three family members, a coleslaw sample that they ate, and a nasal swab from a food preparer in the convenience market where they purchased the food, were indistinguishable by pulsed-field gel electrophoresis.

Campylobacter Species

Campylobacter, one of the most frequent foodborne microorganisms, is the leading gastrointestinal pathogen in several countries. In the US, 95% of the estimated 1.4 million annual *Campylobacter* infections are caused by *Campylobacter jejuni* and 5% are caused by *Campylobacter coli*. Most infections are self-limited and do not require antibiotics.

An increasing incidence of antibiotic resistance was reported in recent years among *Campylobacter* strains isolated from food products and humans. While rarely reported in North America before 1992, fluoroquinolone-resistant *Campylobacter* was subsequently described with increasing frequency in animal carcasses and retail meat. In several countries, this coincided with the use of fluoroquinolones in animal food. In the US, after sarafloxacin and enrofloxacin were approved for animal feed in 1995 and 1996, respectively, an increasing prevalence of fluoroquinolone-resistant *Campylobacter* strains was reported in commercial food. A study conducted in the US found that approximately 20% of the *Campylobacter* isolates from chickens purchased in 1997 at supermarkets in the Minneapolis–St. Paul metropolitan area were fluoroquinolone-resistant. At the same time, fluoroquinolone resistance increased between 1992 and 1998 from ~1% to slightly more than 10% in *Campylobacter* strains isolated from humans. Another study reported that between 1995 and 2001, fluoroquinolone resistance among *C. jejuni* human isolates collected at several Philadelphia-area hospitals increased from ~21% to more than 40%, even though resistance to erythromycin for the same period remained low, under 5%. Similar trends were reported in other countries. More than 30% of the *C. jejuni* isolates from chickens in the Netherlands and France were resistant to nalidixic acid and ciprofloxacin, and resistance rates as high as 32% and 77% were found in Japan and Thailand, respectively.

In a population-based study conducted between 1998 and 1999, Kassenborg *et al.* reported that eating chicken or turkey at commercial food establishments increased approximately 10-fold the risk of a fluoroquinolone-resistant *Campylobacter* infection over the next 7 days. This pointed toward poultry being a major cause of fluoroquinolone-resistant *Campylobacter* infections in the US, a finding echoed by reports from other countries.

Miflin *et al.* examined the antibiotic resistance profile of *Campylobacter* species isolated on Australian broiler farms and noted, as the most salient feature, the lack of ciprofloxacin-resistant strains. The authors speculated that this observation could be explained by the fact that fluoroquinolones were never approved for poultry farming in Australia.

In 2000, the FDA proposed the withdrawal, and in 2005 withdrew its approval for the use of fluoroquinolones in live poultry. The first study to examine the temporal trends of resistance in two poultry producers that voluntarily stopped fluoroquinolone use in 2002 did not find a significant decrease in the prevalence of fluoroqinolone-resistant strains in

2004 and 2006. Despite several limitations, the study indicated that the ban might not have been sufficient by itself to reduce the prevalence of resistant strains, and suggested that additional interventions might be required.

Salmonella Species

Salmonella is one of the major foodborne pathogens worldwide, and in the US it is responsible for 800 000 to 4 million infections annually. Poultry and poultry products, eggs, vegetables, and fruits are some of the food products most frequently implicated. The most common resistance patterns in *Salmonella* are to antimicrobials often used in animal husbandry, and infections with resistant strains have higher morbidity and mortality than those caused by susceptible isolates.

Worldwide, *Salmonella* isolates of food origin show a remarkable variation in their antimicrobial resistance pattern. A 2009 study that collected *Salmonella* strains contaminating meat samples at markets and stores from Seoul, South Korea, reported that all 18 isolates were resistant to erythromycin, 22.2% were resistant to streptomycin, and 16.7% were resistant to tetracycline and chloramphenicol. Five isolates showed resistance to at least two antibiotics, and one was resistant to eight distinct antibiotics.

A study conducted in the wake of an almost 5-fold increase in the incidence of *Salmonella newport* infections reported to the Los Angeles County Health Department in May 1985 revealed that most strains were chloramphenicol-resistant and harbored an identical ~25-MDa plasmid. Epidemiological and microbiological analyses traced the pathogen to infected hamburgers, to the abattoirs where the animals were slaughtered, and to the ill dairy cows. The isolation of chloramphenicol-resistant strains on these dairy farms was associated with the local use of chloramphenicol. These findings indicated that food animals may be a major source of antimicrobial-resistant *Salmonella* infections in humans, and that antimicrobial use on farms contributes to these infections.

The NARMS revealed various trends, in time, for the incidence of resistance to specific antibiotics among foodborne microorganisms. For example, between 1997 and 2009, the prevalence of gentamicin-resistant *Salmonella* isolated from turkeys decreased from 21% to 15%. During the same interval, the prevalence of ceftriaxone-resistant isolates in chickens increased from 0.5% to almost 13%, and the prevalence of chloramphenicol-resistant isolates in cattle increased from 4% to 21%. Multidrug resistance, defined as resistance to five or more classes of antimicrobials, became more prevalent during the same period in isolates originating from several food sources – for example, it increased from 1.4% to ~8% in chicken and ~8% to 20% in cattle isolates.

Between August 1984 and March 1985, the largest *Salmonella* outbreak recorded in the US occurred in northern Illinois and was linked to 2% pasteurized milk originating from a single dairy plant. Its two waves caused >16 000 culture-confirmed infections, but the total number of cases is thought to have exceeded 168 000. The *Salmonella typhimurium* strain involved in this outbreak showed a very rare and unusual pattern of resistance to multiple antibiotics. The use, during the preceding months, of antimicrobials to which the pathogen was resistant, increased the risk of symptomatic infection approximately 5-fold during the outbreak and lowered the dose of pathogen needed to cause clinical disease.

In the 1990s, a new resistance pattern was reported in the US and Europe, with the emergence of a strain known as R-type ACSSuT or *Sa. typhimurium* DT104, resistant to five different antibiotics: ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline. This multiresistant pathogen can acquire resistance to even more antimicrobials, opening further therapeutic challenges. In food animals, *Sa. typhimurium* DT104 was first reported in 1984 in the UK, initially in cattle and later in poultry and pigs. Subsequently, the pathogen disseminated throughout Europe and was first reported in the US in 1985. Occasionally, *Sa. typhimurium* DT104 was implicated in life-threatening human infections. For example, in 1998, a previously healthy woman died in Denmark from an infection caused by this strain, and the infection was traced to contaminated pork.

In June 1998, Mølbak *et al*. isolated an unusual quinolone-resistant and multidrug-resistant *Sa. typhimurium* DT104 strain from five patients, in one of whom the infection was fatal, and from pork at a slaughterhouse in Denmark. By using microbiological and epidemiological approaches, the authors demonstrated that quinolone-resistant bacteria could spread from food products to humans and cause therapeutically challenging infections.

Another relatively recent multiresistant *Salmonella* strain, Newport MDR-AmpC exhibits, in addition, resistance to amoxicillin/clavulanic acid and ceftiofur, and reduced susceptibility to ceftriaxone. Although no Newport MDR-AmpC strains were reported in 1996, they represented 1%, 22%, and 15% of the human isolates in 1998, 2002, and 2004, respectively. A population-based case-control study conducted at 8 FoodNet sites between 2002 and 2003 identified beef, eggs, and chicken as the most probable sources for contamination and revealed that infections were more likely after using antimicrobials to which the strain was resistant, during the 28 days preceding the diarrhea. Consumption of uncooked ground beef, or runny scrambled eggs or omelets prepared at home, during the 5 days preceding the onset of the disease, emerged particularly important risk factors for infections with multidrug-resistant strains.

Koningstein *et al*. identified human antibiotic use as a risk factor for resistant foodborne *Salmonella* infections. This heightened risk, present for at least 1 year subsequent to antibiotic treatment, was evident for several classes of antimicrobials, although its magnitude differed for specific compounds. In an analysis of 32 *Salmonella* outbreaks that occurred in the US between 1984 and 2002, Varma *et al*. found that more hospitalizations occurred during outbreaks with resistant than with susceptible bacterial isolates. Holmberg *et al*. examined 52 *Salmonella* outbreaks between 1971 and 1983, 17 of which were caused by resistant isolates, and reported that antimicrobial resistance is associated with an >20-fold higher fatality rate as compared to antimicrobial susceptibility. Furthermore, human outbreaks with antimicrobial-resistant *Salmonella* strains were shown to be more likely to have a food animal source than outbreaks caused by susceptible strains. Additional epidemiological studies found that individuals infected with resistant *Salmonella* strains

were more likely to have visited or resided on a farm before the infection than individuals with antibiotic-susceptible infections.

Raw milk consumption represents an additional source of antimicrobial-resistant salmonellosis. In the US, the interstate transportation of raw milk that is not destined for pasteurization was prohibited in 1987 and, as of 1995, 28 states permitted its intrastate sale to consumers. In a multidrug resistant *Salmonella* outbreak that occurred in New Mexico in 1983, 12 of the 19 individuals with available food histories ingested raw milk within the 2 weeks preceding the outbreak. Tacket *et al.* found a multiresistant strain in a 72-year-old woman who died in Arizona in 1983 from *Salmonella* enteritis after consuming raw milk, and also identified the pathogen in other individuals from the community who became ill, and in milk samples. Raw or inadequately pasteurized milk consumption was also implicated or suspected in 14 of the 23 *Campylobacter* enteritis outbreaks reported to the CDC between 1980 and 1982.

Data from the FoodNet surveillance network revealed that even though the incidence of Shiga toxin-producing *E. coli* (STEC) O157 infections declined in 2010, the incidence of *Salmonella* infections has not decreased significantly for over a decade, and *Salmonella* remained one of the most prevalent causes of foodborne bacterial infections. Preventing contamination during the slaughtering process, properly cooking the food, detecting and investigating outbreaks, and promptly recalling contaminated food, emerge as some of the most significant initiatives of global importance.

Escherichia coli

Escherichia coli represents the predominant facultative anaerobic bacterium from the gastrointestinal tract. It was causally linked to intestinal and extraintestinal infections, the second group including urinary tract infections, pneumonia, meningitis, and encephalitis. Based on their virulence characteristics, six major *E. coli* groups relevant for gastrointestinal disease were described: enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC), and STEC. EPEC is a frequent cause of watery or bloody diarrhea in infants, but also affects adults, and represents an important cause of traveler's diarrhea. ETEC, a pathogen that produces both heat-labile and heat-stable enterotoxins, is the leading cause of diarrhea in the developing world, the leading cause of traveler's diarrhea, and a frequent pathogen in children under 5 years old worldwide. It causes infections accompanied by watery diarrhea and cramps, but fever is generally low or absent. EIEC, a pathogen that resembles *Shigella* in its mechanisms of pathogenesis, penetrates intestinal epithelial cells and multiplies inside them, causing extensive tissue destruction. It is accompanied by severe diarrhea with blood and mucus, and high fever. EAEC, a pathogen that can attach to the intestinal epithelium in an aggregative manner, is mostly associated with persistent diarrhea in infants. DAEC, a less common cause of enteric disease, causes watery diarrhea, particularly in older children. STEC encodes toxins that are similar to the toxin produced by *Shigella dysenteriae*. Owing to its cytotoxic effects on Vero cells, which were first reported in 1977 by Jack Konowalchuk and colleagues, this pathogen also became known as verocytotoxigenic *E. coli* (VTEC). Stx1 and Stx2 are the two immunologically distinct primary types of toxins produced by STEC. Certain isolates produce only one of these toxins, others produce both, and a few produce multiple toxins, including several Stx2 variants. A STEC subgroup, which contains strains that attach to and efface the intestinal epithelial cells, a lesion known as attaching and effacing (A/E), is referred to as enterohemorrhagic *E. coli* (EHEC). This pathogen may cause infections that range from asymptomatic to life-threatening intestinal and extraintestinal disease, including the complication known as hemorrhagic colitis or hemolytic–uremic syndrome. STEC, EPEC, ETEC, and EAEC are the *E. coli* strains most frequently implicated in foodborne outbreaks.

Multiple lines of evidence indicate that *E. coli* strains, often resistant to one or several antimicrobials, are present in commercial food, and their ability to cause human disease represents a medical and public health concern. Extended-spectrum β-lactamase-producing *E. coli* strains in meat animals and retail meat were reported in Europe and in the US and, in addition, sulfonamide-, fluoroquinolone-, and aminoglycoside-resistant *E. coli* isolates in food animals were described in several countries. A survey that examined food products in retail markets from the Minneapolis–St. Paul area between 2001 and 2003 reported that, overall, 24% of the food samples harbored *E. coli*, and antimicrobial resistance was present in 27% of the miscellaneous food items, 85% of the beef and pork samples, and 94% of the poultry samples. Indirect evidence supported the on-farm origin of resistance in these bacteria. A 2011 study on meat samples collected over a three-year period from retail stores in Northern Greece reported ciprofloxacin resistance in 62% and 28% of the *E. coli* strains isolated from chicken and beef, respectively. A study that examined turkey samples from grocery stores in Ontario, Canada, between February 2003 and May 2004, found that 71% of the *E. coli* strains, 81% of the *Campylobacter* strains, and 49% of the *Salmonella* strains were resistant to one or more antimicrobials. Furthermore, 13% of the *Salmonella* isolates and 18% of the *E. coli* isolates showed resistance to five or more antimicrobials.

Genotypic and phylogenetic analyses of *E. coli* isolated from human volunteers in Minnesota and Wisconsin revealed that antimicrobial-resistant human isolates were more similar to isolates from poultry than to antimicrobial-susceptible isolates colonizing humans, pointing toward their probable origin in poultry reservoirs. The additional observation that drug-resistant and drug-susceptible isolates from poultry were highly similar to each other suggested that drug-resistant isolates originated in the sensitive ones, possibly under selective pressure from antimicrobials used on the farm. This finding mirrored previous observations from Europe, where resistant *E. coli* of human origin was found to be highly similar to resistant isolates detected in poultry.

STEC represents the most important group of emerging foodborne pathogens, and *E. coli* O157:H7, a member of this group, has emerged in recent decades as the etiologic agent of some of most challenging foodborne infections. Additionally, the incidence of non-O157 STEC infections was reported to

also be on the rise worldwide. In North America, STEC was first identified as a foodborne pathogen in 1982, in two *E. coli* O157:H7 outbreaks related to the consumption of undercooked ground beef, and infections caused by this pathogen subsequently became more frequent worldwide. Many outbreaks were initially linked to minced beef and, more recently, additional sources, including vegetables, fruits, milk, juices, and recreational water were implicated. The CDC estimates that more than 73 000 *E. coli* O157:H7 infections and more than 37 000 infections with non-O157 serotypes occur annually in the US, and 85% of these are spread by food and water. In addition to gastrointestinal manifestations, which are often self-limiting, one of the most frequent systemic complications caused by this pathogen, and a potentially fatal outcome of the infection, is the hemolytic–uremic syndrome, a medical emergency that predominantly affects children. Although early *E. coli* O157:H7 isolates were usually susceptible to most antibiotics active against Gram-negative microorganisms, the antibiotic resistance profile of this pathogen has drastically changed over time, and resistance was increasingly reported, particularly during the past two decades, and frequently attributed to multiple antibiotics. Although only 1% of the 200 *E. coli* O157:H7 isolates that were examined by the CDC between 1983 and 1985 were resistant to antibiotics, a 2004 study that examined 901 *E. coli* O157:H7 isolates, 663 from bovines at feedlots in the Midwestern US, and 238 from humans, representing outbreaks and sporadic infections, reported that almost 7% of the bovine isolates and more than 12% of the human isolates were resistant to at least one antibiotic.

In 2009, a molecular epidemiology study comparing cefoxitin-resistant *E. coli* isolated from cattle at four feedlots in southern Alberta with *E. coli* isolated concomitantly from infections affecting patients in Canadian hospitals revealed that similar multidrug resistance-conferring plasmids can be isolated from both sources. Some of the isolates shared more than 90% sequence similarity, as determined by deoxyribonucleic acid (DNA) fingerprinting. This finding underscores the need to focus on the therapeutic implications that accompany the transfer of resistant pathogens and resistance determinants across species.

Shigella Species

Shigella, the etiologic agent of bacillary dysentery, was discovered in 1897 by the Japanese bacteriologist Kiyoshi Shiga. The genus includes Gram-negative, facultatively anaerobic, nonmotile rods that were organized into four serogroups: *Sh. dysenteriae*, primarily associated with epidemics; *Shigella flexnerii*, most frequent in endemic areas; *Shigella boydii*, described in many outbreaks in Central and South America; and *Shigella sonnei*, commonly encountered in developed countries. One feature of the *Shigella* infections is the low infective dose. For example, less than 10 *Sh. dysenteriae* cells are sufficient to cause disease.

An increase in the incidence of antibiotic resistance was reported, over time, for many *Shigella* isolates worldwide. A study conducted between 1989 and 1990 in Germany reported that over 80% of 255 *Shigella* strains were resistant to at least one antibiotic. Ashkenazi *et al.* examined 3511 *Shigella* isolates collected between 1984 and 1992 in Israel and, over the study period, reported that resistance to trimethoprim–sulfamethoxazole increased from 59% to 92%, and that to ampicillin increased from 13% to 86%. *Shigella sonnei* showed a higher incidence of resistance to these antibiotics than *Sh. flexneri*. Another study that examined antimicrobial resistance trends in *Shigella* sp. collected between 1984 and 1989 in the Netherlands revealed that 23% of the 3313 isolates examined were resistant to trimethoprim–sulfamethoxazole and 30% were resistant to ampicillin. In recent years, multidrug-resistant *Shigella* isolates, including strains resistant to extended-spectrum cephalosporins, were described worldwide, and isolates resistant to fluoroquinolones and ciprofloxacin were documented in several countries.

One of the clinically relevant features of *Shigella* infections is their high communicability. In 1987, a shigellosis outbreak was recorded at a mass gathering held in a national forest from western North Carolina. The infections were caused by a *Sh. sonnei* strain resistant to ampicillin, tetracycline, and trimethoprim–sulfamethoxazole. After the gathering, the nationwide dispersal of the participants was linked to several outbreaks that occurred over the next 2 months in other communities.

Another challenge is the transmission of resistance determinants from *Shigella* to other bacterial species. Tauxe *et al.* reported the *in vivo* transfer of antimicrobial resistance during a small outbreak caused by a multiply drug-resistant *Sh. flexneri* isolate that occurred in 1983 on the Hopi Indian reservation. The plasmid conferring multiple drug-resistance, isolated from one patient affected during the outbreak, was identical to a plasmid that was isolated, 3 weeks before the onset of shigellosis, from an *E. coli* strain that caused urinary tract infection in the same patient.

Listeria monocytogenes

Bacteria that belong to the *Listeria* species increasingly emerge as significant foodborne pathogens. As listeriae are widely distributed in nature, human contact occurs frequently, but infections are relatively rare. Nevertheless, when human infections occur, their case-fatality rate can be as high as 30%, despite the availability of antimicrobial treatment. *Listeria monocytogenes* is the most frequent member of the species to infect humans, and in rare cases, *Listeria ivanovii*, primarily an animal pathogen, may also cause human disease. The ability to survive at refrigeration temperatures and to grow on minimal nutrients and in diverse environments, including soil, water, plants, and animals, are some of the features that make *Listeria* a particularly challenging pathogen.

The first time that *Listeria* transmission was attributed to food was in 1979 at a Boston hospital, when the involvement of vegetables was suspected. The possibility that this pathogen is transmitted by food was further strengthened in 1981, when an outbreak in Nova Scotia, Canada, which caused 41 infections and 18 deaths, was traced to contaminated coleslaw prepared from cabbage grown on a farm that used contaminated sheep manure as a fertilizer. This marked the first time when the involvement of *L. monocytogenes* in human foodborne infections was demonstrated.

Sporadic as well as epidemic *Listeria* infections are mainly transmitted by the foodborne route. Listeriosis saw a worldwide increase during recent years, and high mortality rates were reported particularly among pregnant women, immunocompromised individuals, the elderly, and the newborn, although the infection affects healthy people of all age groups. Besides gastrointestinal manifestations, listeriosis is also associated with extraintestinal complications such as meningitis, septicemia, and miscarriage. Particularly soft cheeses prepared from raw milk and ready-to-eat meat products pose heightened risk for susceptible individuals.

Several studies reported that *L. monocytogenes* isolates showing resistance to a wide range of antibiotics are present in food products. Samples collected between 1992 and 1993 from poultry slaughterhouses and from raw milk and cheese at markets in northwestern Spain revealed streptomycin-resistant *L. monocytogenes* and chloramphenicol-resistant *Listeria innocua* in the food samples, and tetracycline-resistant *L. innocua* in the poultry and poultry slaughterhouses. A study that examined *L. monocytogenes* in retail poultry in Porto, Portugal, identified the pathogen in 41% of 63 samples, and 73% of these were resistant to at least one antibiotic. Of 114 *L. monocytogenes* isolated between 2000 and 2002 from food products in Denmark, all showed high minimum inhibitory concentration (MIC) values for ceftiofur, but were fully susceptible to other antimicrobials, including ciprofloxacin, erythromycin, cotrimoxazole, and streptomycin. Studies on food samples, including ready-to-eat salads and milk and raw meat products collected in ten open-air markers from Thessaloniki, Greece, found *L. monocytogenes* in more than 14% of the 210 samples, with one strain exhibiting tetracycline resistance. Food and environmental samples collected between 1996 and 2006 in France found four *Listeria* isolates that were antibiotic-resistant, two to erythromycin and one each to tetracycline–minocycline and trimethoprim. A study that included isolates from food sources and food environments in Italy reported, in 2009, that almost 12% of the 126 isolates were resistant to at least one antibiotic, and one strain (0.8%) was resistant to five antibiotics. Isolates were most frequently resistant to clindamycin, but resistance to other antibiotics, including linezolid, ciprofloxacin, ampicillin, vancomycin, and tetracycline, was also found.

In the US, Prazak *et al*. reported that 95% of the 21 *L. monocytogenes* isolates collected in Texas from cabbage and several environmental and water samples were resistant to two or more antibiotics, and 85% were resistant to penicillin, marking the first time when penicillin-resistant *Listeria* was identified in human, environmental, or food samples. The first study to examine *L. monocytogenes* antimicrobial susceptibility on a dairy farm environment, conducted in Tennessee, reported that all 38 strains isolated from four farms exhibited resistance to at least one of 15 antimicrobials tested, and the overall resistance profile of the isolates covered a broad range of compounds. From the 167 *L. monocytogenes* isolates collected between 2002 and 2003 from raw chickens, ready-to-eat meat, and fresh produce from retail food in Florida and Washington, DC, 73% were resistant to sulfonamides, and more than 8% and almost 2% exhibited resistance to tetracycline and ciprofloxacin, respectively.

Relatively few studies have focused on antimicrobial resistance in *Listeria* species in a clinical setting. In France, Morvan *et al*. examined *L. monocytogenes* antimicrobial resistance in the largest clinical collection to date, and found that more than 1% of 4668 isolates collected between 1989 and 2007 were resistant to at least one clinically relevant antibiotic. An analysis of the temporal evolution of antimicrobial susceptibility in historical isolates collected between 1926, when the pathogen was first characterized, and 1988, reported no acquired resistance to 23 antibiotics, but the minimum inhibitory concentrations for several antibiotics increased subsequently, during the more recent time intervals. Even though the incidence of antimicrobial resistance in *L. monocytogenes* human isolates is currently low, the ubiquity of this bacterium, and the increasing incidence of antimicrobial resistance in animal and food isolates, open concerns about resistance among clinical isolates. This is compounded by the ability of listeriae to acquire resistance determinants from other species. Doucet-Populaire *et al*. reported that the Tn*1545* transposon, which confers resistance to kanamycin, tetracycline, and erythromycin, can be transferred *in vivo* between *Enterococcus faecalis* and *L. monocytogenes*, with an efficiency that is 10-fold higher in the presence of small concentrations of tetracycline.

Enterococcus Species

Enterococci are ubiquitous, Gram-positive bacteria found in many environments, including the soil, water, plants, animals, and the human intestinal flora. Certain enterococcal strains exhibit health-promoting characteristics, and are effective in managing childhood and antibiotic-associated diarrhea, whereas others found applications as dairy adjunct or starter cultures, thanks to their fermenting activity. However, enterococci are also among the most frequent nosocomial pathogens. Despite their presence in the food, enterococci have been associated with foodborne outbreaks in very few instances. Nevertheless, their use as probiotics has been controversial, particularly due to the recent emergence of antibiotic-resistant strains identified in clinical isolates worldwide that, in addition to causing disease, could transfer resistance determinants to other bacteria.

Di Labio *et al*. reported that 69%, 99%, and 68% of the *E. coli*, *Enterococcus*, and *Campylobacter* isolates collected from fecal samples collected between March and July 2006 from 500 randomly selected Swiss veal calves, originating from 129 farms, were resistant to at least one antibiotic, mostly compounds used in animal husbandry. Among *Enterococcus faecium* strains, 35% were resistant to quinupristin/dalfopristin, and almost 80% were resistant to nitrofurantoin.

Ampicillin, gentamycin, and vancomycin are among the most frequent antibiotics used to treat enterococcal infections, and this represents only one of the reasons why vancomycin-resistant enterococci (VRE) emerge as a particularly important medical and public health challenge. A VRE reservoir was established in animals by the use of avoparcin, a glycopeptide growth promoter antimicrobial analogous to and showing cross-resistance with vancomycin, which has been used in animal husbandry in many European countries since 1975, before being banned first in Denmark in 1995, Germany in 1996, and subsequently in other countries, culminating with a comprehensive ban by the European Commission in

1997. VRE were first recorded in 1987 in Europe, and within 10 years, the pathogen represented more than 25% of the enterococci associated with bloodstream infections in the US hospitals. Studies conducted in several European countries revealed that in the years following the avoparcin ban, the incidence of VRE in food products and that of human colonization with this pathogen decreased progressively. In Hungary, during the years after the avoparcin ban in 1998, in addition to a progressive annual decrease in the incidence of VRE strains colonizing food animals, a decrease in the vancomycin MIC values was also recorded. At the same time, in several countries, resistant strains were detected for several years after the avoparcin ban, indicating that antimicrobial resistance persists for a long time, even after the antibiotic use is discontinued.

Yersinia Species

Yersiniae have three medically important members: *Yersinia pestis*, *Yersinia enterocolitica*, and *Yersinia pseudotuberculosis*. *Yersinia enterocolitica*, a significant foodborne pathogen, has been isolated from food products and several domestic and wild animals. Swine represent one of the most significant reservoirs. Several studies revealed a high genetic similarity between human and pig isolates. The ability to grow between 0 and 42 °C and at pH values between 4 and 10 enable this pathogen to survive and multiply in a broad range of refrigerated food products. *Yersinia enterocolitica* antibiotic resistance is dependent on the biogroup and shows broad geographic variations. A study conducted in Austria that examined meat samples from markers and slaughterhouses over a three-year period reported that one of 167 isolates, originating from chicken, was resistant to tetracycline, and the remaining ones were sensitive to several antibiotics tested. All *Y. enterocolitica* isolates harboring *ail*, a gene encoding an outer membrane protein required for attachment and for human virulence, that were collected in 2006 from pigs at an abattoir in Switzerland, exhibited resistance to at least one compound, most frequently ampicillin or erythromycin. The isolates were susceptible to many clinically relevant antibiotics. More than 93% of the *Y. enterocolitica* strains isolated in Switzerland between 2000 and 2003 from humans, retail pork, and pork feces were resistant to cephalothin, and more than 68% were resistant to amoxicillin/clavulanic acid. However, only 1% exhibited resistance to tetracycline, 3.6% were resistant to streptomycin, and all isolates were susceptible to several antibiotics including ciprofloxacin, gentamicin, kanamycin, and neomycin. A survey conducted between 2004 and 2007 that examined commercial food samples from retail stores in northwestern Greece found that *Y. enterocolitica* from pork samples showed resistance to broad-spectrum penicillins, cephalosporin, aminoglycosides, and ampicillin. In the US, Funk *et al*. reported that *Y. enterocolitica* isolated from the oral cavity of swine exhibited a high degree of resistance to sulfonamide, and variable resistance to oxytetracycline, two compounds frequently used in pig feeds. A survey of *ail*-positive *Y. enterocolitica* strains obtained in 2000 from pig fecal samples in the US reported that, even though the isolates were susceptible to 13 of 16 antimicrobials tested, all of them were resistant to ampicillin, more than 87% were resistant to cephalothin, and more than 27% to tetracycline.

Resistant Foodborne Pathogens may Cause Intestinal and Extraintestinal Disease

Extraintestinal pathogenic *E. coli* strains were linked to several types of infection, including sepsis, urinary tract infections, neonatal meningitis, and pneumonia. Multiple lines of experimental evidence reveal that bacteria from food products, in addition to their ability to cause gastroenteric infections, are causally linked to extraintestinal infections as well. Epidemiologic and molecular data traced resistant pathogens causing human urinary tract infections to food animals and food products, and these findings are supported by *in vivo* studies on animal models, which showed that strains isolated from food animals and products may case urinary tract infections in healthy animals. In 2006, investigators recovered extraintestinal pathogenic *E. coli* from retail chicken and turkey food samples collected in Georgia, Maryland, Oregon, and Tennessee. Most isolates belonged to the same phylogenetic group as virulent human strains, several of them were resistant to antimicrobials, and some of them carried genes associated with uropathogenic *E. coli*. A study conducted in Montréal, Canada, found *E. coli* isolates in retail chicken samples and other food sources that were genetically indistinguishable from or closely related to strains implicated in urinary tract infections, reinforcing previous observations that food may serve as a reservoir of extraintestinal *E. coli*. An isolate from retail chicken was indistinguishable by pulsed-field gel electrophoresis from a human clinical isolate, a finding that, even though it does not inform about directionality, indicates that transmission between meat products and humans had occurred, and opens intriguing public health questions. This isolate belonged to clonal group ST131, a worldwide pandemic clone that represents a newly characterized extraintestinal pathogenic *E. coli* isolated, in several countries, from food of animal origin, environmental samples, and healthy food animals. ST131 was for the first time reported as a human pathogen in 2008, is frequently resistant to multiple antibiotics, and isolates infecting humans, companion animals, and poultry often exhibit high genetic similarity.

Antibiotics and the Selection of Resistant Pathogens on Farms

The ability of antimicrobials used on farms to select for resistant bacteria has long been recognized. The Swann Committee Report, published in the UK in November 1969, which emphasized the dangers that antibiotics used in animal farming pose to animals and humans, proposed to carefully monitor antibiotic use in animals, and recommended that certain compounds, which are also prescribed in human medicine, should not be used as growth promoters.

In 1976, Levy *et al*. showed that the intestinal flora of chickens fed tetracycline-supplemented food contained, within a week, almost exclusively tetracycline-resistant bacteria, and within 5–6 months, more than 30% of the fecal

samples collected from individuals living on the farm contained a preponderance of tetracycline-resistant bacteria, as compared to less than 7% in the samples collected from neighbors. The authors suggested that tetracycline from the animal feed exerts selective pressure in individuals in contact with the feed and chickens. Marshall *et al*. demonstrated that resistant *E. coli* could spread from the bovine or porcine gut to other animals and humans on the farm, resulting in prolonged colonization. Importantly, this process occurred in the absence of antibiotic selection.

Holmberg *et al*. described a *Sa. newport* outbreak with a strain harboring a 38-kb R plasmid, and resistant to ampicillin, carbenicillin, and tetracycline, which in 1983 affected individuals from four midwestern states. In several patients, the symptoms appeared 24–48 h after starting antibiotic therapy for nondiarrheal conditions, and multiple lines of evidence indicated that they most likely had asymptomatic *Sa. newport* infections, and antibiotic use provided the selective pressure that allowed pathogen growth. Epidemiological and laboratory analyses of the plasmids from animal and human isolates revealed that the patients had been infected, before initiating antimicrobial therapy, from hamburgers that originated in beef cattle receiving subtherapeutic chlorotetracycline for growth promotion and disease prevention. These findings underscore the need for more prudent antimicrobial use in animals and humans.

Cohen and Tauxe reviewed several studies conducted on *Salmonella*, for which the molecular epidemiology of antimicrobial resistance was extensively studied, and found that strains of animal origin exhibited an antibiotic resistance profile that matched the antibiotics used in animal feed. Similar findings were reported by other investigators and, for several pathogens, multiple lines of evidence pointed toward a causal link between antimicrobials used in animal husbandry and resistance in bacteria from food animals, the farm environment, food products, and humans.

Antibiotics are generally agreed to represent the most important factor responsible for selecting resistant bacteria. Antimicrobial resistance, a global public health crisis, is fueled to a great extent, by the vast amounts of antibiotics used in human medicine and for many nonclinical applications, of which agriculture represents merely one example. Selection of resistance was recently shown to occur even at subtherapeutic antibiotic levels. Gullberg *et al*. revealed that antibiotic concentrations much below therapeutic levels, similar to the ones found in some aquatic and soil environments, are able to select for resistant bacteria. As the authors showed, the concentration range where resistant strains can be selected is much wider than previously thought and might need to include values that are several 100-fold below the minimal inhibitory concentration of susceptible strains.

Several authors pointed out that only a fraction of the antibiotics are correctly used for treating human bacterial infections. In addition to often being improperly prescribed, both in children and adults, for medical conditions that would not benefit from them, such as for viral infections, antibiotics are used in agriculture, aquaculture, and animal farming, in amounts that exceed, by far, their clinical use in humans. Estimating the amount of antibiotics used in agriculture and for food animals is challenging. The large amounts of antibiotics used in agriculture and animal husbandry serve three purposes. In addition to their prophylactic or therapeutic use, antibiotics can be continuously administered at subtherapeutic doses as growth promoters or to reduce the incidence of diseases associated with shipping stress. This latest application originates from the discovery that low, subtherapeutic doses of certain antimicrobials provide growth benefits that are distinct from the advantages associated with infection prophylaxis and therapy.

Approximately half of over 1 million tons of antibiotics released into the biosphere during the last 50 years are estimated to have been used for veterinary and agricultural purposes. An estimated 48% of the over 10 000 tons of antibiotics used in 1997 in the European Union and Switzerland were used in animals. One third of this served therapeutic and prophylactic purposes, and 15% was for growth promotion, with large country-to-country variations. Antibiotic growth additives were banned in the European Union in January 2006. In Chile, it was reported that over 100 metric tons of quinolones, 10 times more than the amount used in human medicine, are used annually for veterinary applications, most of it in aquaculture. Between 50% and 80% of the antibiotics produced in the US are used in agriculture. Approximately 70% of the antibiotics used in subtherapeutic concentrations in animals serve prophylactic purposes, and the remainder is used for growth promotion. The Institute of Medicine estimated that over half of the approximately 31 million pounds of antibiotics produced in 1985 were used in animals, for prophylaxis and growth promotion, and the Union of Concerned Scientists estimated that in 1999, 24.6 million pounds of antimicrobials were used in cattle, swine, and poultry, in the absence of disease, for nontherapeutic purposes.

The link between agricultural antibiotic use and resistant bacteria in animals and humans is illustrated by an example from Europe. Avoparcin, a growth promoter that was never approved in the US, was widely used in animal husbandry in Western Europe before being banned in the European Union in 1997. In 1994, $\sim$24 000 kg of active avoparcin were used as a food additive for animals in Denmark, as compared to 24-kg active vancomycin used for human therapy, and in Austria, over 62 000-kg avoparcin were used for animal husbandry annually between 1992 and 1996. Avoparcin exhibits cross-resistance with vancomycin and can facilitate the selection of vancomycin-resistant pathogens. Several authors found a causal relationship between avoparcin use in farming and the emergence of VRE. After avoparcin was discontinued in Germany in 1996, the incidence of VRE strains in poultry meat decreased, and VRE prevalence in the intestinal flora of healthy humans in the community decreased from 12% to 3% between 1994 and 1997. Studies conducted in several countries showed that resistant strains were still detected several years after the avoparcin ban, indicating that resistance may persist in food-producing animals long after an antimicrobial agent is discontinued.

Smith *et al*. pointed out that in the US, where avoparcin was never approved, the prevalence of VRE in the community is $<1\%$ and the presence of the bacterium is usually limited to individuals with a history of hospitalization, whereas its prevalence in the European Union during the late 1990s was between 2% and 12%. This underscores the additional impact

that antibiotic use for agricultural purposes exerts on the human population, as compared to its exclusive use for medical purposes. Based on this argument, the authors suggested that the agricultural use of antibiotics significantly and quantitatively contributes to antimicrobial resistance in the community. Reducing the agricultural use of antibiotics could, therefore, lower the prevalence of resistance in food animals and should also decrease the risk of contamination of commercial food.

Additional Routes to Contaminate the Food Chain

Resistant pathogens found in the food chain may originate not only from food animals, where they emerge under the selective pressure of antibiotics on the farm, but also from individuals who, after becoming colonized, either on the farm or in other locations, such as hospitals, contaminate the food animals, by a process known as reverse zoonotic transmission, or directly contaminate the food products.

Studies conducted in several countries reveal that farm workers and their families are at higher risk for contamination with resistant pathogens. Voss *et al*. provided the first observation linking pig farming to human MRSA colonization, when they reported an over 760-fold higher colonization risk in Dutch farmers as compared to patients admitted to Dutch hospitals. A cross-sectional study on nasal swabs collected between October 2007 and March 2008 from veal calves, farmers, their family members, and farm employees in the Netherlands, found more frequent MRSA ST398 carriage among calves receiving antibiotic treatment and among individuals living and working on farms. The first study that examined MRSA in swine and swine workers from the US reported an overall prevalence of 49% in swine and 45% in employees at two production facilities from Iowa and Illinois, and the first study conducted in China found MRSA in 11% of the swine and in 15% of the farm workers examined. All 60 strains isolated in this study were resistant to cefoxitin, ciprofloxacin, clindamycin, and tetracycline. Price *et al*. found that poultry workers in the US have a 32-fold higher risk for colonization with gentamicin-resistant *E. coli* than individuals in the community, and underscored the need to consider occupational exposure as a route that enables the entry of resistant pathogens into the community.

Although many studies documented the zoonotic transmission of pathogens from farm animals to humans, an additional, somewhat less extensively studied concept is the reverse zoonotic transmission of antimicrobial-resistant pathogens from humans to animals. In the first known human-to-dog MRSA transmission documented in the Netherlands, van Duijkeren *et al*. found the pathogen in the nose of a healthy dog whose owner became colonized while working in a nursing home. Lefebvre and Weese reported that pet dogs became contaminated with MRSA and *Clostridium difficile* during visits to health care facilities, and Ocepek *et al*. provided the first molecular epidemiologic evidence for *Mycobacterium tuberculosis* transmission from humans to cattle.

Individuals colonized with resistant pathogens may also directly contaminate food products, as demonstrated by Kluytmans *et al*., who reported the case of a hospital dietary worker thought to have contaminated food with MRSA, initiating an outbreak that affected multiple patients and health care workers and caused several deaths.

Resistant pathogens may gain access to humans and food animals by additional routes, which have been less extensively studied, but should receive enhanced attention. Chapin *et al*. found high concentrations of multidrug-resistant enterococci, coagulase-negative staphylococci, and viridans group streptococci in the air of a concentrated swine feeding operation in the US, and 98% of the bacterial isolates showed resistance to at least two antimicrobials used in swine husbandry. Some of the alarming findings from this study were the presence of bacteria resistant to clindamycin and virginiamycin. This latter compound, a swine growth promoter, is an analog of quinupristin/dalfopristin, an antibiotic of last resort for treating certain multidrug-resistant Gram-positive infections in humans. Gibbs *et al*. tested the distance that antibiotic-resistant microorganisms can travel by aerosols from a confined swine animal-feeding facility in the midwestern US, and reported that resistant *St. aureus*, the most frequent organism identified, could be recovered up to 150-m downwind from the facility. In the first study to characterize the presence of resistance genes to a major class of antibiotics in natural groundwater as a result of animal agriculture, Chee-Sanford *et al*. reported that tetracycline resistance determinants were present as far as 250-m downstream from waste lagoons and groundwater underlying swine farms. Illustrating an additional route allowing antibiotic-resistant pathogens access to the food chain, Kumar *et al*. reported that chlorotetracycline from animal manure used for fertilization can be detected in corn, green onions, and cabbage, in amounts that are small but increase with increasing amounts of antibiotic in the manure. Other authors also detected antibiotics originating in manure in different plants, and emphasized that the dangers of soilborne antibiotic resistance in humans have been underestimated.

Resistant Bacteria in the Kitchen and the Potential for Cross-Contamination

Although cooking destroys certain pathogens from food, they pose significant threats under several circumstances, such as the consumption of raw food. Cross-contamination of utensils, clothes, fingers, and kitchen surfaces during food preparation also poses substantial dangers. Although many studies examined antimicrobial-susceptible pathogens, the results are relevant for resistant pathogens as well. Kusumaningrum *et al*. reported that the *St. aureus* transfer rate from artificially contaminated sponges to stainless steel surfaces immediately after contamination was approximately 40%, and some bacteria remained on sponges after the transfer, enabling additional cross-contamination. High percentages of *St. aureus* were recovered from dry stainless steel surfaces at room temperature, exceeding the recovery of other organisms tested, such as *Salmonella enteritidis*, *Bacillus cereus* spores, or *C. jejuni*. On dry stainless steel surfaces, *St. aureus* was detected for at least 96 h when contamination was moderate (10^5 CFU/100 cm^2) or high (10^7 CFU/100 cm^2), revealing its ability to survive and cross-contaminate other surfaces. Scott and Bloomfield

reported that significant numbers of *St. aureus* survive on laminated surfaces and cloths, from where the pathogen may be transferred to fingertips or inanimate surfaces even after a brief contact, posing an infection hazard after contact with food. In a study that examined the secondary dissemination of pathogens from contaminated food in the kitchen environment, Gorman *et al*. revealed the ability of *St. aureus*-contaminated chickens to cross-contaminate multiple sites, including preparers' hands, refrigerator and oven handles, counter-tops, and draining boards.

Interactions Between Ecosystems: The Farm and Beyond

Genes encoding antimicrobial resistance can spread between microorganisms more easily than previously thought, by horizontal transfer. This mechanism occurs more frequently than mutations, and allows the transfer of resistance-encoding genetic elements between plasmids, chromosomes, or plasmids and chromosomes, causing susceptible isolates to become resistant. The horizontal transfer of resistance between different *E. coli* strains, sometimes between strains colonizing animals and humans, together with reports of antimicrobial resistance in *E. coli* colonizing healthy individuals, underscore the need to focus on commensal bacteria as a potential reservoir of resistance for pathogenic strains. Several studies described the transfer of antimicrobial resistance between *E. coli* strains colonizing the intestinal flora. Trobos *et al*. revealed that *sul2*, the gene encoding sulfonamide resistance, can be transferred between different *E. coli* strains residing in the human intestine, and showed that this process may occur concomitantly for multiple antibiotic resistance genes. Resistance can also be transferred from *E. coli* to other pathogens such as *Enterobacter*, *Salmonella*, and *Pseudomonas*. McMahon *et al*. documented the transfer of antibiotic resistance genes between *Sa. typhimurium* DT104 and *E. coli* K12 in broth, milk, and ground meat, at temperatures commonly encountered during food processing and distribution. Ampicillin-resistance genes were transferred at temperatures as low as 15 °C. The horizontal transfer of genetic resistance in food products becomes even more relevant if we consider the report of Sørensen *et al*. who found that glycopeptide- and streptogramin-resistant enterococci of chicken and pork origin, ingested by healthy volunteers, may be detected in the stool for up to 14 days, demonstrating their ability to survive in the gastric and intestinal environment, where resistance genes could be transferred to other bacteria from the intestinal microbiota.

In addition to impacting the local farm environment, antibiotic-resistant strains that emerge on the farm also exert a global impact as a result of the interconnectedness between the various compartments of the ecosystem. In this context, understanding the circulation of pathogens within and between farms becomes an important facet of public health initiatives. Davis and Wray showed that mice infected experimentally with *Sa. enteritidis* shed the pathogen, infecting chickens coming into contact with their droppings. Van de Giessen *et al*. found MRSA-infected rats on three of five pig farms that they examined in early 2008 in southern Netherlands and northern Belgium. The authors emphasized the fundamental role that rats play in disseminating this, and possibly other microorganisms, within and between farms, and underscored the need to incorporate this finding into strategies designed to control the spread of pathogens. In Libya, Rahuma *et al*. reported that significantly more houseflies isolated from hospitals harbored antibiotic-resistant bacteria than flies isolated in the streets, and two flies originating from hospitals tested positive for MRSA, whereas Bouamamaa *et al*. found antibiotic-resistant bacteria, including MRSA, in cockroaches and houseflies collected from residential areas in Tangier, Morocco. Wang *et al*. found identical pulsed-field gel electrophoresis profiles in several multidrug resistant *Salmonella* isolates recovered from swine stool and flies on multiple farms from northwestern Taiwan, reinforcing the idea that flies can serve as vectors for antimicrobial-resistant *Salmonella*. Chaslus-Dancla *et al*. found that gentamycin- and apramycin-resistant *E. coli* and *Salmonella* strains isolated from cattle and hospitalized patients in France and Belgium harbored the same antibiotic resistance-conferring plasmid, indicating that plasmid transfer occurred between bacteria infecting animals and humans.

The importance of focusing on the global, ecological perspective of antimicrobial resistance is illustrated by integrated fish farming, a widespread practice throughout Southeast Asia that involves recycling excreta from human and agricultural sources to feed fish in fishponds, where the resulting nutrients support aquatic photosynthetic organisms. Petersen *et al*. examined an integrated chicken-fish farm for 2 months after fish production started and reported that, over time, significantly more bacteria isolated from water-sediment samples became resistant to several antimicrobials. Rhodes *et al*. provided direct evidence that tetracycline-encoding plasmids, previously associated only with fish farms, disseminated between *Aeromonas* and *E. coli* species and between the aquaculture and human environments in at least four countries: Norway, England, Germany, and Scotland. The authors proposed that instead of viewing the fish farm and hospital as two separate components of the environment, they should instead be envisioned as one interactive compartment that facilitates the free exchange of genetic information.

The flow of antibiotic resistance in nature is further illustrated by the identification of antibiotic-resistant pathogens in populations where their presence cannot be simply explained by exposure to the antibiotic. Bartoloni *et al*. showed that in a remote rural Bolivian community of Guaraní Indians located at an altitude of 1700 m, despite minimal antibiotic use and limited exchange with other communities, 67% of the inhabitants that they examined carried fecal *E. coli* with acquired resistance to over one antibiotic. Gilliver *et al*. reported widespread antibiotic resistance in wild rodents previously unexposed to antibiotics, and Sjölund *et al*. showed that eight *E. coli* isolates obtained from Arctic birds were resistant to more than one antibiotic, with four of them resistant to four or more compounds that were tested. A multiresistance pattern that the authors observed was similar to one that is commonly seen in clinical isolates.

To better reflect the circulation of antibiotic resistance between different compartments of the ecosystem, D'Costa

et al. proposed the concept of antibiotic resistome, which encompasses the totality of antimicrobial resistance determinants across the globe, and incorporates not only resistance determinants directly relevant to the clinic but also the ones encountered in bacteria from other ecosystems, including those from nonpathogenic microorganisms such as soil bacteria.

These examples illustrate the need to employ a complex, interdisciplinary framework when exploring antimicrobial resistance in the food chain and implementing effective public health interventions. To reflect the ability of microbial pathogens from the farm environment to infect animals, humans, and the food chain at various stages and by various routes, this perspective should incorporate multiple facets. These include the ability of antimicrobials to select for resistant bacteria in food animals, farm workers, and the farm environment; the zoonotic and reverse zoonotic transmission of pathogens; cross-contamination in the kitchen environment; and the transfer of bacterial resistance across distinct compartments of the ecosystems.

See also: Bacteria: *Listeria monocytogenes*; *Salmonella* Non-Typhi; *Salmonella* Typhi and *Salmonella* Paratyphi; Shiga Toxin-Producing *Escherichia coli* and Other Pathogenic *Escherichia coli*; *Shigella*; *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*. Characteristics of Foodborne Hazard and Diseases: Cost of Foodborne Diseases. Food Safety Assurance Systems: Cleaning and Disinfection. Public Health Measures: Food Safety in Hospitals and Other Healthcare Settings; Surveillance of Foodborne Diseases

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CHARACTERISTICS OF FOODBORNE HAZARD AND DISEASES

Cost of Foodborne Diseases

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Glossary

Contingent valuation method The use of surveys of individuals to elicit their preferences, measured in monetary terms, for a specified improvement in their health outcomes. It circumvents the absence of markets for health outcomes by presenting survey respondents with hypothetical markets in which they are asked their willingness to pay (WTP) for the improvement in question.

Cost-of-illness (COI) method An approach used to estimate the societal costs of a particular illness or injury in a given time frame (such as a 1-year period). The approach typically focuses on two main types of societal costs associated with the particular illness or injury: medical costs and costs of lost productivity due to morbidity or premature mortality.

Disability-adjusted life years (DALYs) method This method makes time a common metric for death and disability by combining the years of life lost due to premature death (YLL) and the years lived with disability (YLD) for varying degrees of severity.

Health-adjusted life years (HALYs) method The HALYs method measures the years of full health lost because of living with morbidity. Within this group, two popular methods are quality-adjusted life years (QALYs) and disability-adjusted life years (DALYs). QALYs assign values to health outcomes on a 0 to 1 scale, with 0 indicating death and 1 indicating perfect health. DALYs have the reverse 0 to 1 scale. This means that food safety interventions would aim to maximize QALYs and minimize DALYs.

Hedonic wage studies Statistical analyses that estimate the effect of intrinsic job characteristics, such as health risks, fringe benefits, or autonomy, on wages. It is a method to estimate the risk premium.

Human capital approach A method for estimating the impact of an individual's illness or premature death on society by measuring the discounted value of his/her productivity loss (labor earnings) due to morbidity or premature mortality.

Premature death/premature mortality A preventable death that occurs before the average life expectancy for an individual's subpopulation.

Productivity loss The monetary value of output that would have been produced in the absence of an illness, disability, injury, morbidity, or premature mortality.

Value of statistical life The value of a statistical life (VSL) is the conventional way to summarize the value of small risk reductions in mortality. These estimates are not meant to be applied to the value of saving any particular person's life.

Willingness to pay (WTP) A measure of the monetary value an individual would place on reducing the risk of death or illness. It is the maximum dollar amount the individual would be willing to pay in a given hypothetical risk-reducing situation.

Introduction

This article discusses the economic costs and burden of foodborne disease and why their estimation is important. Here, we define burden as the prevalence and incidence of morbidity, disability, and mortality associated with acute and chronic manifestations of foodborne diseases. Estimation challenges are briefly discussed followed by an overview of the methods used to estimate foodborne disease costs. The article shows the US situation for Shiga toxin-producing *Escherichia coli* as an example, though some international examples are also provided. The article concludes with a look at future directions.

Individual, Industry, and Public Sector Costs of Foodborne Disease

Societal costs of foodborne disease are incurred by three main groups: individuals/households, the food industry, and the public sector. Taken as a whole, the costs of foodborne disease pose an economic burden on society, particularly in developing countries where the costs to prevent or treat foodborne disease can strain already limited federal and household resources. Persistent problems with foodborne pathogens within a country's food supply can also hinder food exports, which in turn can ultimately affect a country's agricultural revenue. Developing countries have the potential to

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be relatively hit harder by a food safety problem that adversely affects their food exports, compared to developed countries. This is because food exports from developing countries may account for a proportionally larger share of their total export revenue.

When researchers estimate the economic costs or burden of foodborne disease, they often include only a handful of the costs illustrated in Table 1. More easily measurable costs, such as some of the medical costs, costs of lost income (typically called productivity loss), and premature death are frequently included in these analyses. Most types of costs are excluded due to data limitations and lack of suitable analytical techniques, meaning that societal costs may be underestimated. The impact on industry members can be sizeable, particularly when large recalls or product liability suits are involved.

Why is it Important to Estimate the Costs and Burden of Foodborne Disease?

The estimated economic costs and burden of one or more foodborne diseases on a nation, other population subgroups, or the world can be used in three main ways, to:

1. Evaluate the economic costs or burden of a broader group of foodborne diseases.
2. Serve as input for analyses needed in policymaking, such as for a proposed food safety rule. In the US, cost-of-illness (COI) estimates for foodborne disease have been used in food safety rulemaking by several agencies. For example, cost estimates for several foodborne diseases in the US were used to estimate the benefits of the Hazard Analysis Critical Control Points (HACCP)/pathogen reduction rule for federally inspected meat and poultry slaughter and processing plants in the official regulatory impact analysis. In general, a benefit–cost analysis weighs the pros (i.e., benefits) and cons (i.e., costs) of a proposed regulation against a baseline (i.e., without the regulation). The benefits of some regulations are the costs of foodborne disease avoided or prevented because of the rule.
3. Aid comparisons and other analyses, such as to compare/rank estimated costs for different foodborne diseases or compare benefits and costs of alternative pathogen-prevention and control rules, programs, and strategies to determine the most cost-effective interventions so that resources are appropriately allocated (e.g., cost-effectiveness analysis).

Estimation Challenges for Foodborne Disease

In 2009, Buzby and Roberts identified epidemiological and methodological challenges that hinder the estimation of the economic costs and burden of foodborne disease. Figure 1 provides a simple schematic of a subset of these challenges.

Epidemiological Challenges

Four epidemiological challenges relevant here are:

1. Estimating the annual number of illnesses caused by a particular pathogen.

   Data limitations hinder the estimation of the annual number of foodborne illnesses caused by each pathogen. In short, many events must occur for an ill person to be identified as a laboratory-confirmed case of foodborne disease in the US and for the disease to be recorded in a national database – the ill individual must seek medical care, a specimen must be obtained, the laboratory must test for the particular foodborne disease that caused the illness, and the laboratory must confirm the causative agent and report the case to public health surveillance. This sequence of events does not occur in most instances, partly because most people with a foodborne illness do not seek medical care.

   In the US, one major source of data on foodborne diseases is the Foodborne Diseases Active Surveillance Network (FoodNet), which is a collaborative project of the Centers for Disease Control and Prevention (CDC), 10 Emerging Infections Program sites in different states, the US Department of Agriculture (USDA), and the Food and Drug Administration (FDA). FoodNet consists of active surveillance and related epidemiologic studies for foodborne diseases in the US. Using FoodNet data and other sources of information, the total number of foodborne disease cases in the US is estimated to be 76 million annually, according to a seminal article by Mead *et al.* in 1999. This article also estimated that unknown agents accounted for roughly 81% of all foodborne illnesses in the US and 64% of the associated deaths. Data limitations are particularly glaring for unknown agents, yet total costs will be underestimated if they are not included in the analysis.
2. Attributing foodborne disease to particular foods.

   A second epidemiological challenge is that there is limited data on the share of particular illnesses attributed to specific foods. This information is critical in economic analyses that estimate the number of foodborne disease cases which might be prevented by a proposed regulation. For example, the previously mentioned official regulatory impact analysis of the HACCP program for federally inspected meat and poultry plants included assumptions about what portion of selected foodborne diseases (e.g., campylobacteriosis, salmonellosis, and listeriosis) were due to meat and poultry. Internationally, food attribution data and approaches to obtain estimates of the share of foodborne illnesses attributed to specific foods include analysis of outbreak data, expert judgment, microbial subtyping and source tracking methods, case-control studies, among others.
3. Estimating acute illness outcome severity.

   Thorough economic evaluations of foodborne disease require information on illness severity, duration, and outcomes, which range from regaining full health to premature death. Yet, outcome distributions are limited for most pathogens. In the US, FoodNet collects information on three illness severities (illnesses, hospitalizations, and

Table 1 Costs to society from foodborne disease

Costs to individuals and households[a]	*Industry costs*[b]	*Regulatory and public health sector costs for foodborne pathogens*
Human illness costs:	*Costs of animal production:*	*Disease surveillance costs to:*
Medical costs:	Morbidity and mortality of animals on farms	Monitor incidence/severity of foodborne disease
Physician visits	Reduced growth rate/feed efficiency and increased time to market	Monitor pathogen incidence in the food chain
Laboratory costs	Costs contaminated animal disposal (e.g., farm and slaughterhouse)	Develop integrated database from farm to table
Hospitalization or nursing home	Increased trimming or reworking at slaughter and processing plants	
Drugs and other medications	Illness among workers due to handling contaminated animals/products	*Research to:*
Ambulance or other travel costs	Increased meat product spoilage due to pathogen contamination	Identify new foodborne pathogens for acute and chronic illnesses
Income or productivity loss for:		Identify high-risk products
Ill person or person dying	*Control costs for pathogens at all links in the food chain:*	Identify high-risk production and consumption practices
Caregiver for ill person	New farm practices (age-segregated housing, sterilized feed, etc.)	Identify which consumers are at high risk for which pathogens
Other illness costs:	Altered animal transport and marketing patterns (animal identification)	Develop cheaper and faster pathogen tests
Travel costs to visit ill person	New slaughterhouse procedures (hide wash, carcass sterilizing)	Risk assessment modeling for all links in the food chain
Vocational/physical rehabilitation	Altered product transport (increased use of time/temperature indicators)	
Child-care costs	New wholesale/retail practices (pathogen tests, employee training)	*Outbreak costs:*
Special educational programs	Risk assessment modeling by industry for all links in the food chain	Outbreak investigation costs
Institutional care	Price incentives for pathogen-reduced product at each food chain link	Testing to contain an outbreak
Lost leisure time	New processing procedures (pathogen tests, contract purchasing requirements)	Legal suits to enforce regulations that may have been violated[c]
Home modifications		Cleanup costs
Psychological costs:	*Outbreak costs:*	*Other considerations:*
Pain, other psychological suffering	Herd slaughter/product recall	Distributional effects in different regions, industries, etc.
Risk aversion	Plant closings and cleanup	Equity considerations, such as special concern for children
	Regulatory fines	Impact on food exports
Averting behavior costs:	Product liability suits from consumers and other firms	Impact on agricultural revenue
Extra cleaning/cooking time costs	Reduced product demand because of outbreak:	
Extra cost of refrigerator, freezer, etc.	Generic animal product – all firms affected	
Flavor change from traditional recipes	Reduction for specific firm at wholesale or retail level	
Increased cost to buy safer foods	Increased advertising or consumer assurances following outbreak	
Altruism (willingness to pay for others to avoid illness)		

[a]Willingness-to-pay estimates for reducing risks of foodborne disease is a comprehensive estimate of all these categories (assuming that the individuals have included employer-funded sick leave and medical programs in their estimates) and the estimate covers reduced risks for everyone – those who will become ill as well as those who will not.

[b]Some industry costs may fall with better pathogen control, such as reduced product spoilage. Possible increases in product shelf-life may permit shipment to more distant markets or lower shipment costs to nearby markets.

[c]In adding up costs, care must be taken to assure that product liability costs to firms are not already counted in the estimated pain and suffering cost to individuals. However, the legal and court expenses incurred by all parties are societal costs.

premature deaths) but it covers less than a dozen (i.e., *Campylobacter*, *Cryptosporidium*, *Cyclospora*, *Listeria monocytogenes*, STEC O157, STEC non-O157, *Salmonella*, *Shigella*, *Vibrio*, and *Yersinia enterocolitica*) of the more than 200 microbial agents that cause foodborne disease.

4. Estimating chronic complications.

   Many foodborne pathogens cause one or more chronic complications that can have lifetime health consequences or cause premature death (e.g., paralysis, kidney failure, irritable bowel syndrome). Some, like reactive arthritis, are autoimmune diseases caused in response to infections by certain foodborne bacterial pathogens. Obtaining data and information to estimate the costs and burden of these complications is challenging, yet their inclusion in cost studies is important because they can result in high average and total costs relative to acute manifestations.

Methodological Challenges

The main methodological challenges that hinder the estimation of the economic costs and burden of foodborne disease include:

1. Selecting the method.

   Selecting which method to use for the analysis to estimate the impact of foodborne disease is the first methodological challenge. If the resulting estimates are going to be used in an economic analysis of proposed Federal food safety rule, this choice is particularly important. In general, the analysis' goal usually determines which method is used. The main options include monetary (e.g., COI and willingness to pay (WTP)) and non-monetary methods (e.g., versions of the health-adjusted life years (HALYs) method). The Valuation Methods section discusses these in detail. Economic theory suggests that the WTP method should be used to estimate the benefits of a regulation if the goal of these estimates is their use in a benefit–cost analysis of a potential food safety regulation to see if the regulation is worthwhile to implement. This method can best reflect individual risk preferences. The 2003 US Office of Management and Budget recommends the WTP approach to value-anticipated reductions in the risk of premature deaths which could arise from implementing a proposed regulation.

   The COI method may be the better approach if the goal is to estimate foodborne disease costs and have a detailed breakdown of the cost components. For example, this method is valuable when estimating the monetary value of the benefits of implementing a regulation that reduces foodborne disease risks. Monetizing both the major benefits and costs is usually preferred in the US rulemaking. When monetization is not possible due to ethical issues, analytical limitations, and other factors, non-monetary methods are used.

2. Selecting the point or dollar estimate(s) of the value of statistical life.

   Perhaps the most crucial methodological challenge in a cost or burden analysis is selecting the dollar or point estimate(s) of the value of a statistical life (VSL) for premature deaths. The valuation of premature deaths can be by far the largest component of total estimated costs. For example, USDA's Economic Research Service (ERS) estimates suggest that premature deaths accounted for more than 88% of total costs for *Salmonella* and 90% of total annual costs for Shiga toxin-producing *E. coli* O157 (STEC O157) in 2008 dollars. Over the past decade or so, many of the VSL estimates in analyses that support US federal rulemaking have centered around $5 million per statistical life, updated to current dollars. This value is the midpoint of amounts found in a review of labor market studies.

   How economists value premature deaths is evolving over time. Different parts of the US Federal government have historically used different WTP estimates of the VSL. The choice matters when analyzing a proposed food safety rule because final estimates can vary widely, depending on the assumptions used. That is, whether or not a regulation is put into effect could conceivably be on the line. Sensitivity analysis using different VSL scenarios are helpful when there is uncertainty about which estimates are appropriate.

3. Deciding whether or not to adjust for age or preexisting health status.

   A third methodological challenge is the decision whether the VSL estimate in an analysis should be adjusted for age or preexisting health status. Adjusting for age means that WTP for mortality risk reductions are calculated on the expected remaining years of life lost due to the premature death (i.e., the elderly could forego a few years of their remaining lifespan whereas younger individuals could forego many decades of life). As an example of an adjustment for a preexisting health status, an analysis could account for the elderly being more likely than younger individuals to have a co-morbidity that affects their ability to respond, thus potentially making them more susceptible to foodborne illness. Estimated total costs could vary widely if these adjustments are made. Many of these adjustments are controversial. Analysts for the US Environmental Protection Agency are now directed to not include age adjustments.

4. Estimating the anticipated impact of a proposed rule.

   An integral part of an economic analysis for a proposed food safety rule is estimates of the anticipated number of foodborne disease cases and deaths that would be prevented if the rule is implemented. Predicting what might happen in the future is a challenge. Therefore, sensitivity analyses may be used to cover the plausible range of preventable cases. Epidemiologists and risk assessors are often relied on for this technical information.

Valuation Methods

Monetary Methods

Cost-of-Illness Method

Table 2 summarizes the three main methods for estimating the costs and burden of foodborne disease and, in turn, for valuing

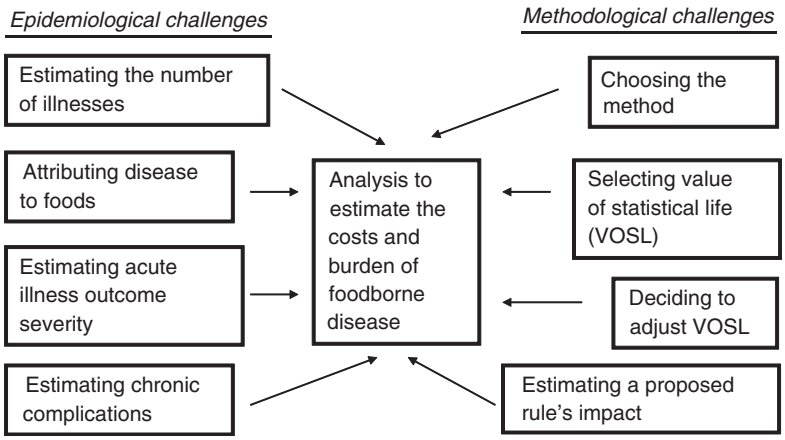

Figure 1 Epidemiological and methodological challenges to estimate the costs and burden of foodborne disease.

the benefits of proposed food safety policies. One main monetary method is the COI method, which estimates the dollars spent on medical costs and the value of lost productivity, such as the wages foregone because of a foodborne disease.

In general, a COI analysis traditionally starts with an estimate of the annual number of cases for a particular disease from all sources, foodborne and other, and then divides this number into severity groups. For example, Figure 2 presents the schematic of a COI study by USDA's ERS on Shiga toxin-producing *E. coli* (STEC O157). The initial acute illness is subdivided into two categories: those who did not visit a physician and survived and those who visited a physician. Some of those who visited a physician were hospitalized and these hospitalizations were divided into subgroups by outcome severity. Some developed hemolytic uremic syndrome (HUS), which is characterized by kidney and neurological failure, and some of these HUS cases also developed end-stage renal disease (ESRD), a chronic condition that results in reduced life expectancy. Those who died prematurely because of their illness are in additional subgroups.

For each severity group identified in a COI analysis for a particular disease, total annual costs are estimated. This entails obtaining appropriate data and calculating the corresponding medical costs, lost productivity costs, value of premature deaths, and other illness-specific costs, such as special education and residential care. In general, medical costs include physician and hospital services, supplies, medications, and special procedures sometimes required for chronic complications (e.g., dialysis for ESRD). Hospitalizations tend to swamp total medical costs.

Productivity loss typically measures the decline in work because workers were ill with a disease and missed work, performed poorly at work, regained only a portion of their preillness productivity, switched to less demanding and lower paying jobs, or were unable ever to return to work, such as from dying prematurely. Productivity losses for those who missed some work but later resumed work are usually calculated differently from those who died or were unable to return to work. The human capital approach is often used for temporary interruptions of work. In essence, this approach uses the present value of the future earnings foregone because of the illness. This productivity loss may be calculated as the product of time lost from work and the corresponding average daily wage rate. Productivity losses for those who died or were unable to return to work can be calculated in several ways, including using a VSL estimate. The total cost of lost productivity and premature deaths is the sum of all costs for all individuals affected, primarily the patients and, in the case of ill children, their parents or paid caretakers. In a COI analysis, subsequent steps often identify what portion of total costs is attributable to foodborne sources. The resulting data may be included in a larger analysis that estimates what portion of cases and deaths would potentially be averted by a proposed regulation.

Willingness-to-Pay Approach

The WTP approach is a second monetary method. It measures the dollars individuals are willing to pay for a reduced risk of encountering a certain health hazard. WTP estimates are often the result of contingent valuation studies, or hedonic wage or labor market studies, which evaluate the small statistical risk of premature death and the increase in wages to compensate for taking this risk (i.e., risk premium). As previously mentioned, the WTP approach is theoretically superior to other approaches and the US Office of Management and Budget 2003 states that it is the preferred approach when valuing reductions in fatality risks that are expected by implementing a proposed regulation. However, it does have some disadvantages as outlined in Table 2 and as a result, some analysts prefer non-monetary methods.

Non-Monetary Methods

Non-monetary methods provide estimates that are not in a monetary unit of measurement (e.g., healthy-time equivalents instead of dollars). They often deal with the health-related quality of life. That is, they convert adverse health outcomes from an illness that compromise both lifespan and functional ability into a common unit of measurement. An umbrella group of non-monetary methods is the HALYs method, which measures the years of full health lost because of living with morbidity. Within this group, two popular methods are quality-adjusted life years (QALYs) and disability-adjusted life years (DALYs). QALYs assign values to health outcomes on a 0

Table 2 Methods to estimate the costs and burden of foodborne diseases

Method	*Advantages*	*Disadvantages*
The *cost-of-illness (COI) method* is an monetary accounting or tally of the annual dollars spent on: ● Medical costs (e.g., physician and hospital services, supplies, medications, medical procedures, transportation to health care, relocation expenses, special education, residential care). ● Lost productivity and other costs like the dollars of employment compensation that was foregone as a result of morbidity or mortality (e.g., lost productivity because workers were ill and either missed work, performed poorly at work, were unable ever to return to work, or died prematurely).	● Provides easy to understand monetary measure of foodborne disease costs or the benefits of a program that reduces foodborne disease. ● Represents real costs to society.	● Estimates may be influenced by income, education, and other factors. ● Intricate disease coding and insurance arrangements can complicate estimation of medical expenses. ● Estimating lost productivity costs may also be difficult because of the various forms of compensation available to employees and because large sections of the population under study may not be in the workforce. ● Provides a partial estimate of economic costs as it excludes more difficult to measure costs to individual/households, industry and the regulatory/public health sector and excludes the vast majority of chronic complications associated with foodborne disease. Therefore, estimates may underestimate the actual benefits of a proposed food safety policy.
Willingness-to-pay (WTP) is a monetary method that estimates the money that individuals are willing to pay to reduce their probability of encountering a health hazard. WTP estimates are often the result of labor market studies, which evaluate the small statistical risk of premature death and the increase in wages to compensate for taking this risk.	● Reflects individual preferences for risk reduction. ● Theoretically superior measure. ● Includes valuation of pain and suffering, lost leisure time, and other costs.	● Estimates are sensitive to the study populations, type of risk, and level of risk so they may not be applicable if used in a different study. ● May not be practical to have a study focused on the risk being evaluated. ● Has some measurement difficulties, especially for nonfatal outcomes.
Non-monetary methods Non-monetary methods look at health-related quality of life, such as in healthy-time equivalents, and are useful in cost-effectiveness analyses. Health-adjusted life years (HALYs) is an umbrella group of non-monetary methods that measure the years of full health lost because of living with a morbidity. Two methods within this group are quality-adjusted life years (QALYs) and disability-adjusted life years (DALYs). Some analyses monetize the estimates in a separate step. For example, the US Food and Drug Administration has been monetizing QALYs in their benefit–cost analyses. *DALYs*. According to WHO, 'the DALY measure combines the years of life lost due to premature death (YLL) and the years lived with disability (YLD) for varying degrees of severity, making time itself a common metric for death and disability. One DALY is a health measure, equating to one year of healthy life lost.' (WHO, 2006)	DALYs are: ● An internally consistent common metric. ● Can develop and incorporate downstream effects on agricultural, social, and trade costs that are traditionally missing from a cost or global burden analysis. ● Can segregate comorbidity (i.e., where several pathologies coexist, and contribute and compete for the cause of death). ● Uses the same value of a human life in rich and poor countries and also levels the playing field between acute and chronic disease because it takes into account the duration of the syndrome and its severity.	● Poorly represents some social costs (e.g., reduced production and trade of cattle because of a food safety issue). ● Still requires subjective value judgments on how to weight or discount for age of onset, disability weights, and future losses.

to 1 scale, with 0 indicating death and 1 indicating perfect health. DALYs have the reverse 0 to 1 scale. This means that food safety interventions would aim to maximize QALYs and minimize DALYs. Estimates from these methods are useful in cost-effective analyses. Some analyses convert the estimates to dollars in a separate step by using a dollar value per HALY, QALY, or DALY conversion factor. For example, the US FDA monetizes QALYs in some of their benefit–cost analyses.

Disability-Adjusted Life Years

According to the World Health Organization (WHO), the DALY measure makes time a common metric for death and disability by combining the years of life lost due to premature death (YLL) and the years lived with disability (YLD) for varying degrees of severity. In essence, one DALY equates to 1 year of healthy life lost. DALYs are the WHO's preferred disease-burden measure. Importantly, DALYs use the same value of a human

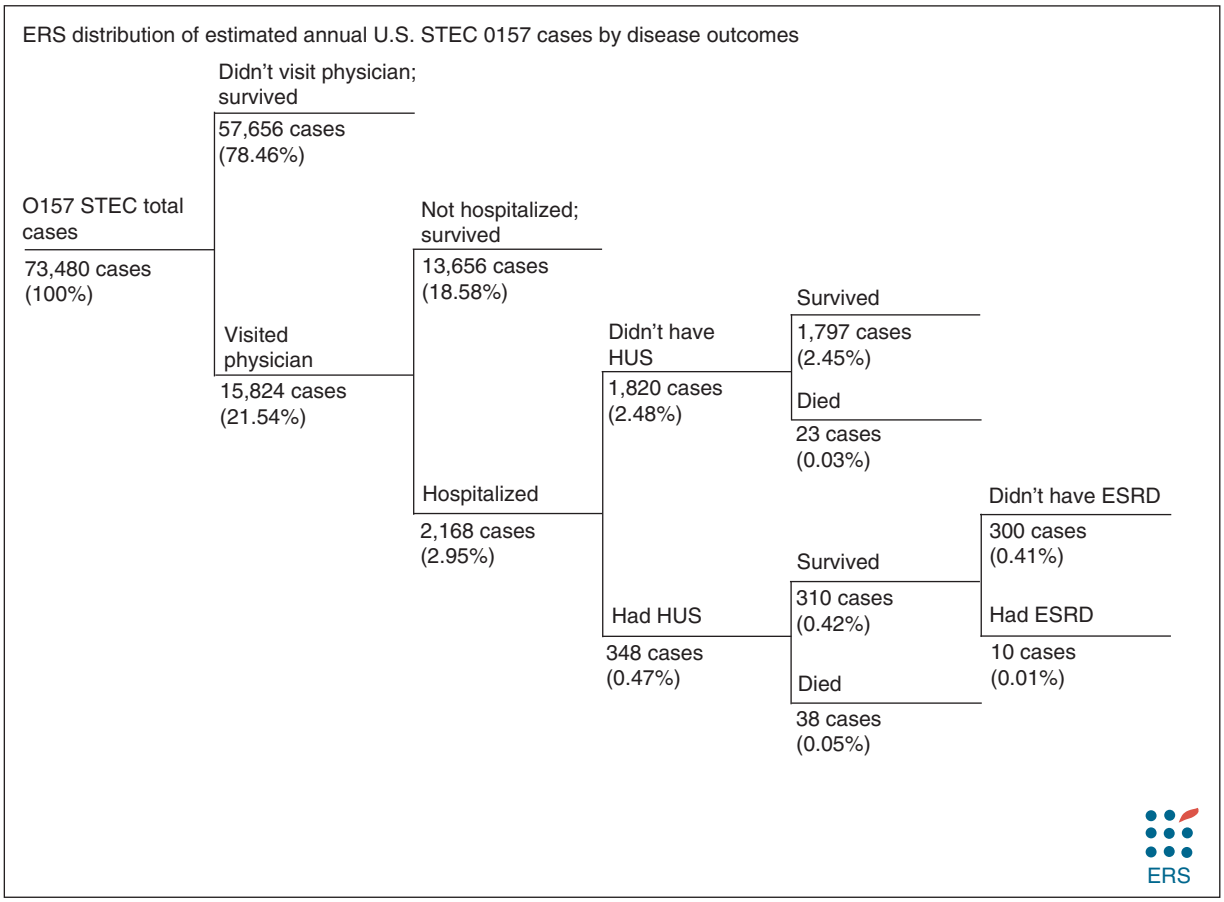

Figure 2 ERS distribution of estimated annual US STEC O157 cases by disease outcomes. *Source*: USDA/Economic Research Service.

life in rich and poor countries and they also level the playing field between acute and chronic disease because they take into account the duration of the syndrome and its severity. The DALY approach is an internally consistent common metric that can segregate co-morbidity (i.e., where several pathologies co-exist, and contribute and compete for the cause of death).

Examples of the Economic Costs and Burden of Foodborne Disease

In the 1980s, the ERS began to conduct some of the earliest studies on the economic costs of foodborne illness in the US. Initially, these analyses incorporated the limited information then available about the incidence of foodborne illness and used the COI method. ERS has continued to update and expand these analyses using improved estimation methods and better data. In 2003, ERS introduced their web-based Foodborne Illness Cost Calculator, which provides information on the assumptions behind foodborne illness cost estimates for *Salmonella* and Shiga toxin-producing *E. coli* O157 (STEC O157). Data users can make their own assumptions in this calculator and calculate their own cost estimates. The calculator uses the COI method for nonfatal illnesses and WTP point or dollar estimates for premature deaths.

ERS, in collaboration with CDC's FoodNet, developed the total cost estimate for STEC O157 in 2005, using a case-control study of STEC O157 patients and FoodNet surveillance data. Table 3 illustrates how estimated costs per case vary by severity level. Estimates include costs for complications that can arise from STEC O157 disease: acute illness costs arising from hemorrhagic colitis (HC, a clinical syndrome manifested by bloody inflammation of the colon), and chronic illness costs for HUS, with and without ESRD. Total costs include medical costs for kidney dialysis and transplants, the value of time lost from work due to nonfatal illness, and the value of premature death, but exclude other potential costs, such as for special education and pain and suffering. This collaboration did not estimate foodborne disease cost because of the lack of a widely-accepted estimate of the percentage of STEC O157 cases that are foodborne. Table 3 assumes two different estimates of the percentage of cases that are foodborne to illustrate how total costs may change with this final assumption, thus emphasizing the importance and need for quality data. Solely for illustrative purposes, this table assumes Mead *et al.*'s estimate from 1999 that 85% of STEC O157 cases are from foodborne transmission, and also assumes the arbitrary number of half of this amount (42.5%). Estimated annual costs of foodborne STEC O157 in the US in 2008 totaled $203.2 million under the 42.5% assumption and $406.4 million under the 85% assumption. One can easily imagine from this simplistic example how costs of foodborne STEC O157 could vary greatly, depending on the assumption of the percent due to foodborne transmission and how this

Table 3 Estimated STEC O157 costs in the US, from all sources and foodborne sources, 2008 Dollars

Severity level	*Total cases*	*Total costs*	*Average cost/case*	*Foodborne*			
				Assuming 42.5% FB		*Assuming 85% FB*	
				Cases	*Costs*	*Cases*	*Costs*
	Number	*Million Dollars*	*Dollars*	*Number*	*Million Dollars*	*Number*	*Million Dollars*
Not hospitalized							
Didn't visit physician; survived	57 656	1.7	30	24 504	0.7	49 008	1.4
Visited physician; survived	13 656	7.4	540	5804	3.1	11 608	6.3
Hospitalized							
Didn't have HUS; survived	1797	13.2	7351	764	5.6	1527	11.2
Had HUS but not ESRD; survived	300	12.4	41 188	128	5.3	255	10.5
Had HUS and ESRD; survived	10	60.9	6 094 462	4	25.9	9	51.8
Didn't have HUS; died	23	107.6	4 680 193	10	45.7	20	91.5
Had HUS; died	38	274.8	7 232 443	16	116.8	32	233.6
Total	73 480	478.1	6506	31 229	203.2	62 458	406.4

Source: Total cases, total costs, and average costs estimates are from the Economic Research Service, http://www.ers.usda.gov/data/foodborneillness/ecoli_Intro.asp (accessed December 2009). For illustrative purposes only, foodborne (FB) cases and costs assume 85% of total cases are foodborne (Mead *et al*., 1999) and arbitrarily half of that amount (42.5%). *Note*: HUS is characterized by red blood-cell destruction, kidney failure, and neurological complications, such as seizures and strokes. Although these figures are for STEC O157 illnesses from all sources, average foodborne illness costs are usually considered to be the same as costs for those illnesses from non-foodborne sources. Columns may not total due to rounding.

Table 4 Sample of estimated costs and burden of foodborne disease

Method	*Author (year of study/method)*	*Foodborne disease*	*Estimated costs*	*Country*
COI	Buzby and Roberts (1997)	Six bacteria, one parasite	$6.5–34.9 billion	US
COI	Roberts and Upton (2000)	STEC O157:H7 outbreak	£11 930 347	UK
DALY	Havelaar *et al.* (2000)	*Campylobacter* sp.	1400 DALY per case	Netherlands
COI	Scott *et al.* (2000)	All foodborne disease	$55.1 million	New Zealand
COI	Lindqvist *et al.* (2001)	Foodborne disease	$123 million	Sweden
COI	Abe *et al.* (2002)	STEC O157:H7 outbreak	¥82 686 000	Japan
COI	Frenzen *et al.* (2005)	STEC O157 (all sources)	$344 million	US
COI	Abelson *et al.* (2006)	All foodborne disease	AU$1249 million	Australia
WTP	Roberts (2007)	All foodborne disease	$1.4 trillion	US
DALY/COI	Haagsma *et al.* (2009)	Select foodborne diseases and irritable bowel syndrome	3757.9 DALY, €65.4 million	Netherlands

might affect a regulatory cost–benefit analysis, particularly if total costs were roughly equal to total benefits. In essence, one weak estimate, if used, can cloud an otherwise well-supported analysis.

Internationally, several studies have estimated the economic costs and burden of foodborne disease. Table 4 provides a small sample that used the COI, DALY, and WTP methods in developed countries. As with the US studies, the international studies' methodology and comprehensiveness have developed over time, with early studies focusing on one or more foodborne diseases. Similar studies have not been conducted in developing countries, largely due to data and resource limitations. However, there are some estimates of selected foodborne and diarrheal diseases and discussions of potential societal costs.

Looking Ahead

The global burden of foodborne disease is unknown. Current national estimates of the costs and burden of foodborne disease may significantly underestimate true values due to incomplete coverage of: (1) the breadth of acute and chronic manifestations of foodborne diseases, (2) the array of associated costs outlined in Table 1, and (3) the unknown pathogens that cause foodborne disease. As a result, economic analyses for policymaking that use these costs or burden estimates will be similarly restricted. Less comprehensive estimates of the costs and burden of foodborne disease could potentially mean that some regulations may not be put in place that would otherwise be adopted, had all foodborne disease costs and burden been accounted for. This also means that funding for public health may be less than what would be justified had total foodborne disease costs been more comprehensive. Additionally, incomplete accounting may mean reduced priority of foodborne disease at the international level.

In 2006, the WHO launched an initiative to estimate the global burden of foodborne disease. This initiative ultimately aims to express the impact of each disease in DALYs – and this reflects the growing trend toward using non-monetary estimation measures. Other trends include increasing dialog and analysis about the merits, pitfalls, and different outcomes from adjusting VSL for age and other factors. For example,

CDC–ERS collaboration is currently estimating the annual cost of premature deaths from foodborne disease using updated death estimates in an analysis, with and without adjustments for age. ERS is also in the process of expanding its cost calculator to incorporate additional foodborne diseases and newly released data from CDC.

For these and other analyses that estimate the economic costs and burden of foodborne disease, the ultimate challenge boils down to obtaining appropriate and reliable data for the various components of the analyses and to develop appropriate methodologies that can best use the limited data and information currently available. Better data could enhance understanding of foodborne disease and help support the need for effective food safety regulations, practices to reduce foodborne disease, and funding for public health to prevent and treat foodborne disease. Globally, foodborne disease will become increasingly important to prevent as, among other factors, the amount of internationally traded food increases and advances in transportation and subsequent decreases in average price per trip allow more people to travel worldwide, potentially increasing the spread of disease, including foodborne diseases.

See also: Public Health Measures: Challenges of Developing Countries in Management of Food Safety; Challenges of Industrialized Countries in Food Safety Management; Monitoring of Contaminants; Surveillance of Foodborne Diseases. Risk Analysis: Estimating the Burden of Foodborne Disease; Risk Management: Application to Biological Hazards

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Economic Research Service, U.S. Department of Agriculture. 'Foodborne illness cost calculator'.

http://www.rti.org/page.cfm?objectid=CA1E1F48-8B6C-4F07-849D6A4C12CBF3C3
RTI International's website provides extensive information on cost of illness studies. Although the majority of this information is not specifically on analyses of foodborne disease costs, the site provides background material on the COI method.

http:/www.who.int/foodborne_disease/burden/en/
The WHO website provides information on the initiative to estimate the global burden of foodborne disease. This website also provides introductory information on DALYs and disability weights, discounting, and age weighting.

CHARACTERISTICS OF FOODBORNE HAZARD AND DISEASES

International Classification of Diseases

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Introduction

The International Classification of Diseases (ICD) allows to categorize diseases, health-related conditions, and external causes of disease and injury in order to be able to compile useful statistics in mortality and morbidity. ICD has evolved over the past 150 years from an International List of Causes of Death to a comprehensive classification system for use in mortality, morbidity, case mix, quality, and patient safety. The ICD has become the international standard diagnostic classification for all general epidemiological and many health management purposes. These include the analysis of the general health situation of population groups and the monitoring of the incidence and prevalence of diseases and other health problems in relation to other variables, such as the characteristics and circumstances of the individuals affected. In this process, ICD has become the tool to allocate the majority of the global health resources. Users nowadays include physicians, nurses, other providers, researchers, health information managers and coders, health information technology workers, policy makers, insurers, and patient organizations. More specifically, its uses include:

- national and international health statistics (mortality and morbidity);
- epidemiology, surveillance, and monitoring;
- individual patient records and electronic health records;
- reimbursement and health system financing;
- reference for treatment guidelines, scientific literature, and research; and
- quality assessment at the level of individual cases up to assessment of health system outcomes and monitoring.

ICD is one core classification of a set of international classifications, the World Health Organization Family of International Classifications (WHO-FIC), that is designed to cover all domains of health. In addition to ICD, it includes core classifications for functioning, disability and health, and health interventions. A set of derived classifications, ICD-O, cover additional detail necessary for special disciplines. Related classifications, such as the anatomical therapeutic chemical (ATC)/defined daily dose (DDD) for drugs, complement the core classification for specific domains.

ICD-10 can be looked up online in English and French at WHO and accessed in other languages through the relevant national institutions and WHO-FIC Collaborating Centres. Overall, ICD-10 is available in 42 languages.

The revision process toward ICD-11 started in 2007, to be ready for publication by 2015. ICD-11 will be scientifically up-to-date and will be designed to provide the necessary detail and functionality to be useful in above-mentioned use cases and for use in electronic environment. An open, Internet-based mechanism allows for broad participation in the process.

ICD is used in various settings with different levels of resolution ranging from a set of 100 to 10 000 codes.

History

The history of the systematic statistical classification of diseases dates back to the nineteenth century. Groundwork was done by early medical statisticians, such as William Farr (1807–83) and Jacques Bertillon (1851–1922). The French government convened the first International Conference for the revision of the Bertillon or International List of Causes of Death in August 1900. The next conference was held in 1909, and the French government called for succeeding conferences in 1920, 1929, and 1938. With the sixth revision of the ICD in 1948, WHO became the custodian of the ICD. The ICD-6 extended the scope of the classification to nonfatal diseases, and WHO has continued to be responsible for periodic revisions of the classification. With a need to create comparability at the international level in public health as well as in clinical research, more and more clinical concepts have been introduced.

Content

The ICD can be used to classify diseases and other health problems recorded on many types of health and vital records. Its original use was to classify causes of mortality as recorded at the registration of death. Later, its scope was extended to include diagnoses in morbidity. The ICD is primarily designed for the classification of diseases and injuries with a formal diagnosis. In addition, the ICD provides for a wide variety of signs, symptoms, abnormal findings, complaints, conditions, and social circumstances, which are the problems or reasons for coming into contact with health services that stand in place of a diagnosis on health-related records. Most of these

categories can be found in Chapters XVIII and XXI of ICD-10. However, most other chapters also include such categories.

Structure

The ICD-10 consists of three volumes, and all three volumes are necessary for correct coding. This section provides an overview of the structure of ICD and how to use it. Before coding with ICD-10 and before interpreting ICD-coded information, users need to study the detailed descriptions in Sections 2.4–3.3 of ICD-10, Volume 2, and undergo some training with the electronic ICD-10 self-learning course.

Volume 1 contains the tabular list, as well as some definitions and a copy of the international agreement about reporting health information, the WHO nomenclature regulations. Volume 2 is a manual with extensive description of the classification and methods for use in mortality and morbidity, including short lists. Volume 3 is the alphabetical index. It contains separate indices for diseases, external causes, and drugs/substances.

The basic ICD is a single coded list of three-character categories, each of which can be further subdivided into 10 four-character subcategories. The three-character categories are grouped into blocks and chapters. The full meaning of a specific category is conveyed by its place in a chapter, block, its title, inclusions and exclusions, and other notes at any level of the hierarchical structure. Essentially in Chapter V, 'Mental and Behavioural Disorders,' definitions indicate the meaning of categories.

For coding, a term is looked up in the index. The index points to a category. The category is verified in Volume 1 taking into account information given in the context of chapters, blocks, categories, and Volume 2.

Chapters

ICD-10 is divided into 21 chapters. Chapters I–XVII relate to diseases and other morbid conditions and Chapter XIX relates to injuries, poisoning, and certain other consequences of external causes. The remaining chapters complete the range of subject-matter nowadays included in diagnostic data. Chapter XVIII covers symptoms, signs, and abnormal clinical and laboratory findings, not classified elsewhere. Chapter XX, 'External Causes of Morbidity and Mortality,' was traditionally used to classify causes of injury and poisoning, but, since the ninth revision, has also provided for any recorded external cause of diseases and other morbid conditions. Finally, Chapter XXI, 'Factors Influencing Health Status and Contact with Health Services,' is intended for the classification of data explaining the reason for contact with health care services of a person not currently sick, or the circumstances in which the patient is receiving care at that particular time or otherwise having some bearing on that person's care.

Blocks

The chapters are subdivided into homogeneous 'blocks' of three-character categories. Within each block, some of the three-character categories are for single conditions, selected because of their frequency, severity, or susceptibility to public health intervention, whereas others are for groups of diseases with some common characteristic. There is usually provision for 'other' conditions to be classified, allowing many different but rarer conditions to be coded. Usually at the end of blocks and chapters, special categories with the postfix 'unspecified' allow coding of cases where the reported detail does not allow assignment to more specific categories.

Categories

Most of the three-character categories are subdivided into four-character subcategories. This serves to identify, for example, different sites or varieties if the three-character category is for a single disease, or individual diseases if the three-character category is for a group of conditions. When the same fourth-character subdivisions apply to a range of three-character categories, they are listed only once, i.e., at the start of the range. A note at each of the relevant categories indicates where the details are to be found.

For Chapters XIII, 'Diseases of the Musculoskeletal System,' and XIX, 'Injury, Poisoning and Certain Other Consequences of External Causes,' supplementary subdivisions for use at the fifth or subsequent character level are proposed.

More Codes per Condition

With ICD-10, diagnostic statements can be coded in more detail using more than one code. The dagger–asterisk system is suitable for cases where a manifestation of a disease is a clinical problem in its own right and needs to be identified in addition to the underlying disease. The primary code is for the underlying disease and is marked with a dagger (†); an optional additional code for the manifestation is marked with an asterisk (*). Other multiple coding systems allow adding the infecting organism, functional activity of tumors, histopathological type of a tumor, or information about toxic agents and causal mechanisms.

Maintenance

Since the publication of ICD-10 in 1992, an updating mechanism allows yearly updates and major revisions every 3 years. Issues that arise in mortality and morbidity use of ICD are reported to WHO-FIC Collaborating Centres, or directly on an online platform. An international consensus process identifies solutions in consultation with classification and scientific experts and makes recommendations for updates to ICD-10.

Implementation

ICD-10 has been implemented in the majority of WHO Member States as the standard for coding diseases in mortality and/or morbidity (statistics, reimbursement, resource allocation, administration, treatment, and research), and ICD is in the process of being implemented in many other Member States. For example, the ICD is used in systematic full

mortality registration in more than 117 countries. However, implementation is not easily defined, because it gives no indication of the level of use of ICD-10 within the whole health sector. For example, a government might decide to implement ICD-10 for coding mortality; however, this represents only a fraction of the use of ICD-10 within a health system, and use in morbidity may relate only to pilot projects or specific diseases. If a country has fully implemented ICD-10 at a national level, this would mean that it has mandated the use of ICD-10 for coding mortality and morbidity across the whole health sector, as in the UK, South Africa, and many other countries. This means that all health care providers (or the appropriate allied personnel) will be required to code every death and every patient discharge, thus using ICD-10 for death registration, claims, and reimbursement purposes.

Modifications and Adaptations

Although some countries found ICD sufficient for clinical reporting, many others felt that it did not provide adequate detail for clinical and administrative uses. As a result, clinical modifications of ICD were developed, as was done for Australia (ICD-10-AM), Germany (ICD-10-GM), or Canada (ICD-10-CA). The USA uses a clinical modification based on ICD-9, the ICD-9-CM. It is used in several other countries that have adopted the US health sector reimbursement system. Recently, the process has been initiated to migrate to a more recent revision of ICD.

ICD and Foodborne Diseases (FBDs)

Traditionally, the term FBD was used for illnesses caused by microorganisms. However, in order to address the full scope of causative agents – of bacterial, viral, parasitic, or chemical nature – and acute and chronic health consequences, the term FBD is used now in a much more general, all-encompassing sense. It also includes foodborne zoonoses.

FBDs are commonly transmitted through ingested food. They comprise a broad group of illnesses caused by enteric pathogens, parasites, chemical contaminants, and biotoxins.

FBDs are an important cause of morbidity and mortality worldwide. They are most commonly associated with self-limiting diarrhea or vomiting. Foodborne contaminants can also contribute to a number of noncommunicable diseases, such as cancer and cardiovascular diseases; however, the contribution of unsafe food to these diseases is often not understood or quantified. Hundreds of different FBDs exist – some have been known for centuries, whereas others have only recently emerged. Standardization in reporting and coding is essential to learn more about the global burden and cost of unsafe food, and allocate adequate resources for FBD control efforts. To provide the necessary evidence base, WHO has started the initiative to estimate the global burden of FBDs.

ICD allows for coding FBDs. In some instances, there are specific categories; in other instances, foodborne conditions are grouped with conditions of other origin. For animal poisons and chemical contaminants, using two codes allows to specify the disease and the relevant group of substances.

The achievable detail in analysis and conclusion depends largely on the quality of reporting. Only fully formulated diagnoses allow correct coding and resulting conclusions, for example, Hepatitis or Hepatitis A.

When analyzing ICD-coded data for FBDs, aspects like attributable fractions, impact of rules for selection of the underlying cause of death, and health system-induced differences in frequencies of encounter or hospitalization need to be taken into account.

ICD has in Chapter I categories for intestinal infectious diseases (and poisoning by related toxins), like salmonellosis or cholera, as well as other FBDs like listeriosis, or hepatitis A, and parasites. The organ system-specific chapters include in many instances conditions that can be foodborne, as for ulcers, or toxic gastroenteritis and colitis. In such instances, the relevant substances (and intent) are identified by adding a code from Chapter XX. The Volume 3, Index to Diseases and Nature of Injury and the Table of Drugs and Chemicals will help the users in finding these conditions in the tabular list (provided in Volume 1). Categories for specific foodborne infections and parasitic diseases are provided in Chapter I and for food poisoning in Chapter XIX. For deaths from food poisoning, appropriate categories are provided in Chapter XX. A new notion of FBDs included lifestyle-related nutrition resulting in obesity and different metabolic syndromes. ICD has categories for such cases, although counting such cases in the group of FBDs is a matter of discussion in the upcoming years.

Examples

Conditions	*ICD-10*
Most infectious diseases	Chapter I
Other possible foodborne conditions	Chapters II–XVIII
Substance toxic effects (injury in general organ specific, as ulcers, are usually located in other chapters)	T36–T65 (Chapter XIX)
Substance toxic effects (intent, mechanism substance)	(Chapter XX)
Accidental (and unknown intent)	X40–X49
Intentional	X60–X69
Assault	X85–X90
Undetermined intent (after an inquiry has been conducted)	Y10–Y19

The table above gives a hint as to where the conditions and agents might be found in ICD. The table does neither indicate that all codes of a given range are relevant to food safety, nor is the list exhaustive. Relevant conditions need to be looked up individually, consulting first the index (in Volume 3) and then verifying the code in the tabular list (in Volume 1). Search terms could include, for example, poisoning and intoxication, or the specific disease, infectious agent or contaminant of interest. Particular attention needs to be given to ICD-10 rules related to coding of perinatal or maternal conditions.

ICD-11

ICD has a broad range of use cases and users. It needs to be suitable for use cases such as mortality, morbidity, case mix, quality, and patient safety, and for use in primary care, secondary

care, and research. Linkages to terminology systems like SNOMED CT® (Systematized Nomenclature of Medicine – Clinical Terms) and alignment and integration with other members of the WHO-FIC have been built into ensure that ICD is the gold standard for defining 'diseases and health conditions' and reporting them, and that ICD is the foundation for global health statistics and captures what health providers are seeing in practice and organizes the health information so that it can be shared – between practitioners, between countries, and over the above-mentioned time. ICD-11 therefore is designed as an information framework that contains a fully specified set of health-relevant concepts and their attributes and relationships. ICD-11 ensures consistency with traditional use cases of earlier ICD versions, because change history is integrated. The revision process toward ICD-11 started in 2007, to be ready for publication by 2015.

Timeline

- May 2011: Open ICD-11 Alpha to the public.
- May 2012: Open ICD-11 Beta to the public.
- May 2015: Present the ICD-11 to the World Health Assembly.

Most important innovation in this revision process is to make use of information technology to enable experts and stakeholders to work collaboratively on a 'Wikipedia-like' joint authoring platform. From the beta phase onward, the platform is open to public for providing their comments, submitting proposals, and taking part in field trials. To assure the quality of the input, however, the ICD-11 Platform differs from the Wikipedia, in that (1) the input is structured and demands scientific references and (2) it is peer reviewed.

New features of ICD-11 include:

1. Collaborative web-based editing.
2. Free access – as an international public good ICD-11 will be freely accessible.
3. Multiple languages – ICD-11 is already produced in multiple languages during the beta phase.
4. Definitions and detailed content – each disease will be operationally defined to avoid ambiguity.
5. Ready electronic health record – ICD-11 will conform to IT standards to link to terminologies and ontologies.

Daily edits to ICD-11 will be continuously made over the next 3 years, and will be made visible online simultaneously. All of the stakeholders that have a vested interest in the product should participate so that ICD best meets the needs of the users, and the classification is fit for purpose. With more input, the tool will better capture the advances in health sciences and give us a more accurate global picture of what is happening in the field of health and medicine.

In relation to FBDs, the information contained in ICD-10 will continue to exist. New ones may be added depending on the input and scientific evidence provided by the experts. Historical crosswalks are built into ICD-11. The possibility of continuity and comparison over time is implemented.

More details on ICD-11, videos, and access to the online browser, for viewing and participating to work on ICD-11 are available at www.who.int/classifications/icd/revision

Conclusion

ICD is the international standard for categorizing and reporting diseases, health-related conditions, and external causes of disease and injury. Use of ICD in food safety will allow compiling international statistics that are comparable internationally, with the ICD-coded data coming from sources, causes of death, or hospital statistics.

An essential prerequisite for every data collection is accurate reporting at the start of the information chain. The documentation should always include a fully specified diagnostic term, for example, hepatitis A or hepatitis E (and not just hepatitis). This allows for proper interpretation of the information and accurate categorization of the case.

ICD coding of FBDs requires some training. An ICD-10 self-learning course can be accessed online or downloaded for use on a local computer.

ICD is accessible online, which includes the ICD-10 and the ICD-11 (which is under development).

Online resources inform users on ways to contribute to the continuous improvement of ICD.

See also: Public Health Measures: Foodborne Disease Outbreak Investigation; Surveillance of Foodborne Diseases

Further Reading

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Relevant Websites

http://www.who.int/foodsafety/foodborne_disease/ferg/
WHO.

http://www.who.int/classifications/network/collaborating
WHO Collaborating Centres for the Family of International Classifications.

http://www.who.int/classifications
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FOODBORNE DISEASES

Contents

Overview of Biological Hazards and Foodborne Diseases

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Glossary

Cysticercosis Infection caused by the tapeworm, *Taenia*; the infection occurs when the tapeworm larvae enter the body and form cysticerci: these resemble cysts whose walls are retracted in one place to form heads; each head has suckers and sometimes hooks. The cysticercus develops from an oncosphere in any organ (frequently in muscles, brain, and eye) of its intermediate host, such as swine and cattle. After entering the definitive human host, the cysticercus protrudes from the cyst and the larva is transformed into an adult worm.

Endemic disease A disease occurring within a particular area.

Enterotoxin A toxin produced by a microorganism specific for the cells of the intestinal mucosa and causing vomiting and/or diarrhea.

Epidemic Outbreak of disease that affects a much greater number of people than is usual for the locality or that spreads to regions where it is ordinarily not present.

Hemolytic-uremic syndrome A disorder that usually occurs when an infection, for example, from *Escherichia coli* O157H7, in the digestive system produces toxic substances that destroy red blood cells, causing kidney injury.

Immunocompromised Having a weakened or impaired immune system.

Mastitis Inflammation of an animal's udder, usually as a result of bacterial infection.

Oocyst A fertilized gamete of parasitic sporozoans that is enclosed in a thick wall resistant to the environment.

Pandemic Worldwide epidemic, a global disease outbreak.

Plasmapheresis A process in which blood taken from a patient is treated to extract the cells and corpuscles, which are then added to another fluid and returned to the patient's body.

Retinochoroiditis Inflammation of the retina and choroid (a brownish membrane between the retina and the white of the eye).

Septicemia Illness with toxicity due to invasion of the bloodstream by virulent bacteria coming from a local seat of infection (also known as blood poisoning).

Sequela A disease or disorder that is caused by a preceding disease or injury in the same individual.

Thrombotic thrombocytopenic purpura Blood disorder that causes blood clots to form in small blood vessels around the body, and leads to a low platelet counts; these clots composed of platelets can block blood flow to the brain, leading to bleeding under the skin forming purple-colored spots called purpura.

Toxicoinfection Type of foodborne illness that occurs due to ingestion of a bacterium that produces toxins within the gut, which are absorbed to produce gastrointestinal symptoms.

Zoonotic disease An infectious disease that can be transmitted from wild and domestic animals to humans.

Introduction

Foodborne diseases can be both acute and chronic, and stem from three sources: biological, chemical, and physical. Bacteria, viruses, and parasites, are the main biological hazards causing acute foodborne diseases. Certain biological toxins can also be considered as causing acute effects, such as most seafood toxins, and these are discussed separately in another overview on chemical hazards. Acute can be defined as an incubation period between ingestion and an adverse effect of a determined time, usually from hours up to months, resulting from a single exposure. The impact of these agents results in toxins absorbed by the gastrointestinal tract or in infections produced in the intestines, but can subsequently affect other parts of the body. The whole human population is exposed to these types of agents, but more frequently where there is poor sanitation and lack of temperature control of food, as it occurs in many parts of the developing world. In developing countries and city slums, exposure to such agents on a continual basis may result in chronic diarrhea, which can be fatal for infants and young children. Foodborne bacterial agents include the following: *Bacillus cereus*, *Campylobacter*, *Clostridium perfringens*; different types of pathogenic *Escherichia coli*, *Salmonella*, *Shigella*, *Staphylococcus aureus*; and various types of vibrios, especially *Vibrio cholerae* and *Vibrio parahaemolyticus.* Less well investigated are illnesses caused by enteric viruses, such as hepatitis A and E viruses, caliciviruses, for example, norovirus (NoV), rotavirus, and possibly adenoviruses and astroviruses. More than 70 species of protozoan and helminth parasites can affect humans who consume contaminated food and water; most of these infections occur because of poverty, limited available sanitation, and improper food storage and preparation habits. Those most often investigated include the protozoa: *Cryptosporidium*, *Cyclospora*, *Toxoplasma*, *Entamoeba*, and *Giardia* and helminths: *Clonorchis*, *Echinococcus*, *Fasciola*, *Opisthorchis*, *Paragonimus*, *Taenia*, and *Trichinella.* Diagnostic and detection methods are routinely available only for a few parasites in food and water, mainly those that occur in industrialized countries, for example, *Cryptosporidium*, *Giardia*, *Toxoplasma*, *Anisakis*, *Trichinella*, and *Taenia.*

Epidemics and Pandemics

The major etiological agents that account for the estimated 1.5 million gastrointestinal deaths each year are enterotoxigenic *E. coli* (ETEC), rotavirus, *V. cholerae*, and *Shigella* spp.; all are known to be endemic in the vast majority of developing countries. Whereas *V. cholerae*, *Shigella*, and rotavirus can be detected by standard assays, ETEC is more difficult to recognize, and therefore is often not appreciated as being a major cause of either infantile diarrhea or of cholera-like disease in all age groups. It also causes traveler's diarrhea in visitors to endemic areas. In fact, ETEC is the most important pathogen of these four pathogens for diarrhea in infants, children, and adults, accounting for 280 million episodes and more than 400 000 deaths annually.

In the historical past, long-lasting plagues and pandemics of infectious disease agents were regularly documented. The latest of these, the seventh cholera pandemic of *V. cholerae* O1, has been the most thoroughly investigated, and has been shown to be transmitted by many related strains through water and food. It began in 1961 in Indonesia, and by 1966, it had affected most of Asia. Cholera incidence then decreased slightly until 1971, when an upsurge was observed in Africa and Europe, which had been free of cholera for >100 years. Cholera rates remained relatively low during the 1980s, with the disease confined to Asia and Africa. However, two major cholera outbreaks appeared in the 1990s: first, a resurgence of cholera in Africa and second, outbreaks starting in Peru that became the first cholera epidemic in Latin America since 1895. The cholera epidemic in Latin America was originally suspected to have come from Asia through the discharge of contaminated ballast water into Peruvian ports, but the isolates from Latin America were closely related to isolates found in Africa in the 1970s and 1990s, indicating that the strain that caused the epidemic in Latin America came from Africa rather than Asia. In addition, a novel serotype, O139, caused major outbreaks on the Indian subcontinent in 1992, and was later shown to be a variant of the seventh pandemic clone; in fact, 9 of the 12 O139 strains actually arose from the O1 precursor strain and were present in seventh pandemic isolates as early as 1979. In 2005, 56 countries had officially notified World Health Organization (WHO) of cholera cases for a total of 131 943 cases and 2272 deaths. The actual number of cases is probably much higher because of poor surveillance systems and frequent underreporting. Food, as well as water, is frequently implicated in the transmission of cholera, and the reporting of cholera resulted in trade embargoes and lost tourism income in many affected countries. Fecally contaminated water may be the main source but increasingly foods have been implicated. Foods are likely to be fecally contaminated during preparation, particularly those associated with water such as fish and shellfish or handled by infected food workers in an unhygienic environment. WHO estimates that the officially reported cases represent only approximately 5–10% of actual cases worldwide. Many of the *V. cholerae* groups/genotypes spread easily and widely to multiple countries or regions. This finding suggests that cholera epidemics or upsurges, which often occurred at the same time in many countries, are caused by the spread of newly arisen genotypes. Additionally, a genotype can also persist for long periods in an endemic region, as was demonstrated in Southeast Asia and Africa. Detailed analysis of how specific diseases are spread may be useful to monitor and control future potential pandemics.

The Burden of Foodborne Disease

With today's improvement in standards of personal hygiene and basic sanitation, safe water supplies, effective vaccination programs, food control infrastructure, and the wide application of newer food processing technologies and hazard analysis and critical control point (HACCP), many foodborne and waterborne diseases (e.g., poliomyelitis, brucellosis, cholera, scarlet fever, typhoid, and paratyphoid fevers) have been either eliminated or considerably reduced in industrialized countries. Nevertheless, most countries experienced important increases in several other foodborne diseases.

Zoonoses are particularly difficult to eradicate or even control because of an ever-present reservoir; these include both domestic and wild animal populations. In most developing countries, foodborne and waterborne zoonotic diseases have been so poorly investigated that even the crude burden of illness cannot be estimated with any degree of certainty. Although the situation regarding foodborne diseases is most serious in developing countries, industrialized countries have experienced a succession of well-publicized outbreaks, which have led to better estimates of foodborne disease cases in recent years. The annual estimates are 2.37 million cases of foodborne gastroenteritis in the UK, 5.4 million cases in Australia, and 11–13 million in Canada. The equivalent number for the USA (48 million; range 28.7–71.1 million in 2011) indicates there is considerable uncertainty in determining such estimates. The above data indicate that from 1 in 3 to 1 in 26 are ill from foodborne disease every year.

Bacteria

B. cereus

Bacillus cereus causes two types of mild foodborne illnesses. The emetic type is characterized mostly by nausea and vomiting and some abdominal cramps occurring 1–6 h after ingesting the food containing a heat-stable emetic toxin, similar in effect to staphylococcal food poisoning but typically milder. The other type is the diarrheal form which causes abdominal cramps and diarrhea after an incubation period of 8–16 h; the effect is similar to *Clostridium perfringens* toxicoinfections. This type produces a heat-labile diarrheagenic enterotoxin and/or a hemolytic enterotoxin, which cause intestinal fluid secretion. The symptoms of those experiencing either type of illness typically resolve themselves within 24 h. *Bacillus cereus* is commonly found in soil, crops, and dust, and therefore spores are frequently present in many foods. However, the spores themselves can be ingested without adverse effects; it requires their germination and toxin production in food to cause the above-mentioned symptoms. The emetic type is most often associated with rice dishes that have been cooked and then held at warm temperatures for several hours, such as fried rice. The diarrheal form is more associated with vegetable dishes or puddings containing cereal products that have been cooked and stored. However, both types of *Bacillus* spores may be present in the same food (or one strain can produce both types of toxin), and a mixture of incubation periods and symptoms may occur, and less typical foods like cooked poultry dishes may also be vehicles. Spices may be overlooked as a source of spores. Generally, illnesses from *B. cereus* (and other *Bacillus* species) are overlooked and rarely reported and they may be much more frequent than currently documented. Thus, there may be foods that may be important vehicles for which there is no record. Since most recognized cases have been confined to developed countries, the extent of *B. cereus* intoxications in much of the world remains unknown.

Campylobacter

Campylobacter jejuni is a major cause of bacterial diarrheal illness in the USA and in many other countries, with an estimated 845 000 foodborne cases per year in the USA alone, third in the number of estimated bacterial foodborne disease cases after *Salmonella* and *Clostridium perfringens*. Part of the reason for the high numbers is the low infective dose. However, this foodborne illness occurs mainly as sporadic cases and not as common-source outbreaks. Most cases of diarrhea, vomiting, fever, and abdominal cramping usually appear within 2–5 days of exposure and resolve within days, but one sequela of concern is Guillain–Barré syndrome (GBS) which is discussed under Section Autoimmune Sequelae to Gastrointestinal Infections, *Campylobacter* grows optimally at 42 °C under low oxygen concentrations, such as would be found in the intestines of warm-blooded birds and mammals. Thus, one of the most frequent sources of *Campylobacter* for consumers is raw meat and poultry, particularly the latter. A large proportion of chicken and turkey carcasses entering the kitchen contain this pathogen in higher numbers than *Salmonella*. Most *Campylobacter* organisms originate in poultry flocks. For instance, an European Union (EU) study shows it is approximately 30 times more likely that a *Campylobacter*-colonized broiler flock will yield positive carcasses for *Campylobacter*, compared with a noncolonized flock. Risks for contamination increase with the age of the slaughtered broilers and time of year. The handling, preparation, and consumption of broiler meat may directly account for 20–30% of human cases of campylobacteriosis in the EU. Outbreaks are not only associated with poultry but also with unpasteurized milk, cheese made from unpasteurized milk, undercooked beef, pork, lamb, shellfish, lettuce, and water, with many of these infections caused by cross-contamination in the kitchen, for example, from raw poultry to cooked poultry or salads or other ready-to-eat foods. Poultry growers have yet to significantly reduce infections in chicks because of their close contact and access to fecally contaminated drinking water. Thorough cooking of raw meats and poultry, avoidance of preparation practices that encourage cross-contamination, and proper hand washing will all reduce the risk of consumer infections.

Clostridium botulinum

Clostridium botulinum is a Gram-positive, rod-shaped anaerobic spore-former that produces oval endospores commonly found in soil, and marine, brackish, and freshwater sediments. Most spores are sufficiently heat resistant that the canning industry stipulated many decades ago that commercial canning for low-acid canned foods (e.g., foods having a pH >4.6) must be subjected to a 12-D log reduction heating step to ensure there are no surviving spores that could germinate and outgrow to flourish in the anaerobic environment. Although fairly frequent in the past, outbreaks involving commercial canned products (cans/tins and jars) are rare today. However, as recently as in 2007, eight cases of botulism were reported in US residents who had eaten commercially prepared hot dog chili sauce. In this outbreak, the spores apparently survived the retorting process through a processing failure. However, it is not the spores or organism themselves that are the concern but the neurotoxin(s) which are produced and ingested with the food. Seven different toxins (A–G) can be produced but

the most common affecting humans are A, B, and E. On ingestion the toxins can manifest themselves in several different types of symptoms from diarrhea to constipation, but the main effect is flaccid muscular paralysis that can cause respiratory failure, and eventual death. Some specific symptoms are double vision, blurred vision, drooping eyelids, slurred speech, difficulty swallowing, or muscle weakness. Symptoms generally begin 18–36 h after eating a contaminated food, but they can occur as early as 6 h or as late as 10 days. If untreated, the whole body may become paralyzed affecting the face, arms, breathing muscles, trunk, back, and legs. All Type A and some Type B strains are proteolytic with the food often spoiling with strong odors making it less likely to be consumed; whereas some Type B and all Type E strains are nonproteolytic and will not spoil the food. Infant botulism is an unusual syndrome affecting only very young children suspected of eating honey or other sweeteners, but the source of spores in such illnesses is rarely determined. Spores present in the low-oxygen, low-acid digestive system of an infant can outgrow and produce toxins. As soon as infants begin eating solid food, there are changes to the microbial flora of the bowel that start to inhibit growth of the bacterium. Types A and B *Clostridium botulinum* are frequently found in soil samples in North America and other continents. Type E is much more likely to be found in marine or estuarine sediments, fish, and marine mammal intestines. In Alaska, Arctic Canada, and northern Russia, the Eskimo/Inuit have traditional 'fermented' seal or whale fins or other parts stored outdoors with blubber. Improper storage conditions may allow spores that are frequently present to germinate and outgrow in the partly anaerobic environment. Although outbreaks are relatively few today, foodborne botulism remains a serious threat and may be caused by products distributed internationally. In Finland, botulism is rare and most domestic cases have been associated with fish products caused by *Clostridium botulinum* Type E toxin. In 2009, two cases of foodborne botulism were detected among French tourists who purchased fish from Finland, which was stored inappropriately and consumed after having returned home. In 2006, two persons in Finland were diagnosed with botulism after having eaten vacuum-packed smoked whitefish. Previously in Italy, conserved olives have also been implicated as a vehicle for foodborne botulism. There were three outbreaks in three different countries in Europe in 2011, all in a similar time frame (September–November). The foods involved were korma sauce (curry with yogurt, onions, and ground almonds, spices, and nuts); olive tapenades (chopped olives, capers, anchovies, and olive oil); and olives stuffed with almonds, all in glass jars. Improper processing was determined as the likely cause in two of these outbreaks. Fewer serious cases and deaths are now reported, perhaps due to better education in food preparation, more rapid medical care with antitoxin readily available for cases, and a younger generation with more Western food tastes and avoiding such foods that are an acquired taste. In North America, some British Columbian and Alaskan tribes still prepare 'fermented' salmon eggs ('stink eggs'), which have the same risks but few outbreaks have been recorded recently. Type E strains have also been associated with partially preserved uneviscerated fish with a few recorded outbreaks mainly in consumers of Middle Eastern origin. The risk for most consumers, however, is not exotic foods but home-canned vegetables, where the pressure cooking has not been sufficient to destroy the spores. Botulism is also occasionally associated with certain strains of *Clostridium baratii*, *Clostridium butyricum*, and *Clostridium novyi*.

Clostridium perfringens

Clostridium perfringens (formerly known as *Clostridium welchii*) is a Gram-positive, rod-shaped, anaerobic, spore-forming bacterium found as a normal component of soil, decaying vegetation, marine sediment, and the intestinal tract of animals including humans. Thus, it is present in areas subject to human or animal fecal pollution, and has sometimes been used as an indicator of fecal contamination of water and food facilities. Type A *Clostridium perfringens* is a frequent cause of foodborne illness in many countries, including the UK and the USA. It is the second most frequent cause of bacterial foodborne outbreaks in recent estimates for the USA. This strain possesses a chromosomal enterotoxin (*cpe*) gene and vegetative cells are relatively heat-resistant compared with other *Clostridium perfringens* strains. Symptoms of abdominal cramps and diarrhea begin 8–22 h after consumption of foods containing large numbers ($>10^8$) of vegetative *Clostridium perfringens* cells capable of producing an enterotoxin, during sporulation in the gut. This is a type of a toxicoinfection. *Clostridium perfringens*-associated illness is normally mild with a duration of <24 h but some symptoms may persist occasionally for 1 or 2 weeks, and deaths only rarely occur as a result of dehydration and other complications in elderly persons. Most illnesses occur after cooked soups or stews have been left too long at ambient temperatures or in too large quantities in coolers that would allow spores naturally present to germinate and produce vegetative cells. The cooking stimulates spore germination and once the temperature has dropped below 50 °C, the vegetative cells can multiply rapidly, growing to large numbers, especially in high protein foods. Because *Clostridium perfringens* can double in number every 7–10 min under optimal temperature (43–45 °C) and nutrient conditions after spore germination, even low numbers of spores can increase to illness-causing levels in a few hours. Thus, outbreaks are most frequently associated with catering companies and others who prepare and then improperly store large quantities of food; social events at churches or large family gatherings are also occasions where meat or poultry dishes may become vehicles for growth of the pathogen. If such stews, soups, gravies, roasts, and other meat or poultry products are served hot or are rapidly cooled before reheating and serving, the risk of *Clostridium perfringens* food poisoning is much reduced.

A more serious but rare illness, however, is caused by ingesting food contaminated with large numbers of Type C *Clostridium perfringens* strains, resulting in infection and necrosis of the intestines and subsequent septicemia. This illness is known as enteritis necroticans or pigbel disease in developing countries, particularly Papua New Guinea, but was also documented in post-Second World War Germany (where it was called Darmbrand). The main reason for the development of the disease is protein deprivation because of

starvation, episodic meat feasting (mainly pork), and staple diets containing trypsin inhibitors, such as sweet potatoes, combined with poor food hygiene. The enterotoxin is normally destroyed by trypsin protease, but production of this enzyme is inhibited if there is lack of protein synthesis. Consumption of spores alone has typically no adverse consequences for foodborne illness. However, *Clostridium perfringens* and other Clostridia can also cause gas gangrene in damaged tissue without medical aid, such as which occurs during military conflicts where spores from the soil can be trapped deep into body tissue where partially anaerobic conditions are present. If the pathogen can multiply in the tissue, it can spread rapidly to other parts of the damaged limb, and cause a systemic infection resulting in death. Interestingly, one reason for embalming dead bodies is to prevent the action of *Clostridium perfringens* from producing tissue gas, resulting from spores germinating and outgrowing into the increasingly anaerobic conditions of the corpse. Strangely, *Clostridium perfringens* has an apparent beneficial use. It is believed to be one of the starter culture components used as the leavening agent in artisanal salt-rising bread. This bread is rarely made today but has been reported to be made in Scotland and Ireland and by immigrants to Canada and the USA centuries ago. Salt-rising bread is different from other breads because yeast does not play any part in the fermentation process. It is thought that the salt used in the starter suppresses any yeast growth and provides an environment to allow naturally present *Clostridium perfringens* and other microorganisms to produce gas and a flavor different from that produced by yeast and lactobacilli (or baking soda). However, the ferment has not been scientifically investigated to understand its beneficial qualities.

Other clostridia apart from *Clostridium botulinum* may occasionally be implicated in foodborne illness but the one of current interest is *Clostridium difficile*, most commonly linked to hospital-acquired infections. These can be chronic and life threatening in humans and hard to eliminate from already-ill patients. *Clostridium difficile* is recognized as both a gut colonizer and cause of diarrhea in food animals, including cattle and poultry. *Clostridium difficile* epidemic infections also occur in piglets. Because *Clostridium difficile* has been isolated from retail foods intended for human consumption in the USA, Canada, and Europe and from meat products intended for consumption by pets, this may be a future concern for contamination of the food supply. At present, there are no analytical methods for testing foods for this pathogen, but these and standards may eventually have to be established to reduce the risk of infections though foods.

E. coli O157:H7 and Other Pathogenic *E. coli*

Escherichia coli O157:H7 was first recognized as a pathogen in 1982 as a result of an outbreak involving hamburgers in two US states. The organism was found to produce a toxin that is capable of damaging the kidneys (hemolytic-uremic syndrome (HUS)) and affecting the central nervous system (CNS) (thrombotic thrombocytopenic purpura (TTP)), often preceded by bloody diarrhea (hemorrhagic colitis (HC)). The toxin has been called both a verocytotoxin (affecting the vero kidney cells of the green monkey) and a Shiga-like toxin (similar to a toxin produced by *Shigella*). In fact, there are a number of similar toxins that make up the verotoxin/Shiga toxin category. This division of nomenclature remains today with both terms verotoxin-producing *E. coli* (VTEC) and Shiga toxin-producing *E. coli* (STEC) being used to describe the same group of organisms. Other virulence factors that may be necessary for severe infections are an attaching and effacing protein (adhesin/intimin) and a hemolysin. An earlier outbreak in Canada with one death in 1980 was likely caused by the same organism after apple cider (unfermented squeezed apple juice) was consumed at a farmer's market. Verotoxin was found in the intestines of the dead youth but VTEC were not successfully isolated. Outbreaks from apple cider have since been occasionally reported in Canada and the USA. In fact, outbreaks of *E. coli* O157:H7 and similar organisms producing verotoxin/Shiga toxin have occurred in many countries outside North America including Australia, Japan, the UK, and many other European countries. It is interesting that the more northerly countries seem to have the higher case rates, for example, Canada, Scotland, and Scandinavian countries. This may relate to farm practices for cattle and sheep that are the main reservoirs for the pathogen. Supershedders (animals that excrete large numbers of the pathogen in their feces) may be a major transmission factor in the field or feedlot and during and after transportation to the slaughterhouse.

In 1993, a major outbreak of *E. coli* O157:H7 infection affected approximately 500 people in four northwestern US states. Many children developed HUS, and four died as a result. This episode forced the US government to declare that *E. coli* O157:H7 was an adulterant in ground beef (and later in nonintact beef). Another large outbreak caused by this pathogen occurred in Africa in 1992, affecting probably thousands of people, with an undocumented number of cases of HUS; drinking water and cooked maize were the identified vehicles of transmission. In 1996, in an outbreak of *E. coli* O157:H7 in Japan, 6309 schoolchildren and 92 school staff members were affected, with two deaths; the epidemiological investigation identified fresh radish sprouts (kaiware-daikon) as the probable cause. This was the largest outbreak ever recorded from this pathogen. However, subsequently two very large and well-publicized outbreaks occurred in Scotland in 1996–97 (400 ill with 20 deaths of elderly persons) and Wales in 2005 (>170 schoolchildren and others, and 1 child died). Both were caused by butchers who managed catering businesses and also worked with slaughtered animals. The outbreaks were traced to contaminated meat dishes that were delivered to residences and schools. Another well-published outbreak originated from Californian bagged fresh spinach in 2006, with 205 confirmed cases of illness and 3 deaths. The ultimate source was not determined but upstream cattle, feral pigs, and irrigation water may have been involved in field contamination, and the washing and disinfection processes for the spinach were insufficient to prevent *E. coli* O157:H7 from entering the bags. This was one of the three leafy green outbreaks caused by *E. coli* and other pathogens in 2006 involving leafy greens, mainly lettuce, over the previous decade. The leafy greens industry is assessing how to minimize risks to avoid similar problems. Other outbreaks have been linked to

contaminated unpasteurized milk, alfalfa sprouts, salami, recreational water, and contact with infected live animals.

VTEC/STEC that are not of the O157:H7 serotype also pose a risk to human health. Several of these have caused outbreaks (O26, O103, O104, O111 O126, O145, and O157:NM), whereas many more have been reported as causes of sporadic illness. In Europe, infections by non-O157 VTEC/STEC, caused by 37 different O serogroups, are more common than those by O157:H7 strains. In the USA, though outbreaks associated with STEC of serotypes other than O157:H7 are rare, they account for approximately 30% of STEC illness reported to the Centers for Disease Control and Prevention. In 2011, the US Department of Agriculture announced that an additional six serotypes of STEC (O26, O45, O103, O111, O121, and O145) would be considered to be adulterants in nonintact raw beef products. Once implemented, it is likely that decision will have an impact on the meat and other food industries, with the potential for increasing the number of recalls. Industry is concerned that testing for more pathogens that may be controlled by the same means as for *E. coli* O157 may be an unnecessary burden.

Apart from VTEC/STEC, there are other enteric *E. coli* pathogens that have been implicated in foodborne outbreaks traced to fecally contaminated food or water, and also through person-to-person contact. These *E. coli* strains have been classified into four major groups based on their virulence mechanisms: ETEC; enteropathogenic *E. coli* (EPEC); enteroinvasive *E. coli* (EIEC); and enteroaggregative *E. coli* (EAEC). Though considered less significant than VTEC/STEC in the developed world, these pathogens are a major cause of infant mortality in regions with poor sanitation and untreated water supply. ETEC is a frequent cause of traveler's diarrhea in people from industrialized nations during visits to developing countries. Infected infants and children in developing countries suffer from dehydrating diarrhea and create a reservoir of the pathogen in these communities. Both heat-labile and heat-stable enterotoxins are produced when these organisms proliferate in the small intestine to induce watery diarrhea similar to cholera. EPEC and EIEC are most common among young children in the developing world. EIEC is very similar to *Shigella* and has a low infectious dose; infections are endemic in developing countries. EAEC is increasingly recognized as a cause of persistent diarrhea in children and adults in developing countries and is also a cause of chronic diarrhea among immunocompromised persons, such as those infected with the human immunodeficiency virus (HIV). Typically, EAEC strains originate only from human sources. However, an EAEC strain of the serotype O104:H4, possessing the genes for a verotoxin, was responsible for an outbreak in Germany in 2011 that involved approximately 4000 cases of illness and caused 50 deaths. This indicates that pathogenic *E. coli* strains have the ability to exchange genetic material between pathogens from different sources, a concern for future prevention and control measures.

A widespread related opportunistic pathogen, *Cronobacter* (formerly *Enterobacter*) *sakazakii* can cause bacteremia, meningitis, and necrotizing enterocolitis. The main concern is for infants consuming powdered infant formula with some strains able to survive in a desiccated state for more than 2 years.

Listeria monocytogenes

Surveillance of listeriosis has until recently been restricted to developed countries, with most incidence rates by country ranging from 0.3 to 0.5 per 100 000, irrespective of the regulatory system and industry control programs that have been in place. *Listeria monocytogenes* can no longer be called an emerging pathogen as foodborne outbreaks have been documented since 1981, and we already know much about the pathogen and its means of transmission. The incubation period may range from a few days to 2 months before flu-like symptoms progressing to more serious conditions such as meningitis and stillbirths, and usually those infected are hospitalized. Those who are highly immunocompromised are at greatest risk of infection and death, for example, the elderly, fetuses, acquired immunodeficiency syndrome (AIDS), and organ transplant patients. Ready-to-eat foods, such as meat, poultry, and dairy products, are the main vehicles documented in outbreak investigations, particularly deli meats and soft cheeses. These products have been most frequently implicated, but other foods including fresh-cut fruits and vegetables may also be transmission vehicles. Large outbreaks have mostly occurred through consumption of deli meats as cold cuts or in sandwiches, and linked to errors in food-processing plants, such as lack of proper sanitation of contaminated slicing machines, followed by opportunities for growth of the pathogen. Because the organism can grow at cold temperatures and can persist in damp environments, home refrigerators may be both reservoirs for cross-contamination and incubators for the slow growth of the pathogen. Because *Listeria* is ubiquitous in the environment, it is understandable that infrequently sanitized refrigerators can be continual sources of contamination if the opportunities for cross-contamination arise. One major concern in the USA is the illegal sale of raw-milk Hispanic cheeses which may contain several pathogens including *Salmonella*, *Brucella*, and *Mycobacterium*, as well as *Listeria*. In addition to outbreak investigations, case–control studies, risk assessments, food attribution studies, and expert elicitation, can help focus on areas of greatest risk for prevention and control measures throughout the food chain. Many governments have strict control policies for this organism in food because of its high mortality rate, with either a tolerance of no more than 100 cfu g^{-1} or no detection at all in the sample taken (usually 25 g). Recalls are relatively common for ready-to-eat food, especially if the zero tolerance policy is in place. Food processors are encouraged to determine if their foods permit the growth of the pathogen or not, and if so limit the opportunities for its growth by adding organic acids, freezing, or destroy any surviving organisms through postpackaging pasteurization. Despite industry awareness and government regulations and controls, the USA has not managed to achieve the Healthy People 2010 goal of 0.25 cases per 100 000 population (this was 0.34 in 2009), and outbreaks continue to occur. In the USA, food processors are encouraged to determine if their foods permit the growth of the pathogen or not, and if so limit the opportunities for its growth by adding organic acids, freezing, or destroy any surviving organisms through postpackaging pasteurization including high pressure. This was not the policy in Canada in 2008, when a large outbreak in Canada resulted in 58 cases and 22 deaths

(38% mortality rate), mainly affecting elderly persons after they ate commercially packaged deli meats. The cause was both improperly cleaned and sanitized large commercial slicing machines that had been contaminated over a period of time, and also poor oversight by the Canadian Food Inspection Agency, the Public Health Agency of Canada, and Health Canada, indicted in a special investigative report by the Government of Canada. Serious illnesses and death from listeriosis continue to be reported. In 2010, chopped celery was the source of a *Listeria* outbreak among 10 Texas residents, 5 of whom died. The 2011 Colorado cantaloupe outbreak with 30 deaths led to a congressional investigation that indicated that condensation from cooling systems drained directly onto the floor; poor drainage resulted in water pooling around the food processing equipment; inappropriate food processing equipment was difficult to clean; no antimicrobial solution was used to wash the cantaloupes; and there was no means to remove field heat from the cantaloupes before they were placed into cold storage. Another issue was that third-party auditors did not identify any major problems in the previous months. However, most risks involving *L. monocytogenes* of ready-to-eat food, particularly deli meats occur more at retail than at processing; the exposed public is likely to be much less and some small outbreaks may not be detected.

Mycobacterium avium Subspecies *paratuberculosis* (MAP)

Johne's disease, or paratuberculosis, is a chronic intestinal infection in ruminant animals believed to be caused by MAP. The usually fatal and slowly developing infectious disease is characterized by chronic intestinal inflammation, reduction of milk yield, wasting syndrome with loss of weight, muscle atrophy, fatigue, and weakness (cachexia), and in some species diarrhea, after a long preclinical phase. Treatment is ineffective and economically impracticable, which forces the farmer to cull these animals prematurely. Detection methods include serological testing, and culture followed by molecular typing of the isolated strains. The infection primarily affects domestic and free-ranging ruminants (cattle, sheep, goats, deer, elk, and bison), but has also been reported in primates, rabbits, stoats, foxes and other nonruminant wildlife. Wildlife reservoirs contribute to the persistence and spread of infection, which is widespread in domestic animals in Europe, North America, Australia, and other parts of the world. Because the pathogen can also exist subclinically in these animals for years without necessarily causing clinical disease, not only is the disease underreported but also these animals represent a reservoir for other animals as well. For instance, bovine herd prevalence has been reported from 7% to 55% in Europe and 9–22% in Australian dairy cattle. In the USA, 34% of lymph nodes and 80% of hide coats of culled beef and dairy cattle were positive for MAP as detected by polymerase chain reaction, but the rate was under 1% in the final processed carcasses. However, true animal prevalence is difficult to compare across studies as only a very few of them adjusted prevalence for factors such as test sensitivity and specificity. Even if animals do not die, there is economic loss because of premature culling and increased veterinary costs. Subclinically infected cows secrete *M. paratuberculosis* in their milk. MAP may also enter the milk by fecal contamination in the milking parlor, and one of the concerns is that it may survive commercial pasteurization as it is more thermotolerant than *Mycobacterium bovis*. It has been cultured from 2% to 3% of retail pasteurized milk units in the UK and the USA, and in the Czech Republic, it has been found in raw milk from fecal culture-positive cows (10–20% of animals), and 1.6% of commercially pasteurized retail milk. Because the population is exposed to this pathogen through the consumption of milk and meat from infected animals, it has been postulated that this organism may be a cause of, or at least a contributor to, Crohn's disease, a chronic inflammatory condition of the human intestine. Other possible routes of human exposure to MAP are dairy products, infant formula, vegetables, fruits, and contaminated water supplies. However, presently, the role of MAP in Crohn's disease is still debated and there is no consensus within the scientific community. Nevertheless, *M. paratuberculosis* is a pathogen of concern, with subclinically infected dairy cows being a major source of the pathogen. The fact that the milk containing MAP has been pasteurized to the required national and EU standards means that humans are likely being exposed to low levels of this chronic enteric pathogen on a regular basis.

Salmonella

The family Enterobacteriaceae, including the closely related genera *Cronobacter*, *Escherichia*, *Salmonella*, *Shigella*, and *Yersinia*, is responsible for most foodborne illnesses. Of these, *Salmonella* is the most widespread in the environment with over 2800 serovars now recognized. The ones most frequently associated with foodborne outbreaks are *Salmonella enterica* subspecies *enterica* with serovars such as Enteritidis, Montevideo, and Typhimurium (typically written *Salmonella* Enteritidis, etc.). In countries where enteric notifiable diseases and foodborne disease outbreak surveillance statistics are recorded, salmonellosis typically ranks first or second in terms of outbreaks and case numbers in most countries. In the USA, there are estimated 644 786–1 679 667 foodborne cases each year and 378 deaths. Symptoms of salmonellosis are typically moderate with gastroenteritis lasting several days, but sequelae can follow an infection, and *Salmonella* is the largest cause of foodborne disease-related deaths in the USA. Part of this is because the pathogen can persist in different environmental niches that range from wet to very dry and in highly nutritious foods to powders. A wide range of foods, including raw meats, poultry, eggs, milk and dairy products, fish, shrimp, frog legs, tomatoes, cantaloupes, leafy greens, yeast, coconut, sauces and salad dressings, cake mixes, cream-filled desserts and toppings, dried gelatin, peanut butter, cocoa, chocolate, tahini, and other foods have been identified as being contaminated with *Salmonella* spp., and, subsequently, serving as vehicles for the transmission of this pathogen in outbreaks. Where the organism is protected from stomach acids, such as in cheese, chocolate, or hamburger, the infectious dose can be quite low (1–100 cfu). As a result of industrialization, large-scale centralized production, and an increasing export market, large outbreaks have been reported. Recent examples of large outbreaks in the USA and Canada include *Sa.* Enteritidis in mung beans in 2005 (648 cases);

Salmonella Tennessee in peanut butter in 2006 (>600, two deaths); *Salmonella* I 4,[5],12:i:– (no serovar name) in pot pies in 2007 (425 cases); *Salmonella* Saintpaul in tomatoes/peppers in 2008 (1438 cases); *Salmonella* Typhimurium in peanut butter and peanut butter products (>3900 were recalled) in 2008–09 (691 cases, 9 deaths); *Salmonella* Montevideo in meat with pepper in 2009–10 (272 cases); and *Sa*. Enteritidis in shell eggs in 2010 (1500 cases and 550 million eggs recalled).

Eggs were also a key vehicle for *Salmonella* outbreaks in Europe, both as the main ingredient and in buffets and inadequately heated bakery items, as well as through contaminated pork products. Some of these were reported from only one country, as 225 cases (62 hospitalized) of *Sa*. Typhimurium from hard cheese in the Netherlands in 2007; 42 cases of *Salmonella* Kedougou from infant formula in Spain; and 53 cases of *Salmonella* 4, 12:i from dried pork sausage in France in 2008. However, in the EU community food items are regularly shipped across borders, and multinational outbreaks are also frequent. From 2001 to 2005, four outbreaks of salmonellosis (*Salmonella* Newport and *Sa*. Typhimurium) in the UK were traced to lettuce but the source of contamination was never conclusively identified because of the complex distribution system. In Finland, in 2005, however, 60 *Sa*. Typhimurium cases were linked to lettuce from a supplier in Spain. An outbreak occurred from chocolate in 2001–02 with 439 cases, not only in Germany but also in 6 other countries; *Salmonella* Oranienberg was found in 5% of 381 chocolates tested at levels of 1.1–2.8 MPN g^{-P}. In 2005, the largest outbreak documented in Spain occurred when precooked chicken contaminated with *Salmonella* Hadar infected 2883 persons; residents in four other EU countries were also affected. In 2007, 55 persons in England/Wales and a further 19 in 5 other countries were infected with *Salmonella* Senftenberg from fresh basil imported from Israel. Also, in 2007, a *Salmonella* Weltevreden outbreak was documented involving 45 persons in Norway, Denmark, and Finland from alfalfa seeds originating in the Netherlands and possibly Italy. In 2008, an outbreak occurred in Norway, Sweden, and Denmark with 37 cases and 4 deaths from *Sa*. Typhimurium in raw Danish pork products. Also in 2008, a similar *Sa*. Typhimurium outbreak, but involving two separate strains, occurred in Switzerland where approximately 150 persons were affected after eating pork, typically barbecued. One strain was more associated with cases from Denmark and France than with Switzerland based on molecular typing, thus indicating that the strain isolated from pork samples was not persistent in the factory but was introduced by pork imported from other European countries. Today, with the use of more sophisticated molecular strain identification systems, it is now possible to link human and food isolates across different countries. This has been facilitated through PulseNet using pulsed-field gel electrophoresis (PFGE) patterns shared on the Web.

In many *Salmonella* outbreaks multiple strains may be present, but typically only the dominant strain is recorded in most reports. An example of this occurred in September 2001, where an outbreak in Australia from imported peanuts resulted in a wider investigation in Canada, England/Wales, and Scotland. Patients infected with *Salmonella* serotypes known to be isolated from peanuts and reported to surveillance systems were interviewed to determine exposure histories, and PFGE patterns of *Salmonella* isolates were shared electronically among laboratories. From this there were 97 cases of *Salmonella* Stanley and 12 cases of *Sa*. Newport infection; one family in Canada had separate members infected with *Sa*. Stanley, *Sa*. Newport, and *Salmonella* Kottbus. Mainly persons of Asian ethnicity were affected: 66% of patients in Australia, 90% in England/Wales and Scotland, and 79% in Canada. This was because dry-flavored Asian-style peanuts were typically consumed by persons of Asian origin. Laboratories isolated *Sa*. Stanley, *Sa*. Newport, *Sa*. Kottbus, *Salmonella* Lexington, and *Salmonella* Unnamed from Brand X peanuts, and isolates of *Sa*. Stanley from peanuts and human patients were indistinguishable by PFGE. The concentration of *Salmonella* was generally very low, ranging from <0.03 to 2 MPN g^{-1} of peanuts in the shell. This international outbreak was detected because of rapid sharing of electronic deoxyribonucleic acid PFGE images.

Despite government vigilance and industry prevention and control procedures, *Salmonella* outbreaks remain a major issue for all countries, and with global trade, a wide variety of serovars exist to contaminate many different types of foods to cause illnesses all around the world. It is heartening to discover, however, that adherence to HACCP principles, at least in the US broiler industry, is now linked to fewer salmonellosis cases.

Shigella

There are four *Shigella* species that can be transmitted by food (as well as by water): *Shigella dysenteriae*, *Shigella sonnei*, *Shigella flexneri*, and *Shigella boydii*. *Shigella dysenteriae* Type 1 is primarily associated with serious life-threatening dysentery in epidemics (fatality rate 5–15%), and is mostly encountered in developing countries. A major virulence factor produced by *Sh. dysenteriae* is Shiga toxin (Stx), similar to the Shiga-like toxins produced by STEC/VTEC. *Shigella flexneri* predominates in areas of endemic infection, mainly in developing countries. More outbreaks of *Sh. boydii* occur in Central and South America than elsewhere. *Shigella sonnei* has been implicated in more foodborne outbreaks in developed countries than any other species. Outbreaks have been most often associated with food, water, and child care centers. However, most cases are sporadic and of unknown origin; in the USA, sporadic shigellosis is most likely to occur among young children and Hispanics. Common exposures include international travel and contact with ill persons or child care. Based on the many outbreaks identified, drinking untreated water, or recreational exposure to water, and eating ready-to-eat vegetables washed with untreated water remain important sources for the pathogen; more than one-third of US shigellosis cases could be foodborne. *Shigella* has been isolated a wide range of foods including potato salad, ground beef, bean dip, raw oysters, fish, and raw vegetables. Ready-to-eat foods are most commonly contaminated with *Shigella* by an infected food handler who practices poor personal hygiene, or products harvested from sewage-polluted areas. *Shigella* is salt tolerant, and can survive in many types of ready-to-eat foods including fruits and vegetables, and those subject to modified atmosphere or

vacuum packaging. Cold temperature storage increases their chances for survival. Outbreaks have implicated the following foods as vehicles: tossed salad, potato salad, tofu salad, pasta salad, bean salad, bean dip, shredded and iceberg lettuce, parsley, watermelon, fresh pasteurized milk, cheese, and oysters, mostly caused by *Sh. sonnei*. In one *Sh. sonnei* lettuce outbreak in Texas in 1986, an infected food worker at a lettuce-shredding facility likely infected 347 persons across two counties. In 1988, 240 passengers and also flight crew on 13 national and international flights originating in Minneapolis were infected with *Sh. sonnei* after eating cold items prepared by an airline flight catering kitchen. In 2005, in Bangkok, Thailand, 103 schoolchildren suffered from *Sh. sonnei* shigellosis (and possibly also salmonellosis) whose origins were likely a mixed chicken and rice dish served for lunch on 1 day. In 2009, in Sweden, there was an unusual outbreak of *Sh. dysenteriae* Type 2 infections involving 47 cases. The contaminated vehicle was sugar snaps imported from Kenya. Outbreaks in Norway and Denmark were also linked to the snaps. Trace back of the implicated sugar snaps showed that several import companies and distributers were involved, and the international certification and quality standards were questioned as to their utility in preventing such contaminated product from reaching consumers.

St. aureus

Staphylococcus aureus has been recognized as a cause of foodborne illness since the 1940s, mainly through ready-to-eat foods contaminated by the pathogen from the human skin or nasopharynx. Typically, *St. aureus* will grow to numbers in excess of 500 000 cfu g^{-1} and produce heat-resistant enterotoxins which cause nausea, vomiting, abdominal cramps, and diarrhea within a few hours of ingestion. Cream-filled bakery products displayed unrefrigerated in shop windows were a major vehicle for this pathogen allowing rapid growth in the filling where the high sugar content may inhibit growth of other organisms leading to rapid growth and enterotoxin production. This is less likely today with the use of more synthetic creams and better temperature control of the storage areas. Cooked poultry, egg, and seafood products have also been frequently implicated where worker contamination followed by storage at improper temperatures before consumption has occurred. Less frequent foods are fermented meats and cheese where the starter culture has been contaminated and the fermentation does not proceed rapidly. In South America, soft white cheese is frequently eaten and has been implicated in many outbreaks because of the use of raw milk from mastitic animals, manipulation by asymptomatic workers, and the use of unhygienic production and improper storage practices. Another occasional vehicle leading to outbreaks is dry pasta where the dough is typically extruded at warm temperatures on equipment that is difficult to clean, permitting growth of *St. aureus* and subsequent toxin production. The organism which competes poorly with other microorganisms can grow rapidly in ready-to-eat processed foods such as ham to produce one or more heat-resistant toxins. Generally, in most developed countries, illnesses from *St. aureus* intoxication have been declining. Part of the reason may be that it is not considered a major pathogen and is not looked for in many surveillance programs, and therefore may be underreported. One large outbreak in Brazil in 1998 with 4000 cases and 16 deaths, however, indicates that this is a pathogen not to be ignored. Methicillin-resistant *St. aureus* is an important hospital-acquired infection but has not yet been linked to foodborne outbreaks.

V. cholerae

Cholera is a severe infection caused by *V. cholerae*, which primarily affects the small intestine. The main symptoms include profuse watery diarrhea and vomiting. Transmission is primarily through contaminated drinking water or food. Severe diarrhea and vomiting can lead to rapid dehydration and electrolyte loss, which can result in death if rehydration with salts is not available. Cholera is a major cause of illness and death in the world, and seven pandemics have been documented since the early 1800s, with the latest major event in the early 1990s in South America. Despite our greater awareness of the disease today, and its means of spread, cholera shows no signs of diminishing, and in fact, new strains are encountered on a regular basis. *Vibrio cholerae* O1, the causative agent of epidemic cholera, has two biotypes (classical and El Tor). However, three variants of the El Tor biotype have been described after 2000 in Bangladesh, India, Vietnam, and Mozambique; hybrid vibrios have also been described in other regions of Asia and Africa. Also, *V. cholerae* O139 first identified in 1992, continues to cause outbreaks in India. Many of these strains are resistant to drugs such as trimethoprim, sulfamethoxazole, and streptomycin. Thus, this pathogen is generating different genetic recombinants, which may change the epidemiological role of the disease. Many large outbreaks have been reported in different regions of the world between 2000 and 2010, for example, in 2000, there were 140 000 cases around the world but mainly in Africa; outbreaks in India in 2007 and in Iraq in 2007 and 2008, with thousands of cases (lack of potable drinking water being one of the factors); Vietnam, Congo, Zimbabwe in 2008; South Africa in 2009; and Nigeria in 2010 (the Nigerian outbreak was blamed on heavy seasonal rainfall and poor sanitation). The epidemic in Zimbabwe lasted 9 months and spread to Botswana, Malawi, Mozambique, South Africa, Zambia, and other sub-Saharan African countries. By January 2010, there had been at least 98 000 reported cases and 4200 deaths making it the deadliest African cholera outbreak in the past 15 years. The Zimbabwean government declared the outbreak a national emergency and requested international aid. The epidemic had an unusually high fatality rate because Zimbabweans were seriously weakened by hunger, HIV, and AIDS. A major contributing factor to the severity of the outbreak was the collapse of Zimbabwe's public health system. In Malawi, 104 deaths were recorded, making it the worst outbreak since 2001–02 when 960 people died. Starting in 2006 and continuing to this day, cholera spread to many parts of Haiti and other countries. It likely originated from a peacekeeper encampment upstream of the first case, and perhaps could have been controlled earlier but neither the local or international agencies had surveillance and medical care facilities in place soon enough

because of other priorities. *Vibrio cholerae* non-O1 (nonepidemic) naturally occurs in the Gulf of Mexico causing most US cases from contaminated shellfish but the disease is relatively mild.

Vibrio parahaemolyticus

Vibrio parahaemolyticus was first isolated in 1950 from clinical samples and dried sardines during an outbreak of gastroenteritis in Osaka, Japan. Its pathogenicity is correlated with the production of a thermostable direct hemolysin, known as the Kanagawa phenomenon. Since the 1950s, *V. parahaemolyticus* infections have increased globally; they are usually associated with eating raw, improperly cooked, or cooked, recontaminated fish and shellfish. *Vibrio parahaemolyticus* is the leading cause of seafood-associated gastroenteritis in the USA, and typically associated with the consumption of raw oysters gathered from warm-water estuaries. A correlation exists between the probability of infection and warmer months of the year. Improper refrigeration will allow the proliferation of this organism in seafood, increasing the possibility of infection. The largest culture-confirmed outbreak in North America occurred during the summer of 1997 when 209 people became infected (one death) after eating contaminated raw oysters harvested from California, Oregon, Washington, and British Columbia. A more recent *V. parahaemolyticus* outbreak in 2005 with 22 cases occurred in Alaskan cruise ship passengers after they ate Prince William Sound oysters, extending by 1000 km, the northernmost documented source of oysters that previously had caused *V. parahaemolyticus* illnesses. All oysters associated with the outbreak were harvested when mean daily water temperatures exceeded 15 °C. Since 1997, mean water temperatures in July and August at the implicated oyster farm increased 0.21 °C per year; and in 2004, the mean daily water temperatures in July and August at the shellfish farm did not drop below 15 °C.

Vibrio parahaemolyticus causes approximately half of the foodborne outbreaks in some Asian countries, and is the leading cause of foodborne disease outbreaks in Taiwan with most infections from the O3:K6 strain. This strain also accounted for the majority of diarrhea cases in patients in Calcutta, India, between September 1996 and April 1997. Pandemic O3:K6 clone of *V. parahaemolyticus* appeared in Asia around 1996. Since its emergence, it has accounted for most *V. parahaemolyticus* infections in Asia. It then spread to the USA in 1998, and Spain and Chile in 2004, where it has caused hundreds of infections, resulting in the first *V. parahaemolyticus* pandemic in history. This serotype may have a lower infectious dose than other pathogenic *V. parahaemolyticus* strains, accounting for its apparent virulence. *Vibrio parahaemolyticus* has always been a major pathogen documented in Japan because much of the population loves seafood. However, illnesses are usually restricted to relatively small-scale outbreaks involving fewer than 10 cases. From 1996 to 1998, there were 1710 incidents, including 496 outbreaks, with 24 373 cases of *V. parahaemolyticus* reported. The number of foodborne cases of *V. parahaemolyticus* in Japan doubled in 1998 as compared to 1997 and exceeded the number of *Salmonella* cases. Similar to the 1994–95 period, outbreaks were more prevalent in the summer with a peak in August and relatively few outbreaks occurred during winter months. Boiled crabs caused one large-scale outbreak, involving 691 cases. In 1997, the incidence increased to 568 outbreaks and sporadic reports, with 6786 cases, and in 1998, there were 850 outbreaks and sporadic reports. The increased incidence during 1997–98 has been attributed to an increased incidence of serovar O3:K6. However, since the high of 667 outbreaks and 9396 cases in 1999 when *Salmonella* and *V. parahaemolyticus* were the main causes of the food poisoning, the incidence of *V. parahaemolyticus* has decreased dramatically to 17 outbreaks and 168 cases in 2008. An extended outbreak in northern Chile in 1997–98 was associated with consumption of shellfish and the exceptionally warm seawater caused by 'El Nino' may have favored the growth of the *Vibrio*. This was the first report of *V. parahaemolyticus* causing an outbreak in Chile. An outbreak in Vietnam with over 500 cases from 1997 to 1999 was associated with fresh seafood eaten by persons of high socioeconomic status. i.e., those who could afford to eat this delicacy.

In Europe, illnesses from *V. parahaemolyticus* have been rare and surveillance programs limited. However, in July 2004, a *V. parahaemolyticus* outbreak with 80 illnesses occurred among guests at several weddings after eating boiled crabs at the same restaurant in Coruna, Spain. *Vibrio parahaemolyticus* O3:K6 was isolated from stool samples. Live crabs were imported to Spain from the UK, processed under unhygienic conditions, and stored at room temperature for several hours before they were eaten. The emergence of this virulent serotype in Europe is a public health concern and emphasizes the need to include *V. parahaemolyticus* in microbiological surveillance and control programs for shellfish-harvesting areas and ready-to-eat seafood. Disease outbreaks caused by *V. parahaemolyticus* in Puerto Montt, Chile, began in 2004 and peaked in 2005 at 3600 clinical cases. Until 2006, every analyzed case was caused by the serovar O3:K6 pandemic strain. In the summer of 2007, only 475 cases were reported and this decrease was attributed to a change in serotype of many pandemic isolates to O3:K59 and the emergence of new clinical strains. There was evidence that pathogenicity-related genes were laterally transferred from the pandemic strain to one of the different *V. parahaemolyticus* groups comprising the diverse and shifting bacterial population in shellfish in this region. Other *Vibrio* species are also important marine and brackish water-related pathogens, particularly *Vibrio vulnificus*, which can cause severe wound infections and death. Symptoms are vomiting, diarrhea, abdominal pain, and a blistering dermatitis, which can lead to septicemia. With changing weather patterns and warming of seawater, we can expect more *Vibrio* infections and outbreaks in future.

Yersinia and Related Pathogens

Yersinia enterocolitica and *Yersinia pseudotuberculosis* have often been isolated from such animals as pigs, birds, beavers, cats, and dogs. Only *Y. enterocolitica* has been detected in environmental and food sources, such as ponds, lakes, meat, ice cream, and milk. Most isolates are not pathogenic; the exceptions are serotypes O:3, O:5,27, O:8, and O:9, which tend

to show different geographical distributions. These virulent strains most commonly found in pigs and raw milk can cause yersiniosis, characterized by gastroenteritis with diarrhea and/or vomiting, fever, and abdominal pain. The symptoms can be sufficiently painful that appendicitis is thought to be the cause and in many infected persons the appendix has been mistakenly removed. Both *Y. enterocolitica* and *Y. pseudotuberculosis* have been associated with reactive arthritis, at a frequency of 2–3%, which may occur even in the absence of frank symptoms. *Yersinia enterocolitica* has been transmitted through contaminated unpasteurized milk and milk products, raw pork, tofu, meats, oysters, and fish. Outbreaks have been associated with raw vegetables; the surface of vegetables can become contaminated with pathogenic microorganisms through contact with soil, irrigation water, fertilizers, equipment, humans, and animals. Another feature of *Yersinia* spp. is that they can grow at refrigerated temperatures. If product is contaminated during manufacture or preparation, subsequent refrigeration can increase the pathogen load. The food most frequently associated with *Y. enterocolitica* outbreaks and sporadic cases is pork. In Scandinavia, *Yersinia* infections are more frequently reported than in most other countries, and this may reflect a high proportion of meat being pork in these countries. For instance, in Norway, yersiniosis is the third most commonly reported cause of acute enteritis after campylobacteriosis and salmonellosis. One outbreak of *Y. enterocolitica* in 2007 was attributed to 11 persons eating a traditional Norwegian Christmas pork dish, brawn, which was probably undercooked. Small outbreaks have also occurred as when children were exposed to raw pork during the making of chitterlings from pork intestines, a traditional winter holiday food in certain US black families (soul food). *Yersinia enterocolitica* is transferred from raw chitterlings to infants, particularly to bottle-fed infants, through contact with the hands of the food preparers, and less frequently by direct chewing of the prepared intestines. However, such pork dishes from intestines are common in many countries with similar risks of infection.

Till date, no foodborne outbreaks caused by *Y. pseudotuberculosis* have been reported in the USA, but human infections transmitted via contaminated water and foods have been reported in Japan, Canada, and Europe. At least four outbreaks involving carrots contaminated with *Y. pseudotuberculosis* occurred in Finland in recent years; these mainly affected schoolchildren. The carrots had been stored over winter and a few had spoiled allowing the pathogen to grow. Over a 2-month period in 1998 in British Columbia, Canada, 74 cases of *Y. pseudotuberculosis* were associated with consumption of homogenized milk but no processing, handling, or storage errors were identified during the outbreak investigation. Outbreaks of *Y. pseudotuberculosis* linked to fresh produce have been detected repeatedly in Finland. From 1997 to 2006, there were nine outbreaks documented, including one with carrots as a vehicle where it was shown that shrews were the environmental source of the *Yersinia* either during harvesting or over the winter storage period. Kawasaki disease in Japan, characterized by fever, rash, conjunctival infection, cervical lymphadenitis, inflammation of the lips and oral cavity, and erythema and edema of the hands and feet, has been linked to *Y. pseudotuberculosis* infections but the etiology is not well established. *Yersinia pestis*, the plague *Bacillus* or *Francisella tularensis*, causing tularemia, have not been implicated directly in foodborne illness, but hunters and those in the endemic areas may contract these diseases from handling dead or alive infected animals, particularly from skinning rodents, or from flea and deerfly bites.

Viruses

Avian Influenza (AI) and Other Respiratory Viral Diseases

Viral diseases causing influenza and other respiratory problems were prominent in the 2000–09 decade. These attracted considerable attention because of their high numbers of cases, the speed with which the diseases traveled around the world, and the unacceptable fatality rates. None of these was transmitted directly through food, though food animals were involved. However, because viruses rapidly mutate, there is a growing concern that a major zoonotic viral disease can occur that will involve domestic food animals and then be transmitted rapidly person to person. Unfortunately, there is no simple methodology available to isolate and identify viruses in outbreaks and some are not culturable, all of which has limited understanding of the extent of foodborne outbreaks caused by these agents.

AI (or bird flu) is transmitted by the highly pathogenic influenza A virus subtype H5N1 virus. It was first identified in 1987, which subsequently led to global spread in 2003 with the deaths of millions of ducks, geese, and chickens in more than 50 countries, but mainly in Southeast Asia where small flocks of poultry are widely owned on small farms and even in households. A major concern was that it would spread through wild bird populations, especially ducks and geese that migrate long distances. In fact, there was some evidence for this; a few flocks were infected, but did not seem to be a major factor in causing a pandemic. There was no evidence that poultry meat could spread AI to the human population. In periodic outbreaks, infected birds are destroyed to regain public confidence in the poultry products and to revoke bans placed on exported products by other countries. AI has claimed at least 200 human lives in Asia, Turkey, Romania, and Russia. The main concern is that the AI virus could mutate to pass directly from person-to-person rather than from poultry-to-person. The H5N1 virus kills up to 60% of the persons it infects, but most infections occur after direct contact with an infected bird and the disease does not appear to spread well between humans. As long as human-to-human transmission remains rare, the virus cannot cause an influenza pandemic. However, if the virus can be first adapted to pigs, it then could develop the ability to spread among humans. Since 2007, in Indonesia, AI outbreaks have diminished in poultry and in people but pigs are still carrying signs of recent infection. This may mean the virus is still evolving and could eventually spread rapidly through pigs and become another version of the 2009 'swine flu' pandemic. This was a respiratory infection caused by the swine influenza A (H1N1), and contained genetic material from human, swine, and avian flu viruses. Occasionally, pigs transmit influenza viruses to people, mainly hog farm workers and veterinarians. However,

H1N1 flu spread quickly and easily around the world, and in June 2009, the WHO declared H1N1 influenza a global pandemic. However, like AI, H1N1 cannot be transmitted through swine or poultry meat or any other food.

Severe acute respiratory syndrome (SARS) is a respiratory illness caused by a virus. SARS was first reported in Asia in November 2002. It spread worldwide over several months before the outbreak ended in July 2003, with 8096 known infected cases and 774 confirmed human deaths (a case-fatality rate of nearly 10%). The severe illness was marked initially by systemic symptoms of muscle pain, headache, and fever, followed in 2–10 days by the onset of respiratory symptoms. The virus was later isolated from wild animals (palm civet, raccoon dogs, ferret badgers, cats, and bats) which were asymptomatic; the palm civets were sold as food in local markets in Guangdong, China. Because it was thought that the SARS virus crossed the species barrier from palm civet to humans, more than 10 000 of these animals were destroyed in Guangdong Province, alone. In 2005, an almost identical SARS-like coronavirus was found in Chinese bats. It was eventually deduced that the SARS virus originated in bats and spread to humans either directly, or through animals held in Chinese markets; bats are considered the natural reservoir of SARS-like coronaviruses.

Hepatitis A and E Viruses

Hepatitis A is one of five human hepatitis viruses that primarily infect the liver and cause illness, and is widespread throughout the world. However, it is declining in countries with adequate potable water and sewage disposal systems. Hepatitis A is one of the few foodborne diseases, i.e., vaccine treatable. The hepatitis A virus (HAV) is transmitted by food and water contaminated with sewage or even urine. Unlike hepatitis B and C, hepatitis A does not develop into potentially fatal chronic hepatitis or cirrhosis; however, HAV infections can still lead to acute liver failure and death (fulminant hepatitis A). Fresh produce contaminated during cultivation, harvesting, processing, and distribution has been a source of hepatitis A. In the USA and other countries, there have been outbreaks associated with frozen strawberries, blueberries, fresh green onions, and lettuce. Restaurant- or caterer-associated outbreaks typically involve an infected food worker who contaminates the food during extensive handling and preparation. Because the incubation period from infection to detectable symptoms can be ≥ 2 weeks, these infected workers can be asymptomatic excreters for many days or weeks before being identified and removed from preparing and serving food. Shellfish contaminated through polluted water are also an ongoing source of infections; the largest one occurred in Shanghai in 1987 and 1988 when 292 000 persons (with 32 fatalities) were reported in 2 months. These infections were acquired after clams were eaten. These were harvested by boats operating under unsanitary conditions, and from waters containing untreated sewage effluent in previously unharvested clam beds. The contaminated clams were then transported to Shanghai distributors. Clams like other shellfish are filter feeders that can concentrate ingested particles including HAV to cause infections after their ingestion. Steaming of clams was found to be insufficient to kill the virus, although those who ate raw clams were more likely to be infected than those who consumed cooked clams.

Like hepatitis A, hepatitis E is a disease transmitted between persons via the fecal–oral route, but less is known of the disease transmission. Hepatitis E virus (HEV) may be mainly acquired through water but may originate from animal sources. Domestic animals have been reported as a reservoir for HEV, with some surveys showing infection rates exceeding 95% among domestic pigs. Transmission after consumption of wild boar meat and uncooked deer meat has been reported as well. The rate of transmission to humans by this route and the public health importance of HEV, however, is still unclear. While most often developing into an acute, self-limiting disease, HEV can proceed into a fulminant form in 1–2% of those infected, and up to 20% in infected pregnant women. There is no effective treatment and the only action that can be taken is to relieve the symptoms through rehydration and patient care. When the disease has run its course, HEV is no longer detectable and the liver recovers its regular function. Major outbreaks have occurred in New Delhi, India (30 000 cases in 1955–56); Burma (20 000 cases in 1976–77); Kashmir, India (52 000 cases in 1978); Kanpur, India (79 000 cases in 1991); and China (100 000 cases between 1986 and 1988). More recent outbreaks occurred in 2004, one in Chad with 1442 reported cases and 46 deaths and another in Sudan with 6861 cases and 87 deaths. A larger outbreak occurred in northern Uganda during 2007 and 2008 with 7000 infected and 121 deaths. As these outbreaks demonstrate, HEV often becomes established in camps with displaced persons where sanitation is poor. Specific factors that have been identified with the disease include lack of covers on latrines in the camps; poor hygiene in homes; and lack of routine hand washing with soap before eating or after using the toilet; infections may also occur from eating the raw meat of a contaminated animal. Control measures include improving sanitation and providing adequate supplies of potable water at camps. However, increasingly, HEV is being seen in developed nations with reports of cases in the UK, USA, and Japan. In 2008, one small outbreak occurred with UK passengers on a cruise ship where consuming shellfish and drinking alcohol were risk factors (possibly with contaminated ice), indicating a common-source foodborne outbreak.

NoV

NoVs are a group of related, single-stranded ribonucleic acid, nonenveloped viruses that cause acute gastroenteritis in humans. NoV is the official genus name for the group of viruses previously described as 'Norwalk-like viruses' or small round structured viruses because of their morphological features. NoVs are part of the larger *Caliciviridae* family, which also includes the genus *Sapovirus*, formerly described as 'Sapporo-like viruses', which also cause gastroenteritis in humans. There are five NoV genogroups of which three (GI, GII, and GIV) cause human infections; variants of the GII.4 genotype have been the most common cause of NoV outbreaks. The incubation period is typically 24–48 h and the disease is characterized by acute-onset vomiting (projectile vomiting)

and watery diarrhea. Recovery is usually within 72 h. A major means of transmission is through aerosolization of vomitus, but the fecal–oral route is probably the most frequently encountered, either by consumption of fecally contaminated food or water, or by direct person-to-person spread. As the minimal infectious dose is 10 or fewer particles, one infected person can contaminate a large area and extensive outbreaks have been reported. In the USA and probably many other countries, more foodborne disease outbreaks are caused by NoV than any other pathogen, but the extent is hard to estimate because NoV infections are also transmitted by nonfood means. Asymptomatic infections are common and may play an important role in outbreaks caused by food workers. Also, infected persons can continue to excrete the virus for weeks after they have recovered. The most frequent vehicles in NoV outbreaks include lettuce, cold cuts, hors d'oeuvres, and multiingredient ready-to-eat products where there is much handling and no further cooking step. NoVs are relatively resistant to environmental stress, and are able to survive freezing, some cooking temperatures as high as 60 °C, including after being steamed in shellfish. NoV can survive in up to 10 ppm chlorine, but chlorine-based disinfectant compounds are better for disinfecting contaminated vomitus- or diarrhea-contaminated areas than quaternary ammonium compounds or vacuum cleaners which can actually create an aerosol of infectious particles. Contamination of foods by NoV can be lessened when persons suffering from gastroenteritis avoid preparing or serving food, by frequent hand washing with disinfectants (alcohol-based hand rubs are insufficient), appropriate use of gloves, and by discarding any food that is in the same area where someone has vomited.

Rotavirus

Rotavirus is the most common cause of severe watery diarrhea, typically preceded by vomiting, among infants and young children, and is one of several viruses that are attributed to 'stomach flu'. Infections are very common, and, with each infection, immunity develops, to make subsequent infections less severe, and adults are rarely affected. Dehydration is more common in rotavirus infection than in infections caused by most bacterial pathogens, and is the most common cause of death related to rotavirus infection in young children. As with other enteric viruses, rotavirus is transmitted by the fecal–oral route. The ease of transmission through a population is illustrated by the fact that the feces of an infected individual can contain more than 10^{10-12} infectious particles, and the infectious dose is only 10–100 of these particles. Rotaviruses are stable in the environment, and it is difficult to completely sanitize contaminated areas. Outbreaks of rotavirus diarrhea are common among hospitalized infants, young children attending day care centers, and elderly people in nursing homes. Apart from person-to-person spread, water is one known source of the virus, and there have been waterborne outbreaks documented. Food is thought to play a minor role in its transmission, probably $<1\%$ of cases. In the USA, it is estimated that NoV accounts for approximately 97% of the virus-caused foodborne outbreaks, with the remainder attributed to hepatitis A and rotavirus. However, a few foodborne rotavirus outbreaks have been described in Japan, the UK, and the USA. One outbreak lasting 4 days occurred in an English boarding school in 1994, and was associated with students eating chicken tikka masala (the median incubation period was 35 h). In 2000, 85 college students in the District of Columbia became ill over a 16-day period after eating sandwiches and deli meats prepared on campus. Some of the preparation staff were carriers. In both the Japanese outbreak from restaurant-prepared food and the above-described campus one, adults (patrons and cooks, respectively) were infected with frank symptoms, which would not have been expected from previous rotavirus studies, as adults are supposed to develop complete immunity. Increased strain virulence may have been a factor.

Other Viruses

In the past, poliovirus was one of the disease scourges of persons living under unsanitary conditions particularly in cities causing paralysis and premature death, but vaccination has largely eliminated the disease except in a few areas of Asia. Astroviruses are spread by the oral–fecal routes and are probably foodborne from time to time. Many other viruses are likely to be recognized at least as occasionally foodborne but the epidemiology has not yet been demonstrated.

Parasites

Protozoans

Cryptosporidium

Cryptosporidium species affect different species of mammals and birds. Young animals are those most susceptible to infection. Human cryptosporidiosis is predominantly caused by *Cryptosporidium hominis* and *Cryptosporidium parvum*, which differ in host range; the former infects mostly humans under natural conditions, and the latter infects both humans and many farm animals, such as cattle, sheep, and goats. The main symptoms are watery diarrhea lasting 2–4 days and most people recover quickly, but diarrhea can be prolonged lasting 1–4 weeks, such as in child care centers. In such centers, the spread of infection is highest among young children who are not toilet-trained and their caregivers (those who change diapers). Immunocompromised persons, especially AIDS patients, are at greatest risk for life-threatening conditions, particularly if the cryptosporidiosis develops into a pulmonary form. The infective dose is <10 oocysts. Oocysts are very resistant to normal decontamination procedures, and there is no universally recommended drug for the treatment of the disease, but nitazoxanide is now approved in the USA for diarrhea caused by *Cryptosporidium*. The infective oocyst stage of the organism shed in feces is resistant to most chemical disinfectants, like bleach, but is susceptible to drying and ultraviolet light. Hydrogen peroxide seems to work best. Outbreaks have occurred from infected food workers preparing ready-to-eat foods, and from environmental sources such apple cider from apples contaminated by animal feces or the processing water, and salad items. In 2008, a relatively large outbreak occurred in Finland among

government employees eating at the same canteen; 72 of them experienced mild gastrointestinal symptoms, mainly diarrhea after eating meals with mixed salad items shipped from Sweden.

There have also been instances of illness related to oocyst survival in milk because of improper pasteurization. Shellfish in polluted waters have been found to contain the oocysts but no outbreaks from consumption of these have been reported. Waterborne outbreaks are the most frequent in communities where there is no filtration system for the potable water supply, and these can sometimes affect thousands of people. For instance, in Milwaukee, Wisconsin in 1993, *Cryptosporidium* oocysts passed through the filtration system of one of the city's water-treatment plants, and in a 2-week period, 403 000 of the estimated 1.61 million residents became ill with stomach cramps, fever, diarrhea, and dehydration caused by the pathogen; there were 54 deaths attributed to this outbreak, mostly among the elderly and immunocompromised persons, such as AIDS patients. In 2000, three separate drinking water-associated cryptosporidiosis outbreaks occurred in Northern Ireland, each with over 100 cases. One outbreak was caused by the bovine genotype, and two were caused by the human genotype; subgenotyping analyses indicate that two predominant subgenotypes were associated with these outbreaks and had been circulating in the community, probably *Cr. parvum* and *Cr. hominis*, respectively.

Cyclospora

The coccidian parasite *Cyclospora cayetanensis* causes protracted diarrhea in humans, and was first identified among expatriates and travelers in Haiti, Guatemala, Nepal, and Peru, where infections are endemic. Since then, *Cyclospora* has been considered a cause of traveler's diarrhea, linked to consumption of untreated water, lack of adequate sanitation, and soil contact with young children. Asymptomatic carriers have also been identified. One waterborne outbreak occurred in the USA in 1990 where bird feces in a water storage tank might have been the source of the parasite. In the mid-1990s, the first foodborne outbreaks were recognized, when illnesses in both Canada and the USA were associated with raspberries and possibly blackberries imported from Guatemala, likely contaminated during spraying of pesticides. Later, mesclun lettuce and basil were identified as vehicles in other outbreaks, most likely from product imported from Mexico and South America. In 2009, 160 passengers and crew were infected by the parasite after the ship had called in at various ports in South America; no specific vehicle was determined but raw fruits or vegetables brought aboard were suspected. There are no known animal hosts for this pathogen and so human source contamination must be sought during an investigation. In developing countries, transmission most likely occurs through sewage-contaminated water that is sometimes used in the fresh produce industry for irrigation or pesticide application. Unfortunately, the oocysts are relatively resistant to chlorine which makes control difficult.

Toxoplasma

Toxoplasmosis is caused by the protozoan *Toxoplasma gondii*. The parasite infects most genera of warm-blooded animals, including humans, but felines represent the only definitive hosts which shed the infective oocysts, with the domestic cat being the animal of greatest risk for humans. *Toxoplasma gondii* is a major cause of abortion and problems with fertility in livestock, especially among ewes, and therefore a significant cause of economic loss in livestock farming. In humans, during the first few weeks postexposure, the infection typically causes a mild flu-like illness or no illness. Thereafter, the parasite rarely causes any symptoms in otherwise healthy adults. However, those with an inadequate immune system, such as AIDS patients, may become seriously ill, and die. The parasite can cause encephalitis and neurological diseases, and can affect the heart, liver, ears, and eyes (chorioretinitis). Transplacental infections with *To. gondii* may occur in some 45% of seroconverted pregnant women. In 10–20% of non-fatal cases, the infants may suffer from damage to the CNS and retinochoroiditis, leading to blindness. It is believed that infected but asymptomatic infants may also develop some sequelae later in life, most commonly retinochoroiditis. It is estimated that, worldwide, in approximately 3 out of every 1000 pregnancies the fetus/infant is affected by toxoplasmosis. In the USA, toxoplasmosis is estimated to be one of the most costly foodborne diseases because of the high lifetime cost of care for surviving but impaired infected fetuses. Unfortunately, there is no completely effective treatment for humans or animals, although pyrimethamine/sulfadiazine are commonly used.

Up to one-third of the world's human population is estimated to carry a current or previous *Toxoplasma* infection. For instance, the seroprevalence in the USA was 10.8% from 1999 to 2004. Interestingly, 60% of the Inuit of Nunavik (northern Quebec) were seropositive for *To. gondii*. In multivariate analyses, risk factors for seropositivity included increasing age, gender (women > men), lower level of education, consumption of potentially contaminated water, frequent cleaning of water reservoirs, and consumption of seal meat and feathered game. In Doha, Qatar in 2008, approximately 30% of the population was affected where there was a high feral cat population (> 2 million), with the vast majority of the cats living on the streets, scavenging garbage as well as feeding on the rodents near homes and restaurants. The immunoglobulin M (IgM) serological information showed ongoing and repeated exposure to *To. gondii* for all age groups except infants. However, although cats are often blamed for spreading toxoplasmosis (such as children playing in sandboxes where cats have excreted), fecal contamination of hands and consumption of raw or undercooked meat containing tissue cysts are more significant risk factors for human infections. For instance, the seroprevalence in pigs in Guangdong Province, China, in 2008–09 was alarmingly high at 58% in one city region. These results indicate that *To. gondii* infection is a significant health problem in pigs, and pork from these animals represents a public health concern in southern China. This compares with a 2005 USA study where the seroprevalence for pigs was 2.6%, with a herd prevalence of 21.6%, and a mean within-herd prevalence of 2.7%. Analysis of swine management practices indicated that rodent control methods and carcass disposal methods were associated with differences in the number of *To. gondii*-positive samples on the farm.

Entamoeba

Entamoeba histolytica is an anaerobic parasitic protozoan that affects primates. *Entamoeba histolytica* is estimated to infect approximately 50 million people worldwide although there can be many asymptomatic cases. Symptoms can include fulminating dysentery, bloody diarrhea, weight loss, fatigue, and abdominal pain. The ameba can penetrate the intestinal wall and reach the bloodstream and internal body organs, such as the liver, spleen, lungs, and even the heart and brain. There is approximately 1–3% mortality rate in those with overt symptoms. A closely related species, *Entamoeba dispar*, is considered to be nonpathogenic but these species are hard to distinguish morphologically. The cysts are transmitted through consumption of contaminated water or food, such as salads, fruits, or vegetables that have been washed in water containing the cysts; handling objects that have been in contact with contaminated soil or animal feces, and by anal sex. More cases occur in the rainy season than in the dry season. One cyst may be enough to initiate an infection with an incubation period of 2–4 weeks. Although water is the primary vehicle for infection, raw fruits and vegetables may also be contaminated, and normal chlorination treatment for potable water is not effective in destroying the cyst. The most dramatic incident in the USA was the Chicago World's Fair outbreak in 1933 caused by contaminated drinking water. There were 1000 cases and 58 deaths; defective plumbing allowed sewage to contaminate the drinking water. More recently, food workers are suspected of causing a few sporadic infections, but there has been no single large outbreak in industrialized countries. Because asymptomatic persons can excrete large numbers of the protozoan, food workers who have recently returned from endemic areas should be cautioned to follow exemplary hand hygiene, especially when preparing ready-to-eat foods.

Giardia duodenalis *(syn.* Giardia lamblia, Giardia intestinalis*)*

Giardia duodenalis is present in many species of wild mammals, as well as in livestock and companion animals (dogs and cats). Thus, consuming water from an apparently pristine wilderness can contain this parasite and cause diarrhea ('beaver fever'), unless it has first been filtered or boiled. Also, up to 30% of dogs can be affected with diarrhea that can become a source of human infection. Giardiasis is widely distributed throughout the world and may be in both symptomatic and asymptomatic forms at a low prevalence level in the population. Symptoms may include diarrhea, gas or flatulence, greasy stools that tend to float, stomach or abdominal cramps, and occasional nausea. Giardiasis may be expressed as diarrhea within 1 week of ingesting the environmental-resistant cyst. Normally illness lasts for 1–2 weeks, but there are cases of chronic infections lasting months to years. The infectious dose is very low; perhaps even one cyst is sufficient to initiate an infection. Giardiasis is more prevalent in children than in adults, possibly because many individuals seem to have a lasting immunity after infection. However, some adults develop a chronic condition, which is difficult to treat. Infants and other children in child care centers and patients with AIDS are the most vulnerable to infections. Foodborne outbreaks from this parasite are relatively infrequently reported but small episodes may occur but are not typically investigated. In 1979, 29 school employees that ate home-canned salmon developed giardiasis. The wife of the school employee who brought the food had an asymptomatic *Giardia*-infected grandson, whom she diapered before handling the salmon; her subsequent washing was insufficient to remove all the cysts from her hands. In 1985, in Connecticut, 13 persons at a picnic later suffered from giardiasis after eating a noodle salad dish, which was made by the hostess. Either she or her asymptomatic children were the source of the infection. In New Jersey, in 1986, a similar outbreak occurred in which 9 individuals within a family of 25 developed symptoms after consuming a home-prepared fruit salad. The salad preparer had a diapered child and a pet rabbit at home, both later found to be positive for *G. lamblia*. The preparer had clearly come in contact with the parasite after diapering the child and/or cleaning up the rabbit cage. In 1998, 22 members of a church youth group in New Mexico suffered from giardiasis with taco ingredients being the most likely vehicle; the source was either contaminated municipal water or one of the parent preparers. In 2005, over a 3-month period, in California, 41 users of exercise equipment in a gym were identified as *Giardia* cases. There were 12 confirmed cases and 29 other persons with symptoms but no detectable cysts in their stools. The most likely source was the gym water dispenser. The dispenser spigot required substantial hand manipulation to use. Thus, contaminated hands could easily come in contact with the spout, which would be a viable environment for the *Giardia*. Those infected may have been a continuing source, because many continued to work out at the gym despite their symptoms.

Helminths

Most helminth infections fall under the term neglected tropical diseases (NTDs), as they are not reportable, but affect large segments of the world's population, mainly the poor and the hard-to-reach population groups where there is lack of access to health care. NTDs represent the fourth most important group of communicable diseases worldwide, behind lower respiratory infections, HIV/AIDS, and diarrheal diseases. Most of these diseases will often result in debilitating complications, contributing to malnutrition, disabilities, anemia, and stunted growth and cognitive development in children. It is estimated that more than 1.2 billion people could be infected with soil-transmitted helminths and/or schistosomes in the Asia-Pacific Region alone. A number of helminth parasites are transmitted to humans through foods. These include the nematodes *Trichinella*, *Anisakis*, and *Ascaris*; the tapeworms *Taenia*, *Echinococcus*, and *Diphyllobothrium*; and the trematodes *Fasciola*, *Clonorchis*, *Opisthorchis*, and *Paragonimus*. Ascariasis alone affects approximately 10–25% of the world's population, although most of those infected have mild or no symptoms. Severe *Ascaris* infections cause approximately 60 000 deaths each year, mainly in children. Eating uncooked food grown in contaminated soil or irrigated with inadequately treated wastewater is a frequent source of infection. *Anisakis simplex* (herring worm) and *Diphyllobothrium latum* (fish tapeworm) are associated with marine and freshwater

fish, respectively. They can be found in the gut or in other tissue. Most *Anisakis* infections occur in Japan where fish are more likely to be eaten raw, and they may have to be removed surgically from the stomach if pain like appendicitis or a gastric ulcer persists. Many of these helminths affect widespread populations in Asia and Africa and even if they are treated, diseased individuals can be reinfected in endemic regions transmitted through the fecal–oral route.

Trichinella

The nematode *Trichinella spiralis*, and other related *Trichinella* species, are acquired through the consumption of raw and undercooked meat containing encysted larvae. The initial symptoms of trichinellosis include diarrhea, abdominal pain, and vomiting, followed later by facial edema, conjunctivitis, fever, and myalgias. Occasional life-threatening manifestations include myocarditis, and CNS involvement. *Trichinella* species can infect swine, horses, foxes, wolves, bears, walrus, skunk, raccoons, rats, and other small mammals, as well as humans. Humans are most likely to be infected by *Tr. spiralis*, *Trichinella nativa*, or *Trichinella britovi*. The cold-adapted species, *Tr. nativa*, found in Arctic mammals is more resistant to freezing; the most frequent strain in bears is *Trichinella murrelli*. Although humans likely were exposed to *Trichinella* spp. long before the domestication of the pig, this host has been the most frequent source of infection in the past few thousand years. Foxes, wolves, and bears have the highest infection rates, but small mammals such as skunks, raccoons, and rats provide the highest risk of infecting the domestic pig. Domestic swine can be exposed to the parasite by the following three ways: (1) feeding on animal tissues containing *Trichinella* cysts; (2) exposure to infected rodents or other infected wildlife; and (3) cannibalism within an infected herd. Vigorous meat inspection programs have effectively lowered prevalence rates among domestic animals by controlling these transmission routes. Today, in the USA, safe cooking temperatures for pork can be as low as 145 °F, as long as the meat is let rest for a full 3 min before carving and serving. Horse, wild boar, and bear may be infected, and meat from these if eaten raw or undercooked, occasionally causes small outbreaks, but the population exposed to these is small. However, human trichinellosis is regularly reported in Poland, with 35 outbreaks and 702 cases being reported from 2002 to 2007. The primary source of human infection today has changed from pork to wild boar meat because of stringent control of commercial pork operations. Escaped raccoon dogs (prevalence of 4%) have expanded in both Poland, and Germany and pose a risk to hunters and domestic pigs kept on backyard farms. Wild (feral) pigs are also expanding in the USA, and because the hunting of these animals is encouraged they may represent a source of infection in humans.

Taenia

Approximately 50 million people worldwide are infected by either the beef tapeworm, *Taenia saginata*, or the pork tapeworm, *Taenia solium*. Adult tapeworms develop in the small intestine when meat contaminated with larval cysticerci is consumed raw or poorly cooked. Whereas infections with adult tapeworms are not generally severe, infection with some larval stages can result in life-threatening conditions. When eggs of the pork tapeworm, *Ta. solium*, are ingested they develop into larval cysticerci which migrate to various sites in the body including the brain, in which case the condition is called neurocysticercosis (60–90% of *Ta. solium* taeniasis cases), and can be fatal. These infections are most often found in rural, developing countries with poor hygiene where pigs are allowed to roam freely and eat human feces. This allows the tapeworm cycle of infection to be completed and taeniasis becomes endemic in these regions. For instance, because pork is a staple meat in many provinces in China, cysticercosis is highly endemic, with >1 million cases annually; those affected are mainly ethnic minority groups preferring to eat raw pork; it is also associated with an increase in tourism and the promotion of 'ethnic' dishes to attract customers. In some regions of Mexico, prevalence is 3.6% of the general population. This compares with approximately 1000 cases in the USA where most cases are immigrants from Latin America. Approximately 50 000 people die each year from cysticercosis. However, there is limited information on the epidemiological profile of foodborne cestodes and trematodes (which are discussed in the next section), and infections from these are likely to be underestimated.

Trematodes

Approximately 6000 trematode species ('flukes') have been described, but only a few are important human parasites. Infection of humans occurs through the consumption of contaminated freshwater fish, frogs, shellfish, snails, tadpoles, snakes, water plants (e.g., watercress), and other aquatic products eaten raw or insufficiently cooked. Raw, pickled, or undercooked fish and other aquatic products are prepared in various ways. Such dishes have been extant for hundreds of years with high cultural, ethnic, and nutritional significance, making it difficult for many people to change to safer food habits. Examples of typical traditional preparations include raw crab meat spiced with soy sauce in the Republic of Korea (South Korea), raw grass carp dishes in China, and fresh uncooked small- or medium-sized fish, moderately or extensively fermented in Thailand and Laos. In the 1990s, an estimated 750 million people were at risk of infections with foodborne trematodes (>10% of the world's population), which comprise liver flukes (*Clonorchis sinensis*, *Fasciola gigantica*, *Fasciola hepatica*, *Opisthorchis felineus*, and *Opisthorchis viverrini*), lung flukes (*Paragonimus* spp.), and intestinal flukes (e.g., *Echinostoma* spp., *Fasciolopsis buski*, and the heterophyids). More recent estimates are staggering despite medical advances; the at-risk populations for clonorchiasis, paragonimiasis, fascioliasis, and opisthorchiasis are 601, 293, 91, and 80 million, respectively. The global estimate for the number of people infected with *Clonorchis sinensis* is 35 million; mostly in China, for *Paragonimus* spp. >20 million; for *O. viverrini* 10 million infections with 8 million in Thailand and 2 million in the Laos; for *O. felineus* 1.2 million; and for *Fasciola* species 2.4–17 million; whereas for several species of liver fluke 40–50 million. The annual mortality rate is probably much higher than the estimated 10 000. *Clonorchis sinensis* is endemic in China, South Korea, Taiwan, and Vietnam. *Opisthorchis viverrini* is prevalent in Cambodia, Laos, Thailand, and Vietnam,

O. felineus is endemic in the former Soviet Union, Kazakhstan, and the Ukraine. *Fasciola hepatica* is endemic on all continents but is of most concern in mountainous regions in South America, Cuba, Iran, Egypt, and Western Europe, with infections from *F. gigantica* being restricted to Africa and Asia. *Paragonimus* infections occur mainly in tropical and subtropical areas of East and South Asia and sub-Saharan Africa. Intestinal fluke infections from *Echinostoma* spp. occur in China, India, Indonesia, Japan, Malaysia, Russia, South Korea, the Philippines, and Thailand. *Fasciolopsis buski* is endemic to Bangladesh, China, India, Indonesia, Laos, Malaysia, Taiwan, Thailand, and Vietnam. *Heterophyes heterophyes* infections are reported from Egypt, Greece, Iran, Italy, Japan, South Korea, Sudan, Tunisia, and Turkey. The most commonly found intestinal fluke infection in China, South Korea, and Taiwan is *Metagonimus yokogawai*.

Clonorchiasis in China is endemic in several regions, and it is estimated that 15 million Chinese are infected with *Clonorchis sinensis*, with numbers tripling over the past decade. Individuals can become infected through consumption of raw freshwater fish (more men than women), handling freshwater fish and not washing, and water contaminated with infected feces, for example, where privies are built adjacent to fish ponds. This increase is partly attributable to rural residents who move to urban centers where raw fish consumption is more fashionable. The disease may decline in time where people are more inclined to eat more of raw, large aquaculture-raised fish (which do not harbor metacercaria) as well as a disinclination to eat fish from rivers polluted with industrial waste. In South Korea, eating raw vegetables and aquatic species are a valued part of many culinary traditions, and the soil may be contaminated through night soil (human fecal waste used as fertilizer) or polluted water. In Japan, foodborne trematode infections are most common in rural areas, where traditional food habits are more preserved and raw freshwater fishes and game meat are also incorporated into the diet. Owing to general unawareness of foodborne parasitic zoonoses, the cysts caused by many trematode infections (such as paragonimiasis and fascioliasis) are often mistaken for cancers, and examination for these false carcinomas results in large economic losses. There is currently no legislative system applied to these types of infections, and both increased surveillance for and awareness of these is needed. In Laos, it is estimated that 1 744 000 individuals are infected with opisthorchiasis throughout the country, with practically the whole population at risk (4 360 000). A campaign in 2008 delivered praziquantel in one Laotian province, targeting children and adults to control both opisthorchiasis and schistosomiasis. However, reinfection can occur rapidly following treatment when there are no supportive control measures in place, such as stressing the importance of thoroughly cooking all aquatic products and boiling water before consumption. Agricultural reform may increase risk factors, as demonstrated when fascioliasis emerged after irrigation systems had been built in Egypt and Peru. However, environmental change can reduce the risk of infections. In Henan province, China, low prevalences of paragonimiasis were found for two villages, where gold mining had contaminated streams and killed crabs, the second intermediate host of paragonimiasis. Pesticide use in rice paddies and farms can be effective in causing the death of the first and second intermediate aquatic hosts in China and South Korea. Unfortunately, although these reduce infections, the risk of chronic disease through inappropriate chemical action may increase. Foodborne trematodiases are being more frequently diagnosed in developed countries due to increasing travel patterns and consumption of exotic foods, and it is probable that climate change will favor conditions for human fascioliasis in newer regions, as air temperature and rainfall are crucial for the fluke to flourish. One area of concern is the rapid development of aquaculture. In well-controlled operations, the risk of infection is lower than in wild-caught fish. However, in Vietnam, metacercariae have been found in cultured fish or waste-fed ponds, with up to 50% of the fish infected with foodborne trematodes. The implementation of HACCP systems should be encouraged, as has been done for farming carp, where these have prevented *O. viverrini* from entering the ponds by monitoring the water supply, fish feed, and pond conditions, and control measures taken to address each deviation. Also, if cold storage is available for aquatic products, any metacercariae present will be killed.

Transmissible Spongiform Encephalopathies (TSEs) Including Bovine Spongiform Encephalopathy (BSE)

TSEs are a group of progressive conditions that affect the brain and CNS of certain animals, including humans. TSEs are unique diseases in that their etiology may be genetic, sporadic, or infectious via ingestion of infected foodstuffs and via iatrogenic (therapeutic action) means. TSEs cannot be transmitted through the air or through touching or most other forms of casual contact, but through contact with infected tissue, body fluids, or contaminated medical instruments, eating infected tissue, transfusion, or transplantation. Normal sterilization procedures such as boiling or irradiating materials fail to render the agents noninfective. For BSE, the best studied of the TSEs, misfolded or contorted prion proteins carry the disease between animals and cause a deterioration of the brain, characterized by the appearance of vacuoles, or clear holes in brain neurons, that give the infected brain its spongiform appearance. BSE in cattle was initially recognized in the UK in 1986, but the cause was not immediately identified, and BSE was subsequently reported in other European countries, with more than 1000 reported cases in 1992, and by 2000, the total number of infected cattle had increased to 180 000. Smaller numbers of cases were reported from Japan, Canada, and the USA, but all were traced back to affected animals in Europe. BSE – a lethal CNS disease specifically targeting cattle produces changes in temperament, such as nervousness or aggression; abnormal posture; lack of coordination and difficulty in rising ('downers'); decreased milk production; or loss of body condition despite continued appetite. The incubation period ranges from 2 to 8 years. Following the onset of clinical signs, the animal's condition deteriorates until death ensues or the animal is euthanized. This usually occurs within 2 weeks to 6 months. Most cases in the UK affected dairy cows between 3 and 6 years of age. The primary means of transmission of BSE to cattle was by eating feed contaminated with rendered material, for example, spinal cord, from BSE-infected cattle; this practice is banned today, as there is no guarantee that the feed will be

free of spinal cord tissue. There is also a possibility that in rare cases, mother-to-offspring transmission may occur, but this is unconfirmed. There is no evidence that BSE is transmitted directly from animal-to-animal. There have been a total of more than 188 000 cases of BSE documented from 30 countries since 1986, and it is possible more will be identified, although control measures of culling infected animals and their progeny has reduced the pool of infected animals considerably. More than 4.4 million cattle were slaughtered during the eradication program in the UK alone. The origin of the infectious BSE prion has not been determined; it was assumed to be a recent phenomenon, but interestingly cases of a disease with similar characteristics were documented by Publius Flavius Vegetius Renatus in the fourth and fifth century AD in the western Roman Empire.

In sheep and goats, scrapie has been documented for many years (infected animals scrape themselves because of itchy skin); this is a fatal, degenerative disease that affects the nervous systems. European Food Safety Authority (EFSA) has stated that sheep and goat milk and derived products are unlikely to present any risk of TSE contamination if the milk comes from healthy animals. EFSA also considers the risk from eating sheep and goat meat to be low, due to the BSE measures currently in place. However, out of fear of BSE-related illnesses, many European countries banned some traditional sheep or goat products made without removing the spinal cord, such as smalahove (Norwegian origin delicacy of smoked sheep's head at Christmas) and smokie (West African origin of blowtorching the fleece off the unskinned carcass of an old sheep or goat).

Chronic wasting disease (CWD) of deer, elk, and moose in western and mid-western USA and Canada, which may be mainly transmitted through saliva, appears to be spreading eastwards. Although there is no proven link between affected cervids and human TSE, hunters should avoid eating tissues (e.g., brain, spinal cord, eyes, spleen, tonsils, and lymph nodes) from animals taken from areas where CWD has been identified. There is also a transmissible mink encephalopathy. There are probably more TSEs to be diagnosed in animals.

Human prion diseases are rare and include classic Creutzfeldt–Jakob disease (CJD; a dementia), Gerstmann–Sträussler–Scheinker syndrome (a dementia), fatal familial insomnia (hallucinations and dementia), Alpers' syndrome (intractable seizures in infants), and kuru (ritual eating of the body and brains of dead relatives leading to a loss of coordinated muscle movements). In CJD, defective protein can be transmitted by contaminated harvested human growth hormone products, immunoglobulins, corneal grafts, dural grafts ,or electrode implants (acquired or iatrogenic form); it can also be inherited (hereditary or familial form). In 1996, a possible link between consumption of BSE-infected meat and the development of a new variant CJD (vCJD) in human cases was postulated, with the prion identified as the causative agent. Approximately 275 people worldwide have been infected with vCJD, but mainly in the UK, and most of these have already died. It is believed that they became infected by eating products from BSE-infected animals. The finding of a second BSE prion (different protein shape contortion) in 2004 raises the possibility that transmission of BSE to humans has been underestimated, because some of the individuals diagnosed with spontaneous or sporadic CJD may have actually contracted the disease from contaminated beef. The BSE crisis caused economic loss for countries that declared they had BSE in their herds even at small numbers; that included the USA, which could not sell beef to Japan for many years. However, the USA permitted cattle from Canada to continue to be exported to the USA because the beef industries were already well integrated after both countries had reported cases, and they both had control strategies in place. It is interesting that although the risk of contracting vCJD, even in the UK, is very low, for example, 1 case per 10 billion servings of beef and beef products, the horror of a progressive dementia-like disease has created huge economic barriers to previously well-established beef trading arrangements. It is unlikely that BSE can be eradicated for several more years to come, as new cases are still reported in cattle worldwide. An interesting new development is that the eyes of sheep in the early stages of scrapie glow when a beam of light is shone on the retina. This finding could develop into an early diagnostic tool for detecting cattle with BSE, rather than requiring dissection of the brain tissue at death or slaughter. Noticing the symptoms early may help prevent infected meat from getting into the food supply. It is, therefore, crucial for animal health authorities to establish and/or maintain effective BSE surveillance and control programs, such as the one just mentioned. All these more recent developments are an indication that TSEs, including BSE and vCJD, are likely to occupy the veterinary and public health/food safety authorities for many more years to come.

Chronic Effects of Foodborne Disease Infections

Most foodborne illnesses result in acute symptoms including diarrhea, vomiting, abdominal pain, cramps, and sometimes fever and jaundice, and are self-limiting. The majority of these cases recover within a few days to a few weeks with no lasting effects. However, for some pathogens and some infected individuals illnesses can last much longer and may have life-shortening outcomes. If the disease becomes systemic and affects the bloodstream and one or more organs, without rapid and appropriate medical intervention, the patient may either die or have a long recovery period.

Life-Threatening and Chronic Infections

Those foodborne diseases deemed to be most severe include brucellosis, listeriosis, typhoid fever, and botulism, although none are common in the developed world. However, the health consequences of these when they occur can be serious and life threatening. Many parasitic diseases also fit this picture of a long-lasting chronic condition with severe effects. Those most vulnerable are the very young, very old, those already ill, and pregnant women, all of whom may have underlying health conditions or lower immunity than healthy adults. For example, in pregnant women listeriosis can lead to abortion, stillbirth, or malformation of the fetus, and the overall fatality rate is approximately 30%. Also, those who are malnourished due to inadequate food production are at greater risk of severe infections. These conditions may follow

adverse climatic conditions, such as flooding, drought, or extended high ambient temperatures, and the only available nutritious food may be spoiled or in short supply. Also, repeated episodes of foodborne diseases over a period of time can lead to malnutrition, with serious impact on the growth and the immune system of infants and children. An infant whose resistance is suppressed becomes more vulnerable to other diseases (including respiratory tract infections) and is subsequently caught in a vicious cycle of malnutrition and infection, and many do not survive under these conditions. Gastrointestinal infections can be followed by invasion of the bloodstream or organs, and systemic infections can be very serious indeed if they cannot be treated quickly by antimicrobial drugs. Although some *L. monocytogenes* infections are mild (gastrointestinal form or with a flu-like syndrome) and can occur without seeking medical aid, they can lead to life-threatening complications, such as septicemia, meningitis, encephalitis, osteomyelitis, and endocarditis. Early in pregnancy, a *Listeria* infection may lead to miscarriage, even if the mother is only mildly ill. Later in pregnancy, such an infection may lead to stillbirth, premature birth, or a potentially fatal infection in the baby after birth. Infants who survive a *Listeria* infection may experience long-term neurological damage and delayed development. Adults aged over 60 years can also be seriously affected by listeriosis, and death rates may be as high as 10–20% for this age group.

Enterohemorrhagic *E. coli* (EHEC) strains, which include *E. coli* O157:H7, produce verotoxin/Shiga toxin that can cause diarrhea, ranging from mild and nonbloody to stools that are virtually all blood but contain no fecal leukocytes. Complications of EHEC infection can include HUS and TTP. These are sometimes called sequelae but are really a continuation of the virulence factors of the *E. coli*, for example, it takes time for kidney damage to be apparent compared with HC and bloody diarrhea. HUS is characterized by the acute onset of microangiopathic hemolytic anemia (loss of blood through small blood vessels), renal injury, and low platelet count. TTP also is characterized by these features but can include CNS involvement and fever and may have a more gradual onset. Most cases of HUS occur after acute diarrhea, often bloody. Antibiotic treatment of *E. coli* O157:H7 colitis may, in fact, stimulate further verotoxin/Shiga toxin production. This will increase the risk of HUS, which is a potential life-threatening condition and can induce hypertension, proteinuria, and chronic renal failure in 5% of affected patients. After exposure to VTEC/STEC, 38–61% of individuals develop HC and 3–9% (in sporadic infections) to 20% (in outbreaks) progress to overt HUS. The overall incidence of HUS is estimated to be 2.1 cases per 100 000 persons per year, with a peak incidence in children who are younger than 5 years (6.1 per 100 000 per year), and the lowest rate in adults who are 50–59 years of age (0.5 per 100 000 per year). A Canadian prospective study showed an annual incidence of 1.11 cases of diarrhea-associated HUS per 100 000 children under the age of 16 years. The provinces of Ontario, Quebec, and Alberta, respectively, accounted for 40%, 31%, and 18% of the cases. The mortality rate was 4%, and 34% for children who underwent dialysis for a median of 12 days (range 2–60 days). Individuals in countries where rare or undercooked beef is commonly consumed are at greater risk for HUS following *E. coli* infections. There are approximately 400 new cases of HUS per year in Argentina, the country with the highest incidence in the world. In the acute phase, mortality in children is 2–4%.

HUS has been associated with *E. coli* O157:H7 outbreaks in nursing homes, child care centers, and schools. Major vehicles of infection include ground beef, unpasteurized milk and juice, sprouts, leafy greens, and salami. Waterborne transmission occurs through swimming in contaminated lakes or pools, or through contaminated water. Because low numbers of organisms can cause infection, EHEC is easily transmitted from person-to-person and has been difficult to control in child care centers. From 1982 to 2002 in the USA, the HUS case rate was significantly higher among ground beef-associated outbreaks compared with all other foodborne outbreaks (5.5 vs. 2.5). In the large multistate hamburger outbreak of 1992–93, there were 589 cases of which 41 (7%) developed HUS. In more recent outbreak scenarios, HUS occurs in a higher proportion of cases. One example is the *E. coli* O157:H7 spinach outbreak of 2006 where 205 cases were reported in many states and Canada; 103 were hospitalized, 31 developed HUS (30.1%), and 3 died. The percentage of case patients in whom HUS developed (29%) was high compared to previous *E. coli* O157:H7 outbreaks (15–20%). This finding is consistent with studies that associate *E. coli* expressing Shiga toxin 2 with a higher incidence of HUS. HUS is reported not only from outbreaks caused by O157 serotype but also other VTEC/STEC serotype events as well. In an outbreak in Belgium in 2007, O145 and *E. coli* O26 infections occurred among consumers of ice cream produced at a farm. Five children, ranging in age from 2 to 11 years, developed HUS, and seven other coexposed persons contracted severe diarrhea. In three of the five HUS cases, VTEC O145 infections were laboratory confirmed, one in association with VTEC O26. Identical isolates of *E. coli* O145 and O26 were detected in fecal samples of patients and in ice cream leftovers from one of the birthday parties, and on the farm. The ice cream was made from pasteurized milk and was most likely contaminated by a food worker. A secondary consequence of HUS and kidney dialysis is the risk of diabetes, either shortly after HUS is diagnosed or many years later. The toxin can damage the insulin-producing cells in the pancreas and cause an insulin deficiency.

Infections caused by *V. vulnificus* may present as fulminate septicemia, decreased blood pressure (septic shock), often complicated with necrotizing cutaneous lesions. Wound infections can also cause septicemia. The case-fatality rate for patients with preexisting liver disease is more than 50%. Antibiotic therapy is usually successful in limited systemic infections, but the high mortality associated with this septicemia suggests susceptible individuals should be forewarned about eating raw shellfish.

Anthrax most commonly occurs in animals such as pigs, cattle, horses, and goats, but it can also infect people through skin contact with the spores (cutaneous anthrax), by inhaling the spores (pulmonary anthrax), or by eating meat that contains the spores (intestinal anthrax). Symptoms of intestinal anthrax appear in approximately 1–7 days, with severe abdominal pain, nausea, vomiting, severe diarrhea, and bleeding from the gastrointestinal tract (stomach and intestines). If the symptoms are not treated quickly, septicemia may follow with

possibly meningitis and pneumonia. Intestinal anthrax has a fatality rate of 25–60%. Certain regions of the world (South and Central America, Southern and Eastern Europe, Asia, Africa, the Caribbean, and the Middle East) report more anthrax in animals than others. For instance, an outbreak of anthrax killed at least 1500 wild game animals in nature preserves in southeastern Zimbabwe in 2009, and at least 83 hippopotamuses in a popular Ugandan game park in 2010. Natural anthrax is endemic in these and many other countries, including Canada and the USA, where the spores can live for decades in dry soil and be ingested by animals ruminating for remnants of vegetation in the driest months. Most human foodborne anthrax cases come from persons scavenging carcasses of animals that have died of anthrax such as described above with the meat consumed either raw or after minimal cooking. In 2010, an outbreak of anthrax in Bangladesh among hundreds of cows infected more than 500 people. The disease spread due to the slaughtering of infected cows from which the meat was sold and consumed. Vaccination of cattle was being carried out to contain the outbreak.

Brucellosis is an important zoonosis and a significant cause of reproductive losses in animals. In domestic livestock, it is caused by *Brucella abortus* (cattle), *Brucella melitensis* or *Brucella ovis* (sheep and goats), and *Brucella suis* (pigs). In humans, brucellosis causes undulant fever and other serious, debilitating, and sometimes chronic infections. Most cases are caused by occupational exposure to infected animals or the ingestion of unpasteurized dairy products. In the USA, *B. suis* has been eliminated from commercial pigs and *B. abortus* has nearly been eradicated from domesticated ruminants. In many patients, the symptoms last for 2–4 weeks and are followed by spontaneous recovery. Others develop an intermittent fever and other persistent symptoms that typically wax and wane at 2- to 14-day intervals. Most people with this undulant form recover completely in 3–12 months. A few patients become chronically ill.

For persons infected with *T. solium*, the pork tapeworm, after eating infected pork that has been undercooked, nearly 250 000 ova are passed daily from human feces to the environment. Cysticercosis is a systemic infection that results from ingesting the eggs of *Taenia*. The eggs are usually found in fecally contaminated water or food. Other possible routes are through autoinfection as a result of the entry of eggs into the stomach by retroperistalsis (reverse peristalsis) or as a result of accidental ingestion of eggs from the host's own fecally contaminated hands. Humans, in this case, are intermediate hosts. Ova are digested in the stomach and release oncospheres that penetrate the intestinal wall to reach the bloodstream. These oncospheres develop into cysticerci in any organ but are common in brain, subcutaneous tissue, or the eyes. The cysticerci become mature and viable approximately 2 months after egg ingestion, and can persist for more than 10 years without any apparent symptoms. Although most brain infections remain asymptomatic, much later, intense inflammation is provoked around the degenerating and calcified cyst, and can result in persistent headaches, seizures, and altered mental status (neurocysticercosis). Cysticercosis is highly endemic in Central and South America, and some parts of Africa and Asia. In Latin America, an estimated 75 million persons live in endemic areas and 400 000 people have symptomatic disease. In the USA, the disease is found in immigrants from Mexico, Central and South America.

Cholangiohepatitis, or recurrent pyogenic cholangitis (RPC), is characterized by a recurrent syndrome of bacterial cholangitis that occurs in association with intrahepatic pigment stones and intrahepatic biliary obstruction. Infection in the biliary system from the parasitic nematode, *Ascaris lumbricoides*, or from trematodes, such as liver flukes *Clonorchis sinensis* and *O. viverrini*, often results in significant epithelial damage. These flukes reside in the peripheral small bile ducts of the liver and produce chronic inflammation of the bile duct, bile duct dilatation, mechanical obstruction, and bile duct wall thickening. Coliforms may then result in portal bacteremia by bacterial translocation as a result of this epithelial damage. Repeated portal bacteremia may cause biliary stasis, obstruction, and stone formation from deficient glucuronidation as a consequence of extreme malnutrition, which consequently creates conditions for RPC. These flukes also are potentially carcinogenic to humans. Human infection of *F. hepatica* allow the flukes to migrate in the liver (hepatic phase) and reside in the bile ducts (biliary phase). In areas of endemic infection, more clonorchiasis cases are now diagnosed incidentally during radiological examinations and there is increasing evidence for links between clonorchiasis and cholangiocarcinoma, especially where there is chronic infection.

Autoimmune Sequelae to Gastrointestinal Infections

Infections from enteric bacteria may give rise to a number of chronic joint diseases which include reactive arthritis, Reiter's syndrome, and ankylosing spondylitis. It is the elevated antibody levels to these organisms generated during the infection that lead to these sequelae. Case reports and outbreak investigations have demonstrated an association between reactive arthritis and infection with *Salmonella*, *Shigella*, *Campylobacter*, and *Yersinia* species with a frequency of reactive arthritis ranging from 1% to 21%. Symptoms commonly begin approximately 7–30 days after an intestinal illness. The knees and ankles are often affected but also other joints. The duration of symptoms varies considerably but, in most individuals, they subside in less than 6 months. However, some individuals may take in excess of 1 year to recover fully, and a significant portion of affected persons suffer persistent or relapsing illnesses. In addition to environmental factors, a human leukocyte antigen (HLA)-B27 genotype is a predisposing factor in over two-thirds of patients with reactive arthritis. Unfortunately, the use of antibiotics in these patients has not been shown to be effective. Initial treatment consists of high doses of potent nonsteroidal anti-inflammatory drugs.

Some large outbreaks have been followed up to identify cases with prolonged arthritis.

In 1984, in Ontario, Canada, an outbreak of *Sa*. Typhimurium occurred among police officers who were serving as security guards on routes during a papal visit; they had eaten meat sandwiches provided by a caterer when they were on duty. Of the 1608 police officers involved, 432 experienced acute gastroenteritis. Within 3 months following the outbreak, 27 (6.4%) of these officers had developed acute arthritis,

which resolved in 9 of them over the next 4 months. The remaining 18 officers had recurrent symptoms or developed reactive arthritis on reevaluation 5 years later. Four were so incapacitated they had to change their employment. Both HLA-B27 and HLA cross-reactive group antigens were risk factors for the arthritis. In 2000, a large waterborne outbreak occurred in Walkerton, Ontario, with over 2300 people suffering from acute gastroenteritis caused by *E. coli* O157:H7 and *Campylobacter*; there were 27 cases of HUS and 6 deaths. After a followup study 4.5 years after the outbreak with most of the Walkerton population participating but excluding those with a known history of arthritis, arthritic conditions were reported in 15.7% of participants who had been asymptomatic during the outbreak, and in 17.6% and 21.6% of those who had moderate and severe symptoms of acute gastroenteritis, respectively. Compared with the asymptomatic participants, those with moderate and severe symptoms of gastroenteritis had an adjusted relative risk of arthritis of 1.19 and 1.33, respectively. No association was observed between gastroenteritis and the subsequent risk of prescription medication for arthritis. In nonoutbreak cases involving persons aged >1 year with culture-confirmed *Campylobacter*, *E. coli* O157, *Salmonella*, *Shigella*, and *Yersinia* infections in two US states between 2002 and 2004, 4468 were interviewed within 8 weeks of specimen collection. Of these, 575 (13%) developed possible reactive arthritis; incidence was highest following *Campylobacter* (2.1/100 000) and *Salmonella* (1.4/100 000) infections. Risk was greater for females, adults, and subjects with severe acute illness, for example, fever, chills, headache, and persistent diarrhea. Risk was not associated with antibiotic use or HLA-B27. A total of 54 (66%) of 82 subjects examined had confirmed reactive arthritis. Enthesitis (inflammation of the ligamentous attachments to the bone) was the most frequent finding; arthritis was less common. Complications of *Campylobacter* infections apart from GBS are rare, but the infections may be followed by the development of reactive arthritis. In 2002–03, Danish researchers surveyed 1339 patients with *Campylobacter* infections, and 171 (19.9%) reported joint pain. Interestingly, complaints of joint pain were not associated with duration of diarrhea, and the prevalence of HLA-B27 antigen was 11.6% in patients with joint pain compared with 6.5% in patients with gastroenteritis only. In another study in Finland, 7% developed *Campylobacter*-triggered reactive arthritis; HLA-B27 was positive in 14% of reactive arthritis patients. In Scandinavia, where *Yersinia* infections are relatively common, *Y. pseudotuberculosis* serotype O:3 infection is frequently associated with reactive arthritis and the clinical picture is severe.

GBS is the most common cause of acute flaccid paralysis. GBS is an autoimmune disorder of the peripheral nervous system characterized by weakness and numbness in the extremities, evolving over a period of several days or weeks; it can eventually paralyze the entire body. Typically, an ascending paralysis occurs, with weakness in the legs spreading to the upper limbs and the face along with complete loss of deep tendon reflexes. Muscles may become so weak that patients need to be put on a ventilator, to allow proper breathing, or be fed through a tube into the stomach because swallowing is not possible. Symptoms are often worst during the first 2 or 3 weeks. With prompt treatment by plasmapheresis or intravenous immunoglobulins and supportive care, the majority of patients regain full functional capacity, but even without any treatment most people eventually recover completely. However, in some cases, the effects are seemingly permanent and can cause premature death; 3–10% of patients die and 20% are still unable to walk after 6 months, and many have pain and fatigue that can persist for months or years. GBS is typically preceded by a *Campylobacter* infection. Basically, the body's immune system reacting to the infection attacks the myelin sheaths protecting the nerves, and this interferes with the way that nerves send signals between the body and brain. *Campylobacter* is associated with several pathological types of GBS, including the demyelinating (acute inflammatory demyelinating polyneuropathy) and axonal (acute motor axonal neuropathy) forms. Different strains of *Campylobacter* as well as host factors likely play an important role in determining who develops GBS as well as the nerve targets for the host immune attack of peripheral nerves. GBS is unlike other disorders such as multiple sclerosis and Lou Gehrig's disease, and does not generally cause nerve damage to the brain or spinal cord. Although GBS cases have occurred following *Campylobacter* outbreaks from raw milk and water, most diagnosed *Campylobacter* cases are sporadic with no known vehicle association. So, the burden of GBS relating to campylobacteriosis has relied on a few extensive epidemiological investigations. For instance, a Swedish study with patient data from 1987 to 1995 showed that the risk of developing GBS during the 2 months following a symptomatic episode of *Ca. jejuni* infection (30.4 per 100 000) was approximately 100 times higher than the risk in the general population. (0.3 per 100 000). A similar study in the UK from 1991 to 2001 indicated that the incidence of GBS in a cohort of patients presenting with *Campylobacter* enteritis (11.7 per 100 000) was 77 times greater than that in the general population (<0.2 per 100 000).

See also: Bacteria: *Bacillus anthracis*; *Campylobacter*; *Listeria monocytogenes*; *Mycobacterium avium* ssp. *paratuberculosis*; Other Pathogenic Enterobacteriaceae – *Enterobacter* and Other Genera; *Salmonella* Non-Typhi; *Salmonella* Typhi and *Salmonella* Paratyphi; Shiga Toxin-Producing *Escherichia coli* and Other Pathogenic *Escherichia coli*; *Shigella*; *Staphylococcus aureus*; *Vibrio cholerae*; *Vibrio parahaemolyticus*; *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*. Disciplines Associated with Food Safety: Epidemiology; Food Virology. Foodborne Diseases: Overview of Chemical, Physical, and Other Significant Hazards; Overview of Emerging Food Technologies. Helminth-Cestode: *Taenia saginata* and *Taenia solium*. Helminth-Nematode: *Trichinella spiralis* and Other *Trichinella* Species. Protozoa: *Cryptosporidium* spp.; *Entamoeba histolytica*; *Giardia lamblia*; *Toxoplasma gondii*. Risk Analysis: Estimating the Burden of Foodborne Disease. Viruses: Hepatitis A Virus; Hepatitis E Virus; Norovirus

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Overview of Chemical, Physical, and Other Significant Hazards

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Glossary

Dose–response assessment The determination of the relationship between the magnitude of exposure (dose) to a chemical, biological, or physical agent and the severity and/or frequency of associated adverse health effects (response).

Exposure assessment The qualitative and/or quantitative evaluation of the likely intake of biological, chemical, and physical agents via food as well as exposures from other sources if relevant.

Food additive Any substance not normally consumed as a food by itself and not normally used as a typical ingredient of the food, whether or not it has nutritive value, the intentional addition of which to food for a technological (including organoleptic) purpose in the manufacture, processing, preparation, treatment, packing, packaging, transport or holding of such food results, or may be reasonably expected to result (directly or indirectly), in it or its by-products becoming a component of or otherwise affecting the characteristics of such foods. The term does not include 'contaminants' or substances added to food for maintaining or improving nutritional qualities.

Hazard A biological, chemical, or physical agent in, or condition of, food with the potential to cause an adverse health effect.

Hazard characterization The qualitative and/or quantitative evaluation of the nature of the adverse health effects associated with biological, chemical, and physical agents which may be present in food. For chemical agents, a dose–response assessment should be performed. For biological or physical agents, a dose–response assessment should be performed if the data are obtainable.

Pesticide Any substance intended for preventing, destroying, attracting, repelling, or controlling any pest including unwanted species of plants or animals during the production, storage, transport, distribution and processing of food, agricultural commodities, or animal feeds or which may be administered to animals for the control of ectoparasites. The term includes substances intended for use as a plant growth regulator, defoliant, desiccant, fruit-thinning agent, or sprouting inhibitor and substances applied to crops either before or after harvest to protect the commodity from deterioration during storage and transport. The term normally excludes fertilizers, plant and animal nutrients, food additives, and animal drugs.

Risk A function of the probability of an adverse health effect and the severity of that effect, consequential to a hazard(s) in food.

Risk analysis A process consisting of three components: risk assessment, risk management, and risk communication.

Risk assessment A scientifically based process consisting of the following steps: (1) hazard identification, (2) hazard characterization, (3) exposure assessment, and (4) risk characterization.

Veterinary drug Any substance applied or administered to any food-producing animal, such as meat- or milk-producing animals, poultry, fish, or bees, whether used for therapeutic, prophylactic, or diagnostic purposes or for the modification of physiological functions or behavior.

Introduction

This overview will cover chemical and physical hazards that may be present in food. It will also cover some miscellaneous hazards that pose risks through novel mechanisms. Chemicals present the largest group of hazards, because, in principle, all chemical compounds in existence in the natural world would be included as they all possess a degree of acute toxicity. In addition to these, hundreds of thousands of totally new chemicals have been synthesized, some of which are manufactured in large quantities. In reducing this universe of possibilities to a practical size, this overview will focus only on chemicals that are likely to be present in food. These include chemicals intentionally added to food (food additive, veterinary drugs, and pesticides) and those contaminants, which are known to pose potential health risks. Such contaminants represent a broad array of chemicals from organic to inorganic, from natural to man-made, from those known in antiquity to those discovered within the past decade. Also discussed are physical hazards that pose risks of injury, such as cutting or choking. Finally, short discussions of other categories of hazards are provided, including radiological and nutritional hazards.

Chemical Hazards

Unlike microbiological agents, which were only recognized as disease agents in the mid-1800s, the chemicals have long been known to cause illness and in some instances, death. Early hunter and gatherers were aware of poisonous plants and animals and some even devised methods to remove the toxic agent. In the first rational approach to chemicals, around 1450, the Swiss physician and alchemist Paracelsus elucidated his famous principle that states, "All things are poison, and nothing is without poison; only the dose permits something not to be poisonous." Or, more concisely, 'The dose makes the poison.' His insight earned him the recognition as the father of toxicology, but his work also laid the foundation for modern pharmaceutical science. Whereas every chemical poses some potential for causing acute toxicity, the potency, and nature of the adverse effect of chemicals vary markedly. For the vast majority of chemicals in the natural world, human exposures are far below levels that may cause any harm.

Although inhalation and dermal absorption can be important routes of exposure in some situations, ingestion of chemicals through food and drinking water is the most common route of chemical exposure for most populations. However, inhalation and dermal exposures as well as the ingestion of soil should be considered when such exposures might be significant, such as occupational exposure or proximity to an industrial pollution source.

Chemicals with Rapid Onset of Symptoms

Ingestion of a chemical in food may give rise to adverse effects rapidly, i.e., within hours or days (often referred to as poisons) or after some delay, i.e., months or years (chronic toxicants). When the onset of an adverse effect is short, the disease is commonly referred to as a 'food poisoning' or more properly, acute foodborne intoxication. Whereas inorganic chemicals, such as arsenic and mercury, often come to mind, the most toxic chemicals are those of biological origin. For example, botulinal neurotoxin is lethal at 1 ng per kg bodyweight and ricin is lethal at 10 μg per kg bodyweight. It might also be said that biotoxins are responsible for most of the cases of acute foodborne intoxications. For example, intoxications caused by the preformed toxins of *Staphylococcus aureus* and *Bacillus cereus* are common foodborne diseases in many countries. Note, however, that *S. aureus* and *B. cereus* are handled as biological hazards because prevention and control measures are focused on the organisms rather than on their toxins. In most cases, however, biotoxins, including those produced by plants and other organisms, are classified as chemical hazards.

Acute foodborne intoxications have been commonly reported throughout history. For example, ergot poisoning was common during the Middle Ages in Europe where it was called St. Anthony's Fire, because the toxin causes the extremities to atrophy, which feels like burning. The toxin is produced by the fungus *Claviceps purpurea* that grows on grain, and particularly rye. It was made famous because it is the precursor for the hallucinogenic drug lysergic acid diethylamide. Outbreaks were reported in Russia and England in the 1920s with the last documented outbreak of ergot poisoning in 1951 in France after a baker used contaminated rye flour to make bread resulting in 4 deaths and 200 cases of illness, some with permanent dementia. An intoxication of much greater impact occurred in Spain in 1981–82 when some 1000 people died and 25 000 were disabled, many permanently, after consuming an adulterated cooking oil that was sold door-to-door. Even today, acute outbreaks involving the misuse of organophosphate and carbamate pesticides are periodically reported. In addition, poisoning incidents caused by the intentional contamination of food have occurred, usually motivated by economic gain (adulteration) coupled with ignorance. Mass food poisonings perpetrated by terrorists and other disgruntled persons have also occurred and remain serious concerns. Although mentioned here in the context of chemicals, it should be noted that these concerns extend to other types of hazards, including biological and physical agents.

Following Paracelsus' principle, all chemicals possess the potential to cause acute toxicity and disease. This inherent toxic property or potency varies by several orders of magnitude. The most potent toxins are usually in the category of fast poisons and only small doses required to produce adverse effects. One measure of toxic potency is the lethal dose 50 (LD 50), which is the amount of a chemical that causes death in 50% of the animals treated, and is expressed on mg per kg bodyweight basis. However straightforward the experiment might appear, death is not a clear universal endpoint, but rather the result of one or more underlying adverse health effects. For example, different chemicals are known to exert their main toxic effects on different target organs. Furthermore, some adverse effects may be reversible, whereas others may be irreversible resulting in delayed injury and disease. Consequently, the LD 50 is only a crude measure of toxicity and other tests, including *in vitro* tests, may be more useful. In this regard, it has been recognized that certain pesticides might manifest acute intoxications in the dose ranges that can in exceptional cases be found on food. To evaluate this potential, risk assessors have developed the acute reference dose (ARfD) as a metric for assessing the health risk of short-term, high-level exposures to pesticide residues on food. However, the approach can be used for contaminants and other chemicals as well. The ARfD is the amount of a chemical that can be ingested within 24 h or less without any adverse effect, expressed on mg per kg bodyweight per day basis. *In vivo* and *in vitro* testing requirements have been developed. It should be noted, however, that chemicals often have more than one adverse effect and that, in principle, the need to establish an ARfD should be assessed for each effect. Another important distinction is the exposure assessment to the chemical that has to be performed to take into account high consumers, usually those in the 97.5th percentile. Similarly, residue concentrations used to determine exposure should also reflect the higher range of residue distributions.

Finally, the topic of food allergies should be mentioned under 'fast poisons' because often the reaction to an allergen is rapid. Chemical components, especially proteins, of many common foods, for example, eggs, milk, fish, shellfish, nuts, legumes, and cereals, may give rise to allergic reactions in many individuals. For those that are sensitive, the immune response to even the minutest exposure may produce life-threatening anaphylactic shock. Owing to the broad range of individual

sensitivities, a safe level cannot be established; therefore labeling of processed food to warn sensitive consumers has been widely adopted. In regard to the use of new biotechnology, the possible introduction of allergenic proteins is explicitly addressed during the safety review process.

Chemicals with Delayed Onset of Symptoms

The natural response to the presence of overt poisons in the food supply resulted in the banning of acutely toxic chemicals from the food supply. However, such lists were not without controversy. In the US, boric acid was banned for use in food in the early 1900s. However, a major debate erupted over the possible banning of sodium benzoate, a preservative that is still widely used today. Whereas such negative lists have their place and are still maintained in many countries, a more proactive precautionary approach was developed using a positive list of permitted chemicals as the basis for assuring that chemicals added to food were safe. During this time, however, safety largely meant the absence of acute effects. The possible delayed effects from long-term low-level exposure were not generally considered. Beginning in the 1960s, concern for chemicals in food started to focus on the health risks posed by such chronic exposures. Diseases, especially cancer, that might be caused by low-level exposure to a chemical, were perceived by the public as particularly insidious, because they could not be detected with the senses and adverse effects might not be manifested for months, years, or even decades later. Given this time gap, public health authorities were in the difficult position of trying to retrospectively establish clear links between exposure to a chemical and a disease outcome. This was further complicated because it is often difficult to exclude other concurrent sources of exposure such as those from air and water.

In view of these difficulties, laboratory animals were used as surrogates for human exposure, although not without its own limitations and controversy. In most developed countries today, before any chemical may be used in food, subchronic (usually 90 days studies) and often chronic studies (usually lifetime studies in rats and mice) as well as certain specialized studies, such as developmental studies, are required. Based on a review of all available toxicological data from animal and human studies, the maximum amount of a chemical that has no demonstrable adverse health effect is estimated, which is referred to as the no-observed-adverse-effect level (NOAEL). Because of the uncertainty in extrapolating from animals to human and variability in the human population, a safety/uncertainty factor of 100 is used to determine the acceptable daily intake (ADI) for the food chemical, which is the amount that can be consumed daily over a lifetime with no appreciable risk to health. These studies have mostly been supported by the food industry, which was required to obtain approval from food safety authorities before these chemicals could be marketed. The ADI has been the 'gold standard' for safety, applied to food additives, pesticides, and veterinary drugs and has served to protect public health for over the past 50 years. During the past decades, a lot of scientific advances and improvements to the ADI process have been undertaken by applying more dose–response modeling to determine a benchmark dose (BMD) to refine the NOAEL approach and to use the data-driven safety/uncertainty factors instead of the 100-fold default.

However, the determination of safety levels for chemical contaminants, which are naturally present or inadvertently find their way into food, have been the responsibility of governments. Besides toxicological information from experimental animal studies, contaminants that are present in the food supply may be subject to epidemiological studies to elucidate their adverse effects on health and take their toxic potencies into account. For example, the potency of aflatoxin B_1 to induce heptocellular carcinoma in human has been derived from long-term population studies in China. However, for most chemical contaminants, the evidence base is less than adequate and consequently, the health reference value used is the PTWI, in which 'P' means provisional because the value is subject to revision with new data, 'T' means tolerable because safety margins are often less than 100-fold used for food additives, 'W' stands for weekly in recognition that a contaminant may accumulate in the body, and 'I' stands for intake, which used here is equivalent to the term 'exposure,' which is the preferred risk assessment terminology. Depending on the half-life of the contaminant in the body, similar health guidance values for daily or monthly intake can also be established, such as the provisional tolerable daily intake (PTDI) and provisional tolerable monthly intake (PTMI), respectively.

For some contaminants where NOAELs cannot be established, another risk assessment approach has been developed. This relies on dose–response modeling to establish a BMD at lower confidence limit (BMDL), which is then compared to the estimated human exposure. The ratio of the BMDL to the estimated exposure is called the margin of exposure (MOE) and has been used for the risk assessment of several genotoxic carcinogens, such as acrylamide, polycyclic aromatic hydrocarbons (PAHs), ethyl carbamate, and arsenic, as well as the neurotoxin lead.

In the following sections, some of the major classes of chemicals with safety implications in food are briefly reviewed, with more detailed discussions appearing in the relevant articles in other parts of this Encyclopedia.

Food Additives

A food additive is a chemical added directly to food to improve their quality, safety, sensory qualities, and certain other properties. Another type of food additive (indirect) is incidentally added to food as a result of their use in other applications, such as lubricants for food- processing machinery and boiler water additives to reduce scaling. Food additives may be natural or synthetic, but are otherwise subject to a demonstration of safety before they can be used. Some of these, by virtue of their long history of use in food or their low inherent toxicity, are generally recognized as safe and are exempt from testing. However, excessive consumption of some traditional preservatives, such as salt, nitrites, and smoke curing, are now considered to be risk factors for certain diseases, such as hypertension and cancer.

Food additives are more specifically defined by the *Codex Alimentarius* as "any substance not normally consumed as a

food in itself and not normally used as a characteristic ingredient of food whether or not it has nutritive value, the intentional addition of which to food for a technological purpose in the manufacture, processing, preparation, treatment, packaging, transport, or storage of such food results, or may be reasonably expected to result in it or its by-products becoming directly or indirectly a component of such foods.'' Some additives have been used for centuries, such as vinegar, salt, or sulfur dioxide for preservation purposes. Curing or smoking of meats also has a long history of use. However, in the twentieth century many more additives were developed and used for such uses as preservatives, flavor enhancers, anticaking and antifoaming agents, antioxidants, bulking agents, food coloring, emulsifiers, humectants, propellants, stabilizers, gelling agents, and sweeteners.

Before they can be approved for use today, all food additives must have a demonstrated useful purpose and undergo a rigorous scientific safety evaluation by appropriate national authorities such as the European Food Safety Authority (EFSA), US Food and Drug Administration, and Health Canada. At an international level the Joint FAO/WHO Expert Committee on Food Additives (JECFA) has been evaluating the safety of food additives since 1956. It should be noted, however, that as new data becomes available, a reevaluation of a food additive might be warranted as in the case of certain colors. In addition, consumer reports of adverse reactions might also trigger a reevaluation, for example, in the case of monosodium glutamate.

The illegal use of banned or unapproved substances used as additives, such as boric acid and certain textile dyes, continues to be a problem in many developing countries. In some cases, the use of unapproved substance in a product that is in turn used as an ingredient in other food may result in global recalls, as was the case of the adulteration of chili peppers with the unapproved textile dye Sudan Red 1, which resulted in the recall of hundreds of food products.

Veterinary Drug Residues

Veterinary pharmaceuticals have been a key element in increasing the production of animal-derived foods. These are administered to animals to prevent and treat infections, promote growth, or otherwise improve animal health, particularly for those animals that are in close contact over long periods of time. These animals tend to be stressed and are more at risk for communicable diseases. When these compounds are administered to food-producing animals, residues may be present in milk, eggs, or other edible tissues. Unless they are degraded to endogenous chemicals, most of these drug residues are of potential toxicological concern and their presence in food products may produce pharmacological or other effects in consumers. Consequently, veterinary pharmaceuticals used in food-producing animals must undergo not only testing similar to food additives mentioned above but also include studies in the target animal, such as metabolism and elimination studies, often using radiolabeled drug. Once a safe level of the drug and its residues in food is established, unsafe residue levels are avoided by using prescribed treatment protocols, especially allowing sufficient time after treatment for the compounds to be depleted from the animal, i.e., withdrawal period to the established maximum residue limit (MRL).

The use of antibiotics to promote weight gain and to improve feed efficiency has been shown to contribute to problems with an increase in antibiotic-resistant microorganisms. Therefore there is an increasing trend by more and more countries to not approve this specific use for antibiotics that are important for human medicine. Another issue that has raised trade problems is the use of growth and other hormones. These are widely used in some parts of the world, especially in ruminants, to increase muscle tissue, i.e., faster and leaner growth. Another controversial drug is recombinant bovine somatotropin (rBST) hormone, which is made using new biotechnology methods. The hormone is capable of increasing milk production by 10–40% as it does not require 'freshening' the cow through pregnancy. JECFA has confirmed the safety of several anabolics, such as 17β-estradiol, trenbolone, melengestrol, and zeranol, and the protein hormone rBST, and concluded that, under good agricultural and veterinary practices, such substances do not present an appreciable risk to the consumer. A number of countries and notably the European Union still oppose their use in food-producing animals because of alleged human and animal health concerns.

As with food additives, illegal and uncontrolled treatment of food animals with veterinary drugs present is periodically detected. Examples are nitrofurans in poultry and egg powder, chloramphenicol in shrimps and honey, malachite green in salmon and other aquaculture fish, and clenbuterol in pork and other meat. In some countries, the use of clenbuterol in pigs and other food animals to keep their meat lean is widespread and occasional illnesses associated with the consumption of treated meat have been reported. Continued monitoring is therefore necessary to ensure that only approved veterinary drugs are used and that permitted doses and withdrawal periods are observed.

Pesticide Residues

The term pesticide includes insecticides, fungicides, rodenticides, insect repellants, herbicides, antimicrobials, and plant hormones. However, most food safety concerns are directed toward those chemicals, which are designed to prevent, destroy, repel, or reduce insects on food crops, as well as on food-producing animals. More recently, the use of certain herbicides in connection with genetically modified food crops has raised food safety concerns. Many countries have agencies other than health responsible for the approval of pesticides, such as the Environmental Protection Agency in the US, the Pest Management Regulatory Agency in Canada, and the Pesticides and Veterinary Medicines Authority in Australia. At the international level, the evaluation of pesticides is carried out by the Joint FAO/WHO Expert Meeting on Pesticide Residues (JMPR). Before a proposed pesticide can be marketed and used, it is evaluated to ensure that it will not harm human health. Pesticides that pass this evaluation are subject to good agricultural practices, usually including observance of withdrawal periods after application. This is intended to allow residues to deplete to the established maximum residue limit (MRL), which is set for each treated food commodity. If residues are found above that level, the commodity could be subject to

seizure by the government. In setting such limits, there must be a safety finding that the pesticide can be used with reasonable certainty of no harm by considering the toxicity of the pesticide and its breakdown products; how much of the pesticide is applied and how often; and how much of the pesticide residue remains in or on food by the time it is marketed and prepared. Typically, MRLs apply equally to domestic and imported food but many countries will now use the Codex MRL for an imported food in cases where the Codex MRL is higher than the national MRL.

The reported effects of pesticides range from mild and reversible effects to immunotoxicity, teratogenicity, carcinogenicity, and acute fatal poisoning. Some pesticides have raised consumer concerns because of their adverse impact on the environment. For example, the organochlorine compounds, like DDT, aldrin, heptachlor, and others, were widely used in agriculture resulting in a disastrous bioaccumulation in birds (see Rachel Carson's 'Silent Spring') as well as posing threats to other life forms on the planet, including humans. With increased government scrutiny covering both approval and monitoring, the health risks from pesticide residues in food now appear minimal. Routine monitoring of commodities for compliance with MRLs, mainly in developed countries, has indicated a low frequency of exceedences. For example, in 2007, the EFSA found that 96% of the 74 000 samples of almost 350 different food types complied with legal MRLs. In many developed countries, total diet studies have shown that virtually all pesticide residue exposures are well within safe levels.

In a number of situations, however, foods have been found to contain high levels of pesticide residues, for example, when the crops had been harvested too soon after the application of pesticides or when excessive amounts of pesticides had been applied through ignorance or incompetence. Sometimes food has been contaminated because of accidental contact with pesticides during storage or transport. Indirect information suggests that consumers in developing countries may be frequently exposed to higher than approved levels of pesticides in their diets. For example, the large number of acute poisonings among agricultural workers implies a poor knowledge of the handling and application of pesticides. Limited monitoring of data on food imported from developing countries indicate that these foods are sometimes highly contaminated. In addition, biomonitoring of organochlorine pesticide residues in breast milk in developing countries provides further evidence of significant cumulative exposure to these chemicals. However, it should be noted that although DDT is banned in all countries for agricultural use, in many tropical locations, DDT is still legally used for indoor spraying of houses to control malaria.

Although organochlorine pesticides were banned because of their environmental persistence, the use of cholinesterase-inhibiting pesticides (organophosphates and carbamates) expanded significantly. Although their rapid degradation reduced their long-term risk, the acute risk to farm workers and consumers has raised new issues, which are now being addressed. Most of these compounds, as expected, have relatively low ARfDs, which means that excessive residues may pose imminent risks to health. In addition, the regulation of such pesticides on an individual basis ignored the possibility of multiple residues occurring on the same commodity, which has been detected in monitoring programs. Therefore, the methodology for cumulative hazard characterization and exposure assessment is under development. At the same time, the possibility of aggregate exposure has also been raised because many of these pesticides are also used at home for pest control.

Environmental Contaminants

Heavy Metals

Heavy metals are stable elements that are slowly metabolized by the body and therefore tend to bioaccumulate. Most of these metals do not have any basic function in the body but can be highly toxic. If the heavy metals accumulate in the body tissues faster than the body's detoxification processes, a gradual build-up of these occur to block essential enzymatic reactions. These metals include lead, mercury, arsenic, and cadmium. Metals are released into the environment by both natural and anthropogenic sources. Most are released into the environment by mining and industrial processing. These toxic metals may leach into underground waters, moving along water pathways and eventually depositing in aquifers, or accumulate in surface waters resulting in water and subsequently soil pollution.

Lead

Although children have been exposed to lead from the ingestion of soil, i.e., pica, the main exposure of the general population is through food and drinking water. Lead affects the hematopoetic, nervous, and renal systems. It has been shown to reduce mental development in children in a dose-related manner with no defined threshold. When lead pipes or lead-soldered water storage tanks are used, the lead exposure from such water may be appreciable. Similarly, processed food and beverages may be contaminated by lead in pipes or other equipment. Food packed in lead-soldered cans may also contain significant amounts of lead and such cans can still be found in some developing countries. During recent years, many countries, especially the industrialized ones, have initiated efforts to reduce lead in drinking water systems and food equipment and containers and these efforts have led to a significant decrease in lead exposure. The elimination of lead-containing additives in gasoline also resulted in the elimination of lead contamination of foods grown along the motorways as well as reducing airborne exposure. Lead may also be found in leaded crystal glassware and some highly colored ceramic or old ceramic dishes. Spices occasionally have been implicated as lead sources, and lead is sometimes added to certain ethnic foods or food supplements to impart a yellow or orange color, a sweet taste, or to increase the weight of the product.

Mercury

Although there are several natural sources of mercury including volcanoes and leaching from rocks, most mercury is anthropogenic in origin, arising from industrial processes like coal burning for power generation, gold mining, alkali and metal processing, and in the past, production of pulp and paper. Mercury is converted to the more toxic methylmercury

through bacterial action, where it is taken up by small aquatic plant and animal species. It bioaccumulates by binding to protein and is biomagnified up the food chain. Although fish are the most common dietary source of mercury (mainly as methylmercury), food containing high fructose corn syrup (HFCS) is another possible source if the HFCS was made using caustic soda containing traces of mercury.

Methylmercury, the most toxic form of mercury, has been shown to have serious effects on the nervous system, which in severe cases, is irreversible. The fetus, infants, and young children are particularly sensitive. The most well-known incident of methylmercury intoxication occurred in the Minamata Bay, Japan, in the late 1950s and was caused by the industrial discharge of mercury-containing compounds. These were subsequently taken up by fish and shellfish and consumed by the local population. In general, fish are usually the major dietary source of methylmercury although certain marine mammals, such as dolphins and whales, can also contain high levels. Therefore, several countries have recommended that pregnant women choose fish that are low in methylmercury, but high in omega fatty acids, such as sardines and salmon.

Arsenic

Arsenic is a potent toxin, which interacts with many essential biomolecules in the body, including deoxyribonucleic acid. Over 137 million people in more than 70 countries are probably affected by arsenic poisoning of drinking water with at least 20 incidents of groundwater arsenic contamination being reported from all over the world. Four major incidents were reported in Asia, including locations in Thailand and China. However, the construction of shallow wells tapping into contaminated groundwater in Bangladesh caused the largest poisoning of a population in history, with 35–77 million people exposed. However, arsenic in groundwater is not confined to the developing regions; groundwater in many parts of the US and Europe contains relatively high levels of arsenic.

Away from areas with significantly contaminated drinking water, food is the major source of arsenic. Arsenic and its compounds are often found in food, as they are absorbed from the soil by plants, such as rice, wheat, and oats. In most cases, arsenic is in the form of organic arsenic, which has been estimated to be some 500 times less toxic than the inorganic form. Limited data indicate that approximately 25% of the arsenic present in food is inorganic, but this depends highly on the type of food ingested. Inorganic arsenic levels in fish and shellfish are low ($<1\%$). Foodstuffs such as meat, poultry, dairy products, and cereals can contain higher levels of inorganic arsenic. In cereals, arsenic is concentrated in the bran and consequently, certain whole grain foods, including complementary food for infants may contain arsenic. The tolerable daily intake for inorganic arsenic set by the JECFA at 2 μg per kg bodyweight was withdrawn earlier in 2011 amid growing evidence that arsenic can cause cancer even at low levels.

Cadmium

Cadmium occurs naturally and is often present in volcanic soils. Some soils have been contaminated with cadmium through the use of bird guano as fertilizers. Cadmium also occurs as an industrial pollutant, which is released into the air from waste sites and incinerators and through effluents from industrial processes. Inappropriate disposal of cadmium-containing batteries is now a major source of contamination in developed countries. Cigarette smoking is the single most important source of human exposure for the general population. The first case of mass cadmium poisoning was documented in Japan in the1950s, caused by contaminated rice that was irrigated with water contaminated with effluent-containing cadmium. The disease was known as Itai-itai disease (literally, ouch-ouch disease) because of the severe pain in the joints and the spine that it produces. More typically, chronic low-level exposure to cadmium is associated with renal failure because cadmium can accumulate in the kidney over a lifetime. Whereas molluscs and especially oysters contain the highest levels of cadmium, consumption of grains results in the highest exposure because of the larger amounts consumed. Present estimated mean dietary intakes of cadmium for most populations are within its PTWI, but some high-percentile consumers may exceed this limit.

Other Metals

There are many other metals that may be of concern at high levels but are less likely to be associated with food exposure to cause chronic illness. These include aluminum, antimony, bismuth, cerium, chromium, cobalt, copper, gallium, gold, iron, manganese, nickel, platinum, silver, tellurium, thallium, tin, uranium, vanadium, and zinc. Metal toxicity can result in reduced mental and central nervous system function; lower energy levels; damage to organs including kidneys, liver, and lungs; and degenerative diseases that mimic Alzheimer's disease, Parkinson's disease, muscular dystrophy, and multiple sclerosis. Of these, aluminum is perhaps of greatest concern because recent toxicity studies have indicated that aluminum is much more toxic than previously thought. Note that some metals listed above, such as iron, manganese, and zinc, have, at low levels, essential biological functions.

Polychlorinated Biphenyls (PCBs)

PCBs are a group of anthropogenic chemicals with 209 congeners first manufactured in the 1920s, and are now banned worldwide because they are hazardous, persistent, and bioaccumulative chemicals. Classified as persistent organic pollutants (POPs), PCBs are included in the Stockholm Convention on POPs that was adopted to eliminate the production and emission of these and related chemicals. Most commercial mixtures are clear, viscous liquids with high boiling points and high dielectric constants. They were used widely as coolants in transformers and capacitors, as heat exchange fluids, and as flame-retardants as well as other uses. Discharges from industrial operation over many years have occurred in several countries including, former Czechoslovakia, Japan, and the USA. Although no longer produced, sources today include old or fluorescent lighting transformers, electrical devices, and appliances containing PCB capacitors. Because high-temperature incineration is expensive, PCBs have often been illegally dumped into the environment, where they accumulate in the food chain (fish, mammals, and birds) due to their persistent nature.

The major source of human exposure is through the diet. The major dietary sources of PCBs are the fatty fish and foods of animal origin. PCBs may be stored unchanged for years or even decades, mainly in the fat and liver. PCBs have been shown to adversely affect neurological development including cognitive function in children. Information on the acute effects of PCBs in humans was obtained from two large-scale incidents that occurred in Japan (1968) and Taiwan (1979). Common symptoms included dermal and eye lesions, irregular menstrual cycles, a lowered immune response, fatigue, headache, and cough; there were also reports of children with poor cognitive development.

From 1998 to 2001, three incidents involving PCB/dioxin contamination of animal feed occurred in Belgium and one incident occurred in Ireland in 2008. The most serious one occurred in 1999 in Belgium when oil from a discarded transformer was unknowingly used in the formulation of approximately 500 t of animal feed, which resulted in the contamination of poultry, eggs, pork, milk, and beef. The contamination was only noticed after chickens started dying; the true human exposure from this incident is uncertain. However, the health, political, and economic consequences of this and other incidents have targeted PCBs and dioxins as priorities for intensive feed and food monitoring.

Polybrominated Biphenyls (PBBs)

Similar to PCBs, PBBs are biphenyls variously substituted with bromine atom instead of chlorine. PBBs were manufactured for use as fire retardants but now, are limited in use to electrical and electronic products sold in the European Union. One major incident occurred in the USA in 1973 when several thousand pounds of retardant were accidentally mixed with livestock feed that was distributed to farms in Michigan, USA. Some 1.5 million chickens, 30 000 cattle, 6000 pigs, and 1500 sheep consumed the contaminated feed before the error was discovered. Epidemiological studies of people who lived on the quarantined farms; people who received food from these farms; and workers engaged in PBB manufacture revealed various potential adverse effects, but the only consistent effect across the various groups appeared to be acne.

Dioxins

The name dioxin applies to a family of structurally and chemically related dibenzodioxins and dibenzofurans, which are mainly by-products of industrial processes and waste incineration. Dioxins are found at low levels throughout the world in many foods, but especially dairy products, meat, fish, and shellfish. Over 90% of human exposure to dioxins is through food. Major incidents involving elevated levels of dioxins in animal-derived foods have occurred in Belgium, USA, and Ireland. In the case of the USA incident, the source of the dioxins was a type of natural clay used as a binding agent in the formulation of animal feed. Acute effects of exposure to high levels of dioxins include a skin disease (chloracne) and altered liver function. Long-term exposure is linked to impairment of the immune system, the developing nervous system, the endocrine system, reproductive functions, and cancer. Levels of exposure to dioxins in several countries are estimated to be only slightly below World Health Organization-recommended PTMI, which is currently 70 pg per kg bodyweight per month. Data from a number of industrialized countries indicate that the trend in exposure is downward as source-directed measures have reduced environmental emissions. However, it should also be noted that chemicals that have the capacity to be endocrine disruptors may exert their effects through synergistic or antagonistic mechanisms so that the cumulative exposure to such chemicals is the subject of intense scientific debate.

Processing Contaminants

Processing contaminants are undesirable compounds, which are formed in, or appear on, food during treatment or processing, including preparation at home. Perhaps the most deadly is botulinal neurotoxin that is produced by *Clostridium botulinum* under anaerobic conditions, such as in canned food with low acid content that is improperly processed. Although rare, the outcome is almost always fatal and unusually detailed regulations have been promulgated to ensure the safety of such products. Other types of foods and processing methods can also give rise to chemical hazards. For example, grilling of meat on the barbeque can produce polycyclic aromatic hydrocarbons, which include a large group of substances typically found in soot. They exhibit varying degrees of toxicity, including carcinogenicity. Similarly, high-temperature cooking, as in frying, can enhance the formation of nitrosamines, especially in nitrite-cured meat, such as bacon. Nitrosamine may be responsible for thousands of cases of colon and gastric cancer per year globally. Nitrosamines are found in many foodstuffs, especially beer, fish, and fish by-products, and also in meat and cheese products preserved with nitrite pickling salt. Other processing contaminants include chloropropanols, which arise from multiple sources through different and complex pathways. They were initially associated with acid-hydrolyzed vegetable proteins and in particular, soy sauce. However, low levels of chloropropanols can also be formed in other foods that have been subjected to heat treatment such as baking, roasting, grilling, or under certain storage conditions. They can thus be present in cereal-based products such as pastries, biscuits, crackers, bread, as well as meat products, for example, burgers. Ethyl carbamate (urethane) has been used in the past for industrial, medical, and veterinary purposes, but present evidence has established its genotoxicity and carcinogenicity. It is produced during the fermentation process, with the highest levels found in stone-fruit brandies. One of the most recently identified processing contaminants is acrylamide, which was shown to be present in carbohydrate-rich foods that undergo heat treatment, such as baking or frying. It is a potent carcinogen and is found at high levels in commonly consumed foods. The formation of acrylamide derives from the Maillard reaction, which gives food its taste, aroma, and color, normally when foods are heated $>120\ ^{\circ}C$. The food industry and academia are working to reduce the level of acrylamide in food because it is perhaps the most serious health risk posed by any processing contaminant. Furan is another processing contaminant that has also been recently identified in foods, such as canned or jarred foods like soups, sauces, and baby foods. It probably originates when amino acids or sugars are

broken down during cooking. Its formation may also be linked to the presence of vitamin C and polyunsaturated fatty acids during cooking, bottling, or canning food products.

Biological Toxins

Mycotoxins

Mycotoxins are a group of toxic secondary metabolites of microscopic fungi (molds), which can cause a range of serious adverse effects in humans and animals. They have been of a growing national and international concern since the 1970s. Animal studies have shown that besides serious acute effects, mycotoxins are capable of causing carcinogenic, mutagenic, and teratogenic effects. Several hundred mycotoxins have been identified. Aflatoxin is the most well-known and important mycotoxin from an economic point of view. As fungi-producing aflatoxins prefer high humidity and temperatures, crops such as maize and groundnuts grown in tropical and subtropical regions are more prone to contamination. Epidemiological studies suggest that aflatoxins and hepatitis B virus are cocarcinogens and the probability of liver cancer is higher in areas where both aflatoxins contamination and hepatitis B are prevalent. Aflatoxins are found in peanuts, maize, tree nuts, and some dried fruits such as figs. Besides adverse weather conditions (both too wet and dry), postharvest handling plays an important role in the growth of molds such as aflotoxin.

Other mycotoxins of concern include ergot alkaloids, ochratoxin A, patulin, fumonisin B, and the trichothecenes. JECFA has established very low provisional tolerable intakes for ochratoxin A, patulin, fumonisin B, and some of the trichothecenes, such as deoxynivalenol and fumonisin B1. In view of their presence in many foods and their stability during processing, mycotoxins must be considered a major public health concern. Currently, over 100 countries have regulations regarding mycotoxins in the feed industry, in which 13 mycotoxins or groups of mycotoxins are of concern. The Codex Alimentarius Commission has also recommended maximum limits for a number of mycotoxins.

Marine Biotoxins

Harmful algal blooms (HABs) periodically kill fish or disrupt food sources for fish and shellfish. When HABs are not detected, seafood taken from such waters may be harmful for human health. HABs can cover huge areas, often stimulated by the right combination of temperature, salinity, nutrient, sunlight, and tidal conditions. When they discolor the water, they are typically called 'red tides.' Sometimes HABs can be highly localized as in the case of coral reefs where reef damage due to storms or construction can disrupt the marine ecology. Human intoxications by planktonic biotoxins result from the consumption of contaminated seafood, such as shellfish and fish. Marine biotoxins are produced by various species of dinoflagellates under certain conditions. Shellfish, which are filter feeders, accumulate the biotoxins without adverse effects and without any apparent organoleptic changes. Different types of intoxications have been described based on their major symptoms, including paralytic shellfish poisoning, diarrheic shellfish poisoning, neurotoxic shellfish poisoning, amnesic shellfish poisoning, and azaspiracid poisoning. Recent warming of the world's oceans has altered the distribution and range of dinoflagellates that make these toxins, introducing the problem to previously unaffected areas.

Another type of marine biotoxins is ciguatoxin, which causes ciguatera fish poisoning. Ciguatoxin is a neurotoxin, which can produce symptoms lasting for several weeks, months, or years. It is serious disease with a case/fatality rate that may range up to 4.5%. Ciguatera has been associated with the consumption of a variety of tropical and subtropical fish, mainly coral fish, feeding on toxin-producing dinoflagellates, or predatory fish consuming such coral fish. It should also be noted that certain fish species use biotoxins for their protection and can present risks to the consumer if consumed inadvertently or intentionally without proper handling, for example, puffer fish.

A lesser known group of biotoxins are derived from certain cyanobacteria (blue-green algae) in brackish or freshwater. These bacteria produce cyanotoxins, which are some of the most powerful natural poisons known. Although not normally affecting the commercial fisheries, exposure to these toxins has occurred through drinking water and occupational and recreational contact.

Plant Toxicants

Edible plants containing toxic substances are important causes of disease in many areas of the world. Cassava (manioc), which is a major source of carbohydrates in the world, contains a high level of toxic cyanogenic glucosides, which must be removed before consumption. Improper preparation of cassava is associated with a neurological disease (konzo), which is due to the presence of residual cyanide. Many food plants possess toxic parts, are toxic unless processed, or are toxic at certain stages of their life cycle. Most of these are not major contributors to human illness because of traditional society's prior knowledge of their preparation. These include apple, cherry, peach, plum, and almond seeds and leaves (cyanogenic glycosides); potato (solanine); tomato leaves and unripe fruit (tomatine); rhubarb leaves (oxalic acid); and various beans (lectin phytohemogglutenin). When such foods are introduced into countries with no previous knowledge of their preparation or when novels foods with no history of use are introduced, toxic exposures may result.

In some cases, poisonous plants that resemble or that cohabitate with edible plants are also potential hazards. In Europe, misidentification of toxic mushrooms is by far the leading cause of illness and death in this category. Seeds of plants producing pyrrolizidine alkaloids have accidentally contaminated wheat and millet, leading to acute and chronic liver disease. In some situations, populations facing extreme food shortages have eaten seeds (e.g., *Lathyrus sativus*), known to be toxic, out of hunger.

Biogenic Amines

Biogenic amines are decarboxylation products of amino acids and are formed during fermentation (e.g., cheese ripening and wine fermentation) and decomposition of protein, usually fish. These biogenic amines include histamine, tyramine,

cadaverine, putrecine, and related metabolites. Histamine fish poisoning (or scombroid poisoning) is a type of food poisoning caused by elevated levels of histamine being present in the fish such as tuna, sardines, mackerel, swordfish, and marlin. Naturally occurring bacteria in fish produce an enzyme, which converts histidine in the fish to histamine. Symptoms appear very quickly after eating the fish (usually within 30 min to a few hours) and include a peppery taste sensation, tingling of the mouth and lips, a skin rash, headaches, dizziness, and itching of the skin. Symptoms usually last for 4–6 h and rarely exceed 1 day, but can be readily treated with antihistamines. Poisoning caused by biogenic amines in other foods such as cheese, red wine, and sauerkraut are reported only sporadically.

Packaging Contaminants

The packaging of food is intended to protect the product from various hazards in the environment and to avoid formation of microbiological hazards, but occasionally, packaging can inadvertently introduce contaminants into food. Normally such materials undergo testing for extractables to determine the potential for the migration of packaging material components. In some cases, food safety issues are raised after the packaging material is in wide use. For example, bisphenol A is an industrial chemical used to make a hard, clear plastic known as polycarbonate, which is used in many consumer products, including reusable water bottles and baby bottles. It is also used in epoxy resins, which act as a protective lining on the inside of metal-based food and beverage cans. Although the exposure of the general public is very low, the greatest concern has been for newborns and infants because bisphenol A is a potential hormone disruptor, which is associated with increased risks for changes in behaviors and adverse effects on sperm. In another example involving exposure of infants, the carcinogenic substance semicarbazide was found in gaskets in baby food jars at low levels of contamination resulting in reformulation of the gaskets by the food industry.

Physical Hazards

A physical hazard is any extraneous object or foreign matter in a food item, which may cause illness or injury to a person consuming the product. Some of these agents cause cuts (glass shards, needles, and metal shavings), choking (jewelry, wood splinters, and gel capsules), and broken teeth (stones, nuts, and bolts). Complaints of physical hazards outweigh those of other issues because they are readily detected. Sometimes, foreign bodies, even though not a hazard per se, may result in psychological trauma (insects, mice, or their parts). Control methods include raw material inspection and specification, vendor certification and letters of guarantees, optical and magnetic detectors, X-ray technology to detect solid objects, effective pest control in the facility, preventative equipment maintenance and proper sanitation procedures, and employee education. Another risk is the possibility of choking on food products, particularly by infants and children. These occur frequently enough to cause concern but are not typically recorded as foodborne disease incidents. The risk of choking is related not only to the size and shape of the food item (e.g., baby biscuits, small sausages, and candies) but also to the consistency of the food, such as in the case of jelly sweets containing Konjac flour. Finally, it should also be mentioned that nanoparticles are also a type of physical hazard because their potential adverse health risks arise from their very small size and possibly their shape. However, for the purpose of this Encyclopedia, these are covered under food technologies.

Miscellaneous Hazards

Radiological Hazards

Unlike chemical hazards, radionuclides are dangerous because they emit particles that have sufficient energy (or that can liberate sufficient energy) to remove an electron from a neighboring molecule to produce free radicals. Such chemical species are highly reactive and are responsible for most of the adverse health effects associated with ionizing radiation. Radiological hazards that have been of concern include strontium-90, iodine-131, cesium-137, and various isotopes of plutonium. Note that plutonium also is a highly toxic chemical. The long-term health effects associated with radionuclides include cancer, teratogenic effects, and genetic mutations.

People are exposed daily to a wide range of naturally occurring radioisotopes of chemical elements. This background radiation is unavoidable and considered to be one of the inherent risks in life. However, consumers' concern for radionuclides derived from nuclear testing has created a climate of fear of an all-out nuclear war. In 1979, this was heightened by the nuclear reactor accident at the Three Mile Island in the USA, which occurred in spite of government's assurances that such accidents would only happen once in 10 000 years of operation. After the Chernobyl nuclear plant went critical in 1986, large parts of the Ukraine and numerous locals in Western Europe were contaminated by radionuclides with long half-lives, such as cesium-137. This resulted in the widespread contamination of food and subsequent trade restrictions. Following the Chernobyl event, Codex established guideline levels for radionuclide in food following the accidental nuclear release for use in international trade. Although the health risks from radionuclides in food appear to be low, most countries continued to employ a precautionary approach and only accepted food that was within previous background levels.

Although operational risks appear to have been reduced over the past 20 years, natural calamities remain potential threats to nuclear facilities. As a result of the unprecedented earthquake and tsunami in March 2011 that hit Fukushima, Japan, several nuclear reactors at the site released massive amounts of radioactive material into the environment. Radionuclides subsequently were found in a range of foods, including spinach, tea leaves, milk, cattle meat, and seafood. This points to the need for preparedness to maintain the sampling and analytical capabilities to respond to emergencies.

Nutritional Hazards

A nutrient is a chemical that an organism needs to live and grow. However, no single food commodity can supply all of our nutritional requirements. Therefore, the quantity and

quality of various foods in the diet are critical for sustaining and protecting health. Among the essential macronutrients, amino acids are considered the building blocks of the body, carbohydrates provide the bulk of the energy for functioning, and essential fatty acids are needed for metabolism. Certain trace elements are also nutritionally essential in small quantities for proper human growth and metabolism. These include iron, cobalt, chromium, copper, iodine, manganese, selenium, zinc, and molybdenum. In addition, vitamins such as thiamin, niacin, riboflavin, folate, vitamins C and D are essential organic chemicals that cannot be synthesized in the body and therefore must be obtained from the diet.

As with all chemicals, nutrients may pose risks if present in excessive amounts (Paracelsus). However, because they are essential for health, the optimum intake of an essential nutrient is neither too low to cause a nutritional deficiency nor too high to cause an adverse health effect. For macronutrients, such as proteins, carbohydrates, and fats, examples of both under and over nutrition exist, sometimes in the same country or same city. However, micronutrients are largely a problem of insufficient intakes. Micronutrient malnutrition is a term commonly used to refer to vitamin and mineral nutritional deficiency diseases. Diets, which lack adequate amounts of essential vitamins and minerals lead to such diseases. Vitamin A deficiency (blindness and susceptibility to infectious diseases), iron deficiency anemia (mental and physical retardation as well as fatigue), and iodine deficiency disorders (goiter, mental and physical impairment, as well as birth defects) are among the most common forms of micronutrient malnutrition. These deficiency diseases cause illnesses and deaths of millions of people every year, particularly in the developing world and particularly among children. Supplementing traditional diets with vegetables, fruits, fish, and meat will diminish the adverse effects of micronutrient malnutrition. Adding iodine to salt is considered to be the most common and cost-effective method of preventing iodine deficiency and in many countries, vitamin A is added to milk. In these cases, the application of food safety management tools, such as hazard analysis and critical control point, are needed to ensure that the products are fortified to prescribed levels.

Although micronutrients are mainly a problem of insufficient intake, it should be noted that the margins between safety and toxicity may be relatively small and exposure to levels significantly above the recommended daily intakes may cause disease.

Infants that rely on infant formula as their sole food source are particularly at risk to nutritional hazards. For example, if the formula is manufactured in such a way that it fails to add an essential nutrient, infants may be harmed. In the USA, the omission of sodium chloride from infant formula resulted in irreversible brain damage in infants. In a more recent case in 2003, thiamine (vitamin B_1) was omitted from the infant formula, and a reported 12 infants suffered neurological damage and two of them died.

Adulterants

Economic fraud in food was a common phenomenon throughout history. Laws relating to the purity and identity of food were adopted by most ancient societies. The practice of adulterating foods in order to deceive the purchaser as to their identity, quality, or other properties continues to the present day. Whereas some of these practices only harm the consumer economically, sometimes the adulteration causes injury, disease, or even death. For example, the addition of stones to add weight presents a physical hazard. A recent example causing serious harm was the addition of melamine to diluted milk in China, to give the impression of higher protein content. This incident involved an estimated 300 000 infants and caused the deaths of 6 and the hospitalization of 860. The large recalls of the contaminated products eroded consumer confidence in the safety of the food supply.

At times adulteration is carried out for purposes of extortion or to settle political or personal grievances. Adulteration of food may occur at any stage of the food production system and with a long list of potential adulterants, including physical objects, like glass shards. The modification of a product at the retail level to make it harmful to the consumer is referred to as tampering. This threat has prompted manufacturers to make products that are either difficult to modify or at least difficult to modify without alerting the consumer that the product has been tampered with.

In some cases, people with politically motivated agendas have adulterated food with chemical hazards and other agents. Terrorist threats to food have resulted in various preventive measures by the food industry and both industry and governments have developed programs to detect and respond to such threats, including tracing and recall systems. Because of the criminal nature of these threats, these programs are referred to as food defense and are headed by police or other security agencies.

See also: Food Additives: Food Additives – General. Foodborne Diseases: Overview of Biological Hazards and Foodborne Diseases; Overview of Emerging Food Technologies. Hazards of Food Contact Material: Bisphenol A and Endocrine Disruption; Food Packaging Contaminants; Nanotechnologies and Nanomaterials. Mycotoxins: Mycotoxins – General. Nutriential Hazards: Micronutrients: Vitamins and Minerals. Processing Contaminants: Acrylamide; Biogenic Amines; *N*-Nitrosamines; Polycyclic Aromatic Hydrocarbons (PAHs). Toxic Metals: Arsenic; Cadmium; Lead; Mercury. Veterinary Drugs Residues: Veterinary Drugs – General

Further Reading

International Programme on Chemical Safety (2009) *Environmental Health Criteria 240: Principles and Methods for the Risk Assessment of Chemicals in Food.* Geneva: World Health Organization. Available at: http://whqlibdoc.who.int/ehc/WHO_EHC_240_2_eng_acronyms.pdf (accessed on 11 April 2013).

Relevant Websites

http://iufost.org/new-sib-chemical-hazards-foods
International Union of Food Science and Technology.

http://www.who.int/foodsafety/chem/en/
World Health Organization: Department of Food Safety and Zoonoses.

Overview of Emerging Food Technologies

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Glossary

Animal cloning The production of progeny from a single parent by reproductive cloning using somatic cell nuclear transfer to create animals that are genetically identical.

Food irradiation A process of sterilizing food with a specific dosage of ionizing radiation (gamma rays, electron beams or X-rays) to destroy microorganisms or insects that might be present and to retard enzymic action such as inhibiting sprouting of vegetables and delaying ripening of fruit.

Genetically modified organism A genetically modified organism (GMO) or genetically engineered organism (GEO) is an organism whose genetic material has been altered using genetic engineering techniques.

High pressure processing (HPP) A cold pasteurization technique by which products, already sealed in final packages, are introduced into a vessel and subjected to a high level of isostatic pressure leading to the inactivation of vegetative microorganisms and enzymes in the food and extending its shelf life. Also called high hydrostatic pressure processing, Pascalization and bridgmanization.

Membrane filtration (MF) The process of separating suspended particles from a fluid through a porous material through which the fluid can pass while the suspended particles are retained. In food microbiology a filter, typically made of cellulose acetate, with pores of various maximum diameters is used to retain certain or all bacteria or viruses on the filter.

Microwave technology Electromagnetic radiation in the microwave spectrum (wavelength between that of infrared and short waves) causing polarized molecules in the food to rotate and build up thermal energy in a process known as dielectric heating.

Modified atmosphere packaging (MAP) A technique used for prolonging the shelf-life period of fresh or minimally processed food by decreasing the oxygen and increasing carbon dioxide and/or nitrogen gases in the atmosphere of a sealed container.

Nanotechnology The manipulation or materials at the nano level.

Pulsed electric field pasteurization (PEF) Application of very rapid pulses of high voltage to foods placed between two electrodes with minimum of heat causing loss of cellular contents of microorganisms present in liquid and semi-liquid food products.

Pulsed light (PL) or Pulsed light technology (PLT) The use of very short bursts of high-intensity white light, at roughly 20 000 times the brightness of the sun to destroy cell membranes of microbes with minimal effect on food quality.

Sous vide A method of cooking food sealed in airtight plastic bags (vacuum pack) in a water bath for longer than normal cooking times, up to 72 hours, at an accurately regulated temperature much lower than normally used for cooking, typically around 55 °C (131 °F) to 60 °C (140 °F) for meats and higher for vegetables. The intention is to cook the item evenly from inside to outside. It is then often rapidly chilled for storage at refrigeration temperatures and reheated at the time of service.

Introduction

New food technologies are continually being developed for both the food industry and consumers, and over the years have served to improve the food supply. At the farm level, these technologies are making crops and food animals more productive and healthier by reducing the likelihood and impact of disease. Some of these technologies have been the basis for the so-called the 'Green Revolution,' which has been essential in feeding the world's growing population. But food preservation technologies have been critical for human survival from the earliest civilizations, when foods had to be preserved between seasons when supplies were not available. When foods have been processed by using traditional methods (such as, drying, salting, smoking, fermenting, etc.), there has been little concern by the public about the safety of these products. These traditional methods were developed before the advent of refrigeration to prevent bacterial growth though control of water activity to avoid both spoilage and foodborne illness. However, present day a closer examination of some of these processes has found chemical hazards were inadvertently produced, such as the contamination of smoked foods with polycyclic aromatic hydrocarbons. Other processes, for example, salting, are now known to pose health risks, such as hypertension.

With the modern scientific tools, food science and technologists from government, academia, and industry have been constantly inventing more efficient processing procedures to reduce waste and promote safer, healthier foods. Specific innovators have been the military to ensure the troops have safe, nutritious, and agreeable foods in the field. Industry has also been involved in the development of new food products for the market. In developed countries, these emerging food technologies are subject to an evaluation process to ensure that the derived products are safe. Part of this evaluation must address the unforeseeable effects that might be posed by new technologies, including foods with no or limited food consumption history. In addition, animal health and environmental risks are assessed. As with other emerging technologies, potential trade barriers, and economic loss might arise if one jurisdiction approves the technology whereas another does not.

Risk managers also have to be cognizant of unfounded fears by consumers about new technologies and the need for special efforts to communicate the real risks, if any, versus the potential benefits. Whereas consumers may be wary of some new technologies such as genetically modified (GM) foods, they have embraced other food technologies without fully appreciating their potential risks and/or their role in their safe use. For example, microwave oven cooking was widely adopted by consumers without a full understanding of the required power (wattage) requirements for foods, problems with uneven heating of food, and the risk of pathogen survival. Risk communication as well as consumer education is essential for consumers so that they can understand and use these new technologies to produce safe as well as wholesome and nutritious food.

Technical terminology also has evolved over time. Pasteurization present day is more than a heating process. The broader definition is 'any process, treatment, or combination thereof, that is applied to food to reduce the most microorganism(s) of public health significance to a level that is not likely to present a public health risk under normal conditions of distribution and storage.' This leaves open processes like high-pressure, irradiation, microwave cooking, ohmic heating, pulsed electric field, pulsed light (PL), and *sous vide* (discussed later in this Introduction) to be considered as pasteurized.

Undoubtedly, other food technologies, such as nanotechnology, will emerge in the future through research and scientific developments and a discussion of their perceived benefits and risks will engage risk assessors and managers as well as civil society, because ultimately food is everyone's business. Some of the food technologies that have raised real or perceived safety concerns are presented briefly below. Some of these are also discussed in more detail in the specific articles.

Genetically Modified Organisms

By genetic engineering through the insertion or deletion of genes, new organisms with recombinant deoxynucleic acid (DNA) can be produced, which are referred to as genetically modified organisms (GMOs). Foods derived from GMOs are called GM foods and were first put on the market in the mid-1990s. The advantage of most GM crops is their ability to be resistant to specific herbicides or certain plant pests and pathogens. Present day, the potential of this technology can also be used to improve other aspects of the food supply. For instance, enhanced vitamin production is a feature of GM rice, the so-called 'golden rice' that produces β-carotene. Some of GM crops are successfully marketed worldwide, such as soybean, corn, rapeseed, and cotton. In contrast, one early product, a tomato with retarded postharvest fruit softening was taken off the market because of competition from a conventionally bred, long shelf life variety. Various GMOs are also routinely used as sources of enzymes for the manufacture of processed foods, with functions such as preventing clotting of milk protein during cheese making, improving juice clarity, and converting starch to sugars.

The development and sale of GM foods has been challenged on safety, economic, and environmental grounds. Evaluation of GM foods for human food safety requires a demonstration of substantive equivalence in nutrition compared to its unmodified counterpart. In addition, special studies are required to demonstrate that the GM food contains no new proteins (or increased level of a known protein) capable of causing allergenic reactions. In this regard, two GM products failed initial safety testing because of possible allergic reactions and were discontinued. To date no adverse health effects caused by GM products approved for sale have been documented.

However, environmental concerns and labeling remain major issues for marketing GM foods. Opponents of GM foods are demanding more rigorous environmental testing before any approvals are granted. Whereas public knowledge of GMO technology and GM foods is generally low, consumer acceptance of the technology is highly variable. In Canada, China, and the USA, many GM foods are on the market and no special labeling is required. However, the European Union (EU), Japan, Malaysia, Australia, and other countries have taken a cautious approach to GM food and have established labeling and traceability requirements for GMOs and their derived food products. These requirements dictate a physical separation of GMOs and nonGMOs throughout the whole supply chain. At the production and raw commodity level, this requires a certification scheme; this becomes more complicated for GM ingredients, especially those which are otherwise indistinguishable from their nonGM counterparts, such a vegetable oils. In some cases, conventionally grown crops and food made from them may contain a small amount of GMOs due to cross-pollination. Current analytical methods are capable of detecting approximately 1% of GM food in a mixture and this is used as the *de facto* limit in most legislation. Therefore, if a product is found to contain more than 1% GMO, it is considered to be mislabeled, and subject to regulatory action, even if it poses no safety concern. Another potential problem in labeling GM food is that it could, in principle, be applied at the foodservice level. As with the term 'organic,' some businesses may try to use 'nonGM' as a marketing advantage and thus further contribute to the unfounded fear of consumers to GM foods. GM animals have also been developed, although none is currently on the market. In September 2010, the United States Food and Drug

Administration (FDA) stated it was willing to consider approval of GM salmon, which grow twice as fast as native species because of the insertion of a gene to produce a growth hormone. The growth hormone shortens the time it takes to grow a mature salmon, and therefore will make salmon more plentiful and cheaper for the public. There are concerns, however, that GM fish might escape from growing pens and then breed with native fish, possibly causing catastrophic effects on the life cycle of wild fish, especially for GM salmon to outcompete the wild salmon population for limited food sources.

Particular issues stand out involving GMO hormone production in cattle because of long-term trade disputes and consumer unease. Bovine somatotropin (bST) hormone is a protein hormone naturally found in the pituitary gland of cattle and which is produced to initiate lactation in cows after giving birth. If bST is injected into nonlactating cows, milk production is induced without the need for a long gestation period. It can also be administered to lactating cows, increasing milk production significantly (10–25%). In the past few decades, a GM source of bST has been developed in the USA. Through recombinant DNA technology, a GM *Escherichia coli* bacterium is used to produce large amounts of the hormone, which is called recombinant bST (rbST). The USA is the only developed nation to permit cows to be given rbST and approximately 20% of herds use the hormone. Although the EU countries do not permit the use of rbST, milk produced using the technology may be imported and sold without labeling. There are various arguments against rbST use from uncertainty about its safety to consumers over a lifetime, economic reasons (too much milk may lower the price, and consumer reluctance to purchase such rbST milk or products made with the milk), to animal health (increase in the risk of clinical mastitis, a reduction in fertility, and an increased risk of animals developing lameness). The Codex Alimentarius Commission has not been able to achieve a consensus on the approval of rbST, especially as Canada and the EU have banned its use for animal health reasons.

Through the use of GM microorganisms, the production of hormones, enzymes, and other natural substances can be enhanced by changing genetic codes to produce the desired substances in much greater quantities and also to prevent the normal switch off signal from being activated to allow for continuous culture in fermenters. Where the desired product is only produced naturally by microorganisms that are difficult to grow in culture, the specific gene can be inserted into another microorganism (a bacterium, yeast, or fungus) that is easier to culture. Because the final product is highly purified, like a specific enzyme, there is no altered DNA or other substances to be concerned about in the product. Because the GMOs are contained in the fermentation vessel, there is also no release into the environment, and thus regulatory oversight is less stringent. GM microorganisms are used to produce vitamins, food additives, and processing agents for the food industry. Examples are: vitamins B_2 and C; xanthan thickeners; citric acid acidity regulators; amino acids, such as glutamate to improve flavor; and enzymes used in the production of cheeses, baked goods, fermented beverages, and glucose.

Apart from vaccines, the approved use of any GM bacteria or viruses present day (called genetically engineered microorganisms (GEMs) has been limited. In agricultural research experiments, such bacteria have been incorporated into soil to facilitate crop growth by fixing nitrogen and applied directly onto crops to kill pests by using a modified form of *Bacillus thuringiensis*. Another possible agricultural use of GEMs is the development of ice-minus bacteria (a modified *Pseudomonas syringae,* commonly found in vegetation) that can delay ice formation and death of the plants during freezing temperatures. Some monosodium glutamate and aspartame is industrially produced using fermentation by GM bacteria. However, relatively few specific GM microorganisms have been developed for the food industry itself to date, but it is likely that their use will be considered more in the future.

Animal cloning

Although animal clones used for food have not yet been commercialized, their potential future exploitation needs to be addressed. An animal clone is a genetic copy of a donor animal, which is intended to be used as breeding animals to introduce desirable traits into herds more rapidly than would be possible using conventional breeding. There is no genetic engineering in the process of cloning animals, just producing offspring identical to the parent. The US FDA concluded in 2008 that meat and milk from clones of cattle, swine, and goats, and the offspring of the clones, are as safe to eat as food from conventionally bred animals. Currently cloned animals are rare and costly to produce and they are unlikely to enter the food chain. It is their offspring, even in the future, that are most likely to be used for producing milk and meat, whereas the original clones themselves are reserved for breeding. Australia and New Zealand, Canada, and Japan are considering whether there is a need to regulate food from cloned animals and their offspring. The EU is considering banning any progeny of cloned animals from entering the food chain. Policies in most countries have not yet been formulated, and although there may be some initial concerns over unnatural breeding, the offspring of cloned animals are eventually likely to be treated as any other animal by meat and milk producers and retailers in most countries where cloning occurs, and their products will be sold to the public unlabeled. However, if the public raises sufficient concerns, their use in food would likely be inhibited.

High-Pressure Processing

High-pressure processing or high hydrostatic pressure processing (HPP), sometimes called pascalization, is a method of preserving, in which a product is processed under very high-pressure, leading to the inactivation of vegetative microorganisms and some enzymes in the food. Food products are sealed in flexible containers (usually a pouch or plastic bottle) and loaded into a high-pressure chamber filled with a pressure-transmitting (hydraulic) fluid, often water. High-pressure pumps are used to create pressures, constantly or

intermittently, up to 700 MPa (100 000 psi). Pressure is applied for usually 3–5 min. The application of HPP on a food product will kill mostly vegetative microorganisms; spores are typically not affected and other treatment, like use of acids, may have to be used if survivors create a potential spoilage or safety issue. HPP works especially well on acidic foods, such as juices, fruits, and yogurts, because pressure-tolerant spores will not germinate in low pH foods. However, HPP works equally well for some solid products, such as fish, shellfish, ready-to-eat (RTE) meats, salad dressing, salsas, rice cakes, and guacamole. The food itself is not affected but proteins are denatured causing death of microorganisms. However, because HPP can affect muscle tissues by increasing the rate of lipid oxidation, an off-flavor may occur in meat products. Although the technology may appear less applicable for dry solids, or foods with air pockets because of potential damage, it has been used successfully for strawberries and bread. Where there are air pockets, the process is designed for the compressed air to be dissolved in water or incorporated into the solid food, and then released slowly after the cessation of the pressure step. In 2008, the United States Department of Agriculture approved HPP as an intervention method for *Listeria monocytogenes* in prepacked RTE meat products, and is being used by at least one major US deli meat manufacturer. HPP is being used in the USA, Europe, and Japan on a select variety of high-value foods either to extend shelf life or to improve food safety. One application is to destroy any bacterial pathogens and spoilage organisms present when HPP is used to shuck raw oysters and clams. Destruction of enteric viruses, such as norovirus, cannot be guaranteed through HPP technology. However, demand for HPP foods is expected to grow in the future.

The pressure assisted thermal sterilization process has been used for the application in the production of sterilized prepackaged low acid foods. The packaged foods are immersed in hot water under pressure and preheated with microwaves at a frequency of 915 MHz; this frequency achieves higher penetration depth and more uniform heating than at other oven frequencies. This combination eliminates pathogens and spoilage organisms in less than 10 min to produce a high quality and safe product. Microwaving is just one process that can be used for preheating to make high-pressure treatment more effective so that rapid sterilization is possible; the pressure itself causes adiabatic heating. One example would be preheating a product to approximately 90 °C followed by pressurization; in a very short time there would be homogeneous heating to approximately 120 °C.

Modified Atmosphere Packaging

Modified atmosphere packaging (MAP) is one of the most important techniques used to achieve safety in fresh cut fruits and other products. This can also prolong their shelf life, as an alternative to ultraviolet (UV) light, infrared radiation, irradiation, and high-pressure. Fresh fruit continues to respire, consuming oxygen and producing carbon dioxide and water vapor, and high carbon dioxide and low oxygen levels reduce the respiration rate. Very successful applications of MAP are reported for fresh cut pineapple, apples, kiwifruit, honeydew, bananas, and mangoes. Unfortunately, although low O_2 atmospheres inhibit the growth of aerobic spoilage microorganisms, some pathogens such as *L. monocytogenes* and *E. coli* O157 are not affected and growth may even be stimulated, depending on the type of product packaged with added gases. Therefore, for safety reasons any MAP use has to be supplemented by other 'hurdles' to prevent pathogen survival and growth.

Food Irradiation

The irradiation of food is the process of exposing food to ionizing radiation to destroy nonsporing pathogens and spoilage microorganisms. With regard to insects and parasites, food irradiation acts by randomly damaging DNA so that these organisms do not survive or reproduce. In addition, root crops, such as potatoes and onions, are inhibited from sprouting. Generally, the shelf lives of irradiated fruits and vegetables are extended. Other uses include delaying fruit ripening, increasing juice yield, and improving rehydration. High-dose irradiation has been used to sterilize food.

Food can be irradiated using gamma radiation, X-rays, or high-energy electron beams. Food irradiation using gamma radiation is the preferred method by most processors because its deeper penetration enables treatment of industrial-size pallets, which reduces the need for repackaging and handling. Facilities using cobalt-60 have had excellent safety records and have been in operation for many years for other applications, such as sterilization of medical supplies, but they require high maintenance. In addition to good penetration, X-rays generated by machines have the added benefit of being an electronic source that can be switched off when not in use. However, X-ray systems require much more electrical energy than the other systems. Machine generated electron irradiation uses less energy than the other systems, and also may be turned off. Electrons are particulate radiation and thus do not penetrate beyond approximately 10 cm, depending on product density. However, this is also an advantage as it requires less concrete shields to protect workers and the environment from radiation exposure.

The early safety investigations were driven by the perception that irradiation of food would produce 'unique radiolytic products.' However, to date, the only ones of possible concern are cyclobutanones arising from irradiation of beef fat (2-alkylcyclobutanones (ACBs) and 2-dodecylcyclobutanones (DCBs)). Food irradiation is perhaps the most studied food process ever developed and Codex has adopted the General Standard for Irradiated Food that permits the use of any dose level subject only to Good Manufacturing Practice. Food irradiation has been approved in many countries for more than 30 products, particularly spices, onions, and potatoes, with an estimated 500 000 metric tonnes treated annually worldwide. Independent scientific committees in Denmark, Sweden, the UK, Canada, and the World Health Organization have endorsed the safety of irradiated food. The EU Scientific Committee for Food has also cleared irradiation treatment for dried aromatic herbs, spices, and vegetable seasonings, but the EU still requires a food by food approval process for other foods. Codex requires that any irradiated

product must be labeled with the word 'irradiated' with or without the 'Radura' symbol;

This labeling policy excludes spices or herbs used as ingredients in another nonirradiated product, such as sausage, in part, because no practical analytical methods exist to detect irradiated foods at that level. For the microbial disinfection of spices, the FDA has established that the maximum irradiation dose should not exceed 30 kGy. Labeling is another issue where consumers may want to know what the decontamination process was used. After the 2006 *E. coli* spinach outbreak, the FDA published a final rule that allows the use of irradiation for fresh iceberg lettuce and fresh spinach in 2008; this decontamination technology is not yet being used by the leafy green industry in the USA, where there is more reliance on product testing at the field and decontamination in the wash system before bagging. Pasteurization of in shell eggs is achieved through heated waterbaths but also has the potential for irradiation decontamination. Irradiation of seafood has the potential to extend the shelf life of fish under refrigeration and improve the quality of quick frozen shrimp, elimination of pathogens including vibrios and hepatitis A virus from raw oysters, and improve the hygiene of fish feed. The extension of shelf life due to irradiation depends on several factors, including initial quality of fish, irradiation dose, packaging conditions, and storage temperature. Irradiation should be considered as only one of several 'hurdles' impacting the growth and survival of pathogens which could include cooking, low temperature, water activity, pH, redox potential, and chemical preservatives.

Concerns have been expressed by some consumer groups that irradiation could disguise poor sanitation and food handling practices that could lead to unnecessary fecal contamination. In contrast, some consumer organizations support food irradiation as a means of eliminating bacteria from processed meat and poultry, particularly *E. coli* O157. As almost all cultures have dishes prepared with raw or partially cooked meat, the use of irradiation can make a major public health contribution to safer food. In developing countries, the technology may be useful to extend food supplies and reduce wastage, such as treating bulk foods at ports and using mobile irradiators to prevent onions and potatoes from sprouting. However, the future of irradiation worldwide remains uncertain with very limited use present day because of the lack of consumer acceptance.

Membrane Filtration

Membrane filtration (MF) allows separation of particles from a fluid and if desired, to concentrate those particles. The particles are separated on the basis of their size and shape with the use of pressure and specially designed membranes with different pore sizes. There are different MF methods in order of increasing pore size, i.e., reverse osmosis, nanofiltration, ultrafiltration, and microfiltration. Reverse osmosis is primarily used for water purification. MF is used for clarification, concentration, separation of components, desalting, and purification of a variety of beverages. Reverse osmosis filtration is based on a very dense membrane that rejects virtually all food components except water. This is possible due to a very high system pressure. For instance, liquids can be concentrated to those with higher solids levels. One reason for this concentration is economic to reduce transportation costs. Microfiltration is also applied to improving the food safety of products in order to avoid damaging heat treatment. Some examples of such products are fruit and vegetable juices, nonalcoholic beers, wines and ciders, ice cream, butter, cheeses, fermented milks, skimmed or low-lactose dairy products. Certainly, the dairy industry has been one of the major beneficiaries of this new technology. Pasteurization and sterilization developed to improve the safety and keeping quality of the milk can affect its taste and aroma. Microfiltration with polymeric or ceramic MF membranes is an alternative process to remove pathogenic microorganisms whilst preserving the taste and increasing its shelf life. Ultrafiltration of milk was first used in France for production of Camembert and Brie cheeses, and is used present day for any high-moisture cheese, for example, feta and ricotta. The filtration process is controlled to give the correct amount of fat, protein, and final moisture to which rennet is added. The carbohydrates, soluble vitamins, and minerals normally lost in the whey are now retained. There is no draining or drying, and what is left after the filtration process can be sold as whey protein. All this means that there is less milk required to make the same volume of cheese. Although the cheeses have higher nutritional value at a better price, there are some complaints that the cheese has lost some of its flavor and taste, and its keeping quality is reduced. This filtration will also remove any possible pathogenic microorganisms from the cheeses. However, proponents of raw milk (unpasteurized straight from the cow) do not seem to want to consider this option to reduce their chances of enteric infections even if the dairy farmer could afford the equipment.

Ohmic Heat Processing

Ohmic heating is also known as electrical resistance heating, direct resistance heating, Joule heating, or electro heating, and may be used for a variety of applications in the food industry. This is a thermal processing method where alternating electrical currents are passed through the food which acts as electrical resistance, and the electrical energy is dissipated into heat to give rapid and uniform heating throughout the product. In ohmic heating, heat energy occurs from within foods; unlike microwave heating, it is not reliant on transfer of energy by water particles, and therefore it is an important development for the efficient heating of low water, low particulate foods. Thus, ohmic heating is different from conventional heating where liquids heat faster than solids. With ohmic heating, solids can heat faster than liquids because the heating rate is a function of particle-shape and orientation to

the applied electric field. Therefore, process parameters for ohmic heating depend on food characteristics. Ohmic heating volumetrically heats the entire mass of the food material, thus the resulting product has a far greater quality than, say, food that has been canned. It is possible to cook large particulate foods (up to 2.5 cm) that would be difficult to process using conventional heat exchangers. Juices can be treated to inactivate enzymes without affecting the flavor. Liquid egg can be ohmically heated rapidly without coagulating it. Like thermal processing, ohmic heating inactivates microorganisms by heat. Ohmic heat processing is commercially used in Europe, Japan, and North America for pasteurizing liquid foods containing large particulates, such as soups, stews, whole and diced fruit, and fruit slices in sirups and sauces, fruit juices and heat sensitive liquids, and some prepared meals. One emerging environmentally friendly application of ohmic heating is fruit peeling, which may greatly reduce the use of lye that is now used in such operations. The shelf life of ohmically processed foods is comparable with that of canned and sterile, aseptically processed products.

Pulsed Electric Field Pasteurization

In pulsed electric field pasteurization (PEF), a liquid or semiliquid product is placed between two electrodes and a pulsed electric field is applied. This process involves applying repeated short pulses of a high-voltage electric field, for example, 10–80 kV cm^{-1}, to a fluid pumped between two electrodes. The effect is to enlarge the pores on the plant or animal cell membranes, lysing the cells. There is, however, no substantial temperature rise to kill pathogens. It would require $>30\,000$ V to cause the death of bacterial, yeast, and fungal cells, but it is still not known whether viruses or spores can be inactivated. The formation of large, permanent pores in cellular tissues helps improve juice yield, increase concentrations of functional components, and enhance the characteristics of dried produce. PEF is mostly used in refrigerated or ambient products, and because it is applied for just ≤ 1 s, it does not result in heating of the product. It is for this reason that it has nutritional advantages over more traditional thermal processes which degrade heat sensitive nutrients. However, most enzymes are not affected by PEF that can cause deterioration in the juice under ambient conditions and the treated products have to be refrigerated to preserve their organoleptic quality. Application of PEF technology has been successfully demonstrated for the pasteurization of foods such as juices, milk, yogurt, soups, and liquid eggs. Application of PEF processing is restricted to food products with no air bubbles and with low electrical conductivity. Any gas bubbles trapped in the liquid during extraction can allow electric arcing between the electrodes causing the potential formation of carcinogens. PEF can be used as continuous process, but after processing the products have to be packaged hygienically and kept cool during storage. PEF has not yet been used in Europe on industrial scale and has only been used in the USA in a very limited capacity for orange juice. PEF, however, does have the considerable large-scale potential for improving quality and taste of pasteurized foods compared with traditional preservation techniques.

Pulsed Light

PL or intense light pulses is a method of food preservation that involves the use of intense and short-duration pulses of broad-spectrum 'white light,' i.e., 20% UV, 50% visible, and 30% infrared, with an intensity claimed to be 20 000 times that of the sun at the earth's surface. The material to be treated is exposed to a least 1 pulse of light having an energy density in the range of approximately 0.01–50 J cm^2 at the surface. A wavelength distribution such that at least 70% of the electromagnetic energy is within the range from 170 to 2600 nm is used. Accumulating electrical energy in an energy storage capacitor over relatively long times (a fraction of a second) and releasing this storage energy to do work in a much shorter time (millionths or thousandths of a second) magnifies the power applied. The result is a very high-power during the duty cycle, with the expenditure of only moderate power consumption. Shorter wavelengths in the UV range of 200–320 nm are more efficient inactivation agents than the longer wavelengths, due to their higher energy levels. Because DNA is a target molecule for these UV wavelengths, it is thought that the primary cause of killing microorganisms is through DNA structural changes. For most pasteurization applications, a few flashes applied in a fraction of a second provide a high level of microbial inactivation, i.e., at least 1 pulse of light (typically 1–20 flashes per second with a duration range from 1 μs to 0.1 s. Light pulses may be used to reduce or eliminate the need for chemical disinfectants and preservatives. This technique is ideal for surface decontamination of packaging materials, transparent pharmaceutical products, and works best on smooth, dust-free surfaces. It has also led to significant surface reductions in microorganisms and extend the shelf life of meat, fish, produce, and bakery products. It can inactivate molds in a variety of baked goods and shrimp treated with PL and stored under refrigeration for 7 days remained edible, unlike the untreated shrimp. Both *Salmonella* in chicken and *Listeria innocua* in hotdogs were both reduced 100 fold after PL treatment. Other undesirable organisms such as *E. coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Saccharomyces cerevisiae* have been inactivated by using 1–35 pulses of light with an intensity ranging from 1 to 2 J cm^{-2}. Treatments have shown that reduction of microorganisms is proportional to treatment time, but this is inversely proportional both to the sample's distance from the lamp source and to the treatment depth. This technology could be a solution to bagged leafy greens that caused outbreaks in the past. These have a risk of pathogens such as *E. coli* O157:H7 being present in low numbers in bags. However, PL treatment alone does not increase shelf life in spite of the reduction in the initial microbial load. PL may have to be used in combination with other decontamination strategies to create a 'hurdle' technology. PL has considerable potential to be implemented in the food industry. However, technological problems need to be solved in order to avoid food overheating as well as to achieve better penetration and treatment homogeneity. In addition, a more extensive research is needed to understand how PL affects quality food attributes, for example, possible undesirable side effects are lipid oxidation, vitamin inactivation, formation of toxic products, and discoloration.

Microwave Technology

Microwave ovens are now one of the most frequently used appliances in domestic and commercial kitchens, at least in the developed world. They save time by heating up foods relatively quickly with little loss of quality, especially for vegetables. For many people, these ovens are mainly used to heat drinks and packaged convenience foods. The power source is the magnetron which converts electric power into very short radio waves. At a frequency of approximately 2450 MHz, the wave energy is readily absorbed by water, fats, and sugars, and through very fast vibration (2.5 billion times per second) the high temperatures generated heat the food. The water molecules have polarity and are spun at the frequency of the electric field to absorb the energy converted to heat. Normally, food is cooked or reheated on full power; for lower power levels, such as defrosting or cooking large cuts of meat, the magnetron cycles are on and off. The higher the power of a microwave oven, the faster it will cook food. Also, over time the magnetron losses capacity. Many home food preparers do not know the current power (wattage) of their ovens and estimate cooking time by experience. So it is important for them to know the power of the oven when using cooking directions from various sources for food safety. A 'Time-to-Boil Test' (heating two cups of ice cold water to a boil) can estimate the microwave oven power, for example, 2 min at high power ($\geq$1000 W) and 3–4 min at low power (300–500 W). Also, because the waves do not reach the food in a consistent way, microwave ovens can cook unevenly and leave less heated areas ('cold spots') where pathogens may survive. Few persons use a food thermometer to test food in several locations to ensure it has reached the recommended safe temperature before it is eaten, although probes tend to be used for large pieces of food cooked over long periods of time. This is important because microwaves penetrate the food only to a depth of 2.5–4 cm. In thicker pieces of food, the microwaves do not reach any deeper, and the center is cooked by conduction of heat from the outer areas of the food into the middle. The uneven cooking brought about by this uncertain food penetration by the waves and lack of consistency in heating by the intrinsic microwave oven process means that temperature measurement for cooking or reheating potentially hazardous foods is critical. Covering a food dish with a lid or plastic wrap will allow the moist heat and steam that is created to ensure more uniform cooking and help destroy any microorganisms present. Sensor heating buttons in more modern microwave ovens may give some reasonable degree of effective cooking but cannot be relied to destroy pathogens alone. After the oven is turned off, it is typically recommended for domestic cooking that the food stand for some minutes to equilibrate the heat throughout the food by conduction to avoid hot and cold spots; this is often called standing time. The more risky practices that require careful temperature measurement are cooking whole stuffed poultry and large roasts, deep dish pizzas, defrosting large pieces of frozen food, warming of food rather than cooking it, home canning in jars. There is also the danger of a superheated cup of liquid erupting violently when it is removed from a microwave oven and scalding the hands. There is little cause for concern about excess microwaves leaking from ovens unless the door hinges, latch, or seals are damaged. Microwave ovens are also typically present in hotels, ski condos, beach houses, and time-shares, and are frequently used by the temporary occupants without knowledge of the power (the wattage and voltage are typically labeled in ovens but are rarely consulted by users). A few outbreaks have been associated with microwaved food.

Consumers present day are so used to eating convenient microwavable RTE fresh or frozen foods at home or work that they rarely read the instructions except for heating times. For these, the purpose of the ovens is simply to warm the food up to palatability. Unfortunately, there are also not-ready-to-eat (NRTE) foods in the market with a similar appearance, and these pose one of the biggest concerns to safe microwaving. If these are not cooked properly, pathogens like *Salmonella* may survive, and frozen NRTE microwavable meals have been reported as vehicles in salmonellosis outbreaks. In one large outbreak involving packaged pot pies, only 29% of patients interviewed reported knowing the power of their home microwave ovens, and 68% did not let pies stand the full recommended time after microwaving, as stated above to allow more even dispersion of the heat in the product and reduce the likelihood of cold spots that could allow survival of pathogens like *Salmonella*. Furthermore, 19% cooked more than one pie simultaneously, indicating that they did not follow microwaving instructions. This outbreak led companies to start making clearer microwaving instructions on their packages and what foods were RTE (warming only necessary) and what were NRTE (cooking sufficient to kill any pathogens). A better understanding of oven power would also help consumers follow manufacturer's instructions better. Unfortunately in 2010, another multistate outbreak was documented, this time from cheesy chicken and rice single serve frozen entrées where insufficient home microwaving was likely an important factor in causing the illnesses. From the above information it is quite possible that many outbreaks have occurred from NRTE convenience foods around the world, but without modern molecular tracing methods, they would not be identified.

In addition to domestic ovens, microwave heating is also used commercially for applications like thawing, drying, and preservation, as well as heating and cooking. Small offices, mini cafes, pastry shops, and many fast food restaurants all keep microwaves to provide hot food to clients. One reason for their popularity is that many models heat approximately 45 times faster than conventional cooking methods. Larger commercial ovens use two magnetrons for quicker and more efficient results (1000–2000 W compared with 600–900 W for domestic ovens). Large capacity microwaves, typically used in restaurants, can have a capacity of up to nearly 16 gallons (60 l), with a variety of cooking preparations.

Sous Vide

One recent particular approach to pasteurization with minimum heat is *sous vide* cooking. This has been promoted as method improving the quality of the final product by using lower cooking temperatures. This is achieved by placing the food in airtight plastic bags, preferably with as much air removed from the bags as possible, in a water bath for many

hours to allow gradual even penetration of the product to retain juices and not be overcooked. To be successful in this methodology, accurate temperature measurement is required. The time of cooking will vary with the type and size of the item in the bags. Most time-frames are up to 12 h. However, as large cuts of meat may take up to 72 h, one safety concern is the risk of germination and outgrowth of *Clostridium botulinum* spores and other anaerobes; water bath temperatures should be high enough ($\geq 55\ ^{\circ}C/\geq 131\ ^{\circ}F$) to prevent this from happening. In addition, unless food is to be consumed right away, any food, particularly *sous vide* products with minimum processing conditions, has to be chilled rapidly after cooking to reduce the opportunities for surviving bacteria to multiply.

Nanotechnology

Nanotechnology is a field of science applied to materials 1–100 nm (nm) in size for many different applications, such as electronics, pharmaceutics, cosmetics, medical devices, and textiles. Nanomaterials are very small, for example, the DNA molecule is approximately 2.5 nm wide, and may exhibit different physical and chemical properties compared with the same substances at a normal scale, such as increased chemical reactivity because of the greater surface area. Nanoparticles naturally occur in emulsions of air, food, and water and with no known adverse effects. However, industry is now looking at possible applications in the field of food science and technology including applications in food production, processing, functional foods, and packaging. Preliminary research has indicated there could be benefits for food ingredients, such as to alter the taste, texture, and nutrition of a product. Present day, the food sector is making significant investments in the application of nanotechnology to food production, although there is no substantial commercial application currently underway. Adding specifically labeled nanoparticles to foods can allow identification of these throughout the food chain and may in the future be a contribution to better traceability of foods and their ingredients and speedup recalls.

Materials with structural features at between 1 and 100 nm have been shown to have different properties when compared with their conventional, much larger counterparts. Examples of potential applications of these properties include delivering pesticides to plants more effectively, preventing (or retarding) microbial spoilage of food through packaging materials, modifying a food's texture and taste as a new food additive, and increasing bioavailability of vitamins and minerals. Specifically, 'smart' food packaging is an area where nanotechnology may offer better protection of the products by absorbing oxygen and moisture or by resisting heat, light, and mechanical damage. Another use might be to detect freshness of a food through embedded nanosensors in packaging that can detect minute quantities of chemicals such as those released when a food starts to spoil. Current examples of nanoenhanced food contact materials are polyethylene terephthalate beer bottles with a nanoclay gas barrier and polypropylene food containers with nanosilver for antimicrobial protection. Because the nanomaterials do not become a component of food, these applications may be less of a concern from a human safety standpoint, but environmental concerns would need to be addressed, and they would still have to be approved.

In food formulation, nanoencapsulation is being studied to allow a controlled delivery system for food ingredients and additives in processed food. This can be used to mask unpleasant flavors and protect ingredients from degradation. Existing organic materials that exist in nano form include vitamins, antioxidants, colors, flavors, and preservatives. Visions for the future include low-sodium foods that still taste salty because of nanointeractions with the tongue, and nutrient delivery systems that use nanotechnology to deliver micronutrients or other functional components. Potential applications include foods that can release an appropriate amount of calcium in consumers with osteoporosis, or those with 'smart filters' that trap molecules that might cause allergic reactions.

There are potential health risks, which have not yet been properly evaluated. For example, some nanomaterials are able to cross the blood–brain barrier. The assessment of potentially hazardous characteristics in new products will require a basic understanding of how these materials are handled by the body. In addition, another major challenge is the ability to detect and measure these materials. Because size is the intrinsic difference, traditional analytical methods cannot be used to quantify (i.e., count) nanomaterials. The manufacturing of nanomaterials does not produce a single entity, but rather a range of entities with different sizes and often different shapes. Such analytical challenges make it difficult to investigate the interaction and stability of nanomaterials in food and feed, in the gastrointestinal tract, and metabolism in biological tissues. Therefore, developing and validating sensitive methods to detect, characterize, and quantify nanomaterials in food and animals, including humans, are priorities for research. Another issue is the attitude of the potential consumer toward the application of nanotechnology in food and agriculture trying to weigh the benefits and risks with limited information.

Conclusion

New food technologies will continually evolve over time, and some of these will be adopted by the food industry and accepted by consumers. Not all of those that are scientifically safe and technically sound are economically feasible. New technologies are driven by a variety of stakeholders. Industry tends not to change unless there are economic advantages in doing so, or new regulations force a change. Both the industry innovators and the consumers have to be comfortable with what these technologies can and cannot provide before any marketing is done or the product is put up for sale. Some technologies will not be accepted by consumers even if the technology will make food safer that its unprocessed counterpart, food irradiation being a good example. Well-performed field trials should be conducted to prove that there is a benefit in safer or better quality food to be offered for sale. In addition, there should be an educational phase to alert the consumer of what to expect and what the consumers' responsibilities are. This information exchange has to be

tailored to the concerns and social issues of the day and to the knowledge level of consumers. Recent food scares may reduce the chances of consumer acceptance so that consumer education is essential. Politicians and policy makers are responsible for balancing the societal benefits offered by new food technologies with the assurance of safety that most consumers would consider reasonable and prudent. Ultimately, no new technology can be introduced without the confidence of the consumer in the safety of the food supply. The consumer has also to be sufficiently knowledgeable about the processes they control such as microwave cooking and what relevant safety information labels convey. For instance, as wattage is required for the correct microwave heating of food it is important to raise awareness of consumers on this matter and provide them the necessary information in language they can act upon; some labels on food packages present day give different cooking instructions for high- and low-power microwaves. Thus, risk assessment and risk communication approaches have to be well-researched with the understanding that consumers should have choices based not only on preference, but also on real safety concerns. What was made clear in the pot pie outbreak was the lack of clear communication by the processor to the consumer for cooking instructions. However, messages have not only to be understandable, but also acceptable to the majority of consumers. The broad policy communications are also important relating to the acceptance of new technologies; these are the responsibility of both industry and government. Poor understanding of GM and hormone issues by consumers was partly due to a lack of transparency by the innovators. When these are addressed, new technologies play an important part of the future in providing an abundant, safe, nutritious, and convenient food supply. Finally, it should be mentioned that in developing countries, 'new' technologies may include something as simple as refrigerators. In that case, education for their proper use, for example, safe storage times, and temperatures, cross-contamination, etc. will be essential for protecting public health.

See also: Food Technologies: Food Irradiation; High Pressure Processing; Microwave Heating; Nanotechnology and Food Safety; Pulsed Electric Field Technology. Foodborne Diseases: Overview of Biological Hazards and Foodborne Diseases; Overview of Chemical, Physical, and Other Significant Hazards. Safety of Food and Beverages: Safety of Genetically Modified Foods

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FOODBORNE DISEASES

Prevalence of Foodborne Diseases in Africa

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Glossary

Decompensation The failure of an organ (especially the liver or heart) to compensate for the functional overload resulting from disease.

Enterosorption Adsorption of substances from the gastrointestinal tract onto an orally administered sorbent medium like activated charcoal.

Hazard Analysis and Critical Control Point System (HACCP) It is a system which identifies, evaluates, and controls hazards which are significant for food safety.

Helminth Parasitic worm; a fluke, tapeworm, or nematode.

Hepatoxicity A condition where liver injury has been caused by chemicals.

Immunocompromised Having an impaired immune system.

Mycotoxins Toxic secondary metabolites produced by toxigenic fungi that contaminate various agricultural commodities either before harvest or under postharvest conditions.

Polyparasitism Infection with more than one parasite simultaneously.

Zoonosis A disease that can be transmitted to humans from animals.

Introduction

Millions of people around the world become ill from food- and waterborne disease. Of these, an estimated three million people die every year with 700 000 estimated deaths occurring in Africa due to diarrhea alone, associated with contaminated food and water. Occurrence of such disease can easily escalate to a food safety emergency situation, which can adversely impact national economies and livelihoods through reduced availability of food for domestic consumption, closure of export markets, and/or the high cost of addressing the effects of the threat. Although cases of foodborne illness occur daily in all countries, there is a severe underreporting of such diseases, particularly in developing countries, including Africa. Consequently, the true prevalence of foodborne disease in Africa and the rest of the world is unknown.

Beyond its macroeconomic effects, foodborne diseases impede development at the country level. Without concerted action to estimate and reduce the burden of foodborne diseases, international efforts to achieve the Millennium Development Goals (MDG), particularly those goals relating to children and the poor, will be jeopardized.

Africa – the Scenario

Africa is a continent consisting of 54 countries, each with diverse cultures, religions, languages, and traditions. Owing to the size and geographical positioning of Africa, a number of different climates exist, which largely determine the types of crops and livestock that can be farmed in the different regions. The level of sophistication of the agricultural sector in Africa also differs, with well-established commercial farming and export markets on the one hand and reliance on subsistence farming, on the other. Similarly, well to poorly developed potable water and sanitation systems exist, often within the same country.

Huge food losses are experienced in many parts of Africa due to lack of infrastructure for transportation to markets, either by rail or road, lack of appropriate storage facilities, particularly refrigeration and freezing, inappropriate storage practices, and lack of applicable technologies for processing foods to extend shelf life. These factors drive the need to import basic commodities such as wheat, maize, and other grains, an unaffordable practice for the least developed, poorer countries. Natural disasters aside, exposed populations experience hunger as a result of these combined factors. The need to fill their bellies overrides food safety considerations, leading often to the consumption of contaminated food, simply because none other is available. The latter is particularly true for grains and nuts contaminated with mycotoxins.

In most countries in the region, the surveillance infrastructure for foodborne diseases is weak or nonexistent. With the exception of cholera (which is subject to the World Health Organization (WHO) International Health Regulations), there is no obligation to report foodborne disease internationally, and only a few countries in the region require national reporting of foodborne disease incidents. Where such reporting is required, the systems in place may be weak and not able to fully address the incidents. Consequently, data from Africa are scarce and where available, reflects situations in certain countries only, which may not be relevant to other regions at different levels of development. This absence of data impedes understanding of the importance of the burden of foodborne

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disease on public health and prevents the development of risk-based solutions to its management, including informing policy makers how best to allocate resources for appropriate foodborne disease control.

Food safety is not regarded a priority among most, if not all African governments, particularly for domestic populations, and is often seen as separate from public health. The harsh reality is that Africa experiences many other challenges, which take priority in many instances. In 2012, 90% of malaria-related deaths occurred in Africa, numbering almost 600 000. Primary health care, provision of education, treatment of human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS), supporting undernourished people, and enhancing food security are all important issues for Africa, requiring considerable spend, placing beleaguered budgets under pressure. According to the Food and Agriculture Organization (FAO, 2010), of the 925 million undernourished people in the world in 2010, Sub-Saharan Africa (SSA) had the highest proportion at 30%, which translates into 239 million people. With respect to HIV/AIDS (Note that HIV is not considered as a foodborne pathogen. However, it is relevant to food safety, as it can potentially be transmitted through breast milk. Also, HIV infections create vulnerability to other foodborne infections.), SSA remains the most heavily affected region, with an estimated 23.5 million infected people in 2011, representing 69% of the global HIV burden. In addition, 92% of pregnant women living with HIV/AIDS and 90% of children who acquired HIV in 2011 are found in SSA (UNAIDS, 2012). It is also a well-known fact that food- and waterborne diseases have a far more severe impact on immunocompromised persons and children under the age of 5 years and negatively affects food security status. The number of HIV-infected people together with the undernourished totals an approximate 263 million immunocompromised people in SSA. Considering that Africa has just over 1 billion people, this figure represents approximately 26% (more than one quarter) of the continent's population. Importantly, this number excludes the millions of children under the age of 5 years. Therefore, a significantly higher number than 26% are at risk of contracting food- and waterborne diseases with more severe consequences, placing a heavy burden on health systems and the economy of the country. Food and water safety therefore have a direct impact on human health and can no longer be ignored or viewed separately from matters of public health. African governments should better coordinate agriculture and public health policies and strategies and inclusion of food and water safety should form an essential part of this approach. Besides the positive impact this will have overall, it will result in more effective allocation and use of already scarce resources.

Food- and waterborne diseases can be due to a range of enteric bacteria, viruses, parasites, toxins, and chemical agents. Most food- and waterborne diseases in Africa are due to poor sanitation, poor personal hygiene, poor hygiene and storage practices, lack of education and awareness, lack of potable water, and inadequate infrastructure to support the provision of nutritious, safe food and water. This article discusses selected food- and waterborne microbiological, chemical, and phytotoxic hazards affecting the African region. It also includes an overview of the safety of street food vending as well as proposing potential solutions to the food and water safety issues facing Africa.

Bacteria

Pathogenic bacteria are one of the major causative agents of food- and waterborne diseases in Africa. Cholera is the most well known, mainly because it is obligatory to report it internationally according to WHO International Health Regulations, and so is discussed in further detail. Reported foodborne disease outbreaks in Africa show that the majority are caused by *Salmonella* spp. (**Box 1**), *Shigella flexneri*, *Shigella sonnei*, and *Shigella dysenteriae*; *Bacillus cereus*, *Staphylococcus aureus*, and *Clostridium perfringens*. Those reported in South Africa between 2010 and 2012 indicate that most outbreaks involved catering at mass gatherings, which may or may not be similar to other countries in Africa. Less frequent foodborne diseases are:

- Botulism (*Clostridium botulinum* Type E) from consuming traditionally salted fish in Egypt in 1991;
- Bloody diarrhea (*Escherichia coli* O157) from consuming beef and untreated water in Swaziland in 1992;
- Botulism (*C. botulinum* Type A) from consuming canned fish from discarded, corroded cans in South Africa in 2002;
- Typhoid fever (*Salmonella* Typhi) in Zimbabwe in 2011/2012 and in Zambia in 2012;
- Anthrax (*Bacillus anthracis*) from consuming undercooked meat from infected animals in Zimbabwe in 2011 and Lesotho in 2012.

Although brucellosis cases have been reported, as perhaps consumption of milk, is not very widespread, they have not been directly linked to contaminated food consumption, but rather to handling of infected animals.

Pathogenic bacteria and groups of bacteria that indicate poor hygiene or fecal contamination have been isolated from a wide range of foods obtainable on the continent. Besides the ones listed above that have also been isolated from foods not implicated in outbreaks, others include *Yersinia enterocolitica*, *Campylobacter jejuni*, *Listeria monocytogenes*, *Cronobacter*, *Vibrio alginolyticus*, *Vibrio parahaemolyticus*, *Brucella* spp., *E. coli*, enterococci. Foods from which these bacteria have been isolated range from raw, cooked to preserved (e.g., salted, cured, and smoked) meats including poultry, game/bush meat and fish; raw and cooked vegetables; raw and

Box 1 Common *Salmonella* serotypes isolated from foodborne outbreaks in Africa

Salmonella Typhimurium
Salmonella Enteritidis
Salmonella Heidelberg
Salmonella Virchow
Salmonella enterica serotype Blockley
Salmonella Stanleyville
Salmonella Anatum
Salmonella Weltevreden
Salmonella Roodepoort

pasteurized milk from cattle, sheep, goats and camels, and associated dairy products; a variety of indigenous foods; infant foods including commercially available powdered infant formula as well as weaning foods made in the home; food at retail level including delicatessens; food from restaurants, hotels, and other catering establishments including street foods. Studies have been conducted on food handling and hand hygiene practices in both formal and informal settings. Much data on the prevalence of pathogenic bacteria in foods produced, prepared and sold in Africa is therefore available. However, the precise significance of this data in terms of foodborne disease is largely unknown, even though levels of pathogens found were often at a level high enough to cause disease.

Cholera

Cholera is an acute diarrheal infection caused by ingestion of food or water contaminated with *Vibrio cholerae*. Of the estimated 3–5 million cases that occur globally every year, approximately 100 000–120 000 die. The WHO estimates that the officially reported cases represent only 5–10% of the actual number occurring annually worldwide indicating widespread underreporting of cholera. This may be due to weak surveillance systems and weak laboratory capacity as is the case in many African countries. The actual estimated number of people at risk of contracting cholera is approximately 1.4 billion, with children under the age of 5 years being the most vulnerable, together with immunocompromised people, i.e., the undernourished and those with HIV/AIDS, the numbers of which are collectively significant in Africa (see Section Introduction).

Cholera remains one of the most serious water- and foodborne diseases in Africa and affects hundreds of thousands of people, with thousands of deaths every year. It is viewed as the 'disease of the poor' as it occurs in places with poor sanitation, poor hygiene, crowding, unplanned human settlement, war, and famine, all of which lead to millions of displaced people annually in Africa, thereby contributing to the occurrence and spread of the disease.

Africa was free of cholera until 1971, after which it spread and became endemic to most countries on the continent. Of the numerous cholera outbreaks notified with the WHO since 2000, the majority are from African countries. Between 2001 and 2009, cases from Africa represented 93–98% of total cases worldwide. In 2003, there were 108 000 cases and 1884 deaths, whereas in 2004, the numbers were 71 600 cases and 1663 deaths, with Mozambique having the highest count of cases at 20 000. Even though cases in 2004 showed a marginal decline, this is no indication that the situation has improved. In fact, cholera cases have increased alarmingly and the disease is a reemerging threat, due to new strains of *V. cholerae*, increasing antimicrobial resistance and an ever-increasing size of vulnerable populations living in unsanitary conditions. These factors have resulted in some of the worst outbreaks of cholera experienced on the continent. In 2008 and 2009, 98 424 cases and 4276 deaths occurred in Zimbabwe alone, during which practically the entire country was affected.

More recently, in 2011, cholera cases reported globally increased by 16%. Of the 58 countries reporting cholera outbreaks, 27 countries were in Africa resulting in 188 678 cases, which is a massive 64% increase compared with 2010 during which 115 106 cases were reported. In 2011, 4183 deaths occurred on the African continent, which represents more than half of the 7816 deaths globally. Somalia had the highest number of cases and deaths, i.e., 77 636 and 1130, respectively, accounting for 27% of the deaths reported for the continent that year. Cameroon, Chad, Democratic Republic of Congo (DRC), Ghana, and Nigeria collectively reported 95 405 cases with 2672 deaths, i.e., 64% of the total number of deaths on the continent.

The most recent outbreak in Sierra Leone in 2012 is the worst experienced by that country in 15 years. As of October 2012, there were 20 736 cases with 280 deaths.

Access rates to water and sanitation in West and Central Africa are among the lowest in the world. Out of 24 countries in the region, not a single one is on track to meet the MDG target for sanitation. This is the underlying cause for cholera outbreaks in West and Central Africa. Although measures for cholera response can help contain the spread of the disease and reduce the number of fatalities, it would undoubtedly be much more effective if the underlying cause of this disease is tackled. This need was recognized at the 64th World Health Assembly (WHA) meeting held in 2011, where Resolution WHA64.15 was introduced, which calls for a revitalized focus on cholera. It defines a range of actions required of the WHO and its Member States toward creating an integrated, comprehensive strategy for cholera prevention and control. Most importantly, the strategy calls urgently for tackling the underlying cause of cholera, i.e., provision of adequate sanitation and safe disposal of human waste together with ensuring safe drinking water. Education and constant reinforcement in the use of proper hygiene measures to prevent contamination of food with *V. cholerae* is also essential. Strengthening surveillance and reporting of cholera together with building local capacities for collecting and analyzing data as well as emergency preparedness for unforeseen natural disasters is included. A point worth mentioning is a multipartner initiative aimed at establishing a stockpile of oral cholera vaccine for use in outbreak response. Importantly, it was emphasized that a vaccine should be used as an adjunct to established prevention and control measures as they provide a short-term benefit. The long-term solution is to improve water and sanitation, which is how the developed world successfully controlled cholera. It is clear therefore that cholera is not only a treatable disease but also more importantly, a preventable one.

Parasites

Food parasitology is an emerging discipline as it has been largely underestimated and therefore neglected. FAO and WHO recently convened a Joint Expert Meeting on foodborne parasites, during which it was acknowledged that infectious diseases caused by foodborne parasites have not received the same level of attention as other foodborne biological and chemical hazards. They nevertheless, cause a high burden of

disease in humans and warrant closer attention. Infections may have prolonged, severe, and sometimes, fatal outcomes, and result in considerable hardship in terms of food safety, food security, quality of life, and negative impacts on livelihoods. Notification of public health authorities in many countries, particularly in Africa, is not compulsory for most parasitic diseases, and therefore, official reports do not reflect the true prevalence/incidence of the disease. Many enteric parasites are readily transmitted on food and it is known that foodborne parasitic infections affect many millions of people worldwide. The WHO Foodborne Disease Epidemiology Reference Group (FERG) has assessed the global burden of human foodborne trematodiasis with data for the year 2005. It estimated that 56.2 million people were infected by foodborne trematodes alone, of which 7.8 million suffered from severe sequelae and 7158 died worldwide. Although many publications include data relating to parasites and countries in Africa, they do not provide specific information on food-related sources of such infections as cases of foodborne transmission have been difficult to determine, and linking infection to a contaminated food source remains so for the majority of scenarios, worldwide.

Food normally becomes a potential source of human infection by contamination, during production, collection, transportation, and preparation or during processing, and the sources of zoonotic contamination are usually feces, fecally contaminated soil or water. In many countries in Africa, certain farming, agricultural, and cultural practices positively enhance transmission. These include the use of human waste for fertilization, the use of contaminated water for irrigation, or washing of, particularly, vegetables which may be eaten raw or undercooked to retain the natural taste and preserve the heat-labile nutrients, the consumption of raw or undercooked meat (often as a cultural practice) originating from infected animals, as well as inefficient or absent pasteurization. Indirect transmission also occurs and the majority arises from infected foodhandlers and poor hygiene, often resulting from overcrowded living conditions where clean running water is scarce –this is particularly so in many African countries, where infrastructure in urban areas may be inadequate to accommodate the rapid urbanization occurring in Africa. Hence, services such as proper provision of sanitation and clean running water for personal hygiene and also for preparing food are not necessarily available. In addition, children and adults with little or no health education are also a risk factor in the spread of parasitic diseases – this is more often the case for the rural poor.

Although much literature originating from the developed world offers opinions on food- and waterborne parasites that are regarded as more important, this list may not be relevant from an African perspective. Examples of those that are perhaps of greater direct importance to Africa are *Entamoeba*, *Cryptosporidium*, *Taenia*, and *Echinococcus*. More recently, FAO/WHO have developed a list of parasites based on global ranking and the parasites mentioned above, rank among the eight most important parasites that have already been singled out by FAO/WHO as neglected tropical diseases and identified by FERG as priorities for further burden of illness studies. This does not suggest that other parasites such as *Toxoplasma*, *Trichinella*, *and Ascaris* are of lesser importance to Africa. In fact, **Table 1** provides data that has recently been published on the seroprevalence of *Toxoplasma* in a number of African countries. Focus was placed on pregnant women, HIV-positive individuals, and school children, i.e., the more vulnerable sectors of the population.

In addition to **Table 1**, Abu-Madi *et al*. (2008) also found 29.8% of subjects tested in Qatar were positive for *Toxoplasma*,

Table 1 Recent data on the seroprevalence of *Toxoplasma* in selected African countries

Country	*Population sector*	*Prevalence (%)*	*Reference*
Tunisia	General	58.4	Bouratbine *et al.* (2001)
Egypt	General	57.9	Hussein *et al.* (2001)
	Pregnant women	58.1	
	Pregnant women (rural)	57.6	El-Gozamy *et al.* (2009)
	Pregnant women (urban)	46.5	
Mali	AIDS patients	60	Maiga *et al.* (2001)
	HIV-seropositive blood donors	22.6	
	HIV-seronegative blood donors	21	
	General	27	Ouologuem *et al.* (2012)
Sudan	Pregnant women	34.1	Elnahas *et al.* (2003)
Nigeria	Healthy individuals	20.8	Uneke *et al.* (2005)
	HIV-positive	38.8	
	Pregnant women	40.8	Akinbami *et al.* (2010)
Tanzania	General	46	Swai and Schoonman (2009)
Ghana	Pregnant women	92.5	Ayi *et al.* (2009)
Swaziland	Children	8	Liao *et al.* (2009)
Morocco	Pregnant women	44.3	Barkat *et al.* (2010)
South Africa	HIV-positive individuals	9.8	Kristiah *et al.* (2011)
	HIV-negative pregnant women (Gauteng province)	12.8	
	General pregnant women	6.4	
Mozambique	HIV-positive pregnant women	31.3	Sitoe *et al.* (2010)
	HIV-negative pregnant women	10.9	
Cameroon	Pregnant women	70	Njunda *et al.* (2011)
São Tomé and Principe	Children	63.1	Fan *et al.* (2012)

with the highest prevalence of positives among subjects of African origin who had immigrated to Qatar – the African countries being Algeria, Egypt, Eritrea, Ethiopia, Kenya, Libya, Mauritania, Morocco, Senegal, Somalia, South Africa, Sudan, and Tunisia.

Human and society behavior plays a fundamental role in the epidemiology, emergence and spread of parasitic zoonoses and there are multiple drivers that contribute to changing trends in foodborne parasitic diseases, many of which are also applicable to Africa. These drivers are summarized below:

- Changing eating habits, such as the consumption of raw or lightly cooked food, particularly vegetables in order to preserve heat-labile nutrients and maintain the taste and crispy texture of the food; this includes the demand for exotic foods, such as bush meat. As more Africans become economically active due to current rapid economic growth in Africa, more disposable income is available in many societies and there is a consequential demand for better goods, including healthier foods. This can be seen by the vast array of products on supermarket shelves in many African countries and rapid expansion of retailers from South Africa, Europe, and America into these growing markets.
- Rapid population growth and more rapid urbanization. In this regard, Africa's population growth, estimated to be 1.5 billion people by the year 2020 as well as rapid urbanization, with 40% of Africans currently living in urban areas and this figure estimated to rise to 60% by 2050 is extremely relevant. This rapid pace of urbanization has resulted in an increasing burden on an infrastructure that may already have been weak for some time, due to decades of underinvestment. Current spend on infrastructure in Africa is in the region of US$45 billion per year, whereas US$90–100 billion a year is needed. This has led to inadequate sanitation, inadequate safe drinking water, and inadequate garbage collection disposal services and facilities, resulting in more breeding sites for vectors of parasites.
- An increasingly global food trade and particularly an increasing market in vegetables, fruit, meat, ethnic foods, and even farm animals, some of which originate from countries (developing countries in particular) without appropriate food safety procedures.
- Improved transport logistics and conditions, enabling agents to survive on food products and reach the consumer in a viable form.
- An increasingly transient human population carrying parasitic fauna worldwide and into geographical areas where such parasites may not have been an important consideration before. In Africa, political conflict and drought has resulted in the displacement of millions of people, not only into completely different countries but also into different areas within a country. This has most likely had an effect on movement of such parasitic fauna, of which the extent is unknown.
- The shift from low- to high-protein food consumption in the next 20 years. This is particularly relevant to Africa, where emerging nations are developing economically with a growing middle class demanding more diverse products, resulting in a concomitant greater dependency on meat and fish products.
- A higher proportion of immunologically compromised individuals either as a consequence of increasing elderly populations or the generation of highly susceptible groups with immunosuppressive diseases or treatments. In the African context, the two main groups affected are undernourished people and those living with HIV/AIDS, both of which result in compromised immune systems (see Section Introduction). These groups are far more susceptible to food- and waterborne infections, including parasites. Data in Table 1 attests this fact.
- Changing farming practices, for example, intensification to produce cheaper food or a shift to free-range/organic animal production to respond to consumer welfare concerns.
- Social and political upheavals, both of which are prevalent in Africa, leading to a decrease in veterinary control and a disturbance in modes of production.
- The impact of climate change on, for example, parasite–host habitats, which may present a greater likelihood of contamination due to extreme weather events and create increased pressure on the safety of some food sources. Climate change may also favor the distribution of intermediate hosts, bringing novel vectors into temperate regions or temperature-associated changes in contamination levels.

In addition to the above drivers, poverty plays a significant role in the prevalence of zoonotic parasitic infections. The World Bank estimated that over a third of the world's population resides and lives on less than US$2 per day, a great proportion of which is found in Africa. Limited access to diagnosis and treatment means that most emerging or reemerging zoonoses go unrecognized. This leads to a greater morbidity and mortality from such infections and the lack of treatment and control results in a greater prevalence of such zoonoses.

The issue of coinfections or polyparasitism has been raised and is certainly prevalent in Africa. Data shows that single infections are rare, with polyparasitism being more likely, due to multiple factors, including inadequate sanitation, lack of potable water, poor hygiene, lack of maternal education where children are involved, as well as close proximity to companion animals and livestock. A number of authors in African countries have reported polyparasitism in both adults and children, with children being at most risk, of which some examples are presented below:

- Chunge *et al.* (1991) found that 72.7% of people tested in Kenya in 1985 and 1986 had mixed infections and in a study conducted by Thiong'O *et al.* (2001) also in Kenya, polyparasitism was reported;
- Curtale *et al.* (2005) and Hussein and Khalifa (2010) suggested coexistence between *Fasciola* and *Schistosoma* in two separate studies conducted in Egypt;
- Ouattara *et al.* (2008) reported the coexistence of at least two species of parasites in 80.2% of pupils who were tested in Côte d'Ivoire;
- Samie *et al.* (2009) found that polyparasitism was more common in hospital patients (39.6%) than among school children (23%) tested in South Africa. Almost half (49%) of all individuals tested were infected with many parasites;

- Abu-Madi *et al.* (2010) established polyparasitism in a study conducted on 1299 African immigrants to Qatar originating from Algeria, Egypt, Eritrea, Ethiopia, Gambia, Ghana, Somalia, Sudan, and Tunisia;
- Tomlinson *et al.* (2010) reported on 80% of children tested across 24 schools in Angola, being infected with one or a combination of parasites;
- Ayalew *et al.* (2011) found a prevalence of 66.2% mixed infections in school children in Ethiopia;
- Damen *et al.* (2011) reported polyparasitism among rural pupils tested in Nigeria, albeit to a lesser degree than single infections.

Although chronic helminth infections could have a significant influence over the immune response, the impact on health of such polyparasitism in the context of enteric protozoan infections on African populations is unknown. There is also a need to understand the relative impact of single and coinfections on the susceptibility to HIV transmission and child education and welfare in Africa.

It is estimated that SSA has 60% of the world's available and unexploited arable land and is touted to become a future global supplier of food, together with Latin America and Asia, as emerging economies. Regarding the domestic population on the continent, the high incidence of HIV-positive individuals and undernourished people, both with compromised immune systems, as well as poverty and related factors such as poor hygiene, lack of sanitation, lack of education, and inadequate clean water sources, will most likely exacerbate the impact of food- and waterborne parasites. The added issue of polyparasitism and impact this may have on the African population generally and more specifically on the approximate 263 million immunocompromised individuals due to undernourishment and HIV, is however unknown.

Viruses

Foodborne diseases caused by viruses are not well-documented in Africa. More attention has been placed on waterborne viruses such as the rotavirus (ROV), which affects millions of children on the African continent. Hepatitis A virus (HAV) and Hepatitis E virus (HEV) are endemic in most African countries; however, the true burden of disease is unknown. Both of these viruses are associated with inadequate water supplies and poor sanitation and hygiene, leading to infection and inflammation of the liver. Although norovirus (NOV) is present on the continent, its exact prevalence and circulating genotypes is unknown. Where outbreaks are reported, there is not necessarily a link to a food source but rather, person-to-person contact as was the case in 2010 in South Africa where six premature babies died in a neonatal ward in a hospital in Johannesburg, from an NOV infection. The true number and impact of NOV infections in Africa is therefore underestimated as is the case with all other foodborne viral diseases. The Ebola virus is a most unlikely foodborne virus, but was nevertheless responsible for 37 cases and 21 deaths in Gabon in 1996, when raw chimpanzee meat carrying the Ebola virus was consumed. This section will therefore focus on ROV, HAV, and HEV in Africa.

ROV

ROV is the most common viral cause of severe diarrhea among infants and young children. An estimated 527 000 deaths occur each year with more than 85% of these deaths occurring in low-income countries mainly in south Asia and SSA, and over two million are hospitalized each year with pronounced dehydration. The primary mode of transmission is via the fecal–oral route. Because the virus is stable in the environment, transmission occurs through ingestion of contaminated water or food and contact with contaminated surfaces or objects. However, in most African countries, transmission is mainly through contaminated water. Since 2006, a number of African countries have participated in a WHO ROV surveillance program, that aimed to identify the genotypes in circulation, to gather information as to the prevalence of the disease in Africa and introduce vaccination programs to protect children from ROV infections. This surveillance program has generated much useful information and where vaccinations are used, the incidence of ROV infection has dropped. Sudan, Ghana, and Rwanda introduced vaccines for the first time in 2011; however, there are many countries where vaccination programs have not yet begun. To have a significant impact on preventing ROV infections and associated disease and deaths on the continent, vaccinations are essential for all African countries.

HAV

Globally, there are an estimated 1.4 million cases of hepatitis A every year. Epidemics can be explosive in growth and cause significant economic losses as it can take weeks or months for people recovering from the illness to return to normal daily activities. The impact on formal food establishments identified with the virus, and local productivity in general, can be substantial, but is largely unknown for Africa. HAV is transmitted via the fecal–oral route, most often through contaminated water and from person-to-person. It is also transmitted via food contaminated by infected foodhandlers, uncooked foods, inadequately cooked foods, i.e., if the temperature during food preparation is inadequate to kill the virus (85 °C for 1 min is required to kill HAV), or foods handled after cooking. It is particularly frequent in countries with poor sanitary and hygienic conditions as in many African and other developing countries. Generally, mortality rate is low but in Africa, among undernourished and immunocompromised people as well as those with hepatitis B or C or underlying liver disease (such as aflatoxicosis), superinfection with HAV can cause a considerable increase in mortality rate.

In most African countries with very poor sanitary conditions and poor hygienic practices, most children (90%) have been infected with the HAV before the age of 10 years. Those infected in childhood do not experience any noticeable symptoms. Older children and adults have thus built up immunity against the virus and epidemics and outbreaks are rare. However, as socioeconomic groups vary in many African countries, an associated variation in infection rate can be expected, with a higher percentage of children in lower socioeconomic groups being seropositive. What is being observed is a higher incidence rate of hepatitis A in those higher socioeconomic groups who

have escaped childhood infection thus making them susceptible in adulthood to HAV. An effective vaccine exists which is available in some African countries, particularly in regions where sanitary and hygienic conditions are good.

As no treatment for hepatitis A exists, prevention is the most effective approach against HAV infections. Improved sanitation, food safety, and immunization are the most effective ways to prevent hepatitis A. Other important interventions include providing:

- education on good sanitation and personal hygiene, especially hand washing;
- adequate and clean water supplies; and
- proper waste and sewage disposal.

HEV

Every year there are 20 million HEV infections, over three million acute cases of hepatitis E, and 70 000 hepatitis E-related deaths, many of which occur in parts of Africa, where large hepatitis E epidemics have been reported. Displaced people living in refugee camps due to wars, political, and economical strife as well as those living in overcrowded temporary housing after natural disasters, are particularly at risk. The most common source of infection is fecally contaminated drinking water and most outbreaks occur following monsoon rains, heavy flooding, contamination of well water, or massive uptake of untreated sewage into city water treatment plants. The ingestion of raw or uncooked shellfish has also been identified as the source of sporadic cases in endemic areas, which include a number of countries in Africa. The symptoms of HEV infection are much the same as for HAV infection. Most people recover completely; however, in contrast to HAV, HEV holds a significant risk for pregnant women with a fatality rate of 10–30% among pregnant women in their third trimester of pregnancy. HEV during pregnancy has also been associated with prematurity, low birth weight and an increased risk of perinatal mortality. HEV could also be serious among persons with preexisting chronic liver disease resulting in decompensation and mortality. Chronic HEV infections have been reported in immunosuppressed people as well. The question therefore arises as to a possible synergistic effect between aflatoxicosis and HEV infections (as for hepatitis B) and the impact this may have on human health in Africa, including the impact HEV may have on the immunocompromised HIV/AIDS and undernourished population in SSA. A vaccine has been developed but is not yet available globally. Outbreaks have been reported in a number of countries in Africa with significant numbers of fatalities (Table 2). For people with liver disease and healthy people, a seroprevalence of HEV of between 2% and 60% is indicated, with higher levels found in endemic areas. In yet other cases, HEV has been suspected, but not confirmed, mainly due to insufficient investigation. Therefore, in Africa, it is possible that HEV is responsible for more outbreaks than what are reported and it may have a more significant impact on human health, than what is believed.

Similarly to HAV, there is no treatment for HEV and prevention is indicated as the best approach, with the same prevention measures being effective, with one major difference, i.e., that currently there is no immunization possible against HEV.

Mycotoxins

Mycotoxins are toxic secondary metabolites produced by toxigenic fungi that contaminate various agricultural commodities either before harvest or under postharvest conditions. They are produced mainly by three genera, i.e., *Aspergillus*, *Penicillium*, and *Fusarium*. Foodborne illnesses due to consumption of foods contaminated with mycotoxins are difficult to detect as most often mycotoxicoses are chronic conditions that manifest themselves over a period of time. It is mainly in acute cases, where death occurs rather rapidly that a mycotoxicosis is more readily identified.

Although there are many toxigenic molds, only a few mycotoxins, particularly those affecting cereal grains and groundnuts are considered dangerous to human health. For Africa, these are mainly aflatoxins and fumonisins, where aflatoxins are associated with groundnuts and cereal grains, whereas fumonisins are mainly associated with maize. However, others such as deoxynivalenol, ochratoxin A, and zearalenone have also been found in commodities in some African countries. As aflatoxins are prevalent in cereal grains and groundnuts, they are also found in animal feed, from where they can enter the human food chain, through contaminated animal-derived foods (milk and meat) as aflatoxin M_1 (AFM_1). Any processed food or beverage commodity that is produced from mycotoxin-contaminated raw materials will most likely remain contaminated as most mycotoxins are not destroyed by heat or food processing. Traditionally fermented beers, which are very popular in Africa, are commonly made from maize, wheat, sorghum, and barley, and may contain mycotoxins if contaminated grains are used as fermentable substrates.

Tropical and subtropical conditions are found in most African countries, therefore high temperatures and moisture, monsoons, unseasonal rains during harvest and flash floods can lead to fungal proliferation and production of mycotoxins. These conditions together with poor harvesting practices, improper storage, and less than optimal conditions during transportation, marketing, and processing, contribute to fungal growth and increase the risk of mycotoxin production.

The FAO has estimated that up to 25% of the world's food crops are significantly contaminated with mycotoxins. It is also claimed that 60% of Africa's grain supplies are at risk due to fungal contamination and mycotoxin production. In most countries in Africa, the presence of mycotoxins in food is often overlooked. This is due to public ignorance and lack of knowledge, lack of enforcement of regulations where they exist, absence of monitoring programs, lack of technological capacity and infrastructure, dumping of contaminated food and/or feed onto the continent, and the introduction of contaminated commodities into the human food chain, especially during food shortages, which are common in Africa. This is mainly due to drought, wars, and political and economic instability. Other contributing factors are:

- Many people in Africa have more uniform diets than their counterparts in the developed world, with little diversity; staple diets consist of maize or other cereal crops, all of which are highly susceptible to mycotoxin contamination.
- Food is often bought from local markets with less attention to quality issues.

Table 2 Outbreaks of HEV in Africa

Country	*Year*	*Number of reported cases*	*Number of reported deaths*	*Source*
Botswana	1985	273	4 (HAV and HBV excluded; no seroprevalence for HEV conducted)	Contaminated water suspected
Central African Republic	2002	222	4	Contaminated drinking water suspected
	2004	213	Not reported	Use of untreated water suspected
Chad	1983/1984	38 French soldiers	None	Not reported
	2004	1442	46	Refugee camps – contaminated river water
	2005	Not reported	>50	Refugee camps – contaminated river water
Côte d'Ivoire	1983/1984	623 cases presumed HEV	Not reported	Not reported
Democratic Republic of Congo	2006	341	13	Contaminated water suspected
Djibouti	1993	Mixed HAV and HEV; numbers not reported	Not reported	Contaminated water
Ethiopia	1988/1989	423	None	Military camps – contaminated water
Namibia	1983	201	7 (6 pregnant women)	Settlements – no potable water; many Angolan refugees
	1995/1996	600	Not reported	Contaminated water supply
Nigeria	1997/1998	10	None	Not reported
Somalia	1985/1986	>2000	87 (46% pregnant women)	Refugee camps – not reported
	1989	11 413	346 (fatality rate 13.8% among pregnant women)	Use of contaminated river water
Sudan	2004	6861	90	Contaminated water suspected
	2012	1050	26	Refugee camps –poor water quality and sanitation
Uganda (Kitgum)	2008	>10 196	169	Poor sanitation
(Pader)	2008	'thousands'	'scores'	Internally displaced people most affected – poor sanitation
(Kaabong)	2009	210	12	Internally displaced people most affected – poor sanitation
(Karamoja)	2011	908	Not reported	Inappropriate disposal of fecal matter and inadequate access to safe water

Abbreviations: HAV, hepatitis A virus; HBV, hepatitis B virus; HEV, hepatitis E virus.

- Food insecurity – the need to satisfy hunger often overrides quality aspects, offering people little choice but to consume food that is contaminated with fungi and that may contain mycotoxins.
- Much of the African population relies on subsistence farming, where improper storage conditions often prevail, which are conducive to fungal growth and mycotoxin production.

Aflatoxins

Aflatoxins are the most studied group of mycotoxins in the African context and often cause acute aflatoxicosis, which is a toxic hepatitis leading to jaundice, and in severe cases, death. Acute aflatoxicosis is an underrecognized and underreported cause of liver damage. Aflatoxin B_1 (AFB_1) is the most common type of aflatoxin found in maize and groundnuts in Africa and has been linked to human primary liver cancer. It is synergistic with hepatitis B virus infection, which is common in SSA, where the risk of liver cancer is then increased tenfold. Developing countries have a higher liver cancer incidence rate and approximately 82% of the 600 000 new cases each year occur in developing countries. Although there have been reported outbreaks of aflatoxicosis in Africa, one of the most well documented and most severe outbreaks of acute aflatoxicosis worldwide, occurred in rural Kenya in 2004. The

outbreak implicated more than seven districts and resulted in 317 case-patients and 125 deaths, due to acute hepatotoxicity. Other outbreaks occurred in 2005 and in 2006, with 30 and 9 deaths reported, respectively. The source of the 2004 outbreak was aflatoxin-contaminated home-grown maize, which was bought from local farms in the affected area and which entered the distribution system, resulting in widespread aflatoxin contamination of market maize. Children and young adults were mostly affected and there were relapses after treatment due to continued exposure to contaminated food. Major challenges that were identified for the management of the cases included inadequately equipped and poorly staffed health care facilities, inadequate logistics, and unavailability of clean food for replacement of contaminated food.

There is increasing evidence linking aflatoxins to stunting and underweight in children, resulting in cognitive impairment and increased mortality risks, both of which can lead to lower human capital with long-term economic consequences. This is of concern, considering that children may be exposed to high aflatoxin levels early in life, i.e., *in utero*, via breast milk and weaning foods commonly used in Africa. Weaning foods consist mainly of maize, but other cereal grains and in some cases, groundnuts are also used, all of which are susceptible to aflatoxin and other mycotoxin contamination. Chronic aflatoxin exposure may result in immunosuppression as well, which could interact with malaria and HIV/AIDS. The health effects of aflatoxins are complicated further by possible exposure to multiple mycotoxins, which can coexist in the same commodity, for example, aflatoxins and fumonisins in maize. Therefore, individuals may be exposed to various combinations of mycotoxins.

There are a number of countries in Africa where a strong, formal agriculture and food industry exists. This is accompanied by legislation for maximum levels of, particularly, aflatoxins in various commodities including feed, a formal infrastructure as well as good farming, storage, and distribution practices. This results in a very low prevalence of aflatoxin contamination in relevant commodities. Industry associations in these countries play a significant role in ensuring that maximum legislated mycotoxin levels are not exceeded. However, even in these countries, there are challenges, for example, ensuring that groundnuts rejected by the formal food industry do not land up in lesser-known processing facilities where peanut butter is manufactured from contaminated peanuts and sold cheaply to unsuspecting institutions and enters into the informal distribution sector. Such peanut butter contaminated with high levels of AFB_1 has been found in rural school feeding programs, with potentially disastrous effects on the health of those children.

Fumonisins

Although the focus is mostly on aflatoxins, fumonisins are problematic in Africa as well, even though they are not as well-researched on the continent. The International Agency for Research on Cancer (IARC) has classified fumonisins as class 2B carcinogens, i.e., possibly carcinogenic to humans and the Joint FAO/WHO Expert Committee on Food Additives has recommended a tolerable daily intake for FB1, FB2, and FB3, alone or in combination, of 2 μg per kg bodyweight per day. There have been no documented fumonisin-related outbreaks in Africa and no country in Africa has fumonisin standards for food. Fumonisin has been associated with human esophageal cancer in parts of South Africa, where subsistence farming of maize occurs. It also reduces the uptake of folate and has therefore been implicated in a high incidence of neural tube defects in rural populations known to consume contaminated maize. Some studies on various local grain-based commodities in Africa have shown very high levels of fumonisin, up to 1830 μg kg^{-1}.

Mycotoxin contamination is not only a burden in Africa and other developing nations but it also significantly affects agricultural products in the developed world. It is estimated to cost the USA's grain industry an annual loss of US$2 billion. However, the application of modern agricultural practices including optimal drying and storage practices and the establishment of a legislatively regulated food processing and marketing system, have greatly reduced mycotoxin exposure in these populations. At the mycotoxin contamination levels, generally found in food products traded in these market economies, adverse human health effects have largely been overcome.

Some countries in Africa have addressed the regulation of mycotoxins in food and feed. In 2003, FAO listed 15 out of 54 countries having specific mycotoxin regulations. These countries cover approximately 59% of the inhabitants of the continent and most of the mycotoxin regulations concern aflatoxins. The fact that countries have no specific regulatory limit for mycotoxins does not mean that the problem is ignored. Several of these countries recognized that they have problems due to mycotoxins and that regulations should be developed. However, the establishment of mycotoxin regulations alone, in the absence of a supporting structure and associated activities will have limited effects in enhancing food safety and health protection, especially in countries where farmers grow agricultural produce for their own consumption via subsistence farming, which is the case in many African countries.

A multidisciplinary approach is key to a sustainable solution for the mycotoxin issue in Africa and needs to be viewed, in the overall context of local food safety, health, and agricultural issues. Collaborative efforts between farmers, researchers, and governments are required to prevent or at least significantly reduce the occurrence of mycotoxins in foods. Farmers should participate in research programs and governments should strengthen their food control and health care systems and provide a legislative environment in which farmers can operate. This legislative environment should include educating farmers and consumers as well as creating awareness, conducting surveillance, and regular monitoring of commodities and enforcing regulations. Novel, simple, cheap, yet accurate methods for detecting the presence of mycotoxins in foods, in particular aflatoxins and fumonisins in cereal grains and groundnuts, at levels detrimental to human health, are needed for Africa. Typical educational programs for farmers should focus on implementing good pre- and post-harvest practices including proper drying techniques, maintaining correct storage facilities, and ensuring that grains and oilseeds are not exposed to moisture during transport and

marketing. Promotion of the Hazard Analysis and Critical Control Point (HACCP) System, where feasible, should also be promoted. In this regard, the South African government has made HACCP mandatory for all processors of groundnuts, i.e., peanut sorting and grading facilities as well as peanut butter manufacturers, since November 2010 due to the aflatoxin problems associated with peanuts in South Africa.

The use of physical methods, including cleaning, separation of screenings, winnowing, washing, aqueous extraction, crushing, dehulling, and milling, has been shown to be effective in reducing mycotoxins in cereals. One of the most effective ways of minimizing aflatoxin contamination in peanuts is physical separation of contaminated, moldy, shriveled, discolored, or insect-infested seeds from sound kernels. Effective and simple drying and storage methods include drying on mats and sun drying and subsequent storage of bags elevated off the ground together with pest control. At preharvest level, the time of planting, the use of varieties less susceptible to mycotoxin contamination and the timing of the harvest are all useful interventions. Other interventions include enterosorption and chemoprotection, both of which are expensive and therefore difficult to implement in developing countries. There is also uncertainty as to the efficacy and safety of these two interventions. It is important to keep in mind that the appropriate intervention will depend on the crop and especially the country. Because of this, before implementing an intervention method, consideration is needed toward the sustainability, cultural acceptability, economic feasibility, ethical implication, and overall effectiveness of the proposed method(s).

Awareness campaigns have proven to be highly effective, even more so when organizations such as FAO and WHO work together with countries' governments to raise awareness and educate people on the issues and prevention methods. To be as effective as possible, multiple means of spreading the information must be used to ensure that it gets to all people, even those in remote rural areas.

Mycotoxins are clearly one of the more important issues in Africa. It is therefore evident that proper control of mycotoxin contamination is dependent on the concerted efforts of all role-players along the food production and distribution chain.

Street Foods

Street foods or street-vended foods are foods and beverages prepared and/or sold by vendors in streets and other public places for immediate or later consumption without any further processing or preparation. This definition includes fresh fruits and vegetables that are sold outside authorized market areas for immediate consumption. Street foods hold many benefits:

- Socioeconomically, they form a major component of the informal food distribution sector, providing economic support to small farmers as an outlet for rural produce, providing income and employment for a large number of people, especially women in poverty in Africa and providing an opportunity for development of business skills with very little capital investment.
- They offer a most affordable way of obtaining a nutritionally balanced meal outside the home for many people with limited means, thereby contributing to food security and possibly even to micronutrient supplementation, provided the street foods are safe.
- They are easily accessible.
- They are a source of attractive and different food for tourists.

Consumers of street foods have cited a number of reasons, besides affordability, for purchasing street foods in Africa. These include tastiness, nutritional value, convenience, cleanliness, and interpersonal trust between vendor and consumer. Even though cleanliness was included in consumer considerations in purchasing street foods, specific matters regarding hygienic practices and food safety were not included. Street foods account for a significant proportion of economic employment, daily urban food consumption of millions of low- and middle-income consumers, and for generating millions of US dollars per annum for this industry across Africa.

Even though street foods offer tremendous benefits, they are also a potential health risk as street-food vendors are often poor, uneducated, and untrained in food hygiene and sanitation. The global food crisis has worsened an already problematic food situation in many African countries and as a result, satisfying hunger often overrides the consideration of food safety and quality. The affordability of food security then extends into the affordability of food safety, i.e., how much food safety can be bought by a consumer who has less than US$1 per day to spend on food?

Other contributing factors to the questionable safety of street foods include lack of infrastructure such as clean, running water, unhygienic environment, lack of toilet and handwashing (with soap) facilities, inadequate waste facilities, improper waste management where facilities do exist, undercooking of food, cross-contamination due to bad hygiene practices, lack of refrigeration and other hygienic storage facilities for raw and cooked foods, lack of protection of food from insects and flies, dirty/improperly washed utensils, and insufficient resources for inspection and laboratory analysis. This general lack of hygiene can result in contamination with a variety of microbial pathogens, including fecal–orally transmissible parasites, viruses, and bacteria.

The prevalence of foodborne pathogens in street-vended foods of a country affects not only the local population but also potentially the cross-border traveler which could lead to spread of the pathogen once the visitor returns home.

The types of street foods that are prepared and sold in different African countries are as diverse as the prevailing climate, cultures, and religions themselves. Each of these foods may hold a variety of risks, not all of them necessarily microbiological in nature, although microbiological hazards are the most common ones identified. Foods range from different cereal-based porridges and traditionally fermented beers, all of which pose a potential risk for the presence of mycotoxins; a variety of cheeses often made from unpasteurized milk originating from nonstate veterinary inspected herds of cattle, sheep, goats, and camels thereby posing potential microbiological risks; various meat (including bush meat) and

poultry dishes often originating from animals infected with parasites and bacteria and/or slaughtered under unhygienic conditions or in nonapproved facilities; vegetables and fruits consumed either cooked or raw, with raw vegetables (and sometimes certain fruits) posing a microbiological risk if irrigated or washed with contaminated water; fish dishes where the fish may be infected with parasites or contaminated with high levels of histamine (where relevant) and bacterial pathogens; fruits and vegetables that may contain excessive levels of registered pesticides (above the accepted maximum residue limit) or the use of nonregistered or illegal (banned) pesticides; nuts of which their origin is either unknown or known and not well-understood and could therefore pose a mycotoxin risk; herbs and spices that may be contaminated with bacterial pathogens, thereby becoming a potential source of contamination in the final product, particularly if not cooked well or eaten raw; the reuse of cooking oil (particularly polyunsaturated oils such as sunflower seed oil which are easily oxidized by repetitive and prolonged use at high heat) when deep frying foods, which may result in diarrhea and other health problems in some individuals; extruded snacks which are often sourced from dubious suppliers, who usually sell these cheaply, with consequent potential risk of the presence of either illegal colorants or permitted colorants in excess of maximum regulated levels. Extruded snacks attract mainly children who are at higher risk of suffering ill-effects from such practices.

Finally, natural toxins associated with specific crops may be sold as street food and thus pose a significant health risk in some African countries. A case in point is the consumption of insufficiently processed bitter cassava root, which can cause konzo, an irreversible paralysis of the legs and leaves people unable or struggling to walk. It is caused by the ingestion of cyanide-containing compounds from the bitter cassava root when it is insufficiently processed, combined with a protein-deficient diet, especially proteins that provide sulfur-containing amino acids that clear the body of cyanide. Drought conditions cause an increase in cyanide to high levels, leading to the disease. Displaced people, because of war, are also at high risk of ingesting such cassava. Six countries in Africa, as far as is known, are affected by konzo, particularly the DRC and more recently, Angola. The true incidence of konzo in Africa is unknown and is believed to be underestimated, with unofficial estimates at 100 000 people affected in the DRC in 2000 due to the civil war in that country, with many affected refugees in the Central African Republic.

The presence of pathogenic bacteria in street foods in Africa is a well-researched topic. This is not the case for foodborne parasites and even less so, for foodborne viruses (see Sections Parasites and Viruses). Common bacteria and bacterial groups found include *L. monocytogenes*, *Listeria innocua*, *B. cereus*, *Salmonella* including *Salmonella arizonae*, *Salmonella.* Typhimurium, *Salmonella biafra*, *Salmonella braenderup*, and *Salmonella* Weltevreden, *Sh. flexneri*, *Sh. sonnei*, *Citrobacter freundii*, and other *Citrobacter* spp., *Enterobacter cloacae*, *Cronobacter sakazakii*, *St. aureus* including presence of enterotoxin where tested for, *Aeromonas hydrophila*, *E. coli*, *Cl. perfringens*, *Vibrio metschnikovii*, *Campylobacter* spp., *Alcaligenes* spp., *Klebsiella* spp., *Pseudomonas aeruginosa*, coliforms, micrococci, coagulase-negative staphylococci, streptococci.

Regardless of the numerous pathogens that have been isolated from street foods in Africa, very few foodborne outbreaks have been specifically linked to street foods. The main reasons for this are a severe underreporting of foodborne disease outbreaks; lack of laboratory capacity in identifying responsible pathogens and/or their toxins; inadequate resources in the responsible ministries to conduct inspections and monitoring programs; lack of appropriate guidelines, knowledge and practices in obtaining and transporting samples of food and clinical specimens in the correct manner to maintain the integrity of the samples; lack of capacity and knowledge in conducting a full epidemiological analysis.

The abovementioned is, however, not unique to street-vended foods.

One very important, but often overlooked fact is that street food vendors are quite capable of producing safe food. Studies have shown that regardless of a visibly unhygienic environment in certain African countries and regions within those countries, no significant pathogens have been isolated from selected foods. The main reasons for this are:

- the practice of basic hygiene – more so among women than men;
- the purchase of fresh raw food for the day's needs, every morning from a local, formally registered retailer close to the vendor, cooked and consumed the same day;
- the low amount of leftovers at the end of the day, which are taken home and consumed by the family, avoiding the practice of returning the following day with cooked food that has stood unrefrigerated for many hours at warm temperatures and being resold without adequate reheating; and
- access to toilets and potable water.

It is therefore clear that basic hygienic and other good practices are essential to provide safe street foods and are possible in Africa. Such good practices should, however, be supported by targeted training programs to improve the safety of street-vended foods. An example of a successful integration of the two above-mentioned components to improve the microbiological safety of street foods is provided below:

An FAO funded project on the microbiological safety of street foods in the Gauteng Province, South Africa was conducted in 1999–2000, after which three workshops were held in three main provinces to provide the local health authorities with results of the project. Shortly afterwards, the National Department of Health developed a training manual on safe practices for street food vendors for use by local health authorities. A number of these authorities in different provinces subsequently developed specific programmes for street food vending in their jurisdictions. The Ethekwini Metropolitan Council (in Kwa-Zulu Natal Province) decided to integrate the informal economy into its long-term plan to promote its economic development. Street food vendors now operate in allocated areas, thus minimizing the problem of public nuisance in Durban City and surrounding towns. The Metro ensures that prior to them being permitted to operate a food establishment, street food vendors receive essential food hygiene training, which enables them to comply with minimum hygiene regulations. A certificate of acceptability is then issued according to national food hygiene regulations, which allows for better control and coordination of the sector within the Metro. A similar success story applies to the Ehlanzeni District Municipality in the Mpumalanga Province. Here, local health authorities provided basic

services such as structures, cleaning services, running water, wash basins, storage facilities and toilets for the vendors, leading to safer foods and improved regulation. Most other provinces have since followed suit.

Improving the safety of street-vended foods in Africa poses great challenges, but the risk factors are well-identified and the solutions are clear. It is important for countries to share results of any studies conducted with all relevant stakeholders to develop appropriate strategies for improving the safety of street foods and to continue promoting the sale of street-vended foods. A successful transition from street-food vending being perceived as a nuisance and as unsafe by national health authorities is possible, when these authorities instead promote, support, and improve the safety of street-food vending. Experiences in South Africa have shown that success is possible provided food control authorities, street-food vendors and all other stakeholders, such as consumer groups and academic structures, collaborate to improve the sector.

Conclusion

The true prevalence of food- and waterborne disease in Africa is unknown and severe underreporting occurs. The continent undoubtedly faces numerous challenges in improving the health status of its population. Food and water are not only two essential requirements for life but access to safe and nutritious food is a basic human right. Although not all health-related matters on the continent are caused directly by unsafe food and water, a significant number are. Exacerbating the situation, are a very young population with millions of children under the age of 5 years and more than one quarter of 1 billion people on the continent immunocompromised due to HIV/AIDS and undernourishment.

Whose responsibility is food and water safety in Africa and what needs to be done? Resolving these matters is not an easy task and will involve a multidisciplinary and multiagency approach. The problem is political, economical, social, educational, and scientific and all of these aspects need to be addressed simultaneously and in a coordinated fashion to make a real and sustainable difference in Africa.

International agencies continue to support many projects in Africa, however, Africa needs to take responsibility for improving the lives of its own people. In this regard, the African Union (AU) has undertaken a number of initiatives to act on hunger, poverty alleviation, animal diseases and zoonoses, aflatoxicosis, enhanced and more coordinated responses to Codex Alimentarius Commission meetings, particularly in the areas of pesticides, food hygiene, and food contaminants. Of particular importance is the meeting held by the AU in Rwanda in November 2012, where the groundwork for an African food safety authority was laid. At country level, food safety capacities in all respects need to be strengthened. Political will is essential as this will drive allocation of resources to important areas addressing food and water safety in countries. An event occurred in 2010 in South Africa, i.e., the World Cup Soccer, which had a most remarkable outcome. A heightened awareness of foodborne illness was created and enhanced surveillance and numerous awareness campaigns were conducted by public and private entities, which included the country's health facilities, prior and during the event. This awareness has remained 2 years after the event and currently 2–12 foodborne diseases are being reported monthly in the country. In addition, a website has been developed by health agencies where the public can report a suspected foodborne illness. It acts as a nationwide foodborne disease portal and forms part of a national database registry that can help determine if the reported illness is part of a larger foodborne disease outbreak. To underscore the escalation of importance of food safety and foodborne disease in South Africa, the National Department of Health published guidelines for the management and control of infectious foodborne diseases in the country in 2011. The guidelines are aimed at assisting health care workers with practical, user friendly, and concise information on the diagnosis, treatment, and reporting of foodborne diseases with the ultimate goal of prevention, control, and obtaining accurate estimations of disease burden in communities.

Africa should use the momentum brought by international events being increasingly hosted on the continent to heighten awareness and implement systems to improve relevant infrastructure and the management of foodborne diseases.

See also: Bacteria: *Vibrio cholerae*. Characteristics of Foodborne Hazard and Diseases: Cost of Foodborne Diseases. Disciplines Associated with Food Safety: Food Microbiology; Food Safety Toxicology; Food Virology; Parasitology. Foodborne Diseases: Foodborne Diseases and Vulnerable Groups; Prevalence of Foodborne Diseases in Australia and New Zealand; Prevalence of Foodborne Diseases in Europe; Prevalence of Foodborne Diseases in North America; Prevalence of Foodborne Diseases in South East and Central Asia; Prevalence of Foodborne Diseases in Western Pacific Region. Institutions Involved in Food Safety: Trust in Animals and Food Safety (TAFS) Forum. Mycotoxins: Aflatoxins; Fumonisins; Mycotoxins – General; Ochratoxin A. Natural Toxicants: Naturally Occuring Toxins of Plant Origin. Protozoa: *Cryptosporidium* spp.; *Entamoeba histolytica*; *Giardia lamblia*; *Toxoplasma gondii*. Risk Analysis: Estimating the Burden of Foodborne Disease. Viruses: Hepatitis A Virus; Hepatitis E Virus

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FOODBORNE DISEASES

Prevalence of Foodborne Diseases in North America

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Glossary

Cost-of-illness An approach toward the economic evaluation of the burden of illness that collates, evaluates, and assesses the individual cost elements of illness. For foodborne diseases this usually includes healthcare-related costs, lost production, and value-of-life estimates.

Disease notification Regular reporting by physicians of new illnesses due to an agreed list of infections. Reporting is usually done to a senior public health official at the jurisdictional or national level.

Emerging disease Any infection or toxin that is becoming recognized as a cause of human illness. This includes agents that are newly identified as causing human illness, such as prions, or agents that are recently recognized as being significant pathogens as they become more common or laboratory technology advances make it easier to identify them in human specimens, such as *Campylobacter*.

Foodborne disease Any disease of an infectious or toxic nature caused by or thought to be caused by consumption of food or water.

Phage type A subdivision of a species of bacteria that depends on whether the bacterial cell is killed by certain bacterial viruses (bacteriophages). Individual strains or phage types will reflect the susceptibility of a particular bacteria to a set panel of bacteriophages.

Surveillance The systematic, ongoing collection, collation, and analysis of data, and the dissemination of information based on that data, usually for public health purposes when done in the context of human disease.

Toxin A naturally occurring poison produced by microbes, or naturally occurring in fungi, plants, and animals. Some microbial toxins, such as botulinum toxin, are preformed in the food under certain conditions; other microbial toxins, such as those produced by enterohemorrhagic *Escherichia coli* (EHEC), are formed in the intestine after the bacteria have been introduced. A number of toxins linked to seafood are produced by phytoplankton and are concentrated in the flesh of the shellfish (paralytic shellfish poisoning) or fish (ciguatoxin) during feeding. A common fish toxin, scombrotoxin, is a histamine-like chemical produced by the action of naturally occurring bacteria in poorly temperature-controlled caught fish.

Introduction

In Canada and the United States (US), foodborne diseases are important causes of morbidity and of potential economic loss. The importance of food safety and security is indicated by government and commercial infrastructures for regulating and protecting food and international trade in food animals, their feedstuffs and products, produce, and food products. Historically, each country has developed legislative requirements, beginning in the US, for example, in 1906 with the Federal Food and Drug Act, and monitoring and enforcement agencies including the US Food and Drug Administration (FDA), and the Canadian Food Inspection Agency. The evolution of legislative approaches to regulate food safety and establish standards for quality, signaled recognition of national government responsibility for safety and quality, and paved the way for the establishment of mechanisms for monitoring food quality and foodborne illness. These national structures work with international frameworks, including trade agreements and the international disease reporting requirements of the World Health Organization.

Although over 200 agents of foodborne disease are recognized, this article focuses on microbiological agents, the most commonly identified cause of foodborne human illness in North America. The significance of individual microbiological pathogens varies over time, with season and geography, and this article pays attention to those most commonly reported or which have emerged as important causes of morbidity or mortality since the 1960s. A relatively small number of microbes are responsible for most cases of reported illnesses including historically recognized pathogens such as *Salmonella*, *Staphyloccocus*, *Streptococcus*, *Clostridium*, and *Bacillus* as well as microorganisms more recently recognized as important including *Campylobacter*, enterohemorrhagic *Escherichia coli* (EHEC), and *Listeria*; protozoal parasites such as *Cyclospora*; and viruses, including norovirus.

Information on Foodborne Diseases

Collection of data on foodborne diseases has, until relatively recently, depended on mainly passive systems for collating

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information on individual cases or outbreaks using jurisdictional and national notification and laboratory reporting systems. Data on foodborne disease at the national level in the US and Canada originated in the early decades of the twentieth century. Reporting in the US was initiated in 1923 to monitor the impact of contaminated milk. Reporting by the US on outbreaks of foodborne and waterborne diseases was added in 1938, and the Salmonella Surveillance Activity was established in 1962 to collate information from States, the US Department of Agriculture and the FDA.

In 1924 national notification was initiated in Canada for a limited number of diseases including typhoid and paratyphoid fever, shigellosis, and poliomyelitis. Subsequently, amebiasis and hepatitis A (1927), brucellosis (1928), trichinosis (1929), botulism (1933), salmonellosis (1958), cholera (1974), giardiasis (1983), listeriosis (1990), and verrotoxigenic *E. coli* (1990) were added. National data for Canada, including case and outbreak information and, from 1973, reports on foodborne, waterborne, and enteric disease incidents, summarized reporting incidence and documented reporting trends. Supplementary morbidity and mortality information is based on passive, national, or jurisdictional information systems for collecting data on hospitalizations and deaths.

These historical approaches to surveillance of foodborne diseases have inherent methodological issues that lead to underreporting and late reporting of the actual cases of disease; and removal and addition of pathogens from lists of notifiable diseases interrupt reporting trends. For most foodborne infections and intoxications, only a small proportion of cases are reflected in national statistics, although this varies with the microorganism. This low proportion reflects issues around seeking of medical care, physician testing and reporting, laboratory technology, and reporting policy.

Evolution of active approaches to foodborne disease surveillance is driven by the public health imperative to protect consumers and to reduce the burden of illness. This requires early recognition of, and response to, outbreaks and the need to improve understanding of foodborne disease risks. This itself is a response to changing trends in foodborne diseases and emergence of new pathogens that reflect a number of underlying factors (Table 1). During the 1990s, it was realized that the US and Canada needed to restructure their foodborne disease surveillance systems to address these new and emerging issues. Two areas of innovation have enabled the development of more active surveillance systems. First, developments in diagnostic technologies, which provide rapid results that can be easily digitized, and information technology that facilitates rapid networking of data. Second, evolution of surveillance from a linear process for analysis of reporting trends to an integrated process for linking reporting trends with identification and assessment of risk (Table 2).

Burden of Disease

Recognition that the incidence of foodborne diseases is significantly higher than indicated by national statistics, and a shift to a risk-assessment approach to identifying risks and priorities has led to efforts to more accurately estimate the burden of these diseases. Estimates in the 1980s suggested 2 million cases annually in Canada with associated costs-of-illness of over CAN$1 billion for health care, lost production and value of life. Estimated cases and costs in the US during the same period ranged between 6.0 million cases with costs of US$ 5.8 billion and 12.6 million cases with costs of US$ 10.6 billion. Further estimates for the US have varied as high as 24 to 81 million cases annually and showed notable variation in estimated hospitalizations and deaths (Table 3).

In 1999 it was estimated that 76 million cases of foodborne disease occurred in the US annually, 13.8 million due to known pathogens (Table 3), with 325 000 hospitalizations and 5000 deaths. Noroviruses (previously Norwalk-like viruses), *Salmonella*, and *Campylobacter* species were identified as the most common foodborne illnesses, accounting for 91% of cases due to known pathogens. These estimates reflect several years' data on reported infections and explained, and largely conservative, extrapolations to give national figures. Data for the US were extrapolated to provide estimates of the economic burden of disease and estimates for acute gastroenteritis which suggested 195 million episodes of illness annually in the US (Table 4). Similar estimates for *Salmonella*-related illness indicated 1.4 million cases annually.

An equivalent study for foodborne diseases has not been published for Canada since the 1980s, but estimates for three pathogens were published in 2006. Based on underreporting rates derived from studies of acute gastroenteritis, conservative estimates for annual cases of verotoxigenic *E. coli* (21 000), *Salmonella* (75 000), and *Campylobacter* (283 000) were calculated. Extrapolating results from studies in two Canadian

Table 1 Underlying factors influencing reported trends in foodborne diseases

- Increased scale of production and distribution from local to mass production at centralized facilities and mass transport and distribution networks.
- Changing husbandry practices to include industrialization of cattle, swine, and poultry production at one end of the spectrum, to production using 'organic' or traditional approaches to maintain produce or livestock free of contaminants such as antibiotics and pesticides at the other end.
- Changes in consumer preferences and food consumption patterns.
- A changing global food economy with, for example, access to fresh produce and fruit year-round via global markets.
- Advertising, and nutritional and dietary pressures on consumers to eat certain specific foods or commodities because they are cheap, easy to access, socially enabling, address obesity, imply health benefits, etc.
- Changes in food processing and packaging technologies and changes in demand for novel products.
- Improvements in technologies for the detection of pathogenic microorganisms, e.g. PCR PFGE and DNA sequencing, and sharing of information.

Abbreviations: PCR, Polymerase chain reaction; PFGE, pulsed field gel electrophoresis.

Table 2 National foodborne disease surveillance systems: USA and Canada

USA and Canada pre1990	
Notifiable disease reporting	Passive state (USA) or provincial/territorial (Canada)
Laboratory based surveillance	Reporting
USA since 1990	
FoodNet	Active sentinel – surveillance (10 sites)
PulseNet	National (and international) sub typing network for bacterial pathogens
eFORS[a]	Internet-based national outbreak reporting
NARMS[b]	Monitors for antimicrobial resistance (national)
Canada since 1990	
NESP[c]	Active laboratory reporting of key enteric pathogens
CIOSC[d]	Active internet-based national reporting of suspect or known outbreaks
CIPARS[e]	Monitors for antimicrobial resistance in selected pathogens
C-EnterNet	Active sentinel surveillance (1 site)
PulseNet	As above and linked with the US system

[a]Electronic Foodborne Outbreak Reporting.
[b]National Antimicrobial Resistance Monitoring System.
[c]National Enteric Surveillance Program.
[d]Canadian Intergraded Outbreak Surveillance Center.
[e]Canadian Intergraded Program for Antimicrobial Resistance.

Table 3 Examples of published estimates of annual cases[a] and deaths due to foodborne disease in USA and Canada

Author	*Estimated cases (millions)*	*Estimated deaths*
USA		
Archer and Kvenberg, 1985	24–81	
Bennett *et al.*, 1987	6.5	9000
Todd, 1989b	12.5	523
Mead *et al.*, 1999	76	5000
Canada		
Todd, 1989a	2.2	31

[a]Canadian Integrated Program for Antimicrobial Resistance.

Table 4 Population-based estimates of acute gastroenteritis in USA and Canada[f]

Country	*Annual incidence rates*	*Estimated annual cases (million)*
USA[a,b]	0.72–0.79	195
Canada[c,d,e]	1.17–1.3	40

[a]Imhoff *et al.*, 2004.
[b]Mead *et al.*, 1999.
[c]Sargeant *et al.*, 2007.
[d]Majowicz *et al.*, 2004.
[e]Thomas *et al.*, 2006b.
[f]The range of estimates may include diarrhea with or without vomiting and may include a downward adjustment for cases with concomitant respiratory symptoms.

Provinces indicated approximately 40 million episodes of acute gastroenteritis annually (Table 4) of which a third was estimated to be foodborne. These estimates suggested slightly higher rates for *E. coli* and *Campylobacter* in Canada compared with the US, although the differences may simply reflect the approaches used.

National Trends

Human exposure to foodborne diseases is multifactored. Reported trends are driven by intrinsic factors relating to the microbiological quality of the food, and extrinsic factors, such as ambient temperature and preparation standards that act to amplify the microbiological load. Age and gender factors suggest that the highest incidence of foodborne disease occurs in the very young and elderly. This pattern may be influenced by the association of a specific agent and food preferences linked to age or the individuals' immune status. Thus, in an analysis of *Salmonella*, *Campylobacter*, *E. coli*, and *Shigella*, in Canada, young children and the elderly were more often reported, except for *Shigella* where cases declined in the elderly. An increase observed in 20–40 year olds for *Salmonella*, *Shigella*, and particularly *Campylobacter* infections, was linked to young adults leaving home for the first time and likely less aware of food handling and purchasing issues. For pathogens such as *Vibrio parahaemolyticus* or *Vibrio vulnificus*, cases are more likely in adults consuming seafood, whereas more children have severe symptoms with EHEC infections due to the pathogenesis of the organism. In this section, recent trends

are discussed in the context of factors that have influenced reported incidence.

Historically Established Foodborne Diseases and Preparation Practices

A relatively small number of foodborne diseases are responsible for the majority of reported illnesses (Table 5). Historically established causes include *Bacillus cereus*, *Botulism*, *Clostridium perfringens*, *Staphylococcus aureus*, *Shigella*, and *Streptococcus*. Trends in reporting of *B. cereus*, *C. perfringens*, *S. aureus*, and streptococcal infection are based primarily on cases identified in association with reported outbreaks in Canada and the US and likely underestimate disease incidence. The researchers compensated for this by using a multiplication factor of 10 in the 1999 estimates for the US. No recent comparable estimates exist for Canada. These pathogens are associated with food preparation practices including handling, storage, and temperature control. Declining reporting trends for these diseases possibly reflects the impact of improved food safety awareness, particularly in the catering industry.

Table 5 Estimated annual foodborne illnesses due to known pathogens in the USA

Disease or agent	*Estimated illnesses*
Bacteria	
Bacillus cereus	27 360
Botulism	58
Brucellosis	777
Campylobacter	1 963 141
Clostridium perfringens	248 520
E. coli O157:H7	62 458
E.coli non O157:H7 STEC	31 229
Other *E.coli*	79 420
Listera monocytogenes	2 493
Salmonella typhi	659
Salmonella, nontyphoidal	1 341 873
Shigella	89 648
Staphylococcus aureus	185 060
Streptococcus	50 920
Vibrio (incl. *V. cholerae*)	5 218
Yersinia enterocolitica	86 731
Sub total	4 175 565
Parasitic	
Cryptosporidium parvum	30 000
Cyclospora cayetanensis	14 638
Giardia lamblia	200 000
Toxoplasma gondii	112 500
Trichinella spiralis	52
Sub total	357 190
Viral	
Norwalk-like viruses[a]	9 200 000
Rotavirus	39 000
Astrovirus	39 000
Hepatitis A	4 170
Sub total	9 282 170
Grand total	13 814 925

[a]Now mostly classified as Noravirus.

Source: Adapted from Mead PS, Slutsker L, Dietz V, *et al.* (1999) Food-related illness and death in the United States. *Emerging Infectious Diseases* 5: 607–625.

Reported cases of botulism since the 1980s (mainly toxin types A, B, and E) have rarely exceeded 50 cases annually in the US, with the highest incidence in Alaska, and 20 cases annually in Canada. Although sporadic cases and outbreaks associated with home food preparation and traditional aboriginal peoples' food account for a significant proportion, a number of outbreaks were associated with commercial or restaurant food items since the 1970s including: baked potatoes (skordalia), bean dip, bottled mushrooms, burrito, canned cheese sauce, chili sauce, clam chowder, garlic in oil, salsa, sauteed onions, and seafood products.

Historically Established Foodborne Diseases and Specific Interventions

The targeted elimination of tuberculosis (TB), due to *Mycobacterium bovis*, and brucellosis (*Brucella melitensis*) in cattle has significantly reduced their incidence in humans in the US and Canada over the last 40 years. An estimate of 1554 cases of brucellosis annually in the US has been made; currently less than 150 human cases are reported each year although increased incidence is noted since the late 1990s. TB infection linked to milk is now rare in both countries and when TB or brucellosis are recorded they are usually associated with travel to countries where the diseases are endemic in cattle, sheep, and goats, or the importation of raw milk products from these sources. The widespread adoption of milk pasteurization, in addition to contributing to the decline in human brucellosis and *Mycobacterium bovis* infection, helped to reduce milkborne typhoid, nontyphoidal *Salmonella* and streptococcal infections following its widespread adoption in the US and Canada by the middle of the twentieth century.

Improved Sanitation

The decline in typhoid and cholera during the early twentieth century reflected improvements in sanitation and reduced exposure to human feces via food and water. The US rate for typhoid declined from approximately 35 (per 100 000 population) in the 1920s to an insignificant rate today. Similarly, an average of over 3000 cases a year notified between 1924 and 1929 in Canada dropped to under a 100 cases a year from the 1970s. Most typhoid cases currently recorded are associated with travel to countries where typhoid remains endemic.

Exposure to human feces continues to play an important role in the transmission of enteric viruses, many of which are foodborne. Most enteric viruses are obligate human pathogens, and over 100 different types can be identified in human sewage including enteroviruses, hepatitis viruses, and gastroenteritis viruses. Noroviruses (Norwalk-like viruses) and hepatitis A are most commonly associated with reported human infection. Researchers have attributed 80% of the estimated 38.6 million foodborne illnesses annually in the US to viral causes, mostly noroviruses. Previously these infections would have been recorded as "unknown cause" and the apparent emergence of these viruses largely reflects improvements in diagnostic approaches in the 1990s.

Industrialization of Meat and Poultry Production

Meat production over the last four decades has seen unprecedented levels of intensive farming and centralized processing and distribution. This has significantly contributed to trends in the incidence of key foodborne diseases (*Salmonella*) and may have mitigated for the emergence of others (*Campylobacter, E. coli*). Intensive farming of cattle, swine, and poultry (both meat and egg production) have presented the farming industry with significant challenges to maintaining the health of livestock and the safety of products derived from them.

Feeding the demand for relatively cheap food has arguably moved the control of pricing from the farmer to the retailer and encouraged practices to maximize gain by the producer, including prophylactic use of antibiotics and recycling of nonuseable offal products into feed for the next generation. This later issue is specifically associated with the emergence of BSE in cattle and the human form of this prion disease, vCJD, in the UK in the late 1980s and early 1990s. The US and Canada have been relatively untouched to date with only 21 cases of BSE and 3 cases of vCJD. The emergence of BSE had profound impacts on cattle feed production, on the movement of cattle across borders, and on public scrutiny of the food supply.

Centralized processing and distribution has resulted in the potential for outbreaks to affect huge numbers of consumers. Probably the largest ever documented outbreak of foodborne illness in the US affected an estimated 197 000 people with *Salmonella* infection in 1995. The illness was linked to widely distributed, improperly pasteurized milk from a dairy in Illinois, USA.

The link between intensive meat and poultry production and increased human health risk is arguably supported by a polarization in *Salmonella* reporting trends. The decline of typhoid in the last century was followed by an increase in reported nontyphoidal *Salmonella* infection. Human cases based on laboratory identifications rose significantly from the 1960s to the 1980s. Nontyphoidal *Salmonella* infections are currently one of the most commonly reported foodborne diseases, and annual trends have remained within 40 000 to 45 000 laboratory confirmed cases in the US and 5000 to 7000 in Canada since the mid-1990s (Figure 1). Although *Salmonella* are ubiquitous in the intestinal tracts of animals, and over 2400 serotypes are described, only a handful are associated with most human illnesses.

Annually, several hundred *Salmonella* serotypes are identified from human cases, but only four or five account for upwards of 50% of reported infections. *Salmonella enteritidis* and *Salmonella typhimurium* are the most frequently reported, whereas *Salmonella heidelberg* and *Salmonella newport* were also in the top four serotypes in most years since 1995 in the US. Canada shows a similar pattern except that *Salmonella hadar* replaces *S. newport* in the four common types in most years. A summary of Canadian *Salmonella* isolates from 1996 to 1999 showed that the serotypes most common in humans were also common in cattle and poultry. Nonhuman isolates of *S. typhimurium* were predominantly from bovines, whereas *S. enteritidis*, *S. heidelberg,* and *S. hadar* were predominantly from poultry and *S. enteritidis* almost entirely from chicken and egg.

The increase in *S. enteritidis* in the US since the early 1970s, described as a "national epidemic," is one of the most important trends in *Salmonella* reporting in recent decades, reflecting international spread of a single serotype in chickens. Unlike cattle, where transmission of *Salmonella* is likely from animal to animal in crowded and stressful conditions, the spread of *S. enteritidis* in the late 1980s and early 1990s, was associated with transovarian transmission via infected eggs, possibly from limited breeding stock to poultry flocks and then by bird to bird transmission. Human infection at the same time was linked to infected eggs and poultry meat. The implication of infected breeding stock is further supported by the limited number of strains involved, suggesting a narrow genetic lineage. Declining reporting of *S. enteritidis* infection in the US from 1996 may reflect efforts to reduce infection of flocks as well as advice on reducing amplification and spread during retailing, storage, and food preparation.

Salmonella serotypes of human importance have multiple subtypes useful in epidemiological research, outbreak investigations and in tracking the evolution of characteristics such as antimicrobial resistance. These are demonstrated

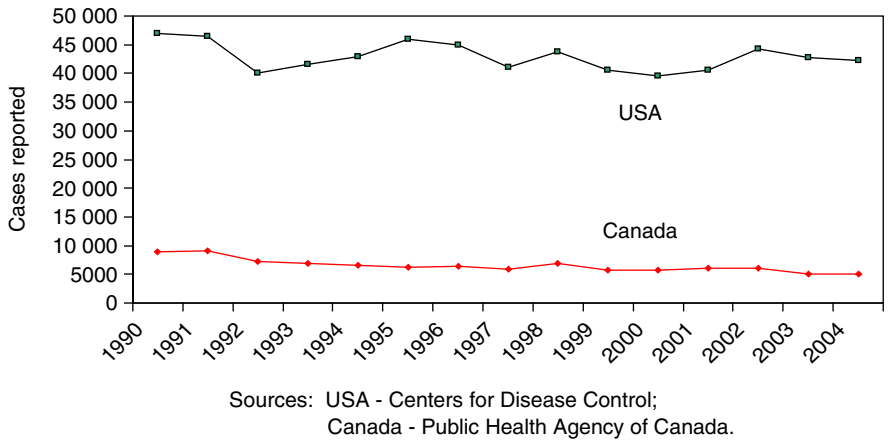

Figure 1 *Salmonella* reports by year for USA and Canada: Notifiable diseases data. *Source*: US Centers for Disease Control; Public Health Agency of Canada.

by traditional subtyping methods (phage typing (PT)) and recent molecular methods (pulsed field gel electophoresis (PFGE)). PT studies of *S. enteritidis* indicate that predominant phage types exhibit some geographic variation: phage types 8, 13, and 13a (and more recently PT4) in the US and Canada; PT4 in the UK; PT4 and PT8 in other parts of Europe. In Canada *S. enteritidis* phage types show similarities between human and poultry sources; PT4, PT8, and PT13 accounting for 40% to 80% of all types from both sources in recent years.

Industrialization of meat and poultry production has influenced the emergence of *Campylobacter* and EHEC since the 1970s. Despite identification as a potential pathogen in the late nineteenth century, *Campylobacter* was not fully recognized as a human pathogen until the 1970s when the development of selective growth media enabled laboratories to routinely test for the bacterium. *Campylobacter* infections, particularly *Campylobacter jejuni*, are now recognized as one of the most common foodborne illnesses. Although the early increase in *Campylobacter* was due to improved laboratory diagnosis, consumption of poultry with high contamination rates has contributed to recent trends. Researchers have estimated 2.4 million *Campylobacter* cases yearly in the US, a million more for *Salmonella* infection. Notifications of *Campylobacter* in Canada have consistently exceeded *Salmonella* for the last 15 years, ranging annually between 10 000 and 16 000 reports. A gradual decline in annual reports since 1994 may reflect improved awareness and food hygiene, although the actual reason is not known.

Although *Campylobacter* is widely found in the intestinal tract of domestic and wild animals and birds, most human illness is linked to meat, poultry and raw milk. Poultry appear susceptible to infection by low numbers of bacteria, and studies in the 1990s indicated that by 4 weeks of age most commercially produced chickens were colonized, probably from environmental sources such as nonchlorinated natural water. Rearing and processing practices contribute to further amplification and spread of the bacterium and a high (but variable) proportion of retail chicken meat is contaminated by the time it reaches the shop shelf. Bacterial counts may increase during transport, slaughter, and processing, although counts on carcasses can be reduced by chilling, by attention to processing plant hygiene and by processes which directly reduce carcass contamination (e.g., irradiation). Canada's C-EnterNet sentinel site studies, however, continued to show retail poultry meat samples ranging from 29% to 43% positive from 2006 to 2008, whereas contamination of other meats (beef and pork) was negligible.

In 1982, a new EHEC strain was linked to separate outbreaks of bloody diarrhea in two US states. The infection was associated with eating beef from a fast-food restaurant chain and *E. coli* O157:H7 was identified from nine cases. A new foodborne disease, described as "hamburger disease" in the press, had been recognized. Interestingly, Canadian researchers had described several *E. coli* strains with similar toxic effects in 1977. These verrotoxin-producing *E. coli* or shigatoxin-producing *E. coli* (STEC) infections were associated with bloody diarrhea (hemorrhagic colitis) and, in a small proportion of cases, hemolytic uremic syndrome (HUS), particularly in young children. HUS is of particular concern because of the life-threatening symptoms and the potentially severe impact on the long-term renal health of the individual, and is recognized as a major cause of renal failure in children.

E. coli outbreaks associated with ground beef products and milk reflects the link to cattle as a major source of EHEC infections. Approximately 3% of calves and up to 80% of adult cattle are positive for *E. coli* O157:H7. Although the organism does not cause symptoms in adult cattle, the spread may be facilitated by feedlot conditions. The seriousness of human illness and high levels of infection in adult cattle, which increases the potential for carcass contamination during slaughter, has resulted in closer scrutiny of ground beef, leading to multiple large recalls of these products. Although a variety of foods are linked to *E. coli* O157:H7 infection, including salad, fruit, vegetables, apple cider, raw milk, yoghurt, sausage, and deer meat jerky, many link to contamination with cattle manure. Current trends in laboratory-confirmed human cases indicate a gradual upward trend in reporting since the mid-1990s in the US, whereas Canadian data suggests a slight decline (Figure 2). Canada's C-EnterNet site

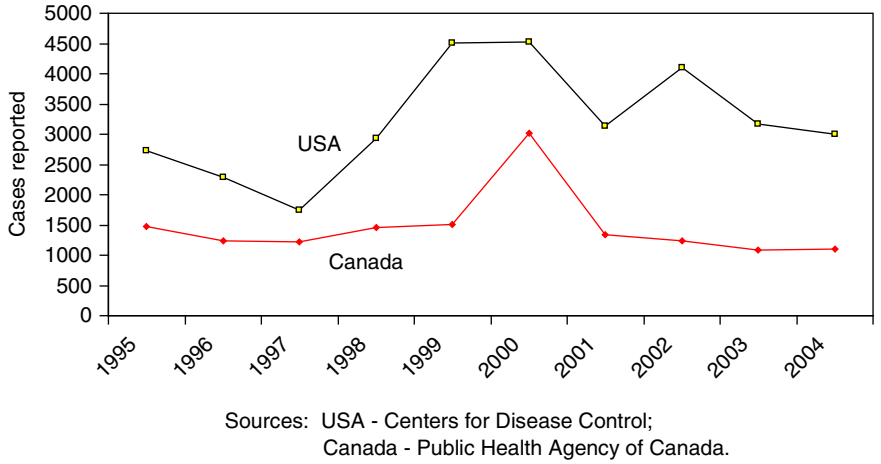

Figure 2 *E. coli* reports by year for USA and Canada: Notifiable diseases data. *Source*: US Centers for Disease Control; Public Health Agency of Canada.

showed significant levels of *E. coli* O157:H7 in dairy and beef cattle manure and farm samples but negligible levels in retail beef samples between 2006 and 2008, suggesting possible successes in minimizing contamination during processing.

Changes in Consumer Demand

What we eat and how we eat it has changed significantly in recent decades, and the increase in eating out, consumption of fast-food and ready-to-eat foods, demand for fresh produce is, to some extent, reflected in foodborne diseases trends. Two pathogens of growing interest, *Yersinia enterocolitica* and *Listeria monocytogenes* are relatively unusual compared to *Salmonella*, *Campylobacter* and EHEC, although their true incidence is unknown. Swine appear to be an important source of *Y. enterocolitica* although a sentinel study in Canada found low and declining levels of the pathogen in pig manure (8–4% of samples) and retail pork (13–3% of samples) between 2006 and 2008 respectively.

Researchers have estimated under 100 000 cases of *Y. enterocolitica* and 2500 cases of *L. monocytogenes* annually in the US. Counts in Canada averaged 624 laboratory confirmed cases (range: 767 to 495) of *Y. enterocolitica* from 1998 to 2004; under 100 cases of listeriosis were recorded annually in Canada through 1995–2004 (average 67.0 notified and 74 laboratory confirmed cases annually. Annual totals have since increased in Canada, including a peak of over 200 cases in 2008 partially linked to a major outbreak of 57 cases and 22 deaths linked to deli meats. Their importance is associated with the relative severity of infection, particularly with *Listeria*. In 1981, the first *Listeria monocytogenes* outbreak fully documenting a food vehicle was linked to coleslaw made from cabbage fertilized with composted sheep manure on a Canadian farm where two sheep had died of *L. monocytogenes* infection. Cold storage of much of the crop may have allowed the bacterium to proliferate, contributing to the outbreak affecting 41 human cases, including 17 deaths (9 were stillbirths or spontaneous abortions) (Sewell and Farber, 2001). *Listeria* and *Yersinia* are psychotrophs, and cold storage is ineffective in limiting growth, and outbreaks associated with products such as soft cheeses, raw and cold meats, and coleslaw requiring cold storage is not unexpected. *Listeria* contamination has been recently demonstrated on retail pork, beef, and chicken, with the highest levels on chicken and beef.

The current choice and year round availability of fresh fruit and vegetable products in US and Canadian supermarkets is likely a response to factors including:

- improved consumer awareness about the benefits of fresh fruit and vegetables through nutrition-education programs and interest in vegetarian lifestyles;
- availability of fresh produce year round from global markets reflecting rapid transport, new preservation technologies, increasing culturally diverse populations; and
- demand for ready-to-eat foods, including prepacked prepared vegetables and fruit, resulting from lifestyle choices and persuasive marketing.

These factors have contributed to increased foodborne illness from fresh produce and derivative products such as juices. Data from US suggest that the proportion of outbreaks linking a known pathogen and fresh produce rose from under 1% in the 1970s to 6% in the 1990s. This trend was more marked for *Salmonella* infections: An analysis of the 73 produce-related outbreaks in the US between 1950 and 2008, found only 7 outbreaks recorded before 1991. New technologies, such as modified atmosphere packaging, not only increase shelf life of fresh produce but may also allow some pathogens to grow faster than spoilage organisms under some circumstances; sourcing of fresh fruit and vegetable from countries with higher risk from fecal contamination; and a trend to eat produce raw have contributed to increased exposure.

Although food handler contamination during preparation was described for some outbreaks, mainly due to *B. cereus*, *S. aureus*, and hepatitis A virus, most implicate animal or human fecal contamination during growth, harvesting, and processing. The link to fecal contamination is further supported by the variation in enteric pathogens linked to produce (Table 6). *Salmonella* is the most common pathogen linked to produce, however, unlike meat-related outbreaks, there is more variation in the serotypes recorded, supporting the implication of

Table 6 Pathogens linked to produce outbreaks in the US and Canada

	Salad	*Sprouts*[a]	*Vegetables*	*Herbs*	*Fruit*	*Juice*
Salmonella	✓	✓	✓	✓	✓	✓
E. coli O157:H7	✓	✓			✓	✓
Other *E. coli*			✓		✓	
Shigella	✓		✓	✓		
Campylobacter					✓	
Bacillus cereus		✓				
Yersinia enterocolitica		✓				
Staphylococcus					✓	
Hepatitis A	✓		✓		✓	
Norovirus	✓				✓	
Cyclospora cayatenensis	✓			✓	✓	
Giardia lamblia	✓		✓		✓	
Cryptosporidium parum			✓			

[a]Includes various types of sprout products such as: alfalfa, clover, soybean, mung bean, cress, and mustard.

fecal contamination from multiple animal sources. The analysis of 73 produce-related *Salmonella* outbreaks, mentioned earlier, recorded 28 serotypes; some due to unusual serotypes associated with animals such as reptiles. Animal and bird feces appears to have been an important contributor in the outbreaks (mostly *Salmonella*) associated with sprouts, either due to contaminated seeds or contaminated irrigation water, with the added impact that the conditions required for sprouting support bacterial multiplication.

The arrival of a new coccidian parasite, *Cyclospora cayetanensis*, was associated with raspberries and blackberries from Guatemala in the US and Canada in the mid-1990s, and later with mesclun lettuce from the US and Peru. *Cyclospora* oocysts in soil contaminated by human feces was the most likely source of contamination, this corresponds with findings that oocysts originating from human feces that are found in soil show higher infectivity than oocysts recovered directly from human feces.

Environmental and Lifestyle Factors

Environmental factors affect foodborne disease trends directly via ambient temperature, or indirectly by influencing human activity. The seasonal trends of *Salmonella*, *Campylobacter,* and EHEC are well documented: the highest incidence occurring during the warmer months, reflecting the potential for greater microbial loading in the environment and in food stored at too high temperature, and people engaging in risky practices. These include, leaving food in the car too long when shopping, and activities including camping, picnicking, and barbequing, where temperature control of food is difficult to maintain. Other climate factors include heavy rainfall causing land run off carrying animal or human feces into irrigation and process water and resulting in surface contamination of vegetable and salad crops or fruit grown on the ground. Outbreaks linked to hanging fruit may be associated with contaminated irrigation, washing or cooling water, or in the use or consumption of contaminated dropped fruit. For example, the juice outbreaks due to *Salmonella*, *E. coli* O157:H7, and *Cryptosporidium* identified in **Table 6** followed the use of dropped apples and oranges.

It is difficult to quantify the impacts of climate change on disease trends; however, modeling ambient temperatures against foodborne illness, and *Salmonella* incidence specifically, indicates a relationship. Descriptive approaches suggest that aboriginal Americans, and northern people in particular, are experiencing effects of climate and environmental change. However, the extent to which environmental change is affecting factors such as lifestyle and disease prevalence in wildlife compared to other factors relating to, for example, socially driven cultural change, is unclear. Some reliance on country foods (game, fish, and sea mammals) anyway results in increased risk from parasitic diseases such as *Trichinella spiralis* and tapeworms compared with the general population. For example, all seven outbreaks of *Trichinella* infection in a Canadian review were associated with meat from wild animals, and there is indication that changes to traditional food practices carry increased risk of botulism.

Botulism is an interesting case study: a disproportionate number of incidents are recorded in Alaskan and Canadian Inuit. Most Canadian cases since the 1970s involved northern people and the US data indicates that 36% of events and 38% of cases recorded between 1990 and 2000 occurred among the relatively small Alaskan population. This compares with botulism in Canada between 1917 and 1973 when most cases were of European descent and linked to home-canned foods. This reflects a decline in home canning by Europeans as commercial availability of nonseasonal products increased, and the influence of environmental and lifestyle factors on aboriginal people resulting in the consumption of improperly preserved sea products or game eaten raw, dried, or fermented. Traditional approaches to fermentation of fish-heads, fish eggs, whale fins, seal flippers, and beaver tails relied on prolonged cold fermentation by burial in permafrost cooled ground. Investigation of outbreaks in Alaskan and Canadian native people since the late 1940s found that introduction of new country foods to a community and modified fermentation practices, including the use of sealed glass jars and plastic containers were the contributing factors. Whale and seal meat have occasionally been associated with *Salmonella* outbreaks in aboriginal people, and although it is unclear whether the animal had *Salmonella* infection or presence of the pathogen resulted from contamination by seagulls.

Fish and shellfish-associated illness has a long history and is caused by diverse agents including bacteria, parasites, and viruses as well as toxins produced by algae, diatoms, and bacteria naturally occurring in the environment, particularly *Vibrio* species. Although reported numbers of cases and outbreaks related to seafood are relatively low (a few hundred cases annually in the US and Canada combined) the popularity of seafood, their importation from many countries, and the serious illness caused by some agents identifies these products as potentially risky. Some seafoodborne agents, including fish and shellfish toxins and *Vibrio* bacteria, are recognized as emerging causes of illness that are potentially influenced by environmental conditions including ambient temperature and marine pollution from coastal land run off of nutrients. These conditions contribute to toxic algal blooms (red tides) producing neurotoxins (neurotoxic shellfish poisoning, paralytic shellfish poisoning, and amnesic shellfish poisoning) and diarrhoetic toxins (diarrhoetic shellfish poisoning). Although many illnesses are mild and under reported, occasional deaths are linked to several toxins and neurotoxic illness may have long-term health impacts; an outbreak of amnesic shellfish poisoning in Canada in 1987 resulted in 107 cases, some with persistent memory loss.

Outbreaks associated with fish in the US are mostly linked to scombrotoxin (57% of fish associated outbreaks: 1983 to 1992) and ciguatoxin (19%). Scombrotoxin, which induces histamine accumulation due to naturally occurring bacteria, is linked to poor temperature control post harvesting. Ciguatera poisoning is related to dinoflagellate toxins accumulating up the food chain, and human illness is primarily linked to higher carnivores. Puffer fish poisoning, resulting from toxins produced by naturally occurring bacteria, is rare in the US and Canada and cases are usually linked to imported fish.

Historically typhoid was the most important shellfish-associated illness, but since the mid-1950s such outbreaks have virtually disappeared. Introduction of purification procedures (depuration) in the 1930s, declining typhoid in the

population and introduction of stringent microbiological criteria for shellfish growing waters, along with monitoring programs, have reduced sewage-related illness. The exception is viral pathogens present in human feces, principally noroviruses, hepatitis A, and non-A, and non-B hepatitis. Introduction of rapid molecular methods to identify noroviruses has contributed to an increase in reported incidents, and indicates that they caused many gastroenteritis outbreaks (up to 50%) previously described as unknown etiology. Currently, noroviruses are estimated to account for two-thirds of all foodborne illnesses (Table 5), although the proportion linked to seafood is unknown. Interestingly, the description of self-limiting gastroenteritis 1 to 2 days after eating oysters in documented typhoid (about 2 weeks incubation) outbreaks in the nineteenth century in the US suggests viral gastroenteritis may have been common then, and was even considered a possible prodrome of typhoid.

Naturally occurring bacteria of the family Vibrionacaea, which include *Vibrio*, *Aeromonas*, and *Plesiomonas* species, account for most bacterial illnesses linked to seafood. Most cases are due to *Vibrio* species associated with filter feeding bivalve molusks such as oysters (usually eaten raw). An estimated 5000 foodborne *Vibrio* infections occur annually in the US (Table 5); in Canada, less than 50 isolates are reported annually, including nonfoodborne infections and infections acquired abroad. Laboratory reported *Vibrio* infections include toxigenic *Vibrio cholerae* (types O1, O139, and recently O141), and more commonly *V. vulnificus* (Gulf coast) and *V. parahaemolyticus* (commonly on the Pacific coast). Unlike toxigenic *V. cholerae* O1 and *V. parahaemolyticus*, *V. vulnificus* rarely causes diarrhea and is of concern because of the high mortality associated with septicemic infection in vulnerable groups. In one report of cases linked to oyster consumption in Florida between 1991 and 1994, the mortality rate was 60%. Concerns about *Vibrio* infections, particularly in the US Gulf States led to the development of the Cholera and Other Vibrios Surveillance System (COVISS), maintained by the Centres for Disease Control and Prevention (CDC) since the late 1980s.

The *Vibrios* primarily associated with human illness share tolerance for salinity (greater in the noncholera *Vibrios*) and are common in warm seawater including estuaries. Their reservoirs include fish, shellfish, plankton and sediment, and their complex ecology affects their persistence in the marine environment and increased prevalence in warmer months. There is potential for introduction of species into new environments via ship ballast water, the likely route for the introduction of *V. cholerae* O1 El Tor (Latin American biotype) into the northern Gulf of Mexico during the Latin American epidemic in the early 1990s. A similar mechanism may also play a role in the dispersal of toxic algae.

Conclusions

Historically, laboratory based and notified diseases reporting has tended to underestimate the incidence of individual disease agents. However, an important characteristic of these approaches is the tracking of specific pathogens over extended time periods permitting the detection of changes in reporting trends, provided there is some consistency in what is reported and how it is reported. Centralized collation of laboratory reports plays an essential role in the detection of emerging pathogens and the identification of outbreaks. Result from outbreak and epidemiological investigations provides valuable information on both the vehicle of infection and characteristics of the population infected. Recent approaches to surveillance have incorporated monitoring for risk factors at the community level and are contributing to improved understanding of the complex dynamics of foodborne diseases as well as helping to quantify levels of risk associated with specific products.

Variations in national foodborne disease statistics reflect differences in methodological approaches to collating or estimating burden, risk, and economic impact of disease as well as differences in the agents included in analyses, and approaches for estimating the proportion of illnesses and deaths due to known and unknown causes. Since the early 1990s a number of countries have attempted to better delineate the national burden of foodborne diseases. The results are used to estimate disease burden, the impact of specific pathogens, and the association of specific foods or food groups with human illness, as well as to assist countries in developing surveillance capacity.

The increasing prominence of foodborne diseases reflects greater public scrutiny of food safety; evolving public expectations of the safety, quality, and benefits of food; food producer and manufacturer response to consumer concerns; and the public health response to the burden, cost, and severity of the illnesses they cause. Changes in animal husbandry, eradication of diseases in economically important food animals, improved sanitation, global expansion of markets for food sources, introduction of new storage and preservation technologies, and development of laboratory diagnostic technologies have contributed to the evolving epidemiology of foodborne diseases. This includes both the emergence of new pathogens and recognition of the role of specific risk factors in human illness. Many factors that influence disease trends are amenable to preventive approaches aimed at reducing infection in animals, poultry, and produce on the one hand, or improved food storage, transport, handling, and food safety awareness on the other.

See also: Bacteria: *Aeromonas*; *Bacillus cereus* and Other Pathogenic *Bacillus* Species; *Brucella*; *Campylobacter*; *Clostridium botulinum*; *Listeria monocytogenes*; *Mycobacterium avium* ssp. *paratuberculosis*; Other Vibrios; *Salmonella* Non-Typhi; *Salmonella* Typhi and *Salmonella* Paratyphi; *Shigella*; *Staphylococcus aureus*; *Streptococcus*; *Vibrio cholerae*; *Vibrio parahaemolyticus*; *Vibrio vulnificus*; *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*. Characteristics of Foodborne Hazard and Diseases: Cost of Foodborne Diseases. Foodborne Diseases: Foodborne Diseases and Vulnerable Groups; Overview of Biological Hazards and Foodborne Diseases; Prevalence of Foodborne Diseases in Africa; Prevalence of Foodborne Diseases in Europe; Prevalence of Foodborne Diseases in South East and Central Asia; Prevalence of Foodborne Diseases in Western Pacific Region. Helminth-Cestode: *Echinococcus granulosus* and *Echinococcus mutilocularis*; *Taenia saginata* and *Taenia solium*. Helminth-Nematode: Anisakid

Nematodes; *Ascaris*; *Trichinella spiralis* and Other *Trichinella* Species; *Trichuris trichiura*. Helminth-Trematode: *Clonorchis sinensis*; *Dicrocoelium dendriticum*; *Fasciola hepatica* and *Fasciola gigantica*. History of Food Safety and Related Sciences: History of Foodborne Disease in Asia – Examples from China, India, and Japan. Institutions Involved in Food Safety: Trust in Animals and Food Safety (TAFS) Forum. Natural Toxicants: Tetrodotoxin. Prions and Agents of TSEs: Bovine Spongiform Encephalopathy in Cattle; Creutzfeldt–Jakob Disease. Protozoa: *Cryptosporidium* spp.; *Cyclospora cayetanensis*; *Entamoeba histolytica*; *Giardia lamblia*; *Toxoplasma gondii*. Public Health Measures: Surveillance of Foodborne Diseases. Risk Analysis: Estimating the Burden of Foodborne Disease. Safety of Food and Beverages: Seafood. Viruses: Hepatitis A Virus; Norovirus

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Prevalence of Foodborne Diseases in South East and Central Asia

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Glossary

Food Any substance, whether processed, semiprocessed, or raw, that is intended for human consumption, and includes drink, chewing gum, and any substance that has been used in the manufacture, preparation or treatment of 'food', but does not include cosmetics, tobacco, or substances used only as drugs. (In the context of this topic level contribution, drinking water is food.)

Food safety Assurance that food will not cause harm to the consumer when it is prepared and/or eaten according to its intended use.

Foodborne disease Any disease of an infectious or toxic nature caused by or thought to be caused by consumption of food or water.

Incidence Number of new cases in a specified population in a defined period of time, divided by the population at risk.

Infection A condition in which the body is invaded by an infectious organism (e.g., bacteria, virus, fungus).

Monitoring Continuous or repeated observation, measurement and evaluation of health and/or environmental or technical data for defined purposes, according to prearranged schedules in space and time, using comparable methods for sensing and data collection.

Outbreak The occurrence of more cases of disease than expected in a given area or among a specific group of people over a particular period of time.

Pathogen An organism capable of causing disease.

Risk A function of the probability of an adverse health effect and the severity of that effect, consequential to hazard(s) in food.

Surveillance The systematic collection, analysis, interpretation and dissemination of health data on an ongoing basis, to gain knowledge of the pattern of disease occurrence and potential in a community, in order to control and prevent disease in the community.

Introduction

Foodborne disease is a worldwide problem despite of various efforts for water, sanitation, and food safety improvement. The global burden of nontyphoid salmonellosis around the world is estimated as 2.8 billion cases and 2.1 million people die from diarrheal disease every year. In 2000 alone, typhoid fever accounted for 2.16 million cases and resulted in 216 000 deaths with 90% of the morbidity and mortality occurred in Asia.

Bacterial pathogens, viruses, and parasites have been etiologically associated with foodborne illnesses outbreaks around the world. *Salmonella* is considered a pathogen of continuing concern while enterohemorrhagic *Escherichia coli* emerged as a foodborne pathogen in the 1980s. Although most foodborne illnesses are self-limiting, some can be life threatening, for example, renal failure (enterohemorrhagic *E. coli*), miscarriage and or stillbirth (*Listeria monocytogenes*), meningitis (*Cronobacter sakazakii*), etc. *Salmonella* serotypes commonly found in the regions seem to be different from those reported in North America and Europe, except for *Salmonella enteritidis* and *Salmonella* Typhimurium.

This article summarizes foodborne diseases prevalent in South East Asia and Central Asia. The two regions consist of mostly developing countries, some of them are still struggling with basic hygiene programs. South East Asia consists of Brunei, Cambodia, Indonesia, Laos, Malaysia, Myanmar, the Philippines, Singapore, Thailand, and Vietnam. Meanwhile, Central Asia includes Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan, and Uzbekistan. Knowledge on pathogens prevalent in South East and Central Asia is mostly derived from reports from clinical isolates of diarrheal patients. *Shigella* is the major causative agent for diarrhea shared in the two regions. In addition to nontyphoid *Salmonella* typhoid and paratyphoid fever also still exist at different levels in some countries. Brucellosis caused by *Brucella* sp. and botulism intoxication due to *Clostridium botulinum* seems to occur more frequently in Central Asia than that in South East Asia, whereas limited report on parasites is available from Central Asia. In many countries, antibiotic-resistant strains of bacterial enteric pathogen are reported, possibly due to unrestricted use of antibiotics.

Food associated with the outbreaks is rarely confirmed during investigation. Given the possibility of unhygienic practices as the most important reason, most of the time no specific food could be etiologically linked to the outbreaks.

Foodborne disease outbreaks in South East and Central Asia are generally underreported. Typically, the number is extrapolated from the limited data because records and or monitoring of foodborne diseases are inadequate. This

article does not discuss foodborne intoxication resulted from chemical hazards such as natural toxicant, seafood toxins, mycotoxins, or environmental pollutant. Sporadic cases of these types of intoxication were still reported in the regions involving local meal such as cassava poisoning, bongkrek acid poisoning, toad poisoning, etc.

South East Asia

Review of foodborne pathogens in this region included data from 10 countries. However, due to the limited data, the extent of discussion of each country may vary. Characteristics shared within the region are unhygienic practices, unsafe water, cultural habit of consumption of raw or undercooked food in some regions, and the presence of inadequately monitored street vendors. All of the above-mentioned factors results in the persistence of enteric pathogens: bacteria, viruses, protozoa, and helminthic parasites.

Shigella, *Vibrio cholerae* O1, and *Salmonella* spp. are most commonly associated with diarrheal diseases in Cambodia, Indonesia, Thailand, Lao PDR, Malaysia, Myanmar, the Philippines, and Vietnam. Cholera in South East Asia, with *V. cholerae* O1 as the predominant strain, contributes to 7% of cholera cases in the world. Typhoid fever persists in some countries even though there is evidence for decreases worldwide. *S. enteritidis* was the most frequently isolated nontyphoid *Salmonella* serotype, whereas rotavirus was the virus most often associated with diarrhea in the region.

Consumption of raw or undercooked food, unhygienic practices such as open defecation, i.e., defecating outside and not in the toilet, and use of night soil for fertilization of fish ponds are risk factors for helminthic parasite infection. Freshwater and brackish water fish, freshwater and brackish water snails, amphibians, terrestrial snakes, aquatic insects, and aquatic plants are the usual vehicles. Various flukes, nematodes, and trematodes have been reported. Echinostomiasis due to *Echinostoma* is believed to be underreported. Echinostomiasis occurs in the Philippines, northern part of Thailand, Indonesia, Malaysia, and Singapore.

Street food vendors and schools have been linked with outbreaks in Brunei, Cambodia, Malaysia, Thailand, etc. With increased awareness on food safety, intervention programs have been developed in many countries and were reported to reduce the numbers of foodborne outbreaks. However, improved awareness of food safety has also prompted countries to conduct more surveys and/or investigations; thus, more outbreaks are reported.

Foreigners traveling in the South East Asia region have been reported as a factor linked to foodborne illnesses. A study of Swedish travelers stated that Thailand and Indonesia were among the 10 countries most often reported for travelers' infections. Eating local food and consuming nonpotable water are likely the reason for illnesses. These two countries together with Brunei, Cambodia, Myanmar, China, Hong Kong, Japan, Laos, Malaysia, Mongolia, North Korea, South Korea, the Philippines, Singapore, Taiwan, Thailand, Tibet, and Vietnam were grouped as East Asia. This group has a risk of nontyphoid salmonellosis in travelers of 270/100 000 and the serotype most commonly found in the group was *S. enteritidis* (15.1%). This was similar to the risk of traveling in West Africa (279/100 000) but lower than that in India and its neighbors (474/100 000), and East Africa (471/100 000). Another source of travelers' diarrhea was reported from a cruise ship traveling in the South East Asia region in which six out of 630 passengers were infected by *V. cholerae* O139 due to consumption of yellow rice in Bangkok.

Brunei

Brunei, the gross domestic product (GDP) per capita of which is the fifth highest in the world, has one of the best health services in Asia. The WHO report in 1999 suggested that cholera was no longer reported there since 1982. Previously the pathogen caused the largest outbreak in 1965 and another one with 24 cases in 1970. In 1999, however, an outbreak involving 72 confirmed and 29 suspect cases happened in schools. Another foodborne outbreak due to consumption of rice–chicken was reported in a seminar but no pathogen was identified.

Cambodia

Diarrhea was found to be the leading cause for morbidity and mortality in this country. It was reported that the illness was generally linked to an inadequate water and sanitation program. The access to drinking water for rural and urban communities was 29% and 69.55%, respectively, whereas access to hygienic facilities was only enjoyed by 8.6% in rural and 49% in urban areas. The number of diarrhea and dysentery cases in the age groups 0–4 years, 5–14 years, >15 years were 17.5% and 4%, 8.3% and 3.3%, 2.8%, and 1.2% of total illnesses, respectively.

Salmonella typhi was isolated from 0.9% the samples of diarrheal patients. Of the isolates, 56% were resistant to multiple antibiotics including ampicillin (56%), chloramphenicol (56%), and trimethoprim-sulfamethoxazole (81%). A couple of outbreaks in 2001 were reported, i.e., a school-related outbreak and an outbreak due to toxic fish.

Indonesia

A study of patients with diarrhea suggested that four major bacterial pathogens were *V. cholerae*, *Shigella flexneri*, *Salmonella* spp., and *Campylobacter jejuni*. The study also showed that 11% of the patients had parasites, consisting of *Blastocystis hominis* (5.7%), *Trichuris trichuria* (2.1%), *Ascaris lumbricoides* (1.5%), *Giardia lamblia* (0.8%), and *Endolimax nana*. Rotavirus, Norwalk-like virus, and adenovirus were also found in 37.5%, 17.6, and 3.3%, respectively. Rectal swabs of people suggested that 18% carried enterotoxigenic *E. coli*, 1% had enterohemorrhagic *E. coli* and 1% harbored *Clostridium difficile*.

The work of Tjaniadi et al. in 2003 confirmed the above findings and concluded that *V. cholerae* O1 was most often isolated (37.1%), followed by *S. flexneri* (27.3%) and *Salmonella* (17.7%). Other pathogens were also isolated at a lower rate, i.e., *Vibrio parahaemolyticus* (7.3%), *S. typhi* (3.9%), *C. jejuni* (3.6%), *V. cholerae* non-O1 (2.4%), and *Salmonella paratyphi* (0.7%). Some of the *V. cholerae*, *S. flexneri*, and *Salmonella* were resistant to various antibiotics, although *S. typhi* and *S. paratyphi* remained sensitive to all antibiotics tested. The absence of

antibiotic-resistant *S. typhi* was confirmed by a survey in five Asian countries published by WHO in 2008.

The endemic area in North district of Jakarta had 180.3 typhoid fever cases per 100 000 per year among the 5–15-year age group whereas cases of diarrhea in infant in this area were reported as high as 759/1000 per year. Cholera was common in infant (4/1000 per year) and was mainly caused by *V. cholerae* El Tor Ogawa (58%) and El Tor Inaba (42%). Children of 1–2 years mostly had shigellosis (32/1000 per year) due to *S. flexneri*. The study also suggested that 75–95% of the *Shigella* was resistant to ampicillin, trimethoprim-sulfamethoxazole, chloramphenicol, and tetracyclin but sensitive to nalidixic acid, ciprofloxacine, and ceftriaxone.

E. coli resistant to ampicillin, gentamicin, cefotaxine, ciprofloxacine, and trimethoprim-sulfamethoxazole was isolated from carriers in hospital and community. The recent use of antibiotics and having medical insurance were directly related to the increase in *E. coli* resistance to antibiotics.

Protozoa and helminthic parasites were linked to gastroenteritis and chronic diarrhea in the country. The *Cyclospora cayetanensis* isolation rate from expatriates with diarrhea in three independent studies were 8.6%–15.1%, 9.1%, and 13.6%. *Entamoeba hystolitica*, *G. lamblia*, *B. hominis*, *T. trichuria*, and *A. lumbricoides* were found at isolation rates of 6.3%, 2.8%, 15%, 3.5%, and 0.8%, respectively. A survey of Indonesian children aged 8–10 years showed that infection by helminthic parasites and protozoa were 84% and 77%, respectively, with asymptomatic children carrying *C. cayetanensis* accounting for 0.6% of the surveyed population. No *Cyclospora* was isolated for both expatriate and Indonesian children under 3 years, and this was attributed to several factors including no exposure to fresh fruits, vegetables, and garnish.

Street food vendors are more likely to cause infection from *S. pratyphi* based on two separate studies conducted in Semarang and Jakarta. While paratyphoid fever is more likely to be foodborne, typhoid fever is associated with a recent typhoid fever case in the home, no washing before eating, and recent consumption of ice cubes. Street food vendors were characterized by poor hand washing practices/facilities, direct contact between food and hands, male workers, and low education. Street food vendors serve various foods, from deep fried foods, soups, grilled satay, or fresh fruit salad. In relation to these vendors, 65% of water, 91% dishwashing water, and 100% of ice were contaminated with fecal coliforms. The risk factors for typhoid fever after two geographically different disasters were different. The absence of clean water and nonavailability of drugs were the main risk factors for typhoid fever following the 2008 tsunami in Aceh, Sumatra, while contact with typhoid patients and lack of hygiene education emerged as the risk factor following 2006 earthquake in Yogyakarta in Java.

Although food produced by the industry is rarely implicated in outbreaks, in 2004 processed milk caused 14 outbreaks in seven cities in six provinces in Java, Sumatera, and Nusa Tenggara islands. The milk was consumed by 2251 school children causing 469 students to become ill and one to die. Processed milk (pasteurized, powdered and commercially sterilized) was thought to be etiologically linked to the outbreaks. Poor hygienic practices, poor handling after processing, as well as poor preparation of milk were concluded as factors contributing to the outbreaks; however, no particular etiologic agent was announced.

Studies of the foodborne outbreaks were rarely published. Cooking methods commonly applied to Indonesian ready-to-eat traditional foods are sufficient to eliminate large number of *S. aureus* and other nonsporeforming bacterial pathogens. Therefore, postcooking handling was thought to be critical and the possible cause of many outbreaks. Between 2001 and 2009 there was an increase in the number of foodborne disease outbreak reported, but it was probably due to a better monitoring and/or reporting system (**Table 1**).

The Indonesia National Agency for Food and Drug Control (NADFC) suggested that pathogenic microorganisms were the major causative agent of the outbreaks, accounted for 37% of the outbreaks, although no specific pathogen was mentioned. Food prepared by street food vendors, together with homemade food and food from food service industries were responsible for most (83%) of the outbreak (**Figure 1**), which was consistent with epidemiological studies of Vollaard et al. in 2004a.

Food safety in Indonesia can be evaluated based on the performance of exported food. Export rejection by the US FDA, for example, was mainly due to *Salmonella* and filth. Dewanti-Hariyadi et al. in 2005 reported that shrimp collected from the ocean and ponds of the northern shore of Java were frequently contaminated with *Salmonella* with isolation rates of 6.25% and 12.5%, respectively. The data suggest that *Salmonella* is an important microbiological contaminant in food and this is in line with data suggesting that *Salmonella* was responsible for 17.7% of diarrheal cases.

Lao People's Democratic Republic (Lao PDR)

Diarrhea is a very important health problem in Lao PDR and 16% of death in children can be attributed to this illness. Investigation of the etiologic agent of diarrheal patients between 1996 and 1997 concluded that *Shigella* and diarrheagenic *E. coli* were the major pathogens, which were responsible for 16.8% and 17.2% of the cases, respectively. Almost all of *Shigella* and diarhegenic *E. coli* were reported to be resistant to ampicillin, tetracycline, and erythromycin. Rotavirus also contributed to 6.1%, whereas *C. jejuni*

Table 1 Number of reported outbreaks and reported cases of foodborne disease in Indonesia 2001–2009[a]

Year	*2001*	*2002*	*2003*	*2004*	*2005*	*2006*	*2007*	*2008*	*2009*[a]
Number of outbreaks	26	43	34	164	184	159	179	197	109
Number of cases	1955	3635	1843	7366	8949	8733	7471	8943	3050
Number of fatalities	16	10	12	51	49	40	54	79	17

[a]Report up to November 2009.

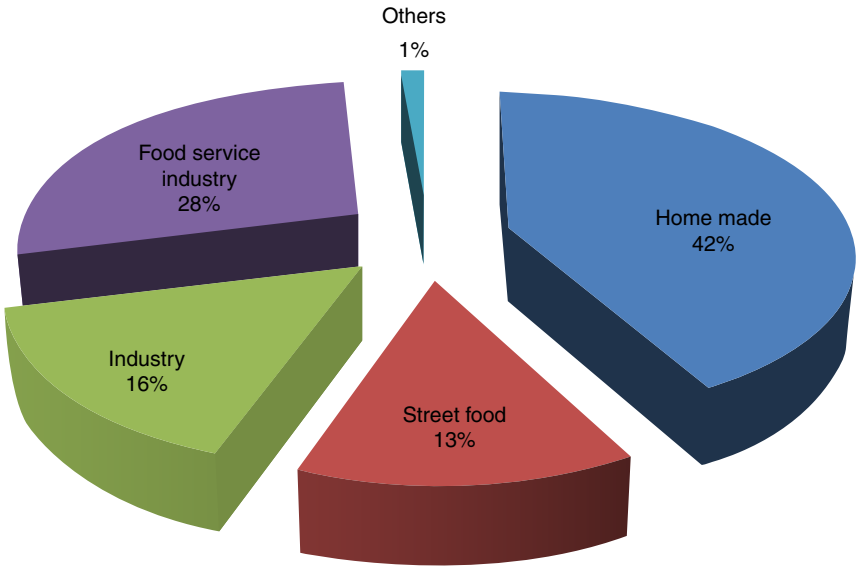

Figure 1 Types of food involved in foodborne disease outbreaks reported to Indonesia NADFC in 2009 ($n = 109$).

Table 2 *Salmonella* serotype isolated in Malaysia 2003–2005[a]

2003			*2004*			*2005*		
Serotype	*No.*	*Percent*	*Serotype*	*No.*	*Percent*	*Serotype*	*No.*	*Percent*
Enteritidis	233	26.7	Enteritidis	206	25.0	Enteritidis	155	28.1
Weltervreden	200	21.9	Weltervreden	165	20.0	Weltervreden	142	25.7
Corvallis	115	12.6	Corvallis	117	14.2	Corvallis	57	10.3
Typhimurium	49	5.4	Typhimurium	43	5.2	Typhimurium	37	6.7
Stanley	32	3.2	Albany	37	4.5	Limete	9	3.3
Tshongwe	29	3.2	Limete	18	2.2	Stanley	8	1.4
Biegdam	19	2.1	Braenderup	15	1.8	Agona	7	1.3
Albany	17	1.9	Tshongwe	15	1.8	Albany	5	0.9
Braenderup	12	1.3	Stanley	11	1.3	Rissen	5	0.9
Newport	10	1.1	Bovismorbificans	10	1.2	Virchow	5	0.9

[a]Thong, 2006.

accounted for 4.4% of the cases. *Salmonella* and *Vibro* cases were considered low.

Between January and August of 2007, there was an average of seven diarrheal cases per 100 000 population. An investigation during a cholera epidemic in Sekong Province suggested that 58.6% of stool samples were positive for *V. cholerae* O1 Ogawa. Water was concluded to be the vehicle of the outbreak.

A human trichinellosis outbreak due to *Trichinella* species was reported in 2004. Pork meals, i.e., uncooked minced pork with mint and fermented pork were the implicated vehicles for of the nematodes.

Malaysia

The prevalence of enteropathogens in humans was reported from stool samples of diarrheal patients that had been previously estimated at a rate of 26.3 incidence per 100 people. The report suggested that of the stool samples examined, 11% had enteropathogenic bacteria, but viruses were not examined. The five most significant pathogens and their rate of isolation in stools are as follows: nontyphoid *Salmonella* 57%, enteropathogenic *E. coli* 14%, *Shigella* 11%, *Campylobacter* 5%, and *Aeromonas* 5%. The four most frequently isolated Salmonella serotypes from 2003 to 2005 were, in order, *S. enteritidis*, *weltervreden*, *corvallis*, and *typhimurium* (Table 2). This finding is consistent with *S. enteritidis* being the most frequently isolated serotype in much of Asia. In addition, where protozoa such as *Cryptosporidium* and *Giardia* have been isolated from humans, they are assumed to be from waterborne sources. Pathogenic bacteria resistant to multiple antibiotics were also reported in Malaysia. *S. typhi* was found to be resistant to ampicillin and chloramphenicol, whereas nontyphoid *Salmonella* were resistant to ampicillin, chloramphenicol, and cotrimoxazole. All *S. flexneri* were resistant to ampicillin and cotrimoxazole, while 80% were resistant to chloramphenicol. Frederick in 2011 also reported that 61.2% of *E. coli* strains

were resistant to multiple antibiotics, such as kanamycin, tetracyclin, chloramphenicol, gentamicin, ampicillin, nalidixic acid, trimethoprim-sulfamethoxazole, cefetoxin, norfloxacin, and ciprofloxacin.

Foodborne disease outbreaks have received more attention in the past few years and have been recognized as important infectious diseases. Soon et al. in 2010 suggested that the number of outbreaks that had been apparently steadily declining between 1999 and 2005 was attributed to food handlers' training programs. However, this optimistic conclusion was confounded by a significant increase in the number of outbreaks since then, and this increase was really an artifact of better investigation. Most foodborne disease outbreaks seemed to occur in schools, although the authors acknowledged there was likely underreporting for outbreaks outside schools. Various foods were implicated in the outbreaks, for example, rice in coconut milk (*nasi lemak*), chicken, beef broth, fish, and starch-based snacks.

Several studies of foodborne pathogens in specific foods have been reported. *E. coli* O157:H7 was isolated from 36% of beef samples, *L. monocytogenes* from 74% of imported beef, 43.5% of local beef and 56% of fermented fish, and *Campylobacter* in 3–18.8% of fresh vegetables. It was also established that *V. cholerae* is carried by shellfish that has been implicated in the cholera outbreaks.

Myanmar

Although data regarding foodborne illness in Myanmar are very limited, it was estimated that 70% of diarrheal diseases was due to food contamination. Diarrheal diseases, foodborne intoxication, typhoid, and paratyphoid were the most frequently encountered illnesses.

In September 2000, a typhoid fever outbreak was reported and a case-control study was established. The risk factors identified were drinking unboiled river water, contact with other patients before illness and failing to wash hands with soap after defecation.

A survey to evaluate indicator bacteria in a popular ready-to-eat food called *mohinga*, i.e., rice noodle served with fish soup, has been conducted ($n=50$). The survey suggested that 100% of the noodle was tested positive for coliform, 80% contained fecal coliform, and 2% contaminated with enteropathogenic *E. coli* or *S. typhi*. Twenty percent of the soup samples had coliform, but fecal coliform or pathogen was absent. Mixing noodles with very hot (90–100 °C) soup decreased the number of bacteria by 1/1000 as compared to that mixed with warm (60–70 °C) soup. Addition of ingredients (chili powder, etc.) increased the total bacterial content in *mohinga* by 2–3 logs more than that prepared without any ingredients.

The Philippines

A survey of 2908 patients with diarrhea during 1983–1984 found that 58.4% had one or more enteric pathogen and rotavirus was the most frequently isolated pathogen (30.6%). The three pathogenic bacteria most often associated with the diarrhea were *Shigella* (11.6%), *Salmonella* (9.2%), and enterotoxigenic *E. coli* (7.8%). Of the samples collected, 302 had multiple pathogens. However, *Salmonella*, enterotoxigenic *E. coli*, and *C. jejuni* were also isolated from healthy patients.

Table 3 Parasites detected in Katipunan, Zamboanga del Norte, the Philippines, February 2008[a]

Parasite	*No. (%)*
Trichuris trichuria	64 (31.1)
Entamoeba coli	49 (23.8)
Ascaris lumbricoides	46 (22.3)
Endolimax naan	14 (19.9)
Hookworm	34 (16.5)
Blastocystis hominis	21 (10.2)
Giardia lamblia	19 (0.2)
Entamoeba hystolytica	14 (6.8)
Cappilaria philippinensis	10 (4.9)

[a]Belizario et al. (2010).

Foodborne nematodes causing severe diarrhea is also a problem in this country. Of the 205 stool samples from people with diarrhea, 73.3% contained parasites: 32% with one parasite and 41% with more than one parasite. Table 3 shows the type of parasites isolated from this study.

Singapore

Cholera cases in Singapore have been declining from 17 cases in 1992 to seven cases a year in 2007. During 1992–2007, a total of 210 cholera cases were reported and *V. cholerae* O1 biotype El Tor as well as serotype Ogawa was reported in 83.8% of the cases. Food associated with the outbreaks included partially cooked green mussels (1993), iced banana-flavored drink with contaminated crushed ice (1999) and imported seafood items (2004). The study concluded that 24% of cholera cases were caused by imported foods.

Salmonellae continue to cause foodborne illnesses and an outbreak in 2000 was predominated by a few strains such as *S. enteritidis*, *S. stanley*, *S. weltervreden*, and *S. typhimurium*. Multiple antibiotic-resistant strains of *S. typhimurium* DT104L were isolated from stool samples and in some foods, particularly ground anchovy. In 2007, *S. enteritidis* has emerged as the most important foodborne pathogen and was responsible for 62.2% of nontyphoid salmonellosis. During an outbreak involving 216 gastroenteritis cases, cream cake was concluded as the food associated and *S. enteritidis* was confirmed as the causative agent.

Thailand

A comprehensive review by Pitisuttithum in 2003 suggested an average of 1493 cases of diarrhea per 100 000 people per year was recorded between 1990 and 1995 in Thailand. During the period, an average of 110 cases of foodborne intoxication, 140 cases of dysentery, 20 cases of enteric fever, and 26 cases of hepatitis were reported each year. *V. cholerae*, *S. flexneri* or *S. dysenteriae*, and *Salmonella* were associated with severe diarrhea, dysentery, and salmonellosis, respectively.

Table 4 Reported foodborne illness in Thailand 2003

Foodborne diseases	*No. of cases*	*Death*	*Morbidity rate*	*Mortality rate*
Acute diarrhea	956 313	146	541.26	0.05
Dysentery	23 113	3	12.44	0
Foodborne intoxications	12 685	11	67.79	0
Enteric fever	9 633	3	3.57	0

In a report made during the Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) regional conference on food safety for Asia and the Pacific in Seremban, Malaysia, Thailand stated that the number of acute diarrhea cases in the country started to decrease in 2003. Unsafe drinking water, lack of personal hygiene, and consumption behavior, for example eating raw or underprocessed food products, were considered as the factors influencing the number of cases. *Shigella*, *Salmonella*, and *E. coli* were reported as the bacterial pathogens most commonly associated with the reported outbreaks during the period. In addition, shigellosis was also linked to poverty. A summary of nationwide foodborne illness is presented in Table 4.

An increase in isolation rate of *Salmonella rissen* from humans, uncooked foods, and ready-to-eat foods was reported in 2008. The isolates that were similar to those isolated in Sweden were entirely tetracycline resistant. The number of foodborne infections doubled in the past 10 years and *Salmonella* as well as *Campylobacter* were the most frequently isolated pathogens from the outbreaks. Table 5 shows the prevalence of *Salmonella* isolated from various foods, food sources, and humans. Of the isolated *Salmonella*, 59% were tetracyclin-resistant, 28% were ampicillin-resistant, 5% were azithromycin-resistant, and 0.5% were ciprofloxacin-resistant. Meanwhile *Campylobacter* (*C. jejuni* and *C. coli)* was isolated at a frequency of 72%, 23%, 64%, and 47% from pigs, pork, chickens, and chicken meat, respectively.

Foodborne disease outbreaks were often linked to school meals. A large outbreak in school causing 1598 students to become ill was reported in 2005. The outbreak was associated with mixed chicken–rice dish with an attack rate of 37% and *Shigella sonnei* as well as *Salmonella* group C were etiologically linked to the outbreak.

A very rare outbreak of botulism was reported in Thailand in 2006. Although no fatalities were reported, the outbreak involved 91 victims, of which 42 needed mechanical ventilators due to respiratory failure. Improperly home-canned bamboo shoots were the source of the intoxication.

As an effort to improve food safety, an intervention program was established by the Department of Health, Bangkok. Pitisuttithum in 2003 evaluated the impact of the intervention and found that compliance of street foods, ready-to-cook, and cooked food to their respective standards had improved from 56.5% to 80.3%, 30.5% to 56.5%, and 82.5% to 90.1%, respectively. Survey at a year postintervention also showed that the number of street foods contaminated by *Salmonella*, *S. aureus*, *C. perfringens*, and *V. cholerae* non-O1 had decreased from 5.5% to 2.1%, 2.6% to 0.7%, 4.5% to 2.8%, and 2.2% to 0.7%, respectively. Similar results were also observed in ready-to-cook and cooked food.

Table 5 *Salmonella* serotypes most commonly isolated in Thailand

Source	Salmonella *serotype*
Pig	Rissen, Derby
Pork	Weltervreden, Rissen, Anatum, Emek
Chicken	Emek, Rissen, Enteritidis
Chicken meat	Weltervreden, Emek, Hadar
Human	Weltervreden, Rissen, Stanley, Enteritidis, Anatum

Vietnam

A study was conducted in Hanoi of 587 children with diarrhea and 249 age-matched healthy controls. Sixty percent of children with diarrhea harbored pathogenic bacteria. In children under 2-years-old, group A rotavirus was predominant (46.7%), followed by diarrheagenic *E. coli* (22.5%). In children over 2 years, enterotoxigenic *Bacteroides fragilis* and *Shigella* were found at the rate of 7.3% and 4.7%, respectively. *E. coli* and *Shigella* isolated from diarrheal children were resistant to ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole. Children with diarrhea are usually associated with poor families, houses lacking potable water and latrines, mothers who do not wash their hands, as well as mothers with low education and or little information on appropriate hygienic habits. Hien et al. in 2008 further identified the diarrheagenic *E. coli* isolated from the diarrheal children. They found attaching and effacing *E. coli* as the predominant strains (9.2%), followed by enteroaggregative *E. coli* (8.8%), enterotoxigenic *E. coli* (4%), enteropathogenic *E. coli* (2.8%), and enteroinvasive *E. coli* (0.8%).

Typhoid fever is still a problem in Vietnam, although a recent report suggested that the number of typhoid fever cases in Vietnam is relatively low, i.e., 24.2/100 000 per year. Infection due to flukes was generally linked to consumption of raw freshwater fish or crabs, and aquatic plants. *Procerovum varium* and *Heterophysopsis contina*, two zoonotic trematode metacercariae, were the most prevalent parasites in farmed grouper fish, and present a risk to fish consumers if the groupers are not adequately cooked.

Central Asia

Similar to South East Asia, acute diarrhea because of *Salmonella* infections was commonly reported in Central Asia. Additionally, typhoid fever is still being reported. *Salmonella* involved in the outbreaks were also reported as resistant to multiple antibiotics. Brucellosis and botulism, which were

rarely reported in South East Asia, have been linked to a few outbreaks in this region. However, not much information is available on the prevalence of helminthic illness in this region. As compared to data obtained from the South East Asia, data on foodborne pathogens in this region is even less available.

Kazakhstan

During 1999–2000, it was reported that 9.5% of the acute intestinal infection cases were caused by *Salmonella* sp. was responsible for 7.5% of the foodborne disease cases in children under 14 during the above-mentioned period. However, the number of *Salmonella* cases constantly declined from 1993 to 2000.

Two examples of rarely reported outbreaks from this country give some details as to the food, the agent, and its cause. In March 2010, 25 patients came down with gastroenteritis, of which 20 were hospitalized. All had been invited to dinner on March 25 in a local restaurant in Kapchigai city, Kazakhstan. Investigation of the outbreak suggested that *S. enteritidis* was the causative agent and pastry with meat was implicated in the outbreak. *S. enteritidis* was isolated from 10 patients and also the leftover pastry. Further investigation revealed that the home-made pastry was prepared in a house with poor sanitary conditions. Another foodborne illness outbreak in Atyrau in Western Kazakhstan reported that 32 patients were hospitalized with diarrhea. All were workers who lived in a company hostel and ate in the same cafeteria. Olivier salad (potato, meat, and mayonnaise) was concluded as the food epidemiologically associated with the illnesses. The outbreak itself was probably caused by improper food preparation practices.

Kyrgyzstan

Foodborne and waterborne diseases, such as bacillary dysentery and other types of diarrhea as well as typhoid fever, are more common in the summer and autumn months and high incidences occurred in the southeastern and southwestern parts of the country. Brucellosis was reported in the extreme southwest and southeast while echinococcosis (hydatid disease) from the southeast part of the country. *Fasciola hepatica* infection has also been reported from different regions of the country.

The WHO Surveillance Programme for Control of Foodborne Infections and Intoxications in Europe 8th Report notified a total foodborne outbreak of 16 765 in 1999 and 14 440 in 2000. In 29–32% of the cases, the causative agent was known. The most frequently notified disease was shigellosis, accounting for 24% of all notified cases in 1999 and 29% of cases in 2000.

Brucellosis was responsible for 6% of illness in 1999 and 8% of the cases in 2000, while botulism accounted for less than 1% of the cases in the 2 years. *Salmonella* was responsible for 2% of the cases in the same years and *S. typhimurium* contributed for 56–68% of the salmonellosis. *S. enteritidis* was also isolated at a lower rate (0.3–2.4%).

Tajikistan

Surveillance on typhoid fever in Dushanbe, Tajikistan, in 1997 reported 8901 cases of typhoid fever and 95 associated deaths. Of the 29 *S. typhi* isolated, 27 (93%) were resistant to ampicillin, chloramphenicol, nalidixic acid, streptomycin, sulfisoxazole, tetracycline, and trimethoprim-sulfamethoxazole. In a case-control study of 45 patients and 123 controls, *S. typhi* infection was associated with drinking unboiled water. Ninety seven percent of the tap water samples were found to be contaminated with fecal coliforms, and samples from the water treatment plants revealed that fecal coliforms were present in the water before and after treatment. There were multiple reasons for this finding: lack of adequate chlorination, equipment failure, and back-siphonage in the water distribution system. After chlorination and coagulation were applied at the treatment plants and a water conservation campaign was initiated to improve water pressure, the incidence of typhoid fever declined dramatically.

The WHO Surveillance Programme for Control of Foodborne Infections and Intoxications in Europe 8th Report 1999–2000 suggested that during 1996–2000, a total of eight foodborne intoxication or infection outbreaks were reported. However, no specific agent was identified following the investigation of these outbreaks involving a total of 106 cases. The WHO report indicated that the most frequently reported disease was acute intestinal infections, i.e., 74 318 cases or 1190.8 incidence per 100 000 population in 1999 and 85 212 cases or 1366 incidence per 100 000 population in 2000. Typhoid fever, bacterial dysentery, and brucellosis were responsible for 7335, 3702, and 337 cases per 100 000 population, respectively, in 1999 and 4415, 2645, and 851 cases per 100 000 population, respectively, in 2000. Brucellosis has been on the rise in Tajikistan and in the bordering country Kyrgyzstan since the mid-1990s. Brucellosis is transmitted mainly through consumption of unpasteurized goat and sheep milk.

Turkmenistan

WHO Surveillance Programme for Control of Foodborne Infections and Intoxications in Europe 7th Report 1993–1998 reported that a total of 33 foodborne disease outbreaks occurred in this country. The outbreaks were comprised of 22 botulism outbreaks involving 55 cases and 11 other bacterial infection outbreaks involving 614 cases. Both outbreaks were derived from improperly home-preserved food such as fish and eggplant. Cryptosporidiosis is also frequently found in children.

Uzbekistan

During 1999–2000, a total of 59 outbreaks of foodborne diseases were reported. Some fatalities occurred during food poisoning incidents in 1999 and 2000; botulism and chemicals accounted for the mortality. Unfortunately, no further details are given.

An investigation of 97 patients with typhoid fever in the Samarkand region of Uzbekistan in 2002 suggested that the endemic typhoid fever was transmitted by contaminated water. The cases of typhoid fever in Samarkand appeared to follow a seasonal pattern, with the majority of infections occurring during dry hot summer months. The study also reported that the use of antimicrobials in the 2 weeks preceding illness onset was also independently associated with illness.

The study also concluded that 15% of *S. typhi* were multiple drug resistant. They were resistant to ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole.

See also: Bacteria: *Brucella*; *Campylobacter*; *Clostridium botulinum*; *Salmonella* Non-Typhi; *Salmonella* Typhi and *Salmonella* Paratyphi; *Shigella*; *Vibrio cholerae*. Characteristics of Foodborne Hazard and Diseases: Cost of Foodborne Diseases; Drug Resistant Pathogens. Foodborne Diseases: Foodborne Diseases in Travelers; Overview of Biological Hazards and Foodborne Diseases. Helminth-Trematode: *Echinostoma*. Public Health Measures: Challenges of Developing Countries in Management of Food Safety

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Prevalence of Foodborne Diseases in Australia and New Zealand

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Glossary

Food vehicle A specific food responsible for an outbreak of foodborne illness or cases of disease.
Foodborne outbreak Two or more cases of illness resulting from a common food or meal.
Gastroenteritis Illness characterized by vomiting, diarrhea, and other symptoms. Gastroenteritis is often defined as three or more loose stools and/or two episodes of vomiting in 24 h.
Incidence The number of persons falling ill with foodborne illnesses during a given period in a specified population.
Notifiable disease A disease that must be reported to health departments under public health laws; where the onus may be on doctors, laboratories, or other specified persons to report cases.
Pathogen An organism that can cause disease in humans or animals.
Prevalence A measure of the occurrence of foodborne disease, indicating the total number of people who have a specific foodborne illness at a given point in time in a specified population.
Rate A measure of the frequency of occurrence of disease, where incidence or prevalence are expressed in terms of a rate or the number of cases per 100 000 or million persons in a given population in a certain time period, such as a year.
Surveillance The systematic collection, collation, analysis, and interpretation of data on cases of foodborne illness, and dissemination to those who have the power to act and prevent further disease.

Introduction

Diseases caused by microbial pathogens and transmitted by food affect all the countries of the world. There are in excess of 200 diseases that may be transmitted by food, although many of these are rare and do not occur in developed countries. Public health agencies invest considerable effort in ensuring safe food supplies. One important component of ensuring that the food supply is safe is surveillance for human cases of foodborne disease, to detect outbreaks, monitor trends, and provide evidence for public health action. One challenge for public health surveillance is that many foodborne illnesses result in mild symptoms that resolve spontaneously and do not require a visit to the doctor. This means that for every reported foodborne infection, there will be many more in the community. One implication of this is that surveillance data alone do not indicate the true magnitude of foodborne disease affecting a community.

In Australia and New Zealand, the most common potentially foodborne diseases are enteric infections, including campylobacteriosis, salmonellosis, listeriosis, shiga-toxin producing *Escherichia coli* (STEC) infection, and norovirus infection. As each of these diseases is caused by different pathogens with different characteristics, the prevention measures are subtly different for each of them.

Humans represent an important sentinel for contaminants in foods. Contamination can enter the food supply at several points in the chain of production and preparation, from: where the food is grown, processed or sold, or during preparation and consumption in domestic or foodservice kitchens. It can be very difficult to identify exactly where the contamination has occurred, except during epidemiological and food safety investigations. Generic food safety advice to cook food properly, keep cold food cold and hot food hot covers the main means of preventing foodborne infections in the home. For industrial sources of foodborne pathogens affecting the food supply, prevention is far more complex requiring intensive quality assurance and risk-based regulatory controls.

Many countries have assessed the prevalence of diseases that may be foodborne, as it is important for policy development. Collaborative groups attempting to estimate the burden of disease and compare findings from country-to-country have been established. Assessing the prevalence of foodborne diseases is a complex exercise; as there are many different etiological agents and many have multiple routes of transmission. In this article, the prevalence and major causes of foodborne diseases in Australia and New Zealand were reviewed.

Description of Surveillance Systems

Australia and New Zealand both have sophisticated systems for surveillance of diseases potentially transmitted by food. A

list of specified diseases are considered 'notifiable,' and cases that come to the attention of the health care system are reported via either general practitioners or laboratories which test for pathogens. Some infections and intoxications caused by food are not specifically notifiable, but may be reportable under public health law when they manifest in outbreaks of disease. In New Zealand and Australia, public health legislation has a requirement for notification of 'food- or waterborne disease outbreaks.' The main reasons for public health surveillance of enteric diseases include: assessment of trends, characterizing risk factors for infection, and detection and investigation of outbreaks. Proper investigation of outbreaks and characterization of risk factors can lead to immediate prevention of further cases, along with system-wide interventions to prevent future cases.

In New Zealand, the Institute of Environmental Science and Research Limited (ESR) collates reported cases of notified diseases through a database called EpiSurv, on behalf of the Ministry of Health. Infected cases who have an occupation that may put the public at risk, for example, foodhandlers and childcare workers, are followed up to ensure they are excluded from work until clear of infection. Information on risk factors and potential sources of infection may also be collected from cases. EpiSurv includes a dedicated module for the reporting of outbreaks. Key data fields collected include case demographics, clinical features and risk factors, and the results of outbreak investigations and analysis, for example, potential outbreak sources and the level of evidence for these sources. Information can be viewed via customizable local and national reports and maps. Data from case notifications and outbreak investigations are monitored to detect related cases both in New Zealand and overseas, and summaries are published in regular surveillance reports.

An annual report on foodborne disease has been published on their website by the New Zealand Ministry for Primary Industries since 2006, and incorporates surveillance data and attribution estimates. The reports also compare rates of reported foodborne disease against 5-year targets for reduction or control. In terms of attribution for foodborne transmission, the primary source for New Zealand is an expert elicitation conducted in 2005. More up to date estimates for poultry-associated campylobacteriosis have been derived from typing data of isolates from human cases and sources in a sentinel site study in the Manawatu region of New Zealand.

In Australia, six States and two Territories have primary public health powers to conduct public health surveillance of infectious diseases and investigate disease outbreaks. State and Territory health departments collect data on notifiable diseases in databases, which are aggregated into the National Notifiable Disease Surveillance System (NNDSS). At the national level, the Office of Health Protection (OHP) in the Department of Health and Ageing manages NNDSS, coordinates national action in response to communicable disease outbreaks and develops public health policy. OHP manages OzFoodNet – a national network of epidemiologists for the investigation of foodborne disease. Under the OzFoodNet program, each State and Territory health department employs one or more epidemiologists to investigate foodborne disease and improve multistate outbreak response. The OzFoodNet program of work is conducted in conjunction with other government agencies, and public health laboratories. OzFoodNet conducts surveillance for foodborne outbreaks and prepares regular reports on foodborne diseases in Australia.

Good microbiological laboratory services are the key to robust foodborne disease surveillance, as there is a need to identify common strains of foodborne pathogens infecting people from contaminated foods. Both New Zealand and Australia have extremely high-quality pathology laboratories, including public health reference laboratories. In New Zealand, ESR provides reference laboratory services to characterize viral and bacterial pathogens, such as *Salmonella*, toxigenic *E. coli*, and noroviruses. In Australia, most States and Territories have jurisdictional reference laboratories that conduct specialized testing for enteric pathogens. In Australia, the Microbiological Diagnostic Unit (MDU) Public Health Laboratory at the University of Melbourne conducts national characterization of a range of different pathogens, particularly *Salmonella*, *Listeria*, and toxigenic *E. coli* that are important for public health. MDU has conducted national surveillance for human and animal pathogens, particularly *Salmonella*, for many years. Similarly, the Institute of Medical and Veterinary Science in South Australia has housed the Australian *Salmonella* Reference Laboratory, which has a role in national surveillance. These laboratory services are critical to identifying and controlling foodborne diseases.

Diseases due to Foodborne Pathogens in Australia and New Zealand

Compared with other developed countries, Australia and New Zealand have higher rates of many illnesses due to potentially foodborne enteric pathogens. For example, the rate of campylobacteriosis in Australia is 10 times higher when compared with the US. There may be several reasons for this, including:

1. better ascertainment of cases of foodborne disease by surveillance in Australia and New Zealand,
2. higher rates of detection of foodborne pathogens in microbiology laboratories in Australia and New Zealand, and
3. differences in the risk factors for foodborne illness in Australia and New Zealand compared with other countries, giving rise to a truly greater incidence of foodborne illness.

Surveillance for foodborne infections differs in New Zealand and Australia, but we can learn from comparing data in the two countries. In particular, notification rates for *Campylobacter* infection have declined dramatically in New Zealand after a concerted risk management program was undertaken by the New Zealand poultry industry and government in 2006–07. Because of the intervention in New Zealand, the reported rate of infection is approaching that of Australia, but both countries are higher than most other countries in the world (**Figure 1**). Although poultry remains an important transmission vehicle for campylobacteriosis, in New Zealand increasing attention is being paid to other transmission routes. Nonfood transmission is likely to be more important in rural areas, including contaminated water and direct contact with livestock.

Figure 1 Rates of notification for campylobacteriosis in Australia and New Zealand, 2002–11. Reproduced from Data for Australia from NNDSS, available at: http://www9.health.gov.au/cda/Source/CDA-index.cfm (accessed on 18 January 2013) and Data for New Zealand from EpiSurv, available at: http://www.surv.esr.cri.nz/surveillance/annual_surveillance.php (accessed on 18 January 2013).

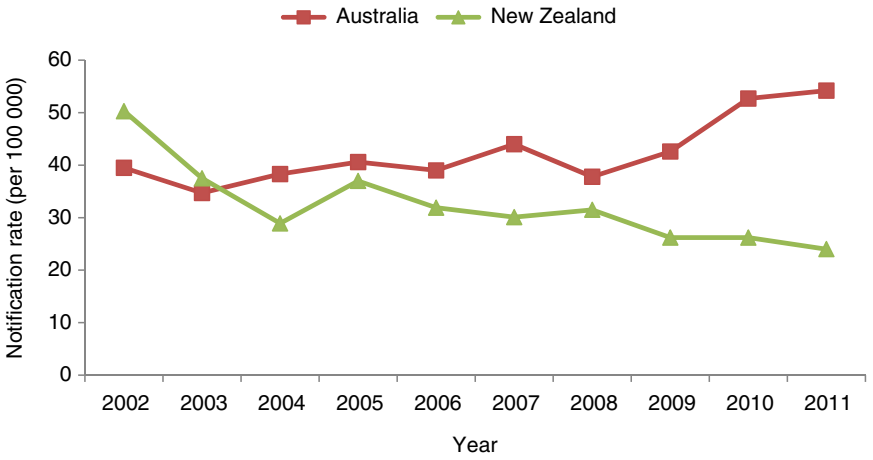

Figure 2 Rates of notification for salmonellosis in Australia and New Zealand, 2002–11. Reproduced from Data for Australia from NNDSS, available at: http://www9.health.gov.au/cda/Source/CDA-index.cfm (accessed on 18 January 2013) and Data for New Zealand from EpiSurv, available at: http://www.surv.esr.cri.nz/surveillance/annual_surveillance.php (accessed on 18 January 2013).

The rates of reported salmonellosis in New Zealand have been generally declining over the past decade (**Figure 2**). In New Zealand the prevalence of *Salmonella* in poultry and eggs is very low compared with other developed countries, largely due to industry control efforts in the 1990s. An analysis of *Salmonella* outbreak data from 2000 to 2009 found that food and foodhandler-associated outbreaks were the most commonly identified transmission routes, but specific foods common to multiple outbreaks were not able to be identified. In contrast, investigation of individual *Salmonella* outbreaks provides important information on sources of infection and preventive measures. A large outbreak of *Salmonella* Typhimurium 42 in New Zealand in 2008 was linked to contaminated flour, which is a novel food vehicle and highlighted that *Salmonella* can be transmitted by many different vehicles.

The rates of salmonellosis in Australia have increased steadily over the last 10 years, particularly since 2008. Much of the increase is thought to be related to *S.* Typhimurium infections associated with raw or minimally cooked egg dishes, as shown by sharp increases in egg-associated outbreaks at this time. Each year, Australian health departments investigate approximately 50–60 outbreaks of salmonellosis that are associated with a wide variety of foods. The OzFoodNet network has assisted with detecting and investigating outbreaks that are wide-spread geographically. Some of these outbreak investigations have identified novel food vehicles, such as dried peanuts, sesame seed products (halva and tahini), and rockmelons.

The highest rates of salmonellosis in both countries occur in young children. Many cases of salmonellosis are acquired in residents while traveling overseas. In Australia, rates of infection are highest in northern Australian populations,

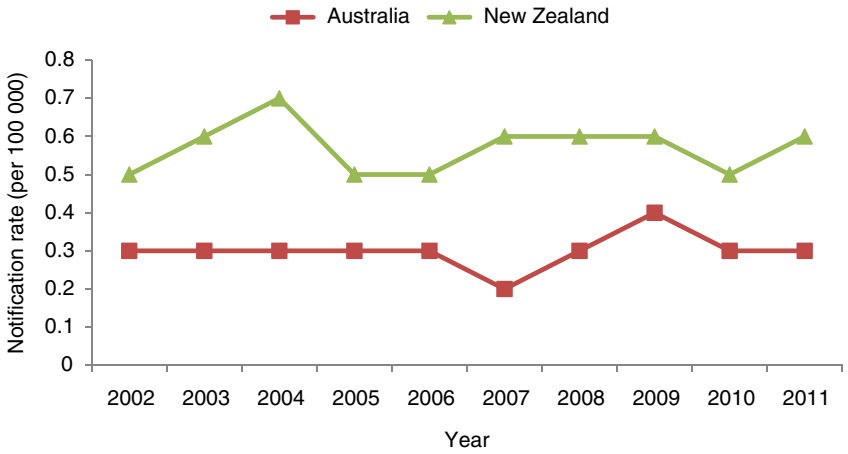

Figure 3 Rates of notification for listeriosis in Australia and New Zealand, 2002–11. Reproduced from Data for Australia from NNDSS, available at: http://www9.health.gov.au/cda/Source/CDA-index.cfm (accessed on 18 January 2013) and Data for New Zealand from EpiSurv, available at: http://www.surv.esr.cri.nz/surveillance/annual_surveillance.php (accessed on 18 January 2013).

particularly in people of indigenous origin. In contrast, higher rates of reported salmonellosis in New Zealand occur in the cooler South Island, and in the European ethnic group.

In New Zealand and Australia, the most common serotype of *Salmonella* is Typhimurium. Neither country has *Salmonella* Enteritidis endemic in egg-laying flocks. The distribution of *Salmonella* strains probably reflects local zoonotic reservoirs. For example, in the southernmost Australian State of Tasmania, the predominant strain of *Salmonella* is serotype Mississippi, which is thought to be acquired from native Australian animals in environmental settings. *Salmonella* Mississippi infections are uncommon in residents of mainland Australia. In New Zealand, *S.* Typhimurium 160 was the predominant strain from 2008 to 2010, and is largely not foodborne, but due to an epizootic in sparrows.

Listeriosis is a serious foodborne infection that results in meningitis, septicemia, pneumonia, and gastroenteritis, although only invasive infections are notifiable under public health legislation. Listeriosis usually affects two main risk groups: unborn babies, and people who are immunocompromised or elderly. The rates of reported *Listeria* infection in New Zealand are higher than in Australia (**Figure 3**). The ratio of perinatal infections to nonpregnancy-related infections appears higher also, with New Zealand in 2010 reporting 26.1% of infections as perinatal compared with 1.4% in Australia for the same year. This may indicate different risk factors for infection affecting the two populations, or may be an artifact of differences in the policies for testing for listeriosis. The majority of infections in both countries occur in people over the age of 65 , or who are on immunosuppressive medications.

STEC is another important foodborne pathogen under surveillance in both Australia and New Zealand. In the Australian State of South Australia in 1995, a major outbreak of *E. coli* O111:H-from contaminated mettwurst (fermented dried sausage) resulted in 23 cases of hemolytic uremic syndrome, one of whom died. This severe outbreak resulted in South Australia initiating robust surveillance for both *E. coli* O157 serogroup and non-O157 serogroups using polymerase chain reaction testing of all diarrheal stools containing blood for genes encoding for production of Shiga toxins. As a result, surveillance for STEC in South Australia is much better than the rest of the country. The rates of disease are lower in other jurisdictions, as STEC are not routinely tested for and usually only when requested. The overall rates of STEC are comparable in both New Zealand (3.5 per 100 000 persons in 2011) and South Australia (3.0 per 100 000), although there are differences in the main serogroups causing disease. In New Zealand, more than 90% of reported cases are infections with *E. coli* O157:H7, whereas in Australia it accounts for approximately 50% of cases.

Other Foodborne Diseases

Several other diseases that are notifiable may also be transmitted by food, but are not discussed in detail here. Examples include shigellosis, typhoid, cholera, botulism, and hepatitis A, which have caused foodborne outbreaks in both countries, but are relatively rare causes of foodborne disease most of the time. In 2009, Australia experienced a large and serious outbreak of hepatitis A due to imported semidried tomatoes, whereas New Zealand experienced an outbreak of hepatitis A due to contaminated blueberries in 2002.

Yersiniosis is a potentially foodborne disease, and is notifiable in New Zealand, where it is the third most commonly reported enteric infection. In contrast, in Australia the incidence was so low that surveillance ceased in 2001. Although outbreaks of norovirus infection are often reported as part of routine surveillance, sporadic cases are not notifiable.

Chemical hazards in food are outside the scope of this article. However, it is worth noting that in New Zealand, illness caused by toxins found in marine species (particularly shellfish) are notifiable (as 'Poisoning arising from chemical contamination of the environment'), whereas outbreaks of histamine intoxication from fish are also reported. Similarly, in some Australian States and Territories it is mandatory to report foodborne diseases of chemical origin, such as ciguatera.

Table 1 Incidence and key features of different studies investigating the incidence of gastroenteritis in Australia and New Zealand

Study features	*Study*				
	Water quality study	*Tank water study*	*National gastroenteritis survey*	*National gastroenteritis survey II*	*Acute gastrointestinal illness study*
Country	Australia	Australia	Australia	Australia	New Zealand
Conducted by	Monash University	Monash University	National Centre for Epidemiology and Population Health & OzFoodNet	National Centre for Epidemiology and Population Health & OzFoodNet	Environmental Science and Research Limited
Year	1997–99	2007–08	2000–01	2008–09	2006–07
Study design	Prospective randomized control trial examining whether reticulated water caused gastroenteritis illness	Prospective randomized control trial examining whether tank water caused gastroenteritis illness	Cross-sectional computer-assisted telephone interview	Cross-sectional computer-assisted telephone interview	Cross-sectional computer-assisted telephone interview
Coverage	Metropolitan community of households with children in Melbourne, Australia	Rural community in South Australia	National	National	National
Time period (months)	18	12	12	12	12
Sample size (persons)	600 families	277 families	6087	7578	3655
Case definition	Two or more loose stools; two or more episodes of vomiting; one loose stool together with abdominal pain, nausea, or vomiting; or one episode of vomiting with abdominal pain or nausea	Two or more loose stools; two or more episodes of vomiting; one loose stool together with abdominal pain, nausea, or vomiting; or one episode of vomiting with abdominal pain or nausea	$\geq$3 loose stools or $\geq$2 episodes of vomiting or, if respiratory symptoms were present $\geq$4 loose stools or $\geq$3 episodes of vomiting in a 24-h period in the previous 4 weeks. Those reporting noninfectious causes were excluded	$\geq$3 loose stools or $\geq$2 episodes of vomiting or, if respiratory symptoms were present $\geq$4 loose stools or $\geq$3 episodes of vomiting in a 24-h period in the previous 4 weeks. Those reporting noninfectious causes were excluded	'Any' diarrhea, vomiting, or both, experienced in the previous 4 weeks, excluding noninfectious causes such as chronic illness, medication, medical treatment, and pregnancy
Incidence (episodes per person per year)	0.80	0.77	0.97	0.74	1.11

Gastroenteritis in Australia and New Zealand

In Australia and New Zealand, there have been several studies examining the incidence of gastroenteritis. Gastroenteritis is the most common outcome of infection from several pathogens that are potentially transmitted by food. The incidence of gastroenteritis in Australia and New Zealand is similar regardless of study design and time of study (Table 1). In general, people experience approximately one episode of gastroenteritis each year.

Attribution

It is very difficult to estimate the proportion of gastroenteritis that is foodborne, as pathogens with different infectious and transmission characteristics result in the same outcome. Some pathogens, such as norovirus, are more commonly spread by one infectious person to another, whereas others are more commonly spread by contaminated food or water. To estimate the proportion of disease due to different pathogens that is transmitted by contaminated food, many international investigators have used expert elicitation, where experts were asked their opinion was about the proportion of each pathogen is foodborne.

For example, OzFoodNet in Australia conducted an expert elicitation, which estimated that 87% of *Salmonella* infections were spread by contaminated food. When the proportion of different etiological agents was considered for all 16 pathogens considered important for foodborne transmission of gastroenteritis, it was estimated that approximately 32% of gastroenteritis was foodborne. Using a similar expert elicitation approach for New Zealand, it was estimated in 2005 that a high proportion of enteric diseases are foodborne, including: norovirus and STEC infection where approximately 40% of infections were foodborne; yersiniosis, campylobacteriosis, and salmonellosis were approximately 60%; and listeriosis at 85%.

Policy Implications

The burden of foodborne disease caused by six microbial pathogens (*Campylobacter*, *Listeria*, *Salmonella*, STEC, norovirus, and *Yersinia*) in New Zealand was estimated as 1510 disability adjusted life years (DALYs) based on data up to 2005. Of the total, 880 DALYs were attributed to campylobacteriosis, with norovirus infection being the second greatest contributor (210 DALYs). The estimate for *Campylobacter* infections, along with other analyses typing investigations that attributed the majority of cases to poultry-associated types, added to the impetus to pursue risk management measures for this disease.

These burden estimates were made before the recent reduction in the incidence of campylobacteriosis over the period 2006–08. More recent data was used to provide a 2009 estimate of the total economic cost of these six diseases. The costs of government and business activity, medical treatment, and lost productivity were estimated as NZ$62.2 million, whereas personal and lifestyle costs (pain, suffering, and disruption) were estimated as NZ$99.7 million. Of the total cost of NZ$162 million, the largest proportion (NZ$50 million) was attributed to infection with norovirus, followed by campylobacteriosis (NZ$36 million).

In Australia, an estimate of costs based on data from 2001 found that 5.4 million cases of foodborne illness were estimated to cost AUS$1.2 billion annually. A separate estimate for the costs of STEC infection in South Australia found that the mean cost per case was AUS$3132, equating to AUS$2.6 million for STEC infections annually each year in Australia.

Apart from the estimates of the value of pain and suffering (which may be made in monetary or DALY metrics), estimates of cost in both countries found that the main contributor was lost productivity from a person not being able to work due to their own illness, or having to look after someone else who was ill. The main reason for the difference in costs between the two countries was that the Australian costing included cases of disease where there was no pathogen identified, but was likely to be foodborne. Foodborne disease of unknown etiology is likely to be extremely common based on efforts to estimate foodborne disease from other countries.

The burden of disease due to *Campylobacter* is high, due to the high number of cases, the severity of resulting gastroenteritis, and serious sequelae of infection such as Guillain–Barré syndrome, reactive arthritis, and irritable bowel syndrome. Poultry meat that is contaminated with *Campylobacter* has been identified as the key risk factor for infection in both countries through case–control studies and source attribution of molecular subtypes. In Australia, chicken meat contaminated with *Campylobacter* has been estimated to result in 50 500 cases of campylobacteriosis each year. The spectacular success of New Zealand in controlling foodborne disease has shown that governments and industry can enter into productive partnerships to prevent foodborne disease. In New Zealand, reported cases and hospitalizations for campylobacteriosis and Guillain–Barré syndrome declined following the regulatory measures, which has resulted in significant economic savings and reduced burden on individuals.

The generally good control of microbial hazards in the food supply of both countries may contribute to changes in the management of higher risk foods, as suggested by the current pressure to relax controls over raw milk consumption. It is important that countries maintain vigilance with foods that may be higher risk for causing foodborne disease. Efforts to improve our understanding about the occurrence and causes of foodborne disease are vital to maintaining a safe food supply.

Conclusion

Each year in Australia and New Zealand, people experience approximately one episode of acute gastrointestinal disease per person per year. It is difficult to estimate the proportion of these that are due to foodborne transmission, partly because even if a case presents to the health system and provides a stool sample, a pathogen may not be detected. Consequently the etiology of a large proportion of these cases is unknown. Nevertheless, for many of the most frequently reported enteric diseases, a high proportion is due to foodborne transmission. The prevalence of foodborne disease in Australia and New Zealand is similar to most developed countries. Both countries have invested substantial resources in investigating

and controlling foodborne diseases. This has resulted in major improvements in identifying the causes of foodborne disease outbreaks. New Zealand has experienced great success in reducing the incidence of campylobacteriosis. The interventions have had a substantial effect in reducing acute and chronic disease, and should serve as a model for other countries.

Acknowledgments

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See also: Bacteria: *Campylobacter*; *Listeria monocytogenes*; *Salmonella* Non-Typhi; Shiga Toxin-Producing *Escherichia coli* and Other Pathogenic *Escherichia coli*. Public Health Measures: Foodborne Disease Outbreak Investigation; Surveillance of Foodborne Diseases

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Prevalence of Foodborne Diseases in Europe

SJ O'Brien, University of Liverpool, Liverpool, UK

Glossary

Disability-adjusted life years It provides a measure of overall disease burden, expressed as the number of years lost due to ill health, disability, or early death.

Foodborne source attribution Refers to the process of partitioning foodborne infections by specific sources, where the term 'source' can include the animal reservoirs or food vehicles.

Meta-analysis Refers to the process of estimating the true effect size from pooled results of, for example, case–control studies, as opposed to the less precise result obtained from a single study under a given single set of assumptions and conditions.

Reservoir Refers to the animal hosts (including humans) from which foodborne pathogens originate. The reservoir can include diseased or asymptomatic carriers/shedders.

Systematic review Refers to a literature review, which is driven by a research question. The process involves identifying, appraising, selecting, and synthesizing all high-quality research evidence relevant to the research question.

Vehicles These are inanimate objects that enable contact between pathogen reservoir and people and include water, food, animals, soil, manure, compost, and other people.

Introduction

Foodborne disease kills people. Reminders of this chastening fact include the devastating outbreak of *Escherichia coli* O104:H4 in Germany in 2011, in which 54 people died and 22% of the 3186 cases of *E. coli* O104 developed the severe complication, hemolytic uremic syndrome (HUS). Outbreaks are striking events, yet the percentage of all cases of foodborne disease that occur as part of outbreaks is fairly small. The first port of call when seeking to understand the prevalence of foodborne disease is the official bodies that have responsibility for monitoring illness in the population. However, the datasets amassed by these organizations tend to underestimate the population burden of illness so, in the past decade or so, there has been a proliferation of new methods across Europe in an attempt to overcome this deficiency and develop better estimates of the true population burden of illness.

The Prevalence of Foodborne Disease in Europe: The Official Estimates

The major sources of collated data on the prevalence of foodborne disease in Europe are the World Health Organization (WHO) Regional Office in Europe and the European Center for Disease Prevention and Control (ECDC). The WHO program covers 53 countries that make up the WHO European Region – from the Republic of Ireland in the West to the Russian Federation in the East, and Israel to the South. The ECDC compiles data from the 27 Member States of the European Union (EU) and three European Economic Association/European Free Trade Association countries. In addition, the European Food Safety Authority (EFSA) is responsible for analyzing data on zoonoses, antimicrobial resistance, and foodborne outbreaks submitted by EU Member States for preparing the EU Summary Report. This is undertaken in collaboration with the ECDC.

The View from the WHO Regional Office in Europe

In 2002, World Health Assembly mandated WHO to develop a global strategy for reducing the burden of foodborne disease. In this strategy, it is recognized that preventing foodborne disease and responding to food safety challenges need a timely, holistic, risk-based approach.

Information about the prevalence of foodborne disease in the WHO European Region is available from the Centralized Information System for Infectious Diseases (CISID). The CISID dataset, compiled from national reports, is underpinned by accurate and up-to-date population data for the WHO European Region and information collected by WHO is useful for risk assessment.

The CISID dataset covers a wide range of foodborne pathogens. In the WHO European Region, salmonellosis and campylobacteriosis are the most commonly reported foodborne diseases. Between 2000 and 2010 the highest incidence of campylobacteriosis was consistently reported from the

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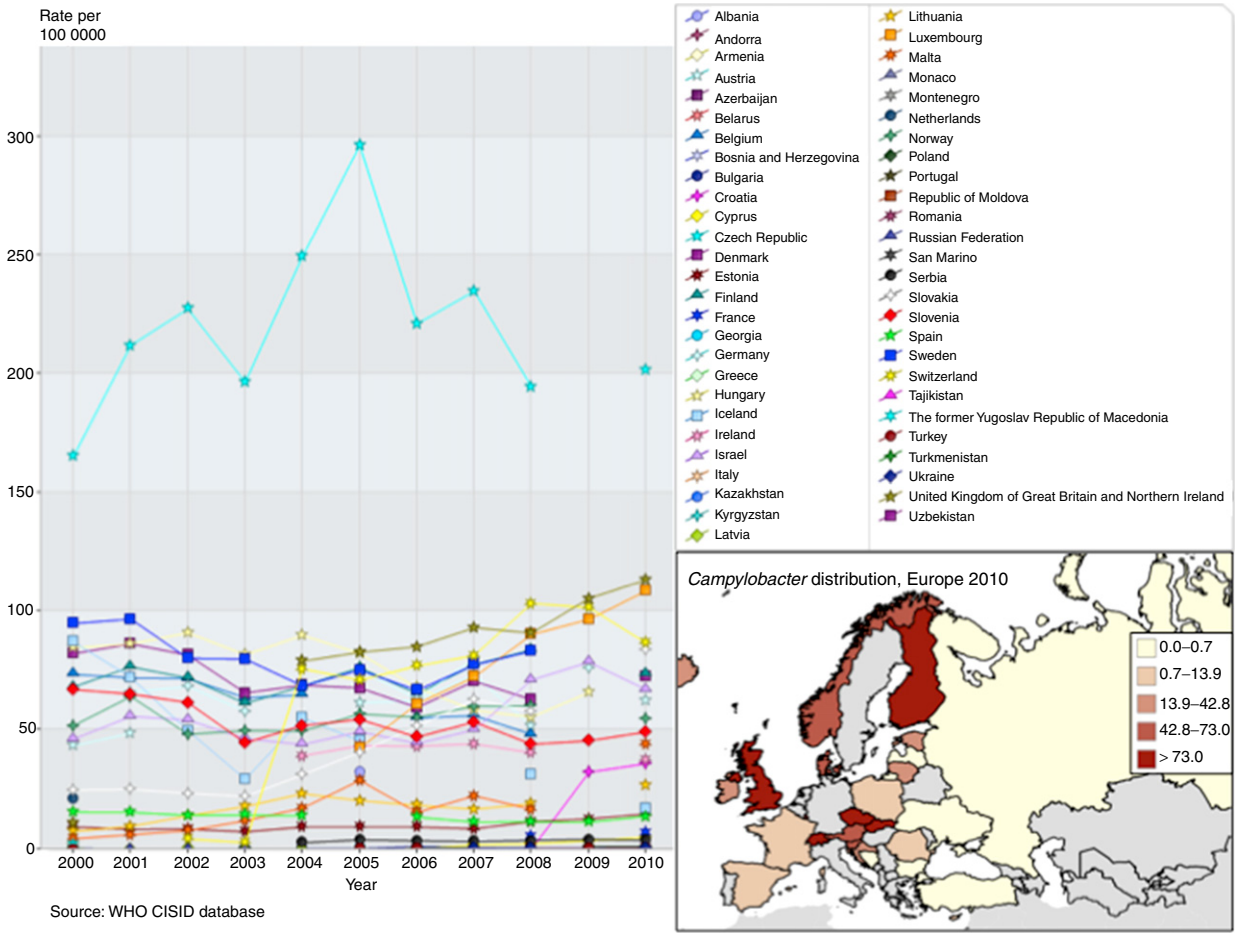

Figure 1 Trend in campylobacteriosis in the WHO European Region, 2000–10.

Former Yugoslav Republic of Macedonia, where rates were almost three times as high as elsewhere in the region (Figure 1).

The incidence of salmonellosis declined over the decades 2000–10 in several countries although *Salmonella* was still a frequent cause of the foodborne outbreaks (Figure 2).

The trend in listeriosis remained relatively stable over the decades 2000–10 (Figure 3), but reporting of enterohemorrhagic *E. coli* (EHEC) was mainly from Western European countries (Figure 4).

Brucellosis remained a significant public health problem in the Central Asian republics. Trichinellosis in the Balkan countries and echinococcosis in both the Central Asian republics and the Balkan countries were causes for concern. Botulism remained relatively frequent in Eastern Europe and is mainly related to traditional ways of preserving foods at home.

The View from the ECDC

Established in 2005, the ECDC is a EU agency that aims to strengthen Europe's defenses against infectious diseases. The Programme on Food- and Waterborne Diseases and Zoonoses was instituted in 2006, and covers a comprehensive range of pathogens. Early priorities included consolidating surveillance for salmonellosis, campylobacteriosis, verocytotoxin-producing *E. coli*/shiga toxin-producing *E. coli* (STEC) infection, listeriosis, shigellosis and yersiniosis; publication of an annual zoonosis report jointly with the EFSA; and developing novel methodology to estimate population exposure to salmonellosis and campylobacteriosis using seroepidemiology. In its 2011 annual epidemiological report, ECDC reported that *Campylobacter* rates are high and it remains the most commonly reported gastrointestinal illness across the EU. In 2009, there were over 201 000 confirmed cases (rate = 53 cases per 100 000 population). The highest rates were reported from the Czech Republic (193 cases per 100 000) and the UK (106 cases per 100 000).

By contrast, the incidence of *Salmonella* infection has decreased progressively since 2004 and this has been linked, at least partly, to effective programs to control infection in the poultry industry. There were over 111 000 cases reported in 2009 (rate = 23 cases per 100 000 population) and *Salmonella* continues to be a common source of outbreaks. *Salmonella* Enteritidis and *Salmonella* Typhimurium remain the most frequently identified serotypes but rates of *S.* Enteriditis infection were 24% lower in 2009 than in 2008 and rates of *S.* Typhimurium had also declined by 12%. Even in the higher incidence countries like the Czech Republic, Slovakia, Hungary, and Lithuania rates have also decreased markedly.

Reported cases of EHEC increased significantly between 2005 and 2009 to just over 3600 cases (rate = 0.86 per 100 000

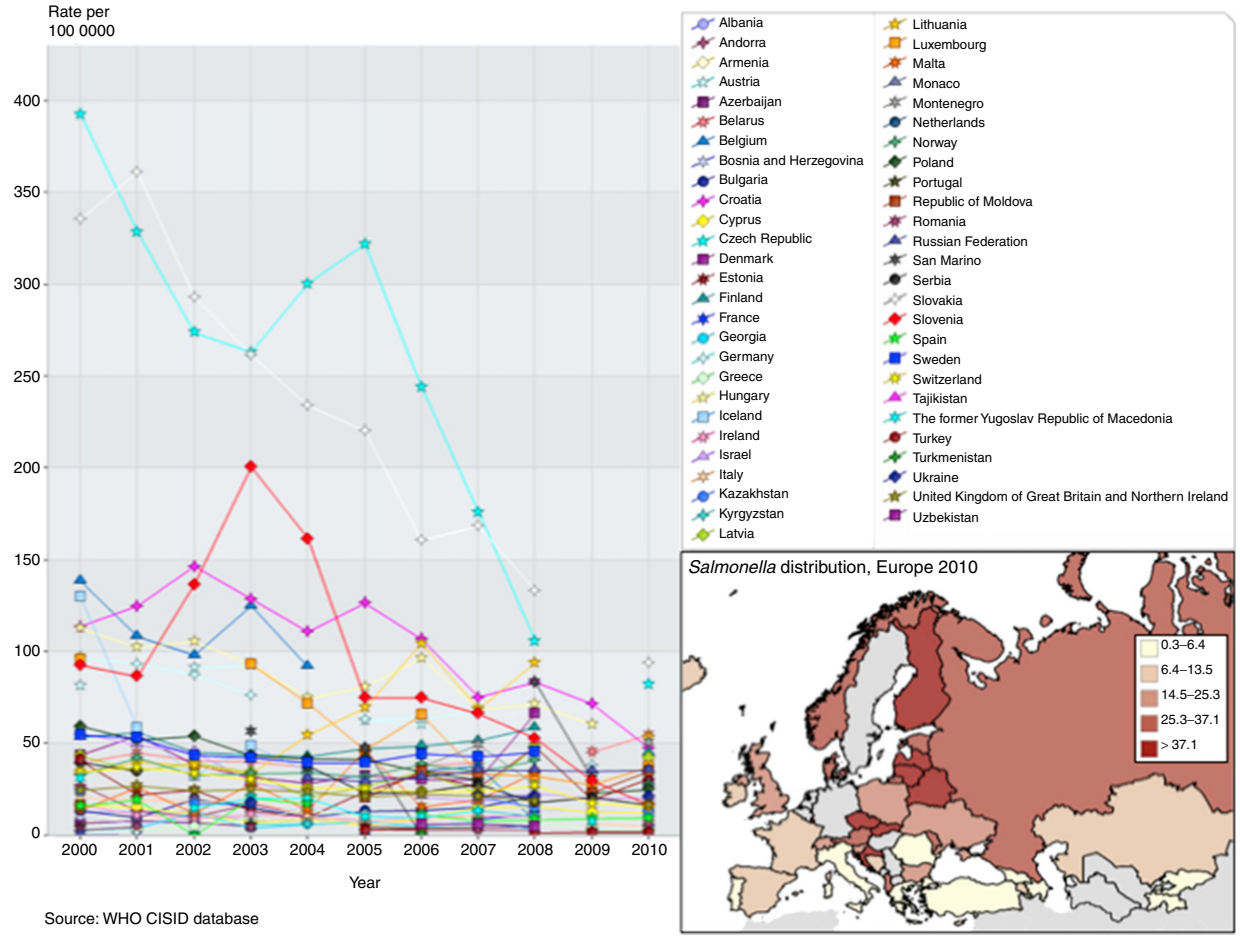

Figure 2 Trend in salmonellosis in the WHO European Region, 2000–10.

population). More than half of the cases were due to STEC O157. There were 242 confirmed STEC cases that developed HUS – a 66% increase in HUS cases compared with 2008. This large increase was, in part, explained by two sizable outbreaks of STEC – one in the UK linked to an open farm and a nationwide outbreak in the Netherlands associated with the consumption of STEC-contaminated beef steak tartare.

The ECDC report shows that some rare or uncommon gastrointestinal infections EU-wide can, nevertheless, affect particular subregions and countries. Brucellosis is reported mainly from Portugal, Spain, and Greece, where it is associated with goat farming. The majority of trichinellosis cases occurred in Bulgaria, Romania, and Lithuania, where it is likely to be associated with eating domestically reared pork and wild boar, and most confirmed echinococcosis cases were from Bulgaria, where increasing proportions of *Echinococcus*-positive cattle and sheep are also being reported. Overall, yersiniosis rates were decreasing but remain high in the Nordic states, Germany, the Czech Republic, and Slovakia, where infection is often associated with pork consumption. Confirmed case rates for listeriosis were highest in 2009 in Denmark (rate = 1.8 cases per 100 000 population), which has experienced a marked increase in cases, especially in people over 70 years of age. The increase was attributed, at least in part, to a surge in consumption of ready-to-eat (RTE) products in Denmark, especially in older people. A similar pattern was witnessed in the UK, where a doubling in cases of listeriosis in people aged more than 60 since 2001 was attributed to a combination of greater consumption of RTE products like sandwiches coupled with an increase in underlying medical conditions like cancer, requiring complex, immunosuppressive treatment.

The View from the EFSA

The EFSA, in collaboration with the ECDC, produces an annual 'European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Foodborne Outbreaks.' In 2010, there were 5262 foodborne outbreaks reported in the EU – similar to the level reported in 2009. These outbreaks involved nearly 43 500 people, of whom approximately 4600 were hospitalized and there were 25 deaths. The evidence implicating a food vehicle was considered to be strong in 698 outbreaks.

Salmonella was the most frequently reported pathogen (30.5% of all outbreaks), followed by viruses (15.0%) and *Campylobacter* (8.9%) (Figure 5). However, there was a considerable proportion of outbreaks in which the causative

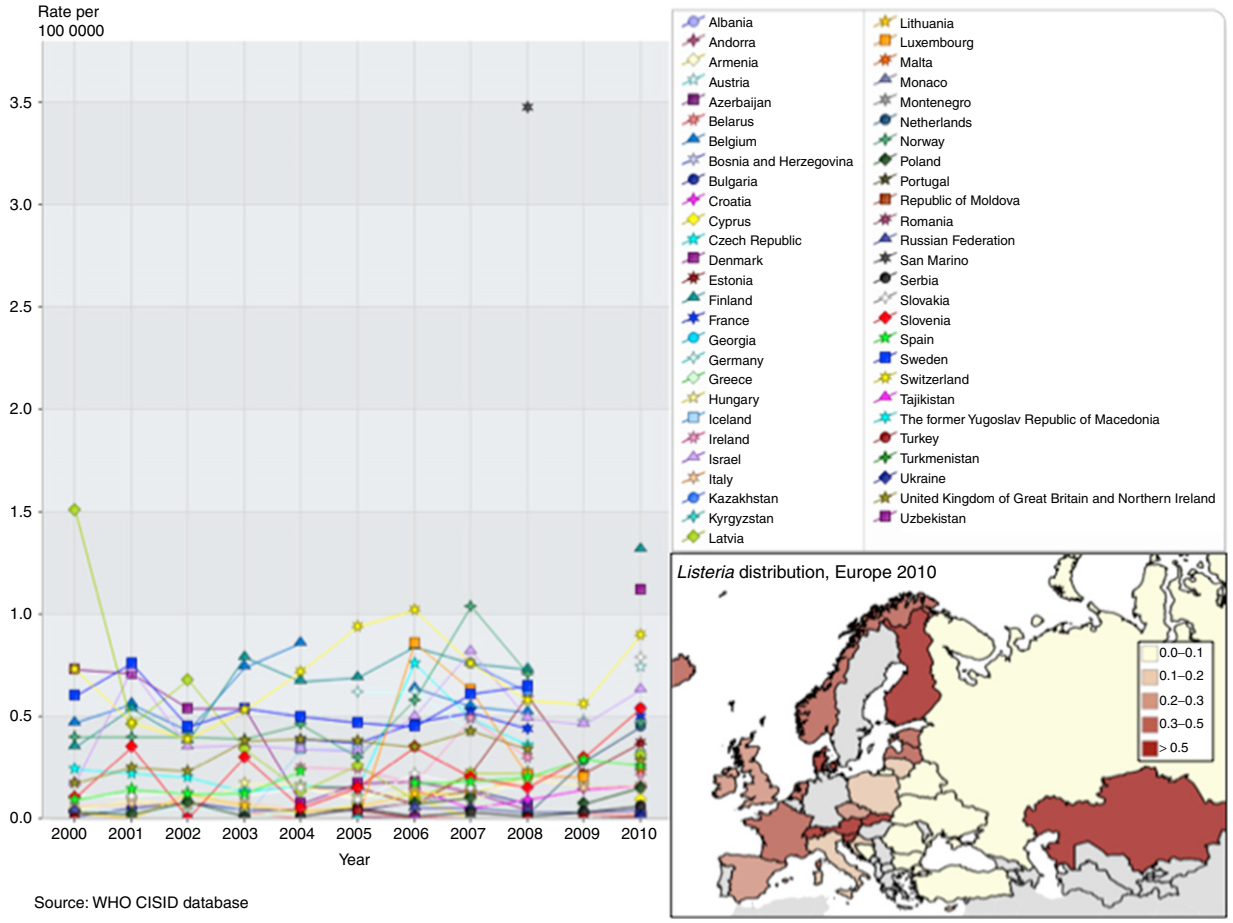

Figure 3 Trend in listeriosis in the WHO European Region, 2000–10.

organism was unknown and a large percentage of *Campylobacter* outbreaks in which the evidence implicating a food vehicle was considered to be weak.

The most frequently reported food vehicles were eggs and egg products (22.1%); mixed or buffet meals (13.9%); vegetables, juices, and vegetable/juice products (8.7%); and crustaceans, shellfish, molluscs, and shellfish products (8.5%). An increase in the numbers of reported outbreaks caused by vegetables and vegetable products was attributed mainly to contamination with viruses.

The View from 'NoroNet'

It is becoming increasingly apparent that norovirus (NoV) is an important foodborne pathogen. Contamination can occur either at primary production, for example, shellfish, salad vegetables, and soft fruits at source or, at retail, for example, through inadequate practises by infected food handlers. Funded initially through a research grant, 'NoroNet' now comprises a voluntary collaboration between virologists and epidemiologists from 13 European countries who have been sharing surveillance and research data on enteric virus infections, mainly NoV, since 1999. There are international partners from North America, Australia, China, India, and New Zealand. The objectives of NoroNet include providing better estimates for the proportion of foodborne NoV infections and determining the high-risk foods associated with illness. Several publicly available analytical tools have been developed including a 'transmission mode tool' to increase the chances of identifying a foodborne source of infection. Using this tool to analyze 1254 outbreaks from nine countries reported between 2002 and 2006 showed that the proportion of NoV outbreaks that were likely to be foodborne was 22%. 'NoroNet' was also instrumental in identifying the latest pandemic strain – the Sydney 2012 virus, which has recently overtaken all others to become the dominant strain worldwide.

Gaps in Our Knowledge

A major drawback of all surveillance systems, be they local, national, or international, is that they underestimate the true population burden of acute gastroenteritis and, in turn therefore, the true burden of foodborne disease. Surveillance systems eavesdrop on the clinical process. The greatest potential loss of information about illness in the population occurs when people do not access the healthcare system. In most countries, an episode of illness has no chance of being included in surveillance statistics unless the patient consults a doctor or nurse. Similarly, information on pathogens is only available if the doctor or nurse requests, and the patient provides, a sample for laboratory testing.

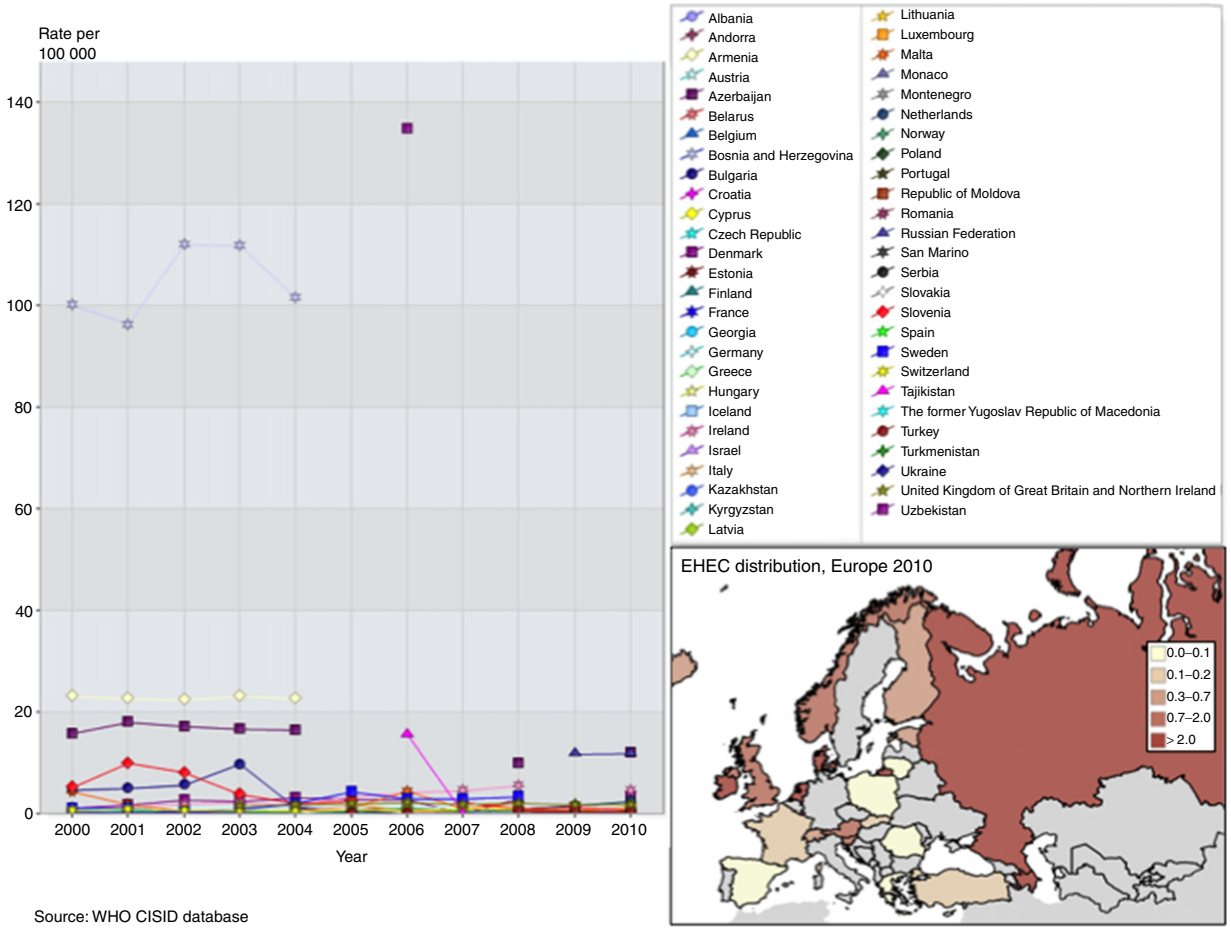

Figure 4 Trend in EHEC infections in the WHO European Region, 2000–10.

National surveillance systems for foodborne disease in Europe operate in different ways. Some are sentinel, symptom-based systems that collect little information on etiology. Others are based on laboratory-confirmed cases of infection. Laboratory testing protocols vary between laboratories within the same country, let alone between laboratories in different countries. Some cover the total population, while others include only a proportion of the total population. Most routine programs are passive, voluntary systems. The degree of underascertainment in many of the systems has not been measured, and all these factors conspire to make meaningful comparisons of disease rates between countries very difficult to accomplish.

The key to determining the real impact of foodborne disease on the population is to understand, first of all, the 'true' population burden of acute gastroenteritis.

Burden of Acute Gastroenteritis

There are several methodological approaches for estimating the incidence of acute gastroenteritis including retrospective cross-sectional surveys (telephone surveys of self-reported illness, door-to-door or postal questionnaire surveys) or prospective, population-based cohort studies (Table 1).

Telephone Surveys

Seven retrospective telephone surveys of self-reported illness have recently been completely performed in the WHO European Region (Table 1). These have the advantage that large samples of the population can be contacted and interviews are relatively short so that participation rates tend to be good. The major disadvantage of telephone surveys and other types of surveys seeking information on symptoms is that the etiology of symptoms is not captured. They are also prone to inaccurate recall, especially if the recall period is fairly long.

Rates of self-reported illness in the general population ranged between 1.4 cases per person per year in Denmark and 0.33 cases per person per year in France. Comparing rates across nations can be difficult. Differences in case definitions, study designs, periods of recall symptoms, and the populations studied can all hamper incidence rate comparisons. For example, one of the studies highlighted in

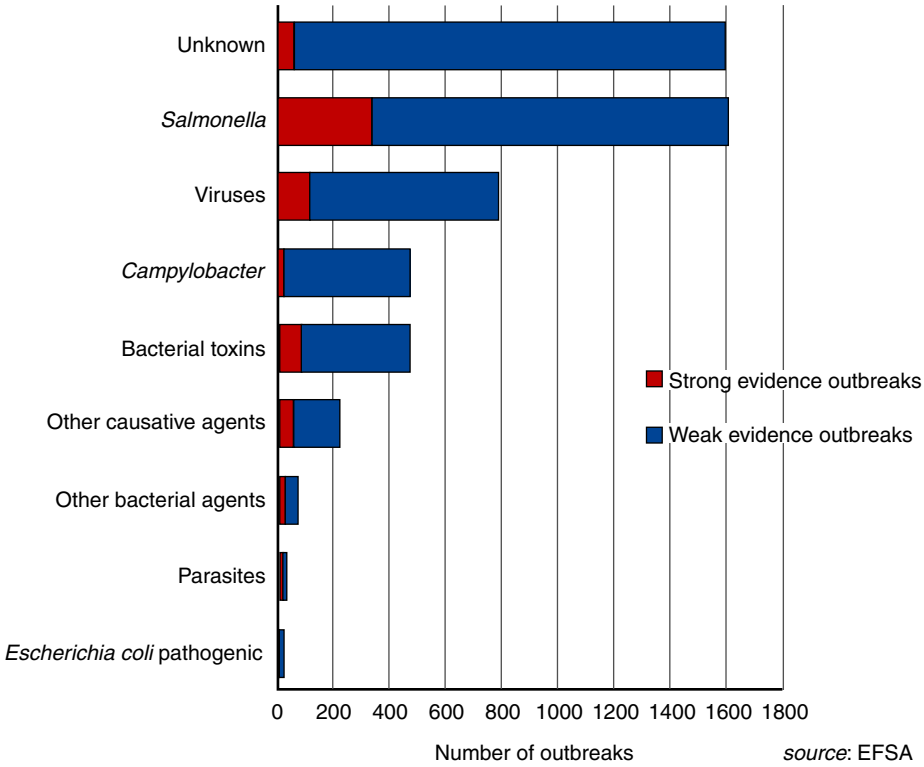

Figure 5 Etiology of foodborne disease outbreaks in the European Union, 2010.

Table 1 Recent population-based studies of the incidence of acute gastroenteritis in Europe

Country	*Study design*	*Study duration*	*Incidence estimate, expressed as rate per person per year (95% CI)*	*Lead author (publication date)*
Poland	Telephone survey	2008–09	0.9 (0.8–1.0)	Baumann-Popczyk *et al.* (2012)
The Netherlands	Questionnaire survey	2009–10	0.96 (0.81–1.11)	Doorduyn *et al.* (2012)
Hesse, Germany	Telephone survey	2004–06	0.86 (0.72–1.03) (children aged ≤15 years) 0.46 (0.37–0.51) (adults aged ≥16 years)	Hauri *et al.* (2011)
Italy	Telephone survey	2008–09	1.08 (0.90–1.14)	Scavia *et al.* (2012)
France	Telephone survey	2009–10	0.33 (0.28–0.37)	Van Cauteren *et al.* (2012)
Israel	Telephone survey	2005	1.49 (not reported) (children aged <17 years)	Ziv *et al.* (2011)
Denmark	Telephone survey	2009	1.4 (1.2–1.6)	Müller *et al.* (2012)
UK	Prospective cohort study	2008–09	0.27 (0.25–0.3) in the community	Tam *et al.* (2012a)

Table 1 only involved children. Nevertheless, using a standardized, symptom-based case definition enabled better comparison of rates between countries, and as the use of this case definition becomes more widespread some of these difficulties in interpreting rates between studies should diminish.

As well as determining disease rates information on healthcare usage in this series of coordinated, cross-sectional telephone surveys of self-reported illness was used to estimate under-reporting and underdiagnosis in the national surveillance systems of the countries taking part. Overall, underreporting and underdiagnosis were estimated to be lowest for Germany and Sweden, followed by Denmark, the Netherlands, UK, Italy, and Poland. Across all countries, the incidence rate was highest for *Campylobacter* spp. and *Salmonella* spp. Adjusting incidence estimates for biases inherent in different surveillance systems provides a better basis for international comparisons than relying on reported data.

Prospective, Population-Based Cohort Study

Prospective studies are uncommon, perhaps because of their expense. Three such studies have been conducted in Europe – one in the Netherlands and two in the UK. The major advantage of cohort studies is the ability to obtain samples from patients with infectious intestinal disease (IID) to confirm etiology, which is important if one of the aims is to calibrate national surveillance systems. A major drawback is that participation rates can be low and losses to follow-up may be high, but there are several strategies to try to overcome both of these important limitations.

In the UK, illness burden has been estimated in a population-based prospective cohort study and prospective study of presentations to primary care (the Second Study of Infectious Intestinal Disease in the Community (IID2 Study)). Up to 17 million people (approximately one in four) in the community were found to be suffering from IID in a year (annual incidence = 0.27 cases of IID per person per year). There were approximately 3 million cases of NoV infection and 500 000 cases of campylobacteriosis. The estimated time taken off from work or school because of IID was nearly 19 million days. Approximately 1 million people presented to their primary healthcare team and the leading causes were NoV infection (130 000 cases) and campylobacteriosis (80 000 cases).

As well as defining illness burden, a secondary objective of the IID2 Study was to recalibrate national surveillance systems, i.e., to estimate the number by which laboratory reports of specified pathogens need to be multiplied to establish the actual number of infections in the community. So, for every case of IID reported to national surveillance centers in the UK, 147 cases had occurred in the community. For *Campylobacter* the ratio of disease in the community to reports to national surveillance was approximately 9 to 1, for *Salmonella* the ratio was approximately 5 to 1, and for NoV the ratio was almost 300 to 1.

Health Economics Assessments

A very powerful way to win the interest of politicians and policy makers is to be able to attach a monetary value to food-related illness. In developed countries, diarrheal disease can be belittled as inconvenient and unimportant alongside noncommunicable diseases like diabetes, heart disease, and stroke. Nevertheless, there can be considerable disruption to society and the economy. For example, the estimated costs of diarrheal disease are in the region of 345 million EUR in the Netherlands, 270 million EUR in Australia, and 2.8 billion EUR in Canada.

Disability-Adjusted Life Years

In the Netherlands, in 2009, the burden of NoV infection alone was estimated to be 1622 (95% confidence interval (CI) 966–2650) disability-adjusted life years (DALYs) in a population of 16.5 million, which is a large amount for what is generally held to be a very mild and self-limiting illness.

Burden of Food-Related Illness

Having worked out the burden of acute gastroenteritis, the next rational step is to apportion illness burden by transmission route, namely foodborne transmission. Once again, several methodologic approaches are available, including epidemiologic and microbiologic approaches, intervention studies, expert elicitation, health economics assessments, and systematic reviews.

Source Attribution Using Outbreak Data

Outbreaks that have been meticulously investigated, i.e., where the evidence linking the outbreak to a food vehicle is strong, can provide useful information for subdividing diarrheal disease by transmission route. However, there are several limitations when interpreting the results. The first is the robustness of evidence incriminating a food vehicle in an outbreak in the first place. For example, in the EFSA Report published in 2010 only 698 of 5262 outbreaks were considered to provide strong evidence of a link to a food vehicle. Second, it has to be accepted that the distribution of food vehicles implicated in outbreaks is the same as the distribution of food vehicles responsible for sporadic cases of infection and this is a major assumption.

In the UK, in an attempt to estimate the impact of disease risks associated with eating different foods, over 1.7 million cases of UK-acquired foodborne disease per year resulted in almost 22 000 people being admitted to hospital and nearly 700 deaths. *Campylobacter* infection caused the greatest impact on the healthcare sector (nearly 161 000 primary care visits and 16 000 hospital admissions) although *Salmonella* infection resulted in the most deaths (more than 200).

In France, it has been estimated that foodborne pathogens cause between 10 000 and 20 000 hospital admissions per year. *Salmonella* is the most frequent cause of hospital admissions, followed by *Campylobacter* and *Listeria*.

Health Economics Assessments

The UK's Food Standards Agency estimates the cost of foodborne illness in England and Wales annually by assessing the resource and welfare losses attributable to foodborne pathogens. The overall estimated cost of foodborne illness annually in England and Wales has remained relatively constant since 2005 at approximately GBP 1.5 billion. For comparison, in New Zealand and the USA the costs are 216 million NZD and 152 billion USD, respectively.

Disability-Adjusted Life Years

In the Netherlands, the foodborne disease burden due to 14 food-related pathogens has been estimated using DALYs. This method for determining disease burden includes estimates of duration and takes into account disability weights for nonfatal cases and loss of statistical life expectancy for fatal cases. In total, there were an estimated 1.8 million cases of diarrheal disease and 233 deaths, of which approximately 680 000 cases and 78 deaths were allocated to foodborne transmission. The total burden was 13 500 DALYs. At a population level, *Toxoplasma gondii*, thermophilic *Campylobacter* spp., rotaviruses, NoVs, and *Salmonella* spp. accounted for the highest disease burden.

Similarly, the public health effects of illness caused by foodborne pathogens in Greece during 1996–2006 have been calculated. Approximately 370 000 illnesses per million people were judged to have occurred because of eating contaminated food. A total of 900 illnesses were found to be severe and three were fatal. The corresponding DALY estimate was 896 per million population. Brucellosis, echinococcosis, salmonellosis, and toxoplasmosis were the most common known causes of foodborne disease and accounted for 70% of the DALY estimate of 896 DALYs per million people.

Expert Elicitation

Expert elicitation employs expert opinion to apportion pathogens according to foodborne transmission or transmission via other routes. An example of this is the Delphi method, which usually involves experts answering questionnaires in two or more rounds. After each round, a facilitator provides an anonymous summary of the experts' forecasts from the previous round as well as the reasons they provided for their judgments. The experts can then modify their earlier answers in response to the replies of other members of their panel. The range of the answers in each round tends to decrease so that the panel will converge toward a 'correct' answer. The Delphi technique is predicated on the basis that forecasts (or decisions) from a structured panel of people are more accurate than those from unstructured groups. Panels do not need to meet in person for the method to work.

Using structured expert elicitation, almost half of the total burden of diarrheal disease in the Netherlands was attributed to food. *Toxoplasma gondii* and *Campylobacter* spp. were identified as key targets for additional intervention efforts, focusing on food and environmental pathways. Not surprisingly, perhaps, a very high proportion of toxin-producing bacteria (*Bacillus cereus*, *Clostridium perfringens*, and *Staphylococcus aureus*) were considered to be predominantly foodborne. By contrast, multiple transmission routes were assigned to the zoonotic bacterial pathogens and protozoan parasite *T. gondii* although the food pathway was considered to be the most important.

Seroepidemiology

An alternative way to assess the incidence of foodborne pathogens is to investigate exposure to them. Pioneered in Denmark and the Netherlands, an approach to studying infection pressure has been developed using serum antibodies to *Campylobacter* and *Salmonella* as biomarkers to estimate seroconversion rates. This shows that infections are much more common than clinical disease, probably because the majority of infections are asymptomatic. A great advantage of this method is that the assessment of incidence is independent of surveillance artifacts. The method confirms that comparing reported incidence between countries can lead to a totally false impression, even within the EU.

Food-Related Illness by Food Commodity

To pinpoint and then prioritize food safety interventions, the burden of food-related illness needs to be allocated to food commodities. Again, several methodologies exist.

Interventions

The most persuasive evidence for the role of contaminated food items probably comes from studies that demonstrate the impact of interventions on human disease burden. For example, in the UK, where two population-based prospective cohort studies have taken place 15 years apart, there has been a marked fall in nontyphoidal salmonellosis in the community. The fall in incidence coincides closely with voluntary vaccination programs in broiler-breeder and laying flocks, and suggests that these programs have made a major contribution in improving public health, demonstrating the success of such concerted, industry-led action.

Natural experiments also illustrate the importance of poultry contamination as a major source of human *Campylobacter* infection. For example, in the Netherlands widespread culling of poultry that took place because of an avian influenza outbreak was followed by a decrease in *Campylobacter* infection in people, particularly in the areas where culling had taken place. Similarly, when contamination with dioxins caused poultry to be pulled from the supermarket shelves in Belgium the incidence of laboratory-confirmed *Campylobacter* infection in people fell.

Microbiological Source Attribution

The main applications of source or reservoir attribution using microbial subtyping have been to *Salmonella* and *Listeria*. Sero- and phagetyping data tend to be used for this purpose. The underlying philosophy is controlling pathogens in the source or reservoir will avert subsequent human exposure, whatever transmission route or vehicle. Comparing results from animal and human surveillance programs provides insights about the major sources of disease in people.

In Denmark, a source attribution model has been developed to quantify the contribution of major animal-food sources to human salmonellosis. This showed that domestic food products accounted for over half of all cases, with over one-third of cases being attributable to table eggs. Nearly a fifth of cases were travel related, and in a similar proportion no source could be pinpointed. Nearly 10% of cases were attributed to imported food products and the most important source was imported chicken. Multidrug- and quinolone-resistant infections were rare in Danish-acquired infection and were caused more frequently by imported food products and traveling abroad.

Source Attribution Using Outbreak Data

Information from well-conducted outbreak investigations can be very useful for the so-called point of consumption attribution as they are gathered at the public health endpoint and can, therefore, be considered to be a direct measure of attribution at the point of exposure. One of the difficulties in using outbreak data, however, is that foods implicated in reported outbreaks are often complex foods, containing several ingredients or food items, any one of which might be the specific source of the pathogen. The method works best for pathogens where outbreaks are relatively common. So, for example, it is more robust for STEC and *Salmonella* than it is for

Campylobacter, because *Campylobacter* outbreaks are rarely recognized. Using EU outbreak data, 58% of *Salmonella* cases that could be allocated to a source were attributed to contaminated eggs and 29% of *Campylobacter* cases that could be allocated to a source were attributed to contaminated poultry. However, for both pathogens the majority of cases could not be attributed to a source, illustrating another limitation of using outbreak data for these purposes.

In the UK, using outbreak data for point of consumption attribution showed that the most important cause of UK-acquired foodborne disease was contaminated chicken and that red meat (beef, lamb, and pork) contributed heavily to deaths. The prioritization exercise that this type of analysis allowed showed that reducing the impact of UK-acquired foodborne disease was mainly dependent on preventing contamination of chicken.

Systematic Review and Meta-Analysis

Several case–control studies of sporadic salmonellosis and sporadic campylobacteriosis have been published, often using different methodologies and conducted in different settings. Systematic reviews consist of a formal process for literature review focused on a specific research question. In a systematic review of case–control studies and meta-analysis of 35 case–control studies of sporadic salmonellosis, traveling abroad, underlying medical conditions, eating raw eggs, and eating in restaurants were the most important risk factors for salmonellosis in the meta-analysis. Similarly, in a systematic review and meta-analysis of 38 case–control studies of sporadic campylobacteriosis, foreign travel, undercooked chicken, consumption of environmental sources, and direct contact with farm animals were all significant risk factors.

In a systematic review and meta-analysis of hepatitis E virus, occupational exposure to swine was found to be a more important route of transmission to humans than eating contaminated pork. This is an important finding requiring further exploration before any public health policy action in relation to food is implemented.

Investigating the Unknown

Most surveillance systems that capture information on etiology elicit information on known pathogens. Yet in the IID2 Study in the UK a known pathogen was assigned to 40% of community cases and 50% of the cases presenting to primary care. In the remainder of the stool samples submitted no pathogen was detected. So what about the rest? There is a number of possible reasons for the high percentage of cases with unknown etiology. First, it is possible that people reported transient changes in bowel habit not caused by IID. Second, these cases might have been caused by organisms not included in the diagnostic algorithms, like enteropathogenic or enterotoxigenic *E. coli*. Third, the cases might have been caused by putative pathogens like enterotoxigenic *Bacteroides fragilis* or *Laribacter hongkongensis*. Several coronaviruses, picobirnaviruses, pestiviruses, and toroviruses have recently been proposed as causes of IID, particularly in children. Whole-genome sequencing techniques, though not yet enabled for widespread use, create enormous prospects for identifying novel pathogens that might be transmitted through the food chain.

Conclusion

Foodborne illness in Europe is an important public health problem no matter what method is used to measure its impact. If success in public health is defined by illness prevented, there is still a long way to go in controlling foodborne disease in Europe.

See also: Foodborne Diseases: Overview of Biological Hazards and Foodborne Diseases; Overview of Chemical, Physical, and Other Significant Hazards

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WHO/Europe: World Health Organization Regional Office for Europe.

FOODBORNE DISEASES

Prevalence of Foodborne Diseases in Western Pacific Region

H Toyofuku, National Institute of Public Health, Saitama, Japan

Glossary

Agent A factor (microorganism, chemical substances, etc.) whose presence or excessive presence is essential for the occurrence of the disease.
Case An occurrence of illness as defined by investigators.
Food Any substance, whether processed, semiprocessed, or raw, which is intended for human consumption, and includes drink, chewing gum, and any substance which has been used in the manufacture, preparation, or treatment of 'food' but does not include cosmetics, tobacco, or substances used only as drugs.
Foodborne disease Any disease of an infectious or toxic nature caused by or thought to be caused by the consumption of food.
Foodborne disease outbreaks The occurrence of two or more cases of a similar foodborne disease resulting from the ingestion of the same food.
Global Foodborne Infections Network (GFN) It is a capacity-building program that promotes integrated, laboratory-based surveillance and intersectoral collaboration among human health, veterinary, and food-related disciplines. GFN is part of World Health Organization's endeavors to strengthen the capacities of its Member States in the surveillance and control of major foodborne diseases and to contribute to the global effort of containment of antimicrobial resistance in foodborne pathogens.
Notifiable disease A disease that must, by law or by ministerial decree, be reported to a government authority.
Surveillance The systematic collection, analysis, interpretation, and dissemination of health data on an ongoing basis, to gain knowledge of the pattern of disease occurrence and potential in a community, in order to control and prevent disease in the community.
Vehicle An inanimate intermediary (e.g., food) in the indirect transmission of an agent that carries the agent from a reservoir to a susceptible host.

Introduction

In the Western Pacific region, most countries are yet to recognize the true health, social, and economic costs of foodborne disease. As a result of the general lack of understanding of the burden imposed by these diseases, inadequate attention has been paid to developing effective national food safety programs.

According to the World Health Organization (WHO)-Western Pacific Region Office (WPRO), the region stretches over a vast area, from China in the north and west, to New Zealand in the south, and French Polynesia in the east. One of the most diverse of the WHO regions, the Western Pacific constitutes some of the world's least developed countries as well as the most rapidly emerging economies. It includes highly developed countries such as Australia, Japan, New Zealand, the Republic of Korea and Singapore, and fast growing economies such as China and Vietnam. There are 37 countries and areas in the Western Pacific region.

Even though Brunei, Lao People's Democratic Republic, Malaysia, Philippines, Singapore, and Vietnam belong to the WPRO, foodborne diseases in these countries are not addressed in this article. Similarly, foodborne diseases in Australia and New Zealand is covered by article 413, In this article, foodborne diseases reported in Japan, Republic of Korea, China, Taiwan, Hong Kong, Guam, and other countries are discussed.

It is important to note that foodborne outbreak investigation and reporting systems at the national level are not similar among countries in this region. Therefore the differences in the numbers reporting outbreaks and the causative agents may not necessarily reflect the levels of food safety situations among countries.

General

Because of large fish consumption in this region, the fishborne disease, especially had *Vibrio parahaemolyticus* as a leading etiological agent. However, in most of the countries, especially Japan and Korea, both numbers and cases of *V. parahaemolyticus* outbreaks decreased. In contrast, virus, especially foodborne norovirus infection increased in these countries.

Country Specific

Japan

Population: 128 million (2011).

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Table 1 Number of foodborne outbreaks and patients by etiological agents, in Japan, 2001–11

Year		*Bacteria*									*Virus*		*Chemical substances*	*Natural toxins*	*Others*	*Unknown*	*Total*
		Salmonella	Staphylococcus aureus	Vibrio parahaemolyticus	Bacillus cereus	Clostridium perfringens	Clostridium botulinum	Campylobacter	*Pathogenic* Escherichia coli	*Others*	*Norovirus*	*Others*					
2001	Number of outbreaks	361	92	307	9	22	0	428	223	28	269	1	8	89	1	91	1 928
	Patients	4 949	1 039	3 065	444	1 656	0	1 880	2 671	49	7 358	13	112	327	1	2 298	25 862
2002	Number of outbreaks	465	72	229	7	37	0	447	97	23	268	1	9	123	2	70	1 850
	Patients	5 833	1 221	2 714	30	3 847	0	2 152	1 641	95	7 961	22	154	372	25	1 562	27 629
2003	Number of outbreaks	350	59	108	12	34	0	491	47	9	278	4	8	112	1	72	1 585
	Patients	6 517	1 438	1 342	118	2 824	0	2 642	1 559	111	10 603	99	218	308	1	1 575	29 355
2004	Number of outbreaks	225	55	205	25	28	0	558	45	11	277	0	12	151	5	69	1 666
	Patients	3 788	1 298	2 773	397	1 283	0	2 485	939	115	12 537	0	299	433	8	1 820	28 175
2005	Number of outbreaks	144	63	113	16	27	0	645	49	8	274	1	14	106	8	77	1 545
	Patients	3 700	1 948	2 301	324	2 643	0	3 439	1 839	484	8 727	1	111	285	8	1 209	27 019
2006	Number of outbreaks	124	61	71	18	35	1	416	43	5	499	5	15	138	7	53	1 491
	Patients	2 053	1 220	1 236	200	1 545	1	2 297	1 081	33	27 616	80	172	511	23	958	39 026
2007	Number of outbreaks	126	70	42	8	27	1	416	36	6	344	4	10	113	8	78	1 289
	Patients	3 603	1 181	1 278	124	2 772	1	2 396	1 576	33	18 520	230	93	355	20	1 295	33 477
2008	Number of outbreaks	99	58	17	21	34	0	509	29	11	303	1	27	152	17	91	1 369
	Patients	2 551	1 424	168	230	2 088	0	3 071	616	183	11 618	12	619	387	47	1 289	24 303
2009	Number of outbreaks	67	41	14	13	20	0	345	36	0	288	2	13	92	17	100	1 048
	Patients	1 518	690	280	99	1 566	0	2 206	341	0	10 874	79	552	290	19	1 735	20 249
2010	Number of outbreaks	73	33	36	15	24	1	361	35	2	399	4	9	139	28	95	1 254
	Patients	2 476	836	579	155	1 151	1	2 092	1 406	23	13 904	796	55	390	29	2 079	25 972
2011	Number of outbreaks	67	37	9	10	24	0	336	49	11	296	6	12	69	68	68	1 062
	Patients	3 068	792	87	122	2 784	0	2 341	1 681	73	8 619	118	222	171	522	1 016	21 616
Total	Number of outbreaks	2 101	641	1 151	154	312	3	4 952	689	114	3 495	29	137	1284	162	864	16 088
	Patients	40 056	13 087	15 823	2243	24 159	3	27 001	15 350	1199	138 337	1450	2607	3829	703	16 836	302 683

The reporting of investigated foodborne outbreaks has been mandatory for Japan since 1948.

During 2001–11, a total of 16 088 outbreaks of foodborne disease were reported (Table 1). These outbreaks caused a reported 302 683 persons to become ill. Among 15 224 (94.6%) outbreaks for which the etiology was determined, bacterial pathogens cause the largest percentage of outbreaks (66.4%) and the largest percentage of cases (48.6%).

Among bacterial pathogens, *Campylobacter jejuni/coli* accounted for the largest number of outbreaks and *Salmonella* accounted for the largest number of outbreak-related cases. Viral pathogens, predominantly norovirus, caused 23.1% of outbreaks and 48.9% of cases; the proportion of outbreaks attributed to viral agents increased from 14.7% in 2001 to 35% in 2006, and kept over 30% since then. Natural toxins caused 9.3% of outbreaks and 2.6% of cases.

During the period 2001–11, there was a continuation in the decline of the total number of *Salmonella* outbreaks from 465 outbreaks in 2002 to 67 outbreaks in 2011. Also, the total number of outbreaks caused by *V. parahaemolyticus* decreased to 9 outbreaks in 2011 after an increase to 205 in 2004. The total number of outbreaks caused by *Campylobacter* increased from 428 outbreaks in 2002 to 645 outbreaks in 2005, then afterward decreased to 337 outbreaks in 2011. The total number of outbreaks caused by norovirus increased from 268 outbreaks in 2002 to 499 outbreaks in 2006, then afterward decreased to 297 outbreaks in 2011.

The average number of foodborne outbreaks were 1462.8 per year, (95% confidence interval (CI) 1267.9–1657.8) and the number of affected patients were 27 515 per year (95% CI 23 990.5–31 040.4). The number of outbreaks in 2001 and 2002 (1928 and 1850, respectively) were significantly higher than the average, due to higher numbers of both *Salmonella* and *V. parahaemolyticus* outbreaks. The number of affected patients in 2006 and 2007 (39 026 and 33 477, respectively) were significantly higher than the average, due to higher number of norovirus patients.

In a study using the active surveillance method in Miyagi Prefecture from April 2005 to March 2006, the estimated incidence of foodborne infections per 100 000 per year in that region was 237 cases for *Campylobacter*, 32 cases for *Salmonella*, and 15 cases for *V. parahaemolyticus*, whereas the reported number of cases caused by these three pathogens in the foodborne outbreak-reporting system in Miyagi were 6.06, 0.51, and 1.36 per year per 100 000 population. The authors emphasized the need for a complementary system, such as a laboratory-based active surveillance in Japan, to the present passive surveillance of foodborne illnesses in Japan to identify and prioritize food safety issues more precisely, to validate the results of relevant microbiological risk assessments, and to monitor the effectiveness of risk management options.

The two most frequently isolated *Salmonella* serotypes during 2008–11 were, in order, *Salmonella* Enteritidis and *Salmonella* Infantis (Table 2). This finding is consistent with *S.* Enteritidis being the most frequently isolated serotype in much of Asia.

Regarding enterohemorrhagic *Escherichia coli* (EHEC) serotypes, nearly all the EHEC foodborne outbreaks were associated with O157, followed by O26 since 2000 (Table 3).

The most common single foodstuff category reported as food vehicle in 2011 was fish and shellfish, responsible for 137 (10.9%) outbreaks. Meat and meat products were the next most common category (6.1%), followed by combined food (5.8%), and fruits and vegetables and their products (3.9%).

Regarding a source attribution of *Salmonella* foodborne diseases, based on the outbreak investigation results, 45–60% of the *Salmonella* foodborne outbreak incidences were considered to be associated with eggs between 1998 and 2002, however, the percentage dropped to 24.2% in 2003. The number of *Salmonella* foodborne outbreak incidences associated with beef, pork and poultry meat, and raw vegetables, which have been frequently reported in other countries, were very limited.

Table 2 Top 15 *Salmonella* serotype isolated from humans in Japan, 2008–11

2008		*2009*		*2010*		*2011*	
Enteritidis	346	Enteritidis	232	Enteritidis	312	Enteritidis	277
Infantis	107	Infantis	90	Infantis	67	Infantis	91
Typhimurium	85	Thompson	69	Thompson	55	Saintpaul	78
Saintpaul	70	Saintpaul	68	Typhimurium	47	Thompson	58
Braenderup	65	Typhimurium	53	Saintpaul	31	Typhimurium	55
Thompson	60	Bareilly	32	Braenderup	30	Montevideo	38
Montevideo	50	Schwarzengrund	28	I 4:i:–	27	Braenderup	34
Stanley	23	Montevideo	27	Nagoya	25	Schwarzengrund	29
Litchfield	19	I 4:i:–	18	Agona	20	Litchfield	21
Schwarzengrund	18	Litchfield	15	Bareilly	20	Hadar	18
Newport	16	Manhattan	14	Virchow	18	Agona	16
Agona	12	Poona	12	Schwarzengrund	17	Newport	14
Nagoya	12	Paratyphi B	11	Montevideo	17	Manhattan	13
Virchow	11	Newport	11	Paratyphi B	14	Nagoya	12
Hadar	10	Nagoya	11	Manhattan	14	Derby	10
Others	194	Others	191	Others	191	Others	204
Total	1098	Total	882	Total	905	Total	968

Table 3 Serotypes of enterohemorrhagic *Escherichia coli* foodborne outbreaks, patients and death in Japan, 2000–11

Year	O157			O26			O111		
	Number of Outbreaks	Patients	Death	Number of Outbreaks	Patients	Death	Number of Outbreaks	Patients	Death
2000	14	110	1	1	1	0	1	2	0
2001	24	378	9	0	0	0	0	0	0
2002	12	259	1	0	0	0	0	0	0
2003	10	39	0	1	141	0	0	0	0
2004	18	70	0	0	0	0	0	0	0
2005	24	105	0	0	0	0	0	0	0
2006	23	166	0	1	13	0	0	0	0
2007	25	928	0	0	0	0	0	0	0
2008	17	115	0	0	0	0	0	0	0
2009	26	181	0	0	0	0	0	0	0
2010	27	358	0	0	0	0	0	0	0

In 2011, eating out at restaurants was the most important setting reported for foodborne outbreaks (51%), followed by households (7%) and Japanese-style inns (4.5%).

Regarding foodborne trematode infection, 150 000 infections of *Metagonimus yokogawai* and 50–100 infections of *Paragonimus* spp. were presented at the WHO/FAO Workshop in Hanoi in 2002.

Korea

Population: 50 004 441 (2012).

In Korea, foodborne disease must be reported to the local public health center.

During 2002–11, a total of 2357 outbreaks of foodborne disease were reported (Table 4). These outbreaks caused a reported 75 275 persons to become ill. Among 1436 (60.9%) outbreaks for which the etiology was determined, bacterial pathogens cause the largest percentage of outbreaks (71.6%) and the largest percentage of cases (72.6%). The total number of outbreaks decreased to 249 in 2011 after an increase to 510 in 2007 from 77 in 2002. The total number of patients decreased to 7105 in 2011 after an increase to 10 833 in 2006 from 2939 in 2002.

In 2011, enteropathogenic *E. coli* (EPEC) caused the most poisoning incidents, and the rest were like the following – Norovirus > *Salmonella* > *Campylobacter* > *Staphylococcus aureus* > *V. parahaemolyticus*.

Viral pathogens, predominantly norovirus, caused 24.9% of outbreaks and 26.0% of cases; the proportion of outbreaks attributed to viral agents increased from 2% in 2001 to 37.7% in 2008, and kept over 20% since then. Among bacterial pathogens, EPEC accounted for the largest number of outbreaks and outbreak-related cases. Natural toxins caused 2.1% of outbreaks and 0.5% of cases.

According to the 2010 trend of food poisoning outbreak by month and location, 51% of outbreaks and 55% of patient occurred in the summer (June to September). 49.6% out of whole food poisoning incidents occurred in restaurants when sorted by locations, and 58.5% out of whole patients were found in group catering services (2011 KFDA Report).

Regarding facilities that caused EPEC outbreaks, schools (1220 patients, 63% of all the EPEC patients) were the most important setting, followed by restaurants (333 patients, 17%), and business (250 cases, 13%). Norovirus outbreaks occurred more frequently at schools (66% of the norovirus patients) and *Salmonella* outbreaks occurred more frequently at restaurants (66% of the *Salmonella* patients).

The most frequently isolated *Salmonella* serotypes during 2006–09 was *S.* Enteritidis, followed by Typhi and Typhimurium (37 isolates each) and Infantis and Typhi (in 2007), and Typhimurium and Typhi (2008 and 2009) (Table 5). This finding is consistent with *S.* Enteritidis being the most frequently isolated serotype in much of Asia.

In addition, the Korea Center for Disease Control and Prevention published cases of notifiable diseases. Among the data, Table 6 indicated cases of which a certain percentage was considered as foodborne. Cholera cases decreased to 3 in 2011 after an increase to 16 in 2006 from 10 in 2004. Typhoid fever cases also decreased to 167 in 2011 after an increase to 233 in 2007 from 174 in 2004. Paratyphoid fever cases and *Vibrio vulnificus* cases were reported as approximately 50 during this period. *Shigella* cases decreased to 117 in 2011 from 280 in 2004. EHEC cases decreased to 37 in 2006, then increased to 71 in 2011. Brucellosis cases decreased to 22 in 2011 after an increase to 215 in 2006 from 47 in 2004.

Parasite

Clonorchiasis is still highly prevalent among inhabitants in the riverside areas of southern Korea. A study in villages along the four major rivers, Nakdong-gang, Seomjin-gang, Youngsan-gang, and Guem-gang in southern Korea, from January to December 2006, with a total of 24 075 stool samples found 2661 infected people (11.1%) for *Clonorchis sinensis*, 431 (1.8%) for heterophyids, 226 (0.9%) for *Entamoeba* spp., 57 (0.2%), for *Giardia lamblia*, 30 (0.1%) for *Trichuris trichiura*, and 18 (0.07%) for echinostomes.

In all, 700 000 infections of *Clonorchis sinensis*, 300 000 infections of *M. yokogawai*, 1000 infections of *Paragonimus* spp., and 257 infections of other species of intestinal foodborne trematode infection were presented at the WHO/FAO Workshop in Hanoi in 2002.

China

Population: 1 307 593 000 (2010).

Table 4 Number of foodborne outbreaks and patients by etiological agents, in Korea, 2002–11

Year		*Bacteria*									*Virus*		*Chemical substances*	*Natural toxins*	*Unknown*	*Total*
		Salmonella	Staphylococcus aureus	Vibrio parahaemolyticus	Bacillus cereus	Clostridium perfringens	Clostridium botulinum	Campylobacter jejuni	*Pathogenic* Escherichia coli	*Others*	*Norovirus*	*Others*				
2002	Number of outbreaks	24	8	10	0	0	0	0	2	2	0	1	0	2	28	77
	Patients	548	370	188	0	0	0	0	63	279	0	137	0	23	1 331	2 939
2003	Number of outbreaks	17	13	22	3	1	1	1	6	6	14	2	0	2	47	135
	Patients	416	808	732	198	12	3	215	1 502	226	1 442	164	0	11	2 180	7 909
2004	Number of outbreaks	23	11	15	2	4	0	3	21	13	13	5	0	3	52	165
	Patients	839	763	300	84	680	0	175	2 043	1156	922	485	0	15	2 926	10 388
2005	Number of outbreaks	22	16	17	1	0	0	1	15	1	6	2	1	1	26	109
	Patients	753	863	663	24	0	0	175	1 883	45	719	25	8	3	550	5 711
2006	Number of outbreaks	22	32	25	5	2	0	1	38	1	51	3	1	1	77	259
	Patients	576	1924	547	59	160	0	53	2 832	5	3 338	33	14	4	1 288	10 833
2007	Number of outbreaks	42	38	33	1	4	0	7	62	0	97	2	0	3	221	510
	Patients	1497	843	634	50	81	0	449	1 945	0	2 345	32	0	22	1 788	9 686
2008	Number of outbreaks	22	15	24	14	6	0	6	36	0	69	1	2	2	157	354
	Patients	387	556	329	376	434	0	73	1 278	0	2 105	26	34	50	1 839	7 487
2009	Number of outbreaks	17	12	12	0	5	0	7	37	0	32	0	0	6	100	228
	Patients	477	864	106	0	527	0	405	1 671	0	568	0	0	126	1 255	5 999
2010	Number of outbreaks	27	19	18	14	5	0	15	28	0	31	2	1	6	105	271
	Patients	677	372	223	401	171	0	380	1 926	0	1 994	8	3	33	1 030	7 218
2011	Number of outbreaks	24	10	9	6	7	0	13	32	2	31	3	0	4	108	249
	Patients	1 065	323	133	98	324	0	329	2 109	20	1 524	21	0	27	1 132	7 105
Total	Number of outbreaks	240	174	185	46	34	1	54	277	25	344	21	5	30	921	2 357
	Patients	7 235	7 686	3855	1290	2 389	3	2 254	17 252	1731	14 957	931	59	314	15 319	75 275

Table 5 Top 15 *Salmonella* serotypes isolated from humans in Korea 2006–09

2006		*2007*		*2008*		*2009*	
Enteritidis	135	Enteritidis	163	Enteritidis	199	Enteritidis	263
Typhi	37	Infantis	62	Typhimurium	49	Typhimurium	109
Typhimurium	37	Typhi	44	Typhi	44	Typhi	37
London	28	Othmarschen	41	I 4,5,12:i:–	20	I 4,5,12:i:–	11
Braenderup	19	Typhimurium	27	Infantis	8	Braenderup	9
Schwarzengrund	19	Paratyphi A	10	Hillingdon	7	Infantis	9
Hillingdon	17	Braenderup	7	Rissen	7	Paratyphi B	8
Paratyphi A	14	Corvallis	6	Weltevreden	7	Rissen	7
Infantis	11	Hillingdon	6	Paratyphi B	6	Paratyphi A	5
Montevideo	9	Weltevreden	6	Anatum	4	Hillingdon	4
Rissen	8	Anatum	5	Derby	4	Ohio	4
Give	6	Derby	5	Nerport	4	Othmarschen	4
Virchow	6	Haardt	5	Schwarzengrund	4	Arizonae	3
Weltevreden	6	Paratyphi B	4	Paratyphi A	3	Panama	3
Corvallis	5	Virchow	4	Virchow	3	Schwarzengrund	3
Others	65	Others	67	Others	0	Others	0
Total	422	Total	462	Total	369	Total	479

Table 6 Cases of notifiable diseases in Korea, 2004–11

Disease	*2004*	*2005*	*2006*	*2007*	*2008*	*2009*	*2010*	*2011*
Cholera	10	16	5	7	5	–	8	3
Typhoid fever	174	190	200	223	188	167	188	167
Paratyphoid fever	45	31	50	45	44	36	55	56
Shigella	487	317	389	131	209	180	228	117
EHEC	118	43	37	41	58	62	56	71
Vibrio vulnificus	57	57	88	59	49	24	73	51
Brucellosis	47	158	215	101	58	24	31	22
Cryptosporidiosis	–	1	–	–	–	–	–	–
Botulism	4	–	1	–	–	–	–	–

Bacteria

In China, the number of outbreaks of foodborne disease and the number of patients affected has increased in the past decades. During 1994–2005, 12 687 outbreaks of foodborne diseases were reported, resulting in 280 380 persons becoming ill and 2297 persons dying. Among events for which the etiology was determined, microbial pathogens were responsible for 4515 events (28.25%), resulting in 146 852 persons becoming ill (42.75%) and 349 dying.

Wang *et al*. identified 1082 foodborne outbreaks, with 57 612 illnesses and 51 deaths through literature review in China during 1994 and 2005. Among the 1082 outbreaks for which the etiology was determined, *V. parahaemolyticus* caused the greatest number of outbreaks (19.50%), followed by *Salmonella* (16.73%), *Bacillus cereus* (13.40%), *Proteus* (11.46%), and *S. aureus* (7.76%). Of the 57 612 cases, *Salmonella* was the most important agent, accounting for 22.16% of illness, followed by *Proteus* (11.56%), a mixture of bacteria (11.2%) and *B. cereus* (9.97%). *Clostridium botulinum* caused the most death (62.75%) and the highest mortality rate (32 deaths in 254 cases).

Among the 181 *Salmonella* foodborne outbreaks, *S.* Enteritidis accounted for the majority (28.73%), followed by Typhimurium (14.36%), Dublin (5.5%), and Blegdam (4.4%). Of the 72 events of *E. coli* foodborne outbreaks, EPEC was the most common classes (70.83%) and other classes of *E. coli* only occupied a small portion. There was no data about EHEC-related infections.

Of the 1082 bacterial foodborne disease events, 31.05% (336 events) occurred in food services units and 26.25% (283 events) took place at home. *Vibrio parahaemolyticus* and *E. coli* outbreaks occurred more commonly in food services (55.45% and 47.22%, respectively). *Salmonella* and *Clostridium botulinum* outbreaks occurred more commonly at home (50.83% and 86.67%, respectively). *Bacillus cereus* and *Shigella* outbreaks occurred more frequently at school (35.86% and 58.33%, respectively).

Cui *et al*. reported the incidence of hepatitis A. In China, hepatitis cases have been reported as part of the National Notifiable Disease Reporting System since 1959, and have been reported separately by serotypes since 1990. The incidence of hepatitis A has declined by 90% since 1991, from 55.7 per 10 000 population in 1991 to 5.9 in 2007.

Li *et al*. reported the proportion of hemorrhagic colitis caused by *E. coli* O157:H7 as 0.97–5.89% in Xuzhou in Jiangsu in June, 2007.

According to the WHO Global Foodborne Infections Network country databank, Typhimurium (26.2%) is the most common *Salmonella* serotype isolated from humans in 2006, followed by Enteritidis (17%), Senftenberg (9.5%), and Derby (9%).

Ran *et al*. conducted a multiprovince (Shanghai, Beijing, and Guangxi) laboratory confirmed nontyphoidal *Salmonella* infections survey in China and found that the *Salmonella* isolation rate was low (of the 39 172 patients with diarrhea evaluated, 662 (2.7%) grew nontyphoidal *Salmonella*).

Cholera incidence rate decreased and stayed at the rate of 0.01 cases per 100 000 population since 2006 after an increase to 0.07 per 100 000 population in 2005. The incidence rate of dysentery decreased to 18.9 per 100 000 in 2010 from 63.04 per 100 000 population in 2000. The death rate also decreased to 0.003 per 100 000 population in 2010 from 0.04 per 100 000 population in 2000. The incidence rate of typhoid and paratyphoid fever was reported only in 2009 at 1.28 per 100 000 population. The incidence rate of Brucellosis increased to 2.7 per 100 000 in 2009 from 0.41 per 100 000 in 2002 (Table 7).

Parasite

The foodborne parasitic zoonoses (FBPZ) causes death and serious diseases in mainland China, where approximately 150 million people are suffering from FBPZ problem.

Clonorchiasis caused by the oriental liver fluke *Clonorchis sinensis* is one of the major parasite zoonosis and 12.5–15 million people are estimated to be infected in China, and the southern province Guangdong has the largest number of infected people (nearly 5.5 million).The increased prevalence of clonorchiasis in humans could be related to factors such as unhygienic practice, poor knowledge, inappropriate farming/fish practices, and eating raw fish.

Approximately 400 angiostrongyliasis cases have been reported in China since 1984, and those predominantly between 1979 and 2008. Humans become infected by ingesting freshwater and terrestrial snails and slugs.

Human paragonimiasis is caused by the consumption of raw or undercooked stream crabs that are infected with *Paragonimus westermani* or drinking water contaminated by metacercaria. This disease is prevalent in 24 provinces and less than 1 million infections were estimated countrywide. People in Shanghai and Chongqing have the highest prevalence, at 5.1% and 4.1%, respectively.

Echinococcosis, including cystic echinococcosis caused by the cestode *Echinococcus granulosus* and alveolar echinococcosis caused by *Echinococcus multilocularis*, is one of the most serious parasitic zoonoses in China. It was estimated that 380 000 people were infected with echinococcosis and 50 million people were at risk of infection.

Human trichinellosis is caused by *Trichinella spiralis* and *Trichinella nativa*, and the infection occurs through the consumption of raw or undercooked meat. Twenty million people were estimated to be infected in China. Yunnan and Inner Mongolia have a serious high mean prevalence of 8.3% and 6.3%, respectively.

Human cysticercosis caused by the larval stage of *Taenia solium* occurred and approximately 7 million people were infected in China.

Human infection with *Toxoplasma gondii* has been reported nationwide with a mean infection rate of 7.9%, approximately 100 million people were estimated as being infected.

Human cases of cryptosporidiosis have been recorded in 14 provinces, with young children being more susceptible to infection than adults (infection rate = 2.1% in children). For example, *Cryptosporidium* oocysts were found in 57 of 548 children with diarrhea (10.4%) in the Zhejiang province.

Human infection with *G. lamblia* has been documented all around China, and was estimated to be approximately 30 million infections.

China, Hong Kong Special Administrative Region

Population: 7 053 000 (2010).

Food poisoning is one of the statutory notifiable diseases in Hong Kong. A total of 6955 food poisoning outbreaks were notified from 2001 to 2011. The number of food poisoning outbreaks increased steadily from 2003 to 2006 and then decreased afterward.

Among the outbreaks, 78.3% were caused by bacteria, followed by viruses (10.1%), mostly norovirus and natural toxins (7.6%). Ciguatera poisoning, a locally notifiable disease, is commonly reported in Hong Kong, and there was an average of 26 ciguatera incidents affecting more than 10 persons annually. In 2004, only 65 incidents (7.9%) affecting 247 patients (7.8%) were attributed to ciguatera poisoning (Table 8).

Table 7 Incidence rate and death rate of infectious diseases in China, 2000–10

		2000	*2001*	*2002*	*2003*	*2004*	*2005*	*2006*	*2007*	*2008*	*2009*	*2010*
Cholera	Incidence rate (1/100 000)	0.02	0.22	0.05	0.02	0.02	0.07	0.01	0.01	0.01	0.01	0.01
	Death rate (1/100 000)	0	0	0	0	0	0	0	0	*	*	*
Dysentery	Incidence rate (1/100 000)	63.04	39.52	35.4	34.52				27.99	23.65		18.9
	Death rate (1/100 000)	0.04	0.01	0.02	0.02				0.01	0		0.003
Typhoid and paratyphoid fever	Incidence rate (1/100 000)			–	–				–	–	1.28	–
	Death rate (1/100 000)			–	–				–	–	0	–
Brucellosis	Incidence rate (1/100 000)			0.41	0.48				1.5	2.1	2.7	2.53
	Death rate (1/100 000)			0	0				0	*	*	0

Abbreviation: *, No death was reported.

Table 8 Number of food poisoning outbreaks and patients by etiological agents, in Hong Kong, 2001–11

Year		*Bacteria*	*Virus*	*Chemical substances*	*Natural toxins*	*Others*	*Total*
2001	Number of outbreaks	529	45	44	33	20	671
	Patients	2 082	393	90	85	57	2 707
2002	Number of outbreaks	546	55	29	28	12	670
	Patients	2 204	235	66	100	35	2 640
2003	Number of outbreaks	333	58	7	22	2	422
	Patients	1 851	270	11	92	6	2 230
2004	Number of outbreaks	637	71	12	93	8	821
	Patients	2 441	283	24	337	46	3 131
2005	Number of outbreaks	740	103	22	106	2	973
	Patients	2 838	466	50	242	5	3 601
2006	Number of outbreaks	838	183	22	50	1	1 094
	Patients	3 218	768	37	119	2	4 144
2007	Number of outbreaks	500	47	28	39	7	621
	Patients	1 689	169	49	71	14	1 992
2008	Number of outbreaks	494	58	17	36	14	619
	Patients	2 105	241	29	97	75	2 547
2009	Number of outbreaks	318	42	8	39	0	407
	Patients	1 281	178	15	66	0	1 540
2010	Number of outbreaks	246	15	4	47	4	316
	Patients	900	54	7	83	12	1 056
2011	Number of outbreaks	267	24	5	37	8	341
	Patients	1 030	101	7	72	39	1 249
Total	Number of outbreaks	5 448	701	198	530	78	6 955
	Patients	21 639	3158	385	1364	291	26 837

Taiwan

Population: 22 805 547 (2006).

In Taiwan, data from incidences of foodborne disease outbreaks have been collected by the Department of Health (DoH) since 1981, and detailed epidemiological data were systemically collected only after 1986. The DoH established Taiwan's Food and Drug Administration in January 2010.

During 1986–95, 852 outbreaks of foodborne illness, including 26 173 cases, were reported in Taiwan, of which 555 (65%) were caused by bacterial infection. Bacterial pathogens, particularly *V. parahaemolyticus* (35.5%), *S. aureus* (30.5%), *B. cereus* (18.7%), *E. coli* (6.5%), and *Salmonella* spp. (5.6%) were the main etiologic agents.

In 1994, 102 outbreaks of foodborne disease involving 4726 cases were reported to the Taiwan DoH. Of these outbreaks, 72.5% (74/102) were caused by bacterial pathogens, with *V. parahaemolyticus* responsible for 56.7% (42/74), *S. aureus* 20.3% (15/74), *B. cereus* 14.9% (11/74) and *Salmonella* spp. other than *Salmonella* Typhi and *Salmonella* Paratyphi 8.1% (6/74).

In Taiwan, the average prevalence of reported foodborne illness from 1995 to 2001 was 6.86 per 100 000 population. During this period, 1171 outbreaks of foodborne illness, including 109 884 cases, were reported in northern Taiwan, of which 735 (62.8%) were caused by bacterial infection. Bacterial pathogens, particularly *V. parahaemolyticus* (86.0%), *S. aureus* (7.6%), and *Salmonella* spp. (4.9%) were the main etiologic agents. *Vibrio parahaemolyticus* has been a leading cause of problems in Taiwan for many years.

During 2002–11, 3088 outbreaks of foodborne illness, including 46 259 cases, were reported in Taiwan, of which 1167 (97%) out of 1203 etiology-identified outbreaks were caused by bacterial infection. Bacterial pathogens, particularly *V. parahaemolyticus* (48.7%), *S. aureus* (6.3%), *B. cerius* (4.9%), and *Salmonella* spp. (4%) were the main etiologic agents. *Vibrio parahaemolyticus* has been a leading cause of problems in Taiwan for this decade, however, both the number of outbreaks and patients decreased to 52 outbreaks with 596 cases in 2011 from 86 outbreaks with 2014 cases in 2002.

Cases of *B. cereus* outbreaks were the largest in 2010 and 2011. Interestingly no *Campylobacter* outbreaks were recorded during this period; however, Wang *et al*. reviewed 104 *Campylobacter enteritis* infection at National Taiwan University hospital in Taipei from January 2000 to December 2006, and found *C. coli* and *C. jejuni* from 24% and 80% of the patients, respectively.

Among food poisoning of which incriminated food was identified in 2009, fresh vegetables and fruits and their products was the most important food (48 out of 53 (90.6%) incriminated food identified known outbreaks and 1172 cases out of 1276 (91.8%) cases from those outbreaks), followed by mixing prepared foods (29 outbreaks with 525 cases).

In 2010, mixing prepared foods was the most important food (39 out of 83 (47.0%) incriminated food identified outbreaks and 2026 cases out of 3480 (58.2%) cases from those outbreaks), followed by meal box (17 outbreaks with 1118 cases) (Table 9).

Among the notifiable diseases, the following number of cases were reported in 2009 and 2010, respectively: typhoid fever 80, 33; paratyphoid fever 6, 12; acute hepatitis A 234, 110; EHEC infection 0, 0; cholera 3, 5; botulism 1, 11; and toxoplasmosis 7, 5.

Table 9 Number of foodborne outbreaks and patients by etiological agents, in Taiwan, 2002–11

Year		*Bacteria*									*Chemical substances*	*Natural toxins*	*Unknown*	*Total*
		Salmonella	Staphylococcus aureus	Vibrio parahaemolyticus	Bacillus cereus	Clostridium perfringens	Clostridium botulinum	Campylobacter jejuni	*Pathogenic* Escherichia coli	*Others*				
2002	Number of outbreaks	6	18	86	4		0		0	1	2	11	138	262
	Patients	30	853	2014	160		0		0	50	216	228	2 370	5 566
2003	Number of outbreaks	11	7	82	11		0		0	0	3	5	138	251
	Patients	177	114	1405	564		0		0	0	177	60	3 134	5 283
2004	Number of outbreaks	8	9	64	7		0		0	0	4	11	178	274
	Patients	206	403	864	166		0		0	0	19	221	2 270	3 992
2005	Number of outbreaks	7	12	62	9		0		0	1	2	6	151	247
	Patients	89	138	775	104		0		0	80	238	62	2 140	3 530
2006	Number of outbreaks	8	18	58	10		1		2		2	3	168	265
	Patients	169	254	626	423		5		322		79	90	2 568	4 401
2007	Number of outbreaks	11	23	38	7		0		1	0	1	3	159	240
	Patients	95	236	365	123		0		10	0	6	394	2 080	3 223
2008	Number of outbreaks	14	14	52	12		3		1	4	1	2	170	269
	Patients	214	372	704	300		8		43	279	4	11	1 257	2 921
2009	Number of outbreaks	22	31	61	11		0		10	6	2	3	221	351
	Patients	472	972	1191	282		0		171	86	57	68	1 953	4 644
2010	Number of outbreaks	27	41	60	46		8		11	5	2	11	296	503
	Patients	777	902	478	1982		11		256	470	10	226	2 229	6 880
2011	Number of outbreaks	11	27	52	36		3		16	1	1	13	266	426
	Patients	67	1048	596	1065		3		199	61	26	126	2 209	5 819
Total	Number of outbreaks	125	200	615	153	0	15	0	41	18	20	68	1 885	3 088
	Patients	2296	5292	9018	5169	0	27	0	1001	1026	832	1486	22 210	46 259

Table 10 Summary of annual notifiable disease reports in Guam, 1996–2009

Disease	*1996*	*1997*	*1998*	*1999*	*2000*	*2001*	*2002*	*2003*	*2004*	*2005*	*2006*	*2007*	*2008*	*2009*	*Total*	*Infected outside of the Guam*
Brucellosis					0	1	0	0	0	0	0	0	0	1	2	
Campylobacteriosis	23	8	16	15	13	10	18	33	29	16	21	14	8	7	231	
Cholera O1 El Tor	1	0	2	0	4 (1[a])	0	0	0	0	3	1	1	0	0	12	1
Fish poisoning (Ciguatera)	12	9	1	7	7	5	5	4	0	4	4	2	3	0	63	
Fish poisoning (Scombroid)	2	5	2	0	6	3	15	8	7	5	5	4	2	0	64	
Food poisoning	132	73	35	75	50	24	53 (4[a])	64	47	50	209	35	18	27	892	4
Giardiasis	6	1	9	23	17	9	7	2	5	11	5	2	0	3	100	
Hepatitis A	7	2	1	1[a]	1	2	1	2	1	2	1	0	7	7	35	1
Salmonellosis	39	35	46	37 (1[a])	28 (1[a])	57 (1[a])	46	44	50	46	38	20	23	11	520	3
Shigellosis	43	74	39	19	46	53	37	41	42	20	18	19	20	13	484	
Toxoplasmosis	1	0	1	0	0	1	0	1	1	0	1	0	0	0	6	
Typhoid fever	1[a]	2[a]	0	0	0	3[a]	0	0	1	1[a]	0	0	0	0	8	7
Vibrio cholerae Non-O1	0	0	0	0	2	0	1	0	1	1	0	0	0	0	5	
Vibrio parahaemolyticus	11	7	22	9	7	4	9	4	6	3	3	1	2	1	89	

[a]Diseases infected outside of Guam.

Taiwan Centers for Disease Control in 2002 began routine subtyping and building a deoxyribonucleic acid (DNA) fingerprint database of bacterial pathogens. After a 4 year practice, PulseNet Taiwan, the National Molecular Subtyping Network for Infectious Disease Surveillance, was formally inaugurated by the DoH, Taiwan.

According to the PulseNet Taiwan, till date, 10 238 *Salmonella* spp., 2048 *Shigella* spp., 89 *V. parahaemolyticus* isolates were included in the DNA fingerprint database. Salmonellosis is estimated to be the most prevalent foodborne disease. More than 2000 *Salmonella* isolates were collected each year from the collaborative hospitals for the determination of serotypes, PFGE fingerprints and traits of antimicrobial susceptibility. *Vibrio parahaemolyticus* is the leading causal agent for foodborne disease outbreaks in Taiwan, however, because most of the outbreaks of *V. parahaemolyticus* infections are easily identified, routine PFGE analysis is not applied to this organism.

In Taiwan, EHEC is a notifiable disease. Taiwan has no indigenous case of *E. coli* O157:H7 infection; only an imported case was identified in 2001. *Vibrio cholerae* infection was rare for the past 40 years. Listeriosis is not in the list of notifiable diseases in Taiwan. Although the incidence rate is unknown, *Listeria monocytogenes* is infrequently identified from patients in Taiwan.

Guam

Total population (mid-year): 168 564 (2005).

According to the annual notifiable disease report from 1996 to 2009, *Salmonella* was the leading agent of potential foodborne infections, followed by *Shigella* and *Campylobacter* (520, 484, and 231 cases, respectively in 14 years). Diseases caused by these pathogens were reported constantly during this period. Cases caused by *V. parahaemolyticus* decreased from 22 cases in 1998 to 1 case in 2007. Even though cases are limited, fish poisoning caused by ciguatera and scombroid are also constantly reported (Table 10).

Marshall Islands

Population: 54 000 (2010).

No systematic surveillance data is available. On 25 March 2009, the largest outbreak in the tiny island of Ebye in the Republic of the Marshall Islands was reported in 174 cases among 187 attendees at a local funeral. Even though the etiology of this outbreak could not be conclusively determined as no causal organism or toxin was identified, however, from clinical epidemiological investigations, the most likely agent of this outbreak could be either enterotoxins of *S. aureus* or *B. cereus*.

Fiji

Population: 861 000 (2010).

To enhance public health capacity in Fiji for foodborne disease surveillance, *Salmonella* Surveillance Project was developed. In 2004–05, 86 non-Typhi *Salmonella* and 275 *S.* Typhi laboratory-confirmed infections were reported. *Salmonella enterica* serotype I 3,10:r:– and *S. enterica* serotype Weltevreden were the most commonly isolated non-Typhi serotype.

See also: Bacteria: *Brucella*; *Campylobacter*; *Clostridium botulinum*; *Clostridium perfringens*; *Listeria monocytogenes*; *Plesiomonas shigelloides*; *Salmonella* Non-Typhi; *Salmonella* Typhi and *Salmonella* Paratyphi; Shiga Toxin-Producing *Escherichia coli* and Other Pathogenic *Escherichia coli*; *Shigella*; *Staphylococcus aureus*; *Vibrio cholerae*; *Vibrio parahaemolyticus*; *Vibrio vulnificus*.

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Foodborne Diseases and Vulnerable Groups

J Rocourt, Paris, France

Glossary

Adaptive immunity (or specific immunity) Is composed of highly specialized systemic cells and processes that eliminate or prevent pathogenic growth. It is acquired during life. The immunity is produced by exposure to antigens, by infection or vaccination (active immunity), or by the transfer of antibody or lymphocytes from an immune donor (passive immunity). Responses are designed to maximally eliminate specific pathogens or pathogen-infected cells. It generates an immunological memory and subsequent responses to a previously encountered antigen are more effective and more rapid than those generated by innate immunity.

Immune memory Greatly speeds up the response to pathogens that have been previously encountered (primary response); if the body is reinfected with a previously encountered pathogen, an adapted cell subpopulation will provide a very specific, efficient, and rapid secondary response.

Immune tolerance Is the process by which the immune system does not attack an antigen. Immune tolerance in pregnancy is the absence of a maternal immune response against the fetus and placenta.

Immunosuppression (or immunodepression) Lowers the body's normal immune response to invasion by foreign substances; may result from certain diseases, such as acquired immune deficiency syndrome or lymphoma. Immunosuppression may also be deliberately induced with drugs (e.g., to prevent the rejection of an organ transplantation) or incidental (e.g., as a side effect of radiotherapy or chemotherapy for cancer).

Innate immunity (or nonspecific immunity) Is the first line of defense. It comprises the cells and mechanisms that defend the host from infection by other organisms in a nonspecific manner. It does not confer long-lasting or protective immunity to the host.

Introduction

The development and outcome of foodborne diseases result from a close interrelationship between the virulence of the pathogen, the number of organisms the person is exposed to at a given time, the quality of the host's anatomical and functional barriers, and the immune competency of the person. The latter, in turn, is affected by age, pregnancy, genetic factors, certain diseases (human immunodeficiency virus (HIV) infection and cancer), use of certain medications, and nutritional status.

Most infectious foodborne illnesses are characterized by acute symptoms that are limited to the gastrointestinal tract, including vomiting and diarrhea. These illnesses are generally limited in both duration and severity, and most patients without underlying illnesses or malnutrition recover without medical treatment or require only modest supportive care. However, some individuals are at increased risk of higher incidence, developing more severe outcomes and higher mortality rates; they are classically defined as the very young, the elderly, pregnant women, and the immunocompromised, often referred to as 'YOPI' (young, old, pregnant women, and immunocompromised). Through local and systemic responses, the immune system is a key element in modifying the establishment of infection, controlling disease once it is established, limiting the severity and dissemination of the disease, and assisting in the control of the pathogen. In addition to the physiological impairment of the immune system, there is a great diversity of diseases and therapeutics generating long-term or permanent immunosuppression making people more vulnerable to foodborne disease: primary immunodeficiencies, immunosuppressive therapy following organ transplantation or for treatment of other diseases, HIV infection, cancer, defects of iron metabolism, and cirrhosis or other liver diseases. Immunocompromised people vary in their degree of immunosuppression and susceptibility to foodborne infection and this is clearly demonstrated by the dose–response model used for the risk assessment on *Listeria monocytogenes* in ready-to-eat foods developed by the World Health Organization (WHO)/Food and Agriculture Organization of the United Nations.

To the author's knowledge, no attempt has been undertaken to estimate the global burden of foodborne diseases in vulnerable populations, because of a number of difficulties, which include:

1. Insufficient data from surveillance in countries where such programs exist: In addition to underreporting, information

on underlying health conditions of patients is rarely collected.
2. Estimation for each pathogen of the percentage of illness attributable to foodborne transmission: These percentages have been evaluated in a small number of countries on the basis of various data such as, outbreak investigation, consumption patterns, specific surveys. Using these estimates for countries with different levels of sanitation and hygiene and different consumption patterns would be questionable.
3. A substantial number of foodborne diseases is caused by unspecified agents. (This paper focuses on pathogens; some chemicals are also of particular concern for the vulnerable groups of the population, such as methylmercury, lead, marine biotoxins, etc.) These include known agents with insufficient data to estimate agent-specific illness, known agents not yet recognized as causing foodborne illness and agents known to be in food but of unproven pathogenicity, and very few data are presently available in this field.

However, an abundant international scientific and medical literature, including case reports, surveys, and reviews, has been published on foodborne diseases in these sensitive populations which provides insight on this growing public health problem affecting both developed and underdeveloped countries.

Age-Related Physical or Physiological Susceptibility

Neonates, Infants, and Children

Increased Susceptibility to Foodborne Pathogens: Relative Immaturity of the Immune System and the Vicious Cycle of Malnutrition–Diarrhea

Although partially protected by maternal antibodies during the first months of life (depending on the mother's immunity), neonates and infants are potentially sensitive to a range of pathogens because their immune system is not fully developed. Developmental immaturity, insufficient proliferative responses and dysregulation of cytokine networks (immature cells are unable to make cytokines critical to mounting effective immune responses and of generating a population of memory cells), and reduced antibactericidal properties are major contributing factors. Neonatal innate immunity is also associated with reduced production of immunoglobulin, mucus, and acid, and deficient gut motility necessary for appropriate local responses to pathogens.

In this context, breast-feeding deserves special attention. Breast milk contains large quantities of secretory immunoglobulin A (IgA), lysozyme secreting macrophages, both T and B lymphocytes that release interferon-γ (IFN-γ), migration inhibition factors, and monocyte chemotactic factors, and impacts the initial bacterial colonization of the newborn gut, thus strengthening the various immune responses; in addition, breast milk supplies the ideal mix, density, and physiological form of nutrients to promote adequate infant growth and development. Unlike bottle-feeding, breast-feeding protects from diarrhea by its antibacterial and antiviral properties and, when exclusive, by avoiding exposure to foodborne pathogens. Because antiretroviral (ARV) interventions to either the HIV-infected mother or HIV-exposed infant can significantly reduce the risk of transmission of HIV through breastfeeding, WHO recommends that national au-thorities in each country decide which infant-feeding practice, i.e. breastfeeding with an ARV intervention to reduce transmission or avoidance of all breastfeeding, will be primarily promoted and supported by maternal and child health services. As soon as breast-feeding is no longer adequate as the sole source of food – between 4 and 6 months of age according to the nutritional status of the mother – breast milk needs to be supplemented, and later on (>2 years) substituted by appropriate foods until the child is gradually introduced to family food. Two major risk factors for foodborne diseases may occur at this stage: ingestion of weaning foods contaminated with pathogens and undernutrition.

Acquired immunity develops with exposure to various antigens, building a defense that is antigen specific. Unlike innate immune responses, the adaptive responses are highly specific to the particular pathogen that induces them and provide long-lasting protection. For the child's immune system to develop normally, exposure to foreign antigens is necessary. Acquired immunity takes time to develop after initial exposure to a new antigen. As children age and encounter more and more pathogens, the immune system becomes more responsive against these microorganisms and can better protect against them because an immune memory is formed. The peak isolation rate of diarrhea in infants and children is partly attributed to the increased susceptibility on the first exposure and partly because medical care is quickly sought for infants and incidents are more often reported.

Evaluating children's susceptibility to foodborne diseases involves raising the issue of malnutrition because children have less nutritional reserve than adults, making them particularly at risk for undernutrition and consequently, to diarrhea. In resource-poor countries, malnutrition appears early as a major problem. A small rise in the prevalence of malnutrition between 3 and 6 months indicates early introduction of milk substitutes, a further rise in the malnutrition rate between 6 and 12 months corresponds to late introduction or inadequate amount of complementary feeds, and further increase between 12 and 23 months is likely to be due to low energy intake, because children are not fed enough with household food. Moderately malnourished children have a 30–70% greater rate of diarrhea, 2- to 3-fold increase in the duration of their episodes than better nourished children in the same settings and the risk of death increases gradually with the deterioration of the nutritional status. A vicious cycle between diarrhea and malnutrition appears quickly. Malnutrition leads to weight loss, lowered immunity, higher susceptibility to pathogens, and impaired growth and development in children; this is further aggravated by diarrhea, malabsorption, loss of appetite, and diversion of nutrients for the immune response; as a consequence, this situation generates reduced dietary intake. In addition, fever increases both energy and micronutrient requirements. Protein energy malnutrition impairs cell-mediated immunity (significant decrease in functional T lymphocytes and an increase in null cells), phagocytic function, and the complement system. It also diminishes Ig concentrations and cytokine production. Micronutrient deficiencies (vitamin A which maintains the

integrity of the epithelium in the gastrointestinal tract, zinc and copper) also adversely affect the immune response. Infection induces protein catabolism and negative nitrogen balance, depletion of carbohydrate stores and increased gluconeogenesis, affects lipid metabolism, and redistributes minerals between nutrient compartments. These factors further increase the vicious cycle between malnutrition and infection. Diarrhea is a major cause of complication in children with severe acute malnutrition and increases their odds of death substantially irrespective of other factors. For example, a review of the medical files of children with severe malnutrition admitted at New Halfa Hospital, Sudan, indicated that among the 1097 children admitted during 2007–09, 73% were children <2 years and 71% had diarrhea; of the 61 children who died, 18% had septicemia following diarrhea and respiratory tract infections. Currently, malnutrition is responsible for more than one-half of all deaths of children worldwide, primarily deaths caused by infectious diseases.

Impact of Increased Susceptibility to Foodborne Pathogens on Health

Despite the efforts of the international community, diarrheal diseases still pose a major threat to children. For example, a study of 79 067 deaths of children <5 years from 25 countries in sub-Saharan Africa and Southeast Asia in 1998 to estimate causes of death in high child-mortality countries demonstrated that diarrhea ranked second (after pneumonia) with 25% of cases. The estimated incidence rates in developing countries ranged from 3.5 to 7.0 cases per child per year during the first 2 years of life, and from 2 to 5 cases per child per year for the first 5 years.

In a number of countries in Asia and Africa, weaning foods prepared under unhygienic conditions are frequently heavily contaminated with pathogens and the incidence of diarrheal diseases starts increasing soon after weaning is initiated. Gastroenteritis is usually expressed as a mild diarrhea, but it can be seen as a severe clinical form with enhanced symptoms (nausea and vomiting), possibly leading to dehydration and death in children. Major bacterial pathogens include *Escherichia coli*, *Salmonella*, *Campylobacter*, and *Shigella*. Two pathogens deserve special mention regarding neonates and infants in spite of low disease incidence: *Cronobacter* spp. and *Clostridium botulinum*. Although *Cronobacter* spp. causes invasive infections in all age groups, neonates and infants <2 months are likely to experience higher disease rates, with those born prematurely or with low birth weight being at higher risk for severe infection (necrotizing enterocolitis, bacteremia, and meningitis), with high case-fatality rates (50–80%). In England, over the period 1997–2003, the annual incidence rate for neonates, infants aged 1–11 months, children 1–4 years, and children 5–9 years were respectively 17, 2, 0.7, and 0.2 cases per million population. Outbreaks have been detected most commonly among newborns and very young infants in hospital nurseries and neonatal intensive care units. Contaminated powdered infant formula has been epidemiologically linked with infections. Unlike most adults, infants are susceptible to infection by spores of *C. botulinum*, which, after ingestion, can germinate and then colonize the infant colon producing botulinum neurotoxin in the digestive tract, which then induces a rare but life-threatening disease. In healthy adults, the intestinal microflora prevents growth from ingested *C. botulinum* spores, but in infants the microflora is unable to stem growth. Honey is a well-recognized vehicle.

Diarrheagenic *E. coli*, and more especially enteropathogenic *E. coli* and enterotoxigenic *E. coli* (ETEC), are a leading cause of infantile bacterial diarrhea in developing countries: ETEC causes approximately 210 million cases and 380 000 deaths, each year mostly in children. The diverse range of *E. coli* pathotypes and antigens means that children may be subject to repeated infections by different subtypes without immune protection. A special pathovar of *E. coli*, enterohemorrhagic *E. coli* (EHEC) can result in hemorrhagic colitis with a small percentage of cases developing hemolytic uremic syndrome (HUS), a life-threatening complication characterized by hemolytic anemia, thrombocytopenia, and renal insufficiency. Young children are at the highest risk of developing HUS: In the USA, in 2000–06, the median age of patients who developed HUS was 4 years. Compared with persons who did not develop HUS, those with HUS were younger, more likely to be hospitalized, had a longer length of hospital stay, and were more likely to die. Death occurs in approximately 12% of patients with diarrhea-associated HUS, and 25% of survivors demonstrate long-term renal sequelae.

Besides diarrhea, several nonTyphi serotypes of *Salmonella* Enterica (NTS) are an important cause of childhood bacteremia. Although infection of healthy adults usually results in self-limiting gastroenteritis of short duration that does not require antibiotic therapy, the very young are at increased risk, with higher incidence of disease, of complications, and of death. Invasive NTS has emerged as a leading cause of bloodstream infection in children, with an associated case fatality of 20–25%, especially in Africa. The clinical presentation of invasive NTS disease is often close to that of *Salmonella typhi*. In former Zaire, for example, the case-fatality rate for NTS and *Salmonella* Typhi infections were 22.7% and 29.4%, respectively; infants under 6 months had a higher case-fatality rate than older children (relative risk = 1.7) and death occurred significantly more often when children fell ill with *Salmonella* bacteremia in the late rainy season, coinciding with the peak of malnutrition, than in the dry season. In comparison to NTS, *S. typhi* is a highly adapted, invasive, and human-restricted pathogen that in the nineteenth century caused considerable illness and death in the USA and Europe but now has the greatest impact in developing countries where school-age children, especially those from resource-poor settings with inadequate water and sanitation systems, are disproportionately affected. It is estimated that a total of 400 000 cases occur annually in Africa, with a significant morbidity between 1 and 5 years of age. Additionally, the emergence and spread of *S.* Typhi strains having multiple resistance to nearly all commonly available antibiotics in most developing countries is another major challenge to health care systems by reducing the effective treatment options, increasing treatment costs, and increasing the risk of complications and death.

Campylobacter (*Campylobacter jejuni* and *Campylobacter coli*) is one of the most common causes of bacterial acute gastroenteritis worldwide and an important cause of childhood morbidity. In developed countries, the disease is found mainly in children <5 years and in young adults; in the USA in 1996–2000, the highest incidence was observed among

infants <1 year (56 cases per 100 000 population), followed by 41 cases per 100 000 population among children aged 1–4 years. Bacteremia is detected in less than 1% of patients and is most likely to occur among the very young or very old or in immunocompromised patients. Serious systemic illness caused by *Campylobacter* infection rarely occurs but can lead to sepsis and death.

Shigella is the most important cause of bloody diarrhea worldwide. Review of the English-language literature between 1984 and 2005 indicated a global incidence of shigellosis at 80–165 million cases annually, with 99% of episodes occurring in the developing world; case-fatality rates ranged from 0% to 21% according to studies; *Shigella* was isolated from diarrheal or dysenteric stools with similar frequency in Asia and Africa and with lower frequency in Latin America and the Caribbean. During a prospective population-based study in six Asian countries conducted between 2000 and 2004, over 600 000 persons of all ages residing in Bangladesh, China, Pakistan, Indonesia, Vietnam, and Thailand, *Shigella* was isolated from 2927 (5%) of 56 958 diarrhea episodes. The incidence for children <5 was 13 episodes per 1000 residents per year whereas the overall incidence was 2 per 1000 per year.

Viral gastroenteritis occurs with two epidemiologic patterns, endemic sporadic cases in children and outbreaks that affect people of all ages and are caused by two main agents: rotavirus and norovirus. The illness affects all children worldwide during the first years of life regardless of their level of sanitation, hygiene, quality of water and food, or type of behavior. The incidence of rotavirus diarrhea is high, accounting for approximately 12% of severe gastroenteritis among children <5 years and 80% of recognized viral etiologies. A multicenter study in Europe (Belgium, France, Germany, Italy, Spain, Sweden, and the UK) indicated that 86% of rotavirus diarrhea occurred in children aged between 3 months and 3 years. Cases were more severe than the acute gastroenteritis in general with dehydration, vomiting, fever, and lethargy, resulting in more emergency department consultations and hospitalizations. Rotavirus is one of the main causes of seasonal peaks in pediatric hospitalizations, and contributes significantly to periodic high bed-capacity pressures and associated adverse consequences. In a 2009–10 survey in Burkina-Faso, acute malnutrition was significantly associated with more severe symptoms and a prolonged duration of diarrheal episodes in children. Norovirus is the leading cause of foodborne outbreaks worldwide, especially in closed and crowded environments, such as hospitals, nursing homes, and cruise ships and, as child rotavirus immunization programs are developed, may soon eclipse rotavirus as the most common source of pediatric gastroenteritis: For example, norovirus was the second most common cause of community-acquired acute gastroenteritis in children in Greece in 2008–09, following rotavirus, and the commonest cause in hospitalized children <5 years in Spain in 2006–07. Compared with rotavirus enteritis, norovirus infection is slightly less severe and fever was less frequent. Immunity against norovirus appears to be strain specific, and, given the genetic variability in circulating viruses, individuals are likely to be repeatedly infected during their lifetime.

Hepatitis A and hepatitis E viruses are associated with inadequate water supplies and poor sanitation and hygiene, and spread by close personal contact, with occasional foodborne outbreaks. Hepatitis A is highly endemic in developing nations where infection often occurs in children who are likely to be asymptomatic; only 30% of children <6 years develop symptoms, which usually are nonspecific and flu-like without jaundice. In industrialized countries, the introduction of hepatitis A vaccines led to a sharp decline in the number of reported cases in children and a shift to a higher percentage of cases occurring in older age groups.

Intestinal parasitic infections contribute significantly to the enteric disease burden experienced by children. The two most frequent parasites identified in children diarrhea are *Giardia lamblia and Cryptosporidium (Cryptosporidium hominis* and *Cryptosporidium parvum)*. Although an important percentage of cases may be asymptomatic, cryptosporidiosis in children is most frequently associated with diarrhea, nausea, vomiting, fever, and abdominal discomfort that usually resolves within 2 weeks. Although cryptosporidiosis and giardiasis affect persons in all age groups, the highest incidence is observed for children 1–9 years as illustrated by data from the USA, various countries in Europe, Africa, and Asia. Multiple symptomatic *Cryptosporidium* infections associated with prolonged oocyst shedding occur frequently in countries where the disease is endemic (40% of infected children in a study in India) and may contribute to the long-term effects of cryptosporidiosis on the physical growth of children. Low birth weight, malnutrition, stunting, and lack of breast-feeding have been reported to predispose children to cryptosporidiosis and other various foodborne parasitic diseases as well. Data worldwide, and more especially from Asia and Africa, indicate that *Cyclospora*, *Entamoeba histolytica*, *Microsporidium*, *Blastocystis hominis*, and *Ascaris lumbricoides* are also frequently isolated from feces of children with diarrhea.

Pregnant Women

Because of the changes in hormonal and immunological parameters that take place during pregnancy, pregnant women are especially vulnerable to a number of infections including those caused by foodborne pathogens. More importantly, these foodborne infections may be transmitted to their fetus.

Because the fetus has genetic traits partially maternal and partially paternal and is antigenically different from the mother, the maternal immunological system becomes tolerant of the foreign tissue antigens present in the fetus. To prevent the rejection of such a 'semi-allogenic graft' by the maternal immune system, cell-mediated immunity, which plays a major role in foreign tissue graft rejection, must be downregulated during pregnancy. A number of immunological and hormonal changes (increase in progesterone production) therefore take place in the woman's body for a successful pregnancy. The immunological relationship between the mother and the fetus is a bidirectional communication determined by fetal antigen presentation and by recognition of and reaction to these antigens by the maternal immune system (e.g., cytokines of maternal origin operate on placental development and antigen expression on the placenta determines the maternal cytokine pattern). Placental immune response and its tropism for specific pathogens affect the pregnant woman's susceptibility to

certain pathogens and impact the severity of the disease. Consequently, there is a modulation of the immune system which leads to differential responses depending not only on the microorganisms, but also on the stages of the pregnancy. Pregnant women and their fetuses are especially susceptible to two pathogens: *L. monocytogenes* and *Toxoplasma gondii*. They are intracellular microorganisms and therefore, cell-mediated immunity plays a key role in the control of the disease.

Listeria monocytogenes: Although the infection of the mother may be asymptomatic or characterized by a self-limited, nonspecific flu-like illness of the third trimester, the transplacental transmission may have more serious consequences for the fetus and newborn and may lead to subsequent intrauterine infection causing spontaneous abortion, stillbirth, and severe neonatal septicemia. All kinds of industrially processed ready-to-eat foods which support *L. monocytogenes* growth during extended refrigerated storage are typical food vehicles of listeriosis and this explains why it is mainly reported from industrialized countries. The incidence in France in 2001–08 was 5.6 cases per 100 000 pregnancies, ranking pregnant women among the highest risk groups for listeriosis. In the USA in 2004–09, pregnant women had a markedly higher listeriosis risk (relative risk: 114) than nonpregnant women.

Toxoplasma gondii: When a seronegative pregnant woman is infected by *T. gondii*, the disease is similar to that of any adult infected by the parasite and is not recognized in most cases. However, congenital infection leads to miscarriage, stillbirth, and preterm birth, or to neurological disorders in the infant (mental retardation and visual impairment) although the majority of infected newborns are asymptomatic at birth. The fetus is presumed to be at risk only if the mother has a primary, active infection during pregnancy. Transplacental infection of the fetus is estimated to occur in approximately 45% of infected pregnant women. The birth prevalence of congenital toxoplasmosis throughout the world ranges from less than 1 to 10 per 10 000 live births. A recent review of the literature published during the last decade on toxoplasmosis seroprevalence in women who were pregnant or of childbearing age indicated that foci of high prevalence were present in Latin America, parts of Eastern/Central Europe, the Middle East, parts of Southeast Asia, and Africa. A trend toward lower seroprevalence was noted in many European countries and the USA.

In addition to these particular two pathogens, transplacental infection of the fetus, preterm delivery, or low birth weight were also reported for *S.* Typhi, *C. jejuni*, *Brucella* species (generally *Brucella melitensis*), *Coxiella burnetii* (causing Q fever), and hepatitis A and E viruses. In the case of hepatitis E, although the mortality rate is usually low (0.07–0.6%), the illness may be severe among pregnant women (fulminant hepatic failure), with mortality rates reaching 25% as seen in large outbreaks in China, Southeast and Central Asia, the Middle East, and in Africa.

The Elderly

The elderly constitute a very heterogenous population in terms of physiological functions, health, and lifestyle. All these changes increase susceptibility to a number of foodborne diseases and the elderly people experience more frequently invasive infection, prolonged convalescence, serious sequelae, and higher case-fatality rates. In the USA, the highest morbidity rates were in older adults (>65 years) for all foodborne pathogens except *Shigella*; overall, most deaths (58%) occurred in persons >65 years old. *Listeria* had the highest case-fatality rate (16.9%), followed by *Vibrio* (5.8%), EHEC (0.8%), *Salmonella* (0.5%), *Campylobacter* (0.1%), and *Shigella* (0.1%).

Immune Senescence

With advancing age appear progressive decline and dysfunction of the immune system, in both peripheral and gut-associated immune response. In contrast to the relative immaturity of immune system cells in the neonates, the immune system in the elderly is characterized by a general decrease in cell function, rather than major changes in the number of cells; both qualitative and quantitative changes in the efficiency of humoral and cell-mediated immune responses are observed (such as delayed-type hypersensitivity reactions, T cell-dependent antibody production, response of memory cells, production of interleukin-2, antibody production to new antigens, etc.). Innate immunity is also compromised. Impairment in mucosal immune system contributes significantly to the increased risk of severe illness and mortality. The effect of a weakened immune response often manifests most clearly during periods of important stress (surgery, sepsis, multiple organ failure, malnutrition, dehydration, etc.). In addition to senescence, the alteration of the immune system by treatments that deeply affect its functions such as chemotherapy with cancers and immunosuppressive drugs used with solid organ transplantation is one of the leading causes of death in the elderly.

Physiological Changes in the Gastrointestinal Tract

The gastrointestinal tract changes during aging. Inflammation and shrinkage of the gastric mucosa increase with age, leading to low gastric acidity and a decreased role in limiting the number of microorganisms that enter the small intestine. A reduction of peristalsis delays the clearance of the pathogen from the intestinal tract, giving some pathogens more time to grow and form toxins in the gut, contributing substantially to the increased prevalence and severity of foodborne infections. In addition to these well-known factors, a recent genomic analysis of fecal bacteria showed that significant structural changes occur in the microbiota with aging, especially regarding protective bifidobacteria; reductions in these organisms in the large bowel may be related to greater disease risk.

The presence of underlying conditions also contribute significantly to the morbidity and mortality of foodborne diseases in the elderly. Diabetes exposes people >65 years to a higher risk of foodborne diseases if in insufficient control through persistent hyperglycemia. Prolonged use of antibiotics may stimulate overgrowth of pathogens and the loss of competitive inhibition provided by the natural gut microflora. Many elderly people are malnourished (various degrees of malnutrition and micronutrient deficiencies), which reduces cell-mediated immunity; a number of reasons explain this malnutrition: taking medication, digestive disorders, chronic illnesses, physical disabilities, dental problems, altered senses of smell and taste, early satiety, decrease in pleasure, social isolation, and depression may lead to appetite loss and financial constraints may decrease resources allocated to food.

In addition to health-related underlying conditions, the lifestyles of the elderly may generate additional risk factors for both acquiring and transmitting foodborne diseases. In western countries, retired persons use their increased leisure time to travel which put elderly travelers at a higher risk for diarrheal disease when visiting places where poor hygiene, improper food handling, or storage at inappropriate temperatures may result in increased exposure to food contamination. Conversely, a large proportion of the geriatric population, who require nursing care and related medical or psychosocial services, live in long-term care facilities (LTCFs); characteristics of this environment and its residents facilitate the acquisition and transmission of gastrointestinal infection.

Impact on Health

NTS infection more often presents as a primary bacteremia and is associated with significantly more frequent extraintestinal organ involvement and a high mortality rate (up to 40% in some studies). Among persons with no underlying disease, incidence rates of listeriosis increased with age, from 0.05 cases per 100 000 persons <65 years to 0.98 cases per 100 000 persons >75 years in France in 2001–08; an unexplained increase in the number of listeriosis cases has been observed with the elderly from the UK, EU, and USA for a few years. In the USA in 2000–06, a survey of patients with EHEC infections without HUS showed that, the case-fatality rate was highest among persons aged >60 years; also, among EHEC cases with the HUS symptoms, the case-fatality rate of persons aged >60 years was 33.3%, i.e., 11-fold higher than of the case-fatality rate of children aged <5 years. Thirty percent of deaths of the elderly occur in nursing home residents in the USA. In Australia in 2007, 1010 (54%) of the 1882 reported outbreaks of gastroenteritis occurred in LTCFs. Main bacterial pathogens were *Salmonella*, *Clostridium perfringens* (pseudomembranous colitis), and *Staphylococcus aureus*; the incidence of *Salmonella* infections was higher in LTCF residents than in community residents. In recent years, noroviruses have emerged as a major cause of gastroenteritis outbreaks in LTCFs. Using a modeling approach, 228 deaths associated with norovirus were estimated in England and Wales between 2001 and 2006, which represented 20% of deaths from infectious intestinal disease in patients >65 years. Although food may become contaminated outside the facility, many outbreaks originate from contamination occurring within the facility during food handling, storage, and preparation. In the Western countries where the elderly are a quickly growing segment of the population, deaths related to bacterial gastroenteritis are increasing at a greater rate than in any other age category. This suggests that foodborne diseases will be an expanding burden on the health care system unless better prevention and control measures are implemented.

Diseases and Therapeutics Generating Long-Term or Permanent Immunosuppression

The frequency of community and hospitalized patients with compromised host defenses has increased dramatically over recent decades worldwide. The origin of this trend is multifactorial and includes: (1) the emergence of HIV infection since the early 1980s; (2) the increasing use of solid and hematologic transplantation strategies; (3) patients with cancer treated with chemotherapy; and (4) the use of immunosuppressive medication for conditions such as Crohn's disease, rheumatoid arthritis, and other conditions which can be successfully controlled with iatrogenic immunosuppression. This makes a composite of multiple compromised host defenses including anatomic breeches, cell-mediated, and humoral immunodeficiencies. Because of diminished or absent immune responses, the expected local clinical signs of infection may not be present or detectable; atypical presentations caused by opportunistic pathogens are more prevalent, making the detection and management of infections in such patients more difficult. Despite significant progress in the prevention, diagnosis, and treatment of foodborne infection in the immunocompromised host, it remains an important cause of morbidity, increased length of hospitalization, increased total costs, and mortality.

HIV Infection

HIV infection is the most common immunodeficiency state worldwide. Numerical and functional deficiencies in $CD4^+$ T lymphocytes and deficiencies of other cells of the immune system is followed by an increased susceptibility to opportunistic and common pathogens. The development of malnutrition in HIV/acquired immune deficiency syndrome (AIDS) includes disorders of food intake, nutrient absorption, and intermediary metabolism. Interactions between HIV, immunity, and nutrition are complex and related to each other: HIV causes immune impairment leading to malnutrition which leads to further immune deficiency, then contributing to rapid progression of HIV infection to AIDS. In addition, coinfection with HIV and malaria is very common in sub-Saharan Africa, HIV and malaria being among the leading causes of morbidity and mortality in this region. HIV-associated immunosuppression contributes to more frequent and more severe malaria episodes. Malnutrition, immune system, malaria, and diarrhea are interconnected in a complex negative cascade which expands the burden of diarrheal disease.

HIV-infected subjects have a predilection for chronic diarrhea, which is most pronounced in those with lowest CD4 cell counts. A wide range of etiologic agents are responsible for acute and chronic diarrheal disease, and the prevalence of such agents varies greatly by geographic region, season, patient age, immune status, the use of cotrimoxazole for opportunistic infection prophylaxis and of highly active antiretroviral therapy (HAART), and socioeconomic conditions. Gastroenteritis secondary to *Salmonella*, *Campylobacter*, and a variety of enteric viruses can result in persistent infection, with more severe and prolonged diarrheal disease and an increased risk of bloodstream invasion and metastatic infection. Invasive strains of NTS have emerged as an important cause of bloodstream infection in HIV-infected adults and children, often associated with multiple recurrences, and with high case-fatality rates (20–25% in Africa). The increasing prevalence of antibiotic resistance complicates the management of this disease. Protozoa infections are more common than helminth infections. Cerebral toxoplasmosis was one of the first opportunistic infections to be described in HIV-infected patients. After acute infection, *T. gondii* continues to exist in tissue in

humans; in people with immunodeficiencies such as AIDS, rupture of cysts results in disease reactivation, including encephalitis or disseminated toxoplasmosis. Toxoplasmosis remains one of the main causes of early death among HIV/AIDS patients in some countries. Cryptosporidiosis remains the most common opportunistic enteric protozoal disease in HIV-infected patients. Although the first case of human cryptosporidiosis was reported in 1976, full recognition of cryptosporidiosis as a human disease originated from its association with AIDS patients in the late 1970s. The prevalence of intestinal colonization due to *Cryptosporidium* is significantly higher among HIV-infected persons presenting with diarrhea and low CD4 lymphocyte count <200 cells μl^{-1}. Chronic diarrheal cases were frequently found to have polyparasitic infection. Other parasites frequently identified in HIV/AIDS patients include *Isopsora belli*, *Cyclospora*, *Blastocystosis*, *Entamoeba*. A number of studies (e.g., in Germany, Spain, Italy, and Puerto Rico) demonstrated that HAART has resulted in a reduction of bacterial and parasitic infections by a restoration of the cell-mediated immunity and also by a direct inhibitory effect on the proteases of parasites.

Cancer and Organ Transplantation

Several bacterial and viral foodborne infections pose a threat to cancer patients. Patients are immunocompromised because of their disease or their medical therapy. Similarly, patients with solid organ transplants can become immunosuppressed from a pharmacological regimen used to prevent rejection of a transplanted organ and thus susceptible to foodborne infections. Multiple studies have identified malignancy and organ transplantation as a risk factor for NTS or *Campylobacte*r bacteremia, causing prolonged duration, atypical localization, and higher mortality. In Saudi Arabia, *Cryptosporidium* infection rates of 84% and 74.3% were observed in patients receiving immunosuppressive drugs for organ transplantation and malignancy, respectively. Norovirus infections are increasingly being recognized as important causes of diarrhea in hematopoietic stem cell transplant recipients. A study in France on the incidence rates of listeriosis among people with the same underlying condition during the period 2001–08 indicated they varied widely according to the nature of cancer, ranging from 0.19 cases per 100 000 cases of kidney cancer to 55 cases per100 000 cases of chronic lymphocytic leukemia. Decreasing risk ranking of patients with cancer was the following: chronic lymphocytic leukemia, liver cancer, myeoloproliferative disorder, multiple myeloma, esophageal, stomach, pancreas, lung, and brain cancer. Organ transplantation belonged to the group of underlying conditions with the highest incidence (>5 cases per 100 000). These patients had a >100-fold increased risk of listeriosis compared with persons <65 years with no underlying conditions. The case-fatality rate of listeriosis ranged from 20% to 40%, with the highest for cases with lung and pancreatic cancer.

Other Underlying Conditions

In contrast to this population of patients with acquired immunodeficiencies which is rapidly increasing, patients with hereditary immunodeficiencies, which may affect any part of the immune defenses, including humoral, cell-mediated, and innate immunity, constitute a small but relatively constant proportion of the population for which few foodborne infection cases have been reported. More cases are reported for underlying conditions such as cirrhosis and liver diseases, hemodialysis, and diabetes. Patients with cirrhosis, bacterial peritonitis, bacteremia, and extraintestinal localizations with high in-hospital mortality rates have been associated with *Salmonella*, *Campylobacter*, *Cryptosporidium*, and *Vibrio vulnificus*.

The role of immunosuppressive medication in the increased susceptibility to foodborne disease has been demonstrated in several studies; for example, in a retrospective study in Israel, 80 episodes of NTS bacteremia in children were compared with 55 episodes in adults over a 10-year period; adults were more likely than children to have predisposing diseases and to receive prior immunosuppressive agent medications. In a 13-year retrospective cohort study to describe nonperinatal listeriosis mortality in Los Angeles County (USA) during the period 1992–2004, steroid medication was found to be a statistically significant risk factor for mortality. In addition to treatment of cancer and of organ transplant rejection as illustrated in the previous paragraph, immunosuppressive agents are prescribed in patients suffering from chronic cold hemagglutinin disease, rheumatoid arthritis, the nephrotic syndrome, systemic lupus erythematosus, Crohn's disease, multiple sclerosis, or psoriasis, making these people more vulnerable to foodborne infections.

Acid-suppressing medications, which inhibit secretion of gastric acid, heal the lesions and suppress the symptoms of diseases like peptic ulcer and reflux esophagitis and, as a consequence, interfere with the antibacterial action of gastric secretions. During outbreaks as well as sporadic cases, antacids have been identified as a risk factor for listeriosis, salmonellosis, and campylobacteriosis.

It is well established that chronic psychological factors produce low to moderate degrees of immunosuppression and increase infectious disease incidence. Whether this affects foodborne disease, especially during extreme conditions such as household food insecurity, maternal distress, refugees, or displaced population camps requires further investigations because such situations usually correlate with deficient hygiene and sanitation related to food handling, preparation, and storage.

Prevention of Foodborne Disease in Vulnerable Populations

In addition to 'the five keys to safer food' promoted by WHO, which explains the basic principles that each individual should know all over the world to ensure safe food handling practices and prevent foodborne diseases ('keep clean, separate raw and cooked, cook thoroughly, keep food at safe temperatures, and use safe water and raw materials'), more specific recommendations to vulnerable population according to their health status and/or their potential exposure to foodborne pathogens should be considered to ensure an appropriate level of foodborne disease prevention.

The national food safety/public health agencies of a number of countries have developed recommendations for

such population at increased risk. For example, The US Department of Agriculture's (USDA) Food Safety and Inspection Service and the US Department of Health and Human Services' Food and Drug Administration have prepared 'Guides for People at Risk of Foodborne Illness' to provide practical guidance on how to reduce risk of foodborne illness (http://www.fda.gov/Food/ResourcesForYou/Consumers/SelectedHealthTopics/default.htm). These brochures include:

- Food Safety for People with Cancer
- Food Safety for Transplant Recipients
- Food Safety for People with HIV/AIDS
- Food Safety for Pregnant Women
- Food Safety for Older Adults
- Food Safety for People with Diabetes

and they inform on foodborne pathogens and symptoms of major illnesses, and advice on eating at home or out, on selecting lower risk foods and on safe handling, preparation, storage, and transportation of food. Several organizations – for example, WHO, USDA, the Food Standards Agency of the UK – have issued guidelines to ensure product safety, including information on safe infant formula preparation, storage, and handling (http://www.nal.usda.gov/wicworks/WIC_Learning_Online/support/job_aids/formula.pdf, http://www.food.gov.uk/multimedia/pdfs/formulaguidance.pdf, http://www.who.int/foodsafety/publications/micro/pif2007/en/).

In addition to ensuring the microbiological safety of food for vulnerable groups and providing advice on high-risk foods and food safety, strengthening foodborne disease surveillance is also an essential component to minimize foodborne infections in these populations, especially in hospitals and in aged-care facilities where foodborne outbreaks are becoming a major issue in spite of being preventable.

Conclusion

Many factors can influence the susceptibility to foodborne infection, including increases in diseases that cause immunosuppression, increased use of immunosuppressive agents, and malnutrition. Both the developed and developing countries suffer to various degrees the consequences of foodborne illness.

On a global scale, the leading cause of increased host susceptibility to infection is probably malnutrition. There are 925 million undernourished people in the world today. In the developing world, an estimated 230 million (39%) children <5 years are chronically undernourished and approximately 54% of deaths among children of this age are associated with malnutrition. The prevalence of child malnutrition in the year 2020 is projected to remain high. In sub-Saharan Africa, the number of child deaths is increasing while food insecurity and absolute poverty are expected to increase. Since 1999, the year in which it is thought that the HIV epidemic peaked, the number of new infections has fallen by 19% globally; however, of the estimated 15 million people living with HIV in low- and middle-income countries who presently need treatment, only 5.2 million have access. In spite of growing understanding of cancer cell biology which is gradually leading to better treatments, cancer remains one of the major causes of morbidity and mortality worldwide. As the world's population continues to grow and age, the burden of cancer will inevitably increase; even if current incidence rates remain the same, it is estimated that by 2020, the number of new cases of cancer in the world will increase to more than 15 million (with deaths increasing to 12 million). The predicted sharp increase in new cases will mainly be due to progressively aging populations in both developed and developing countries.

The world's population is aging significantly and rapidly because of age dynamics, declining fertility, and longer life expectancy. From 1950 to 2050, the total global population is projected to grow by 3.5 times; during this period, the number of people >60 years is projected to grow by 10 times and the number of people >80 years is projected to grow by 30 times. The development of shanty towns without sanitation around the megalopolis cities of Asia, Africa, and South America generate overpopulation with poor economic and social conditions. 1.2 billion people are living on less than a dollar a day. Poverty and poor living conditions, including lack of sanitation and infrastructure for wastewater and solid waste management, lack of health education, increase the opportunities for food contamination and resulting disease. Moreover, scientists predict substantial impacts from climate change on the incidence of diarrhea and poor people are most likely to be the first victims and the greatest sufferers of environmental degradation following these changes.

If current trends continue, the number of people vulnerable to foodborne disease will continue to grow globally, making diarrheal diseases an even more important public health problem, especially in the developing world. It is clear that diarrhea is a major obstacle in the realization of Millennium Development Goal no. 4 ('Reduce child mortality'), in spite of being a preventable disease.

See also: Bacteria: *Campylobacter*, *Clostridium botulinum*; *Cronobacter (Enterobacter) sakazakii* and Other *Cronobacter* spp.; *Listeria monocytogenes*; *Salmonella* Non-Typhi; *Salmonella* Typhi and *Salmonella* Paratyphi; Shiga Toxin-Producing *Escherichia coli* and Other Pathogenic *Escherichia coli*; *Shigella*. Foodborne Diseases: Foodborne Diseases in Travelers; Overview of Biological Hazards and Foodborne Diseases; Prevalence of Foodborne Diseases in Africa; Prevalence of Foodborne Diseases in Europe; Prevalence of Foodborne Diseases in North America; Prevalence of Foodborne Diseases in South East and Central Asia; Prevalence of Foodborne Diseases in Western Pacific Region. Protozoa: *Cryptosporidium* spp.; *Giardia lamblia*; *Toxoplasma gondii*. Public Health Measures: Surveillance of Foodborne Diseases. Toxic Metals: Lead; Mercury. Viruses: Hepatitis A Virus; Hepatitis E Virus; Norovirus

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www.who.int/topics/food_safety
World Health Organization (WHO).

Foodborne Diseases in Travelers

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Glossary

Dysentery Pragmatically defined as travelers' diarrhea, which invades the intestinal mucosa resulting in systemic disease with fever and blood admixed to the stools.

Enterotoxigenic *E. coli* The most frequent pathogen associated with travelers' diarrhea in any low-income parts of the world.

Hazard analysis and critical control points (HACCP) A preventive system that identifies, evaluates, and controls hazards that are significant for food safety.

High-risk destinations Countries with a two-week incidence rate of travelers' diarrhea exceeding 20%.

Travelers' diarrhea Three or more unformed stools per 24 h with at least one accompanying symptom, such as fecal urgency, abdominal cramps, nausea, vomiting, or fever.

Foodborne diseases are the most frequent health problem among travelers from industrialized countries visiting low-income destinations. Predominantly this is described as travelers' diarrhea (TD), although more serious gastrointestinal infections, such as typhoid fever or cholera, may also occur. Also hepatitis A, hepatitis E, and lastly poliomyelitis are essentially transmitted by the fecal–oral route. Food intoxication has only rarely been described in recent years in this population, but historically incidents have become known, for example, on a jumbo jet.

TD is usually defined as three or more unformed stools per 24 h with at least one accompanying symptom, such as fecal urgency, abdominal cramps, nausea, vomiting, fever, etc. However, milder forms of TD may also result in incapacitation, as the patients cannot predict how the illness will evolve. In this population, dysentery is usually pragmatically defined as TD which invades the intestinal mucosa resulting in systemic disease with fever and blood admixed to the stools.

Epidemiology

Similarly to children in the developing world, travelers face pathogens unprotected, they are nonimmunes. The most recent attack rates on tourists and business people having visited different parts of the world are shown in Figure 1. Also other populations, for example, military, may be affected. Developing countries are high-risk regions with rates of TD of 20 to more than 60% per 2-week stay. In contrast, visitors to low-risk areas experience TD in less than 8%. However, for travel within Europe or North America the rate is not nil. From comparing rates over time it becomes obvious that there has been a dramatic reduction of TD rates in southern Europe. It also appears that Tunisia and Jamaica through systematic efforts of their authorities, mainly the ministries of health and tourism, have been able to reduce the risk of the illness. In contrast, over the past decades hardly any risk reduction has been observed in most of the other destinations when returning travelers were investigated.

Environmental and Behavioral Factors

Seasonality may have a limited relevance: Among British tourists in Monastir, Tunisia, the rates varied from 16–18% in May–July to 20–23% in August–October and also in Mexico and Jamaica lower rates are observed in winter; in contrast, there are no relevant differences observed in the tropics.

On a specific destination, there are highly significant differences in TD incidence rates in different risk groups. Probably most important is the selection of hotel. As demonstrated in Jamaica, the incidence rates in 18 hotels visited by at least 40 clients for 1 week varied between 0 and 33% ($p < .001$). Experts while visiting these places realize that these rates mirror the hygienic conditions. Five star-rated hotels tend to have a slightly higher TD incidence rate as compared to many three or four star-rated hotels. This is plausible, considering that elaborate food in the more refined places more frequently needs to be touched by fingers.

As expected, duration of exposure is relevant. TD usually occurs during the first week abroad. Although there is still a risk of illness in the subsequent weeks and up to 2 years, it appears that some immunity is gradually developed.

Whether or not avoidance of potentially contaminated food and beverages play a role, is still debated. Although various retrospective studies, probably biased, showed no effect, the only follow-up survey demonstrated a linear increase

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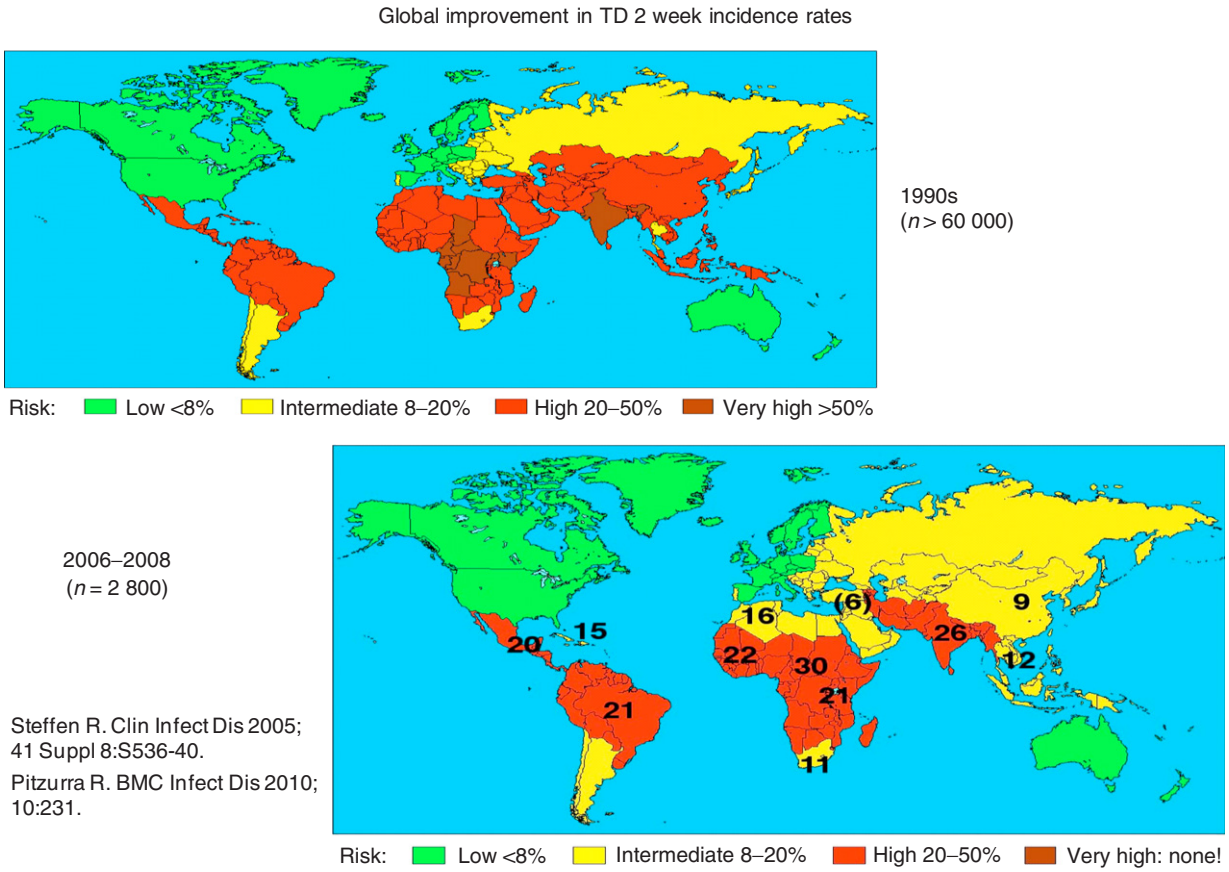

Figure 1 Two-week incidence rates of travelers' diarrhea at various destinations in Swiss travelers, 2006 to 2008. In Turkey and the Arabian Peninsula the color for intermediate risk is deliberate despite the rate of 6%, as this region was high risk in the 1990s and as the sample was very small. Reproduced from Pitzurra R, Fried M, Rogler G, Rammert C, Tschopp A, and Hatz C (2011) Irritable bowel syndrome among a cohort of European travellers to resource-limited destinations. *Journal of Travel Medicine* 18: 250–256.

of TD incidence with increasing numbers of dietary mistakes. Additional behavioral risk factors may be bathing in seawater or having meals in cafeterias or from street vendors. Also, consumption of unpasteurized milk resulted in an excessive odds ratio (OR) of 6.2; similarly, Mexican sauces were identified as often being contaminated. Excessive beer consumption resulted in a significant higher TD rate, whereas there is indication that wine may have some protective effect.

Host Factors

The origin of the traveler is the most relevant host factor. Owing to acquired immunity, the risk of illness is far lower in those living in areas with high endemicity when visiting other developing countries; this has been demonstrated in delegates to conventions, students, and military populations. Similarly, various studies have demonstrated that travelers who recently visited the tropics have a diminished incidence rate of TD, probably because of some developed immunity.

Various studies have demonstrated younger age to be a risk factor. Particularly prone to suffer from TD are infants, toddlers, and those between 15 and 30 years of age. We can imagine that small children are at a particularly high risk because their fingers indiscriminately touch floors and other contaminated materials before being licked, whereas in young adults a greater appetite may result in consumption of a larger inoculum of pathogens. In most studies no significant, or at least no clinically relevant, difference of TD incidence rates has been observed by gender, a recent account illustrating a higher proportion of females consulting Geosentinel sites was most likely biased. A more adventurous travel style of young adults is not the only explanation for the higher risk, because the significant difference is demonstrated even when the population is stratified by hotel and only all inclusive travelers are analyzed who do not consume any meals outside the hotel.

A genetic susceptibility to TD has been demonstrated. At least enteroaggregative *Escherichia coli*-associated diarrhea occurs significantly more often in AA genotype-251 (OR 209, 95% confidence interval (CI) 28–1525), as compared to a T genotype (OR 14, 95% CI 2–105) or event TT genotypes (OR 1). It is unknown whether a higher incidence rate of TD among British travelers is associated with genetic differences or different dietary habits.

Additionally, lack of gastric acidity subsequent to operation or associated with medication such as omeprazole or magnesium hydroxide aluminum hydroxide has been identified as a risk factor. Increasingly the gastric acid barrier is compromised by long-term medication in travelers.

Clinical Picture and Impact

TD usually is not a severe illness. Slightly less than one in four patients voids six or more unformed stools per day. The proportion of patients with classic TD is less than 40% at intermediate risk destinations, but clearly exceeds 55% at typical high-risk destinations. TD is hardly a life-threatening condition, although a few fatalities have been anecdotally reported, hospitalizations are particularly often indicated in small children. The average duration of untreated TD is approximately 4 days; 50% are free of symptoms within 48 h. Patients with fever, other symptoms suggesting severe TD, and those in whom pathogens are identified tend to have a longer duration of illness.

More important is the incapacitation due to TD, which may lead to extreme frustration at times of highest expectations. Approximately one-third of all patients are unable to pursue planned activities, which means that at typical high-risk destinations such as India or Kenya 30 and 20%, respectively, of all travelers will be incapacitated by TD. The duration of incapacitation is usually limited to 12–24 h, which is considerable, if we assume that many trips last only for a week. Additional time is lost when health professionals need to be consulted.

There is a growing interest in sequelae: Already early studies indicated that TD persists in 1% for 4 months or longer. Recently, several studies have indicated that irritable bowel syndrome (IBS) may be associated with TD. Although rates of up to 11% were documented in small initial studies, only 1.5% among 2800 Swiss travelers reported such problems.

Etiology

There has been a consensus for some time that diarrheagenic *E. coli* is the most important cause of TD, but for decades some 20–40% of all TD cases remained without a definable cause. Recently, stool samples from TD patients in Mexico, Guatemala, and India have been analyzed more thoroughly, including assessments in 20 instead of just 5 *E. coli* colonies, evaluation as to whether *Bacteroides fragilis* secrete an enterotoxigenic proinflammatory zinc-dependent metalloprotease toxin (ETBF), and also determination of various *Arcobacter* species. Particularly in Goa, India, bacterial pathogens were detected in 94% of TD stool samples, whereas the overall pathogen detection rate at all sites was 84% (Table 1). This confirms that the vast majority of TD is caused by fecal contamination of food and beverages.

Another recent study has demonstrated that between 10 and almost 20% of patients with gastrointestinal infections abroad may be infected or coinfected by noroviruses. Parasites such as *Entamoeba histolytica* or *Giardia lamblia* are the cause of TD in less than 5%, but they may persist for a longer period on returning home and thus higher rates may be detected in returning travelers. Often multiple pathogens are detected in these thorough scientific TD stool assessments, whereas in routine stool bacteriology far fewer pathogens are detected. In outbreaks in hotels or on cruise ships, often a single pathogen can be identified. In contrast, it appears the airline catering has an effective quality control, as hardly any post-flight outbreaks has been reported in the recent decade.

Table 1 Etiology of travelers' diarrhea – bacterial pathogens[a]

Enterotoxigenic *E. coli* (ETEC)	76%
Heat stabile enterotoxigenic *E. coli* (ETEC-ST)	51%
Campylobacter spp.	9%
Arcobacter spp.	8%
Enterotoxigenic *Bacteriodes fragilis* (EBTF)	7%
Shigella spp.	4%
Salmonella spp.	2%
Aeromonas spp.	2%
Plesiomonas spp.	2%
Vibrio spp.	1%

[a]Based on 201 TD stool samples collected 2007/08 in Guatemala, Mexico, and India (JIA).

Pathogens associated with TD may be resistant to any antimicrobials, although this appears to be less frequent among patients with TD as compared to locals in the same country. It is of concern that pathogens detected in stools of TD patients show increasing rates of resistance not only to quinolones, but also to azithromycin. Most assume that travelers using antimicrobials against TD for a variety of reasons contribute far less to resistance as compared to the local population.

Prevention

Essentially we can consider five different strategies for prevention. The first would be to reduce the risk for the visitors in the host countries. This can be achieved by implementation of the principles of hazard analysis and critical control points (HACCP) in hotels and restaurants, as demonstrated in Jamaica by Ashley *et al.*, 2004. Also, improvement of the infrastructure to provide safe water supplies, safe sewage collection, and disposal may drastically reduce the risk of TD.

The second option is to abandon travel plans to any high-risk country. Anecdotally it appears that some people are afraid to travel, among other reasons, because of the risk of TD. But travel health professionals should probably consider only advising against travel to a developing country for persons at high risk of complications of TD while abroad, be they immunosuppressed or infants and very small children, who often need to be hospitalized in case of such illness.

The third option is the avoidance of potentially contaminated food and beverages. The rule 'boil it, cook it, peel it – or forget it' has been shown to drastically reduce the risk of TD in one prospective study, whereas all retrospective surveys failed to show such a benefit. Anyhow, less than 5% of tourists strictly adhere to this recommendation, the majority of travelers select, for instance, salads from attractive buffets or accept ice cubes in the drinks. Organisms in contaminated ice will survive concentrations of alcohol found in drinks mixed with tequila and whisky. However, almost all enteropathogens are killed at 70 °C and most food items served at 60 °C are safe.

The fourth option is prophylactic medication, and a variety of drugs have been considered. These include probiotics, bismuth subsalicylate (available in the US), and antimicrobials. Only antibiotics offer a satisfactory protection, reducing the incidence of TD by 80% or more. Quinolones, azithromycin, and rifaximine (a nonabsorbed antibiotic, which therefore has

a profile of adverse events similar to placebo) are those most often considered. This is controversial: although in North America such prophylaxis is sometimes prescribed, European doctors are extremely reluctant to prescribe prophylactic antibiotics. This might be exceptionally considered for high-risk groups, such as for patients with preexisting illness in whom dehydration subsequent to TD may be dangerous (e.g., those with a history of stroke or transient ischemic attacks, inflammatory bowel disease, insulin-dependent diabetes mellitus, chronic renal failure, or AIDS), or persons who are particularly prone to suffer from TD, be that for genetic reasons (rare) or due to a lack of the gastric acid barrier. Such prophylaxis may also be considered for very important persons (VIPs) during very short trips.

Currently no vaccine offers satisfactory protection against TD. Typhoid vaccines are specific against typhoid, which is not characterized by diarrhea. The only cholera vaccine available in a limited number of countries (e.g., not in the US) offers some limited cross-protection against enterotoxigenic *E. coli* (ETEC), but the estimated efficacy against TD of all causes is limited to 5–23%. A skin-patch vaccine against ETEC is currently being tested.

Therapeutic Options

As discussed in the section on Prevention, there is no practical way to prevent TD. Thus, therapy – and while abroad often self-treatment – plays an important role. As diagnostic capabilities at many of the favorite tourist destinations worldwide are virtually inexistant, one will select therapeutic agents on the basis of scientific studies, some of which dating back many years.

The leading principle in the management of TD is to avoid dehydration. In healthy adults, an adequate fluid and electrolyte balance can be maintained with tea with sugar (fluids without sugar are not well absorbed by the infected gastrointestinal tract), bottled soft drinks (no cola, as that contains caffeine, which increases intestinal motility and thus diarrhea), juices, and soups, crackers, etc., to replace electrolytes. Electrolyte-containing oral-rehydration solutions such as those formulated by World Health Organization (WHO) will neither diminish the amount or duration of diarrhea, but they are paramount in infants, children, or also in elderly TD patients in whom dehydration may much more rapidly have devastating effects. Because of damage to the intestinal lactase-producing cells by enteric pathogens, dairy products should be avoided during illness, otherwise no diet is indicated.

Most experts agree that travelers to TD risk destinations should carry a travel-kit. Purchase of often cheaper medication at the destination is no longer recommended, because it is known that a substantial proportion may be falsified and ineffective; also storage at adequate temperatures may be a problem in many pharmacies in a tropical climate. It is paramount to give the travelers detailed verbal and written instructions on how the medications in the travel-kit should be used.

For self-treatment of TD, this ought to include an antimotility agent (usually loperamide) and an antibiotic. Many studies have shown that both these medications lead to rapid symptomatic improvement. The most dramatic reduction in the duration of illness is achieved by a combination therapy: two-thirds of the patients are free of symptoms within 4 h. In adult TD patients loperamide up to 16 mg 24 h^{-1} may be used, but such high doses often subsequently result in constipation. Loperamide is contraindicated during bouts of dysentery as defined in the introduction, unless used in combination with an antibiotic. Quinolones, and in Southeast Asia azithromycin, have become the antibiotics of choice for self-treatment. Studies have shown single-dose therapy and 3- to 5-day courses to be equally effective in most situations. It appears that early therapy is slightly more effective.

In contrast, therapy with lactobacilli or other probiotics has not been shown to be effective in modifying the course of TD. Also charcoal has been shown to be ineffective.

Some with concerns about self-therapy have recommended that TD patients should consult a local doctor. A recent study conducted in Phuket (Thailand), Goa (India), and Mombasa (Kenya) illustrated that some physicians caring for patients at tourist hotels use obsolete antimicrobials or combinations, that are not recommended in industrialized nations. Additionally, many have now set up their private clinics in vicinity of the hotels and increasingly hospitalize TD patients (some 80% of the TD consultations) overnight for antimicrobial infusion therapy. This results in costs amounting to several hundred US dollars, which the patients try to get reimbursed from their insurance on return. In some remote areas there is concern about nosocomial transmission mainly of the hepatitis B virus, occasionally also of other pathogens.

Rather often TD symptoms may have their onset only on return, or they may persist. Although state-of-the-art laboratory facilities are usually available in the country of residence, it is generally not practical to perform bacteriological stool examinations, first, because most patients will be cured by empiric therapy as described above before the results of the assessment are available, and second, because most laboratories do not routinely perform all the examinations listed in Table 1. Whenever symptoms are not cured by antibiotics, a stool analysis for parasites is indicated. If the results are negative or targeted therapy does not relieve the symptoms, ultimately colonoscopy may be indicated, as rarely TD symptoms may be the expression of a yet undetected malignancy in the gastrointestinal tract.

See also: Food Safety Assurance Systems: Hazard Analysis and Critical Control Point System (HACCP): Principles and Practice. Foodborne Diseases: Overview of Biological Hazards and Foodborne Diseases; Prevalence of Foodborne Diseases in Africa; Prevalence of Foodborne Diseases in North America; Prevalence of Foodborne Diseases in South East and Central Asia; Prevalence of Foodborne Diseases in Western Pacific Region

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BACTERIA

Contents

Acinetobacter

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History, Background, and Taxonomy

After years of erratic taxonomy of Gram-negative diploid organisms with same morphology, expert microbiologists established these bacteria in the 1970s in several genera: *Moraxella* spp., *Branhamella* sp., *Achromobacter* spp., with further studies identifying the genus *Acinetobacter* spp. These strictly aerobic bacteria grow easily on common media, at temperatures 20–30 °C, (optimum: 33–35 °C). Growth at 41 and 44 °C is a phenotypic character. Phenotypic identification of *Acinetobacter* spp. was based on oxidase–indole–nitrate-negative tests, catalase-positive tests, and oxidative production (or not) of acid from D-glucose, D-ribose, D-xylose, and L-arabinose. Phenotypic studies recognized two species in 1980 (Approved Lists of Bacterial Names), based on sugar use: *Acinetobacter calcoaceticus* and *Acinetobacter lwoffii* (nonacid

producing). Both species names have been used for two decades until application of DNA–DNA hybridization in 1986, discriminating genomic groups. 6 given species names were added to 12 initial genomic species, in 2008, 31 named genomic species and 12 unnamed species, and in 2009 were added *Acinetobacter berezinae* sp.nov. and *Acinetobacter guillouiae* sp.nov. in this evolving taxonomy of the genus.

Epidemiology and Habitat

Acinetobacter spp. are ubiquitous Gram-negative coccobacilli and nonmotile (displaying a 'twitching motility' presumably due to polar fimbriae). *Acinetobacter* spp. are widely distributed in nature, in soil and water, as free-living saprophytes. They are found in virtually 100% of soil and fresh-water samples (less than $1–7.9 \times 10^4$ cfu 100 ml^{-1}). They utilize a wide variety of substrates as sole carbon source in nature, plants, food, and foodstuffs (source for human carriage).

Acinetobacter spp. are recovered from human specimens, interstitial areas, axillae, toe webs, throat, and various secretions of healthy people. They form a small part of the human skin flora in approximately 25% of normal people. Digestive tract colonization has been documented in infants and adults in a prospective study (77% of 73 patients). Rectal colonization of *Acinetobacter* comprises the risk of 'translocation' phenomenon (transfer from gut to form infected sites in lungs or other organs).

Acinetobacter genospecies roles vary: *Acinetobacter baumannii* or *Aci. calcoaceticus* is more common in pathological conditions, whereas *Acinetobacter johnsonii*, *Acinetobacter junii*, and *Aci. lwoffii*, predominate in the environment. In animals, various *Acinetobacter* spp. have been isolated, but they were only occasionally found as agents of animal infections.

Clinical Profile of *Acinetobacter* Infections

"You've heard of MRSA, but what about *Acinetobacter*?" Los-Angeles Times Health, (24 December 2009).

Carriage of *Acinetobacter* spp.

Carriage of *Acinetobacter* spp. by personnel hands, patients, and materials in hospitals contributes to dissemination and outbreaks of nosocomial infections.

Acinetobacter Respiratory Tract Infections

Acinetobacter respiratory tract infections (RTIs) are severe Gram-negative pneumonias with fever, neutrophilia, purulent sputum, and infiltrates on radiograph or computed tomography scan. Bacteria are isolated from bronchial aspirations, brushings, or bronchoalveolar lavage. RTIs occur in mechanically ventilated patients, elderly, and patients with various risk factors.

Community-Acquired Pneumonia

Rare cases have been found in patients with chronic pulmonary diseases, diabetes mellitus, and renal and hepatic insufficiencies, with high mortality rates.

Bacteremic Pneumonia

It is the most severe infection, associated with shock and sepsis in patients with severe underlying pathologies. Mortality rates range from 30% to 75% when sources of the pathogens are trauma, surgical procedures, presence of catheters, intravenous (IV) lines, dialysis, and burns. Respiratory failure at admission increases the risk of bacteremia by threefold in case of malignancies, intracranial hemorrhage, and central venous catheters. Clinical signs of true bacteremic episodes are fever, leukocytosis, and blood cultures positive with the same genotypic isolate of *Acinetobacter*. Incidence of *Acinetobacter* bacteremia ranks second after pneumonias, its prognosis depends on the underlying condition of patients.

Urinary Tract Infections

From 2% to 61% of nosocomial UTIs have been attributed to *Acinetobacter* spp. with mean incidence of 30.5%, occurring in patients with risk factors like indwelling urinary catheter. Removal of the catheter is recommended to control bacteriuria.

Skin and Soft Tissue Infections

Infections of skin structures such as decubitus ulcers, leg ulcers, and onychodystrophy of the hands occurring 2 months after *Acinetobacter* peritonitis have been early identified. Colonization of burns, postoperative wounds with *Acinetobacter* spp. are seen in specific units, competing with *Pseudomonas aeruginosa* in most burned patients.

Meningitis

Nosocomial meningitis is infrequent manifestation of *Acinetobacter* infection after neurosurgical procedures. Cases of primary meningitis occur especially in children. *Acinetobacter* meningitis may result from the introduction of the organism directly into the central nervous system following intracranial surgery, myelography, ventriculography, lumbar puncture, or transnasal aspiration of craniopharyngioma. Relatively indolent, *Acinetobacter* meningitis is identified by meningeal signs, high fever, lethargy, or headache. An exceptional outbreak of *Acinetobacter* meningitis was described in a group of children with leukemia, after administration of intrathecal methotrexate.

Pediatric *Acinetobacter* Infections

Pediatric *Acinetobacter* infections occur by acquisition of the organism in infants with peripheral IV catheters. Environmental cultures revealed that air conditioners increased the incidence of cases by airborne dissemination of the bacteria. *Aci. baumannii* infection was related to mechanical ventilation in 15 preterm infants. In Japan, *Acinetobacter* septicemia was

seen in intensive care unit (ICU) neonates during 30 months (mortality rate 11%): Strains were isolated from the hands of staff, sinks, and fecal samples.

Miscellaneous

Rare infections such as suppurative thyroiditis, necrotizing enterocolitis, *Acinetobacter* pericarditis with tamponade (in a patient with systemic lupus erythematosus), and septic pericarditis. Rare cases of *Acinetobacter* infections have been recognized by genotypic identification of strains, as seen after earthquake (Turkey, 1999) and in war zones (Vietnam conflict, Gulf war soldiers, 2003–2004).

Therapeutic Problems

Antibiotic Resistance

The earliest problem has been the 'natural' resistance of the bacteria to few antibiotics existing in the 1970s (ampicillin, carbenicillin, cephalothin, tetracyclines, and chloramphenicol). As new antibiotics were developing in 1980s (second- and third-generation cephalosporins, aminoglycosides, and fluoroquinolones), *Acinetobacter* acquired mechanisms of resistance to the major antibiotic classes: They can produce a wide variety of β-lactamases: plasmid-mediated (TEM, SHV-type, and CARB-5 hydrolyzing penicillins), chromosomally mediated (cephalosporinases, extended spectrum β-lactamases (ELSB, CTX-M), emerging carbapenemases: (VIM, KPC, OXA-24–26–40–58….).

Imipenem Resistance

Spread of carbapenem-resistant strains constitutes a major threat for treatments of multidrug-resistant (MDR) *Acinetobacter*. Other mechanisms like alteration of drug targets (penicillin-binding proteins), efflux pumps, and permeability problems are growing. *Acinetobacter* developed resistance to initially active aminoglycosides, inactivated by production of a series of aminoglycoside-modifying enzymes. Fluoroquinolones, used to treat *Acinetobacter* infections, underwent chromosomal mutations in the *gyr*A and *par*C genes and are fluoroquinolone resistant in patients in admitted ICUs. Few therapeutic alternatives, combinations include sulbactam and fosfomycin, and rifampicin and colistin.

Pathogenesis and Virulence Factors

Seen as relatively low-grade pathogens, in fact *Acinetobacter* spp. exhibit significant pathogenic importance. Virulence mechanisms of *Acinetobacter* strains include: (1) presence of a polymer (slime), a polysaccharidic capsule, formed of L-rhamnose, D-glucuronic acid, and D-mannose, found mainly in *Aci. baumannii* isolated from catheters and tracheal devices. Polysaccharidic structure protects bacteria from phagocytosis; (2) adhesiveness to human epithelial cells mediated by the capsule in relation to presence of polar thick fimbriae (5 nm), correlated with twitching motility; (3) lipopolysaccharide component of cell wall and presence of lipid A are major virulence factors in the pathogenicity of *Acinetobacter*; (4) outer membrane proteins can stimulate inflammatory response; (5) extracellular enzymes (lipases, esterases, amylase, and collagenase), periplasmic enzymes (butyrate and esterase), and membrane-bound enzymes (proteases, leucine, and alanine aminopeptidases) damage tissue lipids and proteins; and (6) iron regulation and role of siderophores are other factors associated with virulence (*Acinetobacter* spp. are able to grow under iron-deficient conditions).

Sources and Food Origins of *Acinetobacter* spp. Relation with Human Contamination

Intestinal Carriage: Potential Sources of Contamination of Food

Intestinal flora of neonates has been shown as a reservoir of the organism and risk factor for bacterial carriage. Bacterial overgrowth in the stomach of ICU patients due to diminished acid secretions can allow *Acinetobacter* to multiply and reach intestinal areas (carriage and contamination). Mouse gastritis due to *Aci. lwoffii*, by means of urease activity and fimbriae helping to adhere to gastric epithelial cells result in gastric acid suppression favoring *Acinetobacter* gut colonization.

Acinetobacter Presence in Food

Some data are available on the contamination and spoilage of food due to *Acinetobacter*, and also its possible role in human infection.

Meats

As psychrophilic-like *Pseudomonas*, *Acinetobacter* can multiply at low temperatures on surfaces of dry carcasses. Owing to their composition, muscles are sensitive to microbial alteration. In chicken carcasses and other poultry meats, it has been shown that *Acinetobacter* spp. contribute to the spoilage process of refrigerated meat, economically important spoilage of bacon and chickens. Preparations containing mince meats can have high microbial load, contaminating equipments and fresh pork sausages (Sulzbacher *et al.*). A survey in the University of Nebraska has identified *Acinetobacter* spp., as contaminants of beef and occurrence in radurized product.

Milk Products

In raw milk and cheese, even stored in refrigerated conditions, microbial development in tubular heat exchanger (pasteurizing milk) has been shown through ascending temperature ($\sim$20–90 °C), with change of dominant microbiota formed of *Acinetobacter* to thermophilic bacilli. The lipolytic activity of *Acinetobacter* in milk and dairy products results in different alterations of smell and taste. Among recently designated *Aci. berezinae* sp. and *Aci. guillouiae* sp., food origin of few strains was raw milk.

A positive application of *Acinetobacter* as lipase producers has been described as they are capable of efficient degradation of fats and oil in kitchen wastewater treatment.

Fishes

Among the microorganisms found on surfaces and in the intestine of fresh fishes, when caught in warm waters, *Acinetobacter* is common. Dominant microflora in molluscs consists of Gram-negative bacteria (*Pseudomonas*, *Acinetobacter*, and *Flavobacterium*). Taken in waters of northern areas, Gram-negative bacilli predominates, including *Acinetobacter*.

Vegetables

Acinetobacter strains have been found in vegetables. In tropical climates like in Hong Kong, 51% of local vegetables are colonized with *Aci. baumannii*. In raw sewage effluent, 10^6 cfu 100 msl^{-1} have been found, and in vegetables 30 isolates out of 177 vegetables. Fresh water (with sediment), sewage, soil (barley field), and activated sludge (from Sweden, Australia, Portugal, and Moravia) were colonized with *Aci. baumannii*.

Food-Animal Transport

Food-animal transport and use of antimicrobial agents may produce selection of drug resistance among aerobic bacteria such as *Acinetobacter*. Industrial food animal production includes concentrated animal-feeding operation, as a source of bacterial transmission.

Transmisison to Humans

Acinetobacter spp. most frequently isolated in food are *Aci. johnsonii*, *Aci. junii*, *and Aci. lwoffi*. Contaminations from food may occur during industrial preparation, under poor hygienic conditions. Home food preparation could be a potential source of occasional infection of skin, gut, and throat, but rare cases are found in the literature, knowing that *Acinetobacter* spp. are bacteria of low virulence. Conditions which allow the agent to express its pathogenicity are hospitals where patients are at the high risk of infection. In Korean food, an *Aci. baumannii* producing a PER-1 enzyme (ELSB) has been isolated, responsible for a nosocomial outbreak in an ICU In 1000 autochthonous strains from fresh and fermented artisanal products (Piemond area), 98 were bacteriocin-producing strains with hypothetic presence of *Acinetobacter* as a bacteriocin producer. (In genetically modified foods prepared for fragile new born babies, contamination risks by *Acinetobacter* spp. remain potential).

Other Aspects in Relation to Food and Other Conditions

Unusual and Special Situations

Sludge Cigarettes

Environmental species (*Acinetobacter baylyi*, *Acinetobacter towneri*, and *Acinetobacter tandorii)* were recently isolated from activated sludge. *Acinetobacter* strains isolated from wounded soldiers of Iraq were sent to France and analyzed for the presence of pathogens in tobacco and persistence in cigarettes: Identifying DNA of *Acinetobacter* spp. in cigarettes confirmed their role for fulminant epidemics of pneumonia in a surgical ICU.

Oysters

Microbial flora of pacific oysters (*Crassostrea gigas*) has been shown as containing *Acinetobacter* in treated sea water. Microbial potential presence in oysters subject to ultraviolet radiation-treated water remained comparable to levels held in untreated sea water.

Body Lice

Occasionally, *Aci. baumannii* has been found in infected animals and insects: *Aci. baumannii* strains have been isolated from 22% of body lice, fleas, or ticks, contaminated by biting homeless people having silent bacteremia, or as in tropical countries with high and humid temperature where biting insects contribute to contaminations.

Military Environmental Conditions

During Operation Iraqi Freedom, significant numbers of MDR *Aci. baumannii* infections occurred. Search for potential sources of contamination from foods resulted in isolation of several strains in soil bags (from 31 locations in field hospital soil), one strain in drinking water source, and one strain isolated from soil outside a field nutrition care section. These data demonstrate that despite practice of strict hygiene and controlled food (from the US), sparse isolates in military zone were responsible for outbreaks of *Acinetobacter* infections. Thirty-seven *Acinetobacter* isolates were obtained from equipment room, operating room, heater/air conditioners, sinks, tent walls, and most instruments. Other studies have been carried out with *Aci. baumannii* isolates from Iraq soldiers (Naval Medical Center) including molecular genetics.

Acinetobacter Metabolism

Enzymatic Activities

The high metabolic power of these bacteria expresses enzymatic activities, either destructive or active in assimilation, or dispersion of metabolites in the environment. From these activities, food spoilage, and/or persistence of *Acinetobacter* in industrial foods can occur but is not yet studied enough. Destructive enzymatic activities in *Acinetobacter* by lipases: The strain *Acinetobacter* BD413 expressed in high levels an extracellular lipase in early stationary phase, but rapidly degraded by an endogenous protease.

Proteases

Recognized for food bacterial spoilage, protease activities are based on the ability to utilize different proteins: *Acinetobacter* exhibits powerful proteolytic activities, hydrolyzing all test-proteins (gelatin, casein, and water-soluble proteins of beef muscle), among 122 tested bacteria.

Degradation of Aromatic Compounds

Acinetobacter radioresistens S13, able to grow on phenol or benzoate as sole carbon and energy sources, has been capable to produce biodegradation kinetics for mixtures of acetate,

benzoate, and phenol: the substrate utilization benzoate > acetate > phenol, showed that benzoate was the preferred substrate.

Inhibitory Effects of Radiolytic Products Have Been Carried Out Against Multidrug Resistant Organisms

Acinetobacter could not grow when incubated at 5% on the surface of fresh meat irradiated to 1500 krad.

Beneficial Aspects of *Acinetobacter*

Positive properties were found in its industrial applications: (1) production of surface polysaccharide-containing biopolymers of industrial importance (emulsan®); (2) ability of *Acinetobacter* spp. to sequester inorganic phosphate (intracellular polyphosphate) with application in phosphate removal from wastewater environments; and (3) degradation by *Acinetobacter* of ochratoxin A produced by *Aspergillus ochraceus* and other fungi. (Ochratoxin, a carcinogenetic toxin, in rats and mice is involved in Balkan endemic nephropathy).

Conclusion

Acinetobacter is a bacteria of low virulence. It is a major risk for vulnerable people, for example, patients in hospitals. It is found in a variety of food; however, it has been rarely associated with foodborne disease. Therefore, its role as a foodborne pathogen is undefined. However, *Acinetobacter* is a promising beneficial 'cleaning bacteria' as it has application in a number of areas such as phosphate removal, aromatic wastes, spillages, scraps, and degradation of ochratoxin.

See also: Foodborne Diseases: Foodborne Diseases and Vulnerable Groups. Food Safety Assurance Systems: Personal Hygiene and Employee Health

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BACTERIA

Aeromonas

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Glossary

Anthroponosis An infectious disease in which the etiological agent is carried by humans and is transferred to other humans and animals.
Foodborne disease Any disease resulting from the consumption of contaminated food, pathogenic bacteria, fungus, viruses, or parasites that contaminate food, as well as chemical or natural toxins such as poisonous mushrooms.
Organism life cycle A period involving all different generations of a species succeeding each other through means of reproduction, whether through asexual reproduction or sexual reproduction (a period from one generation of organisms to the same point in the next).
Taxonomy The science of classification, in microbiology the arrangement of microorganisms into a classification.
Zoonosis Any infectious disease that can be transmitted between species (by various ways, by a vector or by their products, and food) from animals to humans or from humans to animals (less common).

Introduction

Aeromonas is a Gram-negative, facultative anaerobic rod that morphologically resembles members of the family *Enterobacteriaceae*. Diseases produced by some species of this genus are called aeromoniasis (ICD-10 A05.8).

The Pathogen

In taxonomy bank of the NCBI 29 species of *Aeromonas* have been described (Table 1), many of them associated with human diseases. The most important pathogens are *Aeromonas hydrophila*, *Aeromonas caviae*, and *Aeromonas veronii* biovar *sobria* (Table 1). The organisms are ubiquitous in fresh and brackish water. *Aeromonads* are estuarine bacteria and are ubiquitous in fish and shellfish, meats, and fresh vegetables. They group with the gamma subclass of the Proteobacteria (g-proteobacteria).

Aeromonas hydrophila, the most clinically important species of the genus, is a widespread representative of *Aeromonas* found in water, water habitants, domestic animals, and foods (fish, shellfish, poultry, and raw meat). The microorganism has the potential to be a foodborne pathogen, especially strains from hybridization group (HG1), associated with clinical cases of illness. *Aeromonas* species have been recognized as potential or emerging foodborne pathogens for more than 20 years. Additionally, as it affects fish, amphibians, and reptiles, *Aeromonas* is also considered a zoonotic bacteria. Aeromoniasis is rare in wild or domestic mammals and birds.

Table 1 Main species included in the genus *Aeromonas*, characterized and reported in the National Center for Biotechnology Information (NCBI) Taxonomy Browser (2012) (http://www.ncbi.nlm.nih.gov/taxonomy)

Aeromonas allosaccharophila	*Aeromonas media*
Aeromonas aquariorum	*Aeromonas molluscorum*
Aeromonas bestiarum	*Aeromonas piscicola*
Aeromonas bivalvium	*Aeromonas popoffii*
Aeromonas cavernicola	*Aeromonas rivuli*
Aeromonas caviae	*Aeromonas salmonicida*
Aeromonas cf. bestiarum/salmonicida	*Aeromonas sanarellii*
Aeromonas diversa	*Aeromonas schubertii*
Aeromonas encheleia	*Aeromonas sharmana*
Aeromonas enteropelogenes	*Aeromonas simiae*
Aeromonas eucrenophila	*Aeromonas sobria*
Aeromonas fluvialis	*Aeromonas taiwanensis*
Aeromonas guangheii	*Aeromonas tecta*
Aeromonas hydrophila	*Aeromonas veronii*
Aeromonas jandaei	

Clinical Presentation

Two major diseases have been associated with *Aeromonas*: gastroenteritis and wound infections, with or without bacteremia or sepsis. Gastroenteritis typically occurs after the ingestion of contaminated water or food (aeromoniasis is a foodborne disease), usually presenting with selflimiting diarrhea, with children being the most susceptible population, whereas wound infections result from exposure to contaminated water. Although some potential virulence factors (e.g., endotoxins, hemolysins, enterotoxins, and adherence factors)

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have been identified, their precise role is still unknown. Aeromonas species cause: Opportunistic systemic disease in immunocompromized patients (although still few reports have enoughly support this), diarrheal disease in otherwise healthy individuals, and wound infections.

The pathogen produces different virulence factors including exotoxins, cytotoxins, adhesions, and others which are considered responsible for intestinal and extraintestinal infections in human beings and also for a wide variety of infections in animals, but the exact role of each, particularly in human disease has not been fully elucidated. As a psychrotroph, *A. hydrophila* grow in foods during refrigeration. Aeromonads are not resistant to food processing regimes and are readily killed by heat treatment. The disease spectrum associated with this microorganism includes gastroenteritis, septicemia, traumatic and aquatic wound infections, and infections after medical leech therapy. Multiple resistance of the bacterium to many antimicrobials is a fact of high significance.

Importance as Food Pathogen

Aeromonas has become increasingly recognized as human enteropathogen. This foodborne bacteria has been cultured from fish, pigs, broilers, eggs, milk, and vegetables, among other food products. *Aeromonas* sp. also multiply rapidly at $+4\ ^{\circ}C$ which is a significant risk in food storage. Furthermore, *Aeromonas* sp. have been recovered from fresh water sources, and some isolates are resistant to chlorination which makes it a further risk factor. No large food- or waterborne outbreaks have been reported so far with *Aeromonas* sp., but human isolated cases are commonly reported in the literature in association to water and food contamination. In 1995, in Sweden, a foodborne outbreak involving *A. hydrophila* was reported. A group of 27 people consumed a typical Swedish food 'landgång' which is a type of 'smörgåsbord' containing shrimps with mayonnaise, liver paté, ham, sausage, and legume salad which was purchased from a food store. A total of 22 of the 27 persons became ill within 20–34 h of consumption of the food and reported the symptoms ranging from severe acute diarrhea, abdominal pain, headache, fever, and vomiting.

Prevention and Control

Many scientific studies are done to assess the influence of different factors on survival of *A. hydrophila* and other species of the genus. The ability of the pathogen to grow at refrigeration temperatures may have great impact on refrigerator stored foods. Many factors for control of growth of *A. hydrophila* have been studied. Current strategies of control include: Hurdle technology (temperature, pH, NaCl, and $NaNO_2$), washing, oxidizing, smoking, modified atmosphere, use of probiotics, polyphosphates/NaCl, heating, use of plant extracts, high hydrostatic pressure, cooling/chilling, chlorine treatment, and alcohol treatment, among many others.

See also: Food Safety Assurance Systems: Microbiological Testing, Sampling Plans, and Microbiological Criteria. Institutions Involved in Food Safety: Trust in Animals and Food Safety (TAFS) Forum; World Health Organization (WHO); World Organisation for Animal Health (OIE). Public Health Measures: Surveillance of Foodborne Diseases

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BACTERIA

Arcobacter

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Glossary

Foodborne pathogens Microorganisms that are transmitted to humans via foods and cause diseases in humans.
Gastroenteritis Inflammation of stomach and intestinal tissues.
Identification Differentiation of bacteria at the species and/or subspecies level by characterizing by phenotypic and/or genotypic methods.
Isolation The growth of bacteria in/on a suitable growth medium to obtain the desired bacterial species.
Plating medium A solid growth medium solidified by addition of agar, that includes essential nutrients for bacterial growth.
Selective supplement Combinations of antimicrobial agents used to inhibit the unwanted bacteria in an isolation medium.

Background

The first isolation of an *Arcobacter* species was from bovine fetuses in the late 1970s. It was identified as an aerotolerant *Campylobacter* sp. (*Campylobacter cryaerophila*) because of its DNA base composition and general morphology, differing from other *Campylobacter* spp. because of its aerotolerance and its ability to grow at temperatures down to 15 °C. Similar bacteria were soon isolated from sources such as aborted bovine and porcine fetuses, cow's milk and bovine preputial sheath washings. *Campylobacter nitrofigilis* was isolated from a salt marsh, and appeared to be a nonpathogenic free-living species. After a comprehensive taxonomic study, the name *Arcobacter* was proposed in 1991 for this group, as a second genus in the family Campylobacteraceae. Consequently, the aerotolerant campylobacters became *Arcobacter cryaerophilus* and *Arcobacter nitrofigilis*, respectively. The genus *Arcobacter* later expanded to include two more members, *Arcobacter butzleri* and *Arcobacter skirrowii* isolated from various sources such as from human and animal diarrhea, aborted fetuses of animals, human blood, and from preputial fluids of bulls. Recently, another species, *Arcobacter cibarius*, was isolated from the skin of broiler carcasses and the same year, an obligately halophilic strain, called *Arcobacter halophilus*, was isolated from a hypersaline lagoon, indicating the ubiquitous distribution of *Arcobacter* spp. and a tendency to adapt to diverse environmental conditions. Three more species have been identified in the past few years: *Arcobacter mytili* has been isolated from mussels and brackish water; *Arcobacter thereius* from the liver and kidney of aborted pig fetuses and the cloaca of duck; and *Arcobacter marinus* from the marine environment. An unclassified organism, '*Candidatus* Arcobacter sulfidicus' producing filamentous sulfur has also been isolated from coastal seawater.

Characteristics of the Organisms

Like *Campylobacter* spp., *Arcobacter* spp. are Gram-negative, slightly curved, S-shaped, or helical rods 0.2–0.9 μm wide and 1–3 μm long. A characteristic darting or corkscrew-like motility is provided by a single polar, unsheathed flagellum. Arcobacters produce whitish or grayish smooth-rounded colonies varying in size, depending on the species. They produce oxidase and catalase and some strains are α-hemolytic. They can multiply at 15 and at 37 °C, but many are unable to multiply at 41.5 °C. Arcobacters can grow in aerobic conditions, but the best growth is observed under microaerobic conditions with 3–10% O_2, 5–10% CO_2, although *A. skirrowii* grows better when hydrogen is also present. They are unable to ferment carbohydrate but utilize organic acids and amino acids as carbon sources. Their most important distinctive features compared to *Campylobacter* spp. are their ability to grow in air at 30 °C and their growth at temperatures down to 15 °C.

Pathogenicity, Clinical Manifestations, and Virulence Factors

Three species of *Arcobacter*, *A. butzleri*, *A. cryaerophilus*, and *A. skirrowii*, are associated with various diseases in humans and animals. *Arcobacter butzleri* is the species most commonly reported as a cause of gastroenteritis and bacteremia in humans, with *A. cryaerophilus* and *A. skirowii* less frequent. Diarrhea is the most common symptom reported. They have been found particularly as a cause of diarrhea in malnourished children in developing countries. Although *Arcobacter* spp. and *Campylobacter jejuni* cause diarrhea with similar symptoms, *A. butzleri* infections are more frequently associated with a persistent,

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watery diarrhea, whereas *C. jejuni* more commonly causes bloody diarrhea. *Arcobacter butzleri* infections are also associated with abdominal pain with and without diarrhea, nausea and vomiting, or fever. However, some patients may remain asymptomatic. *Arcobacter skirrowii* has been implicated as a cause of chronic diarrhea.

Arcobacter butzleri, *A. cryaerophilus*, and *A. skirrowii* are associated with diseases in cattle, sheep, and pigs, such as enteritis, mastitis, and reproductive disorders, including abortions. *Arcobacter butzleri* is also associated with diarrhea in nonhuman primates, such as macaques.

Although arcobacters are associated with various diseases in humans and animals understanding of the mechanism(s) of pathogenicity of these microorganisms and their toxin production is still limited. Some strains have cytotoxins and cytolethal distending factors, some hemagglutinate human and animal erythrocytes, and some can adhere to cell lines and have invasive potential.

Epidemiology of *Arcobacter* spp.

The transmission of *Arcobacter* spp. to humans is considered to occur mainly through contaminated food and drinking water; close contact with pets; and perhaps through person-to-person-transmission. *Arcobacter* spp. have been isolated from the intestinal tract of healthy mammals including cattle, pigs, sheep, dogs, and horses, and also from pork, beef, lamb, poultry meat, and shellfish. Like campylobacters, arcobacters are more prevalent on poultry meat than red meat and thus, poultry carcasses may be a major reservoir of these bacteria. Four species of *Arcobacter*, *A. butzleri*, *A. cryaerophilus*, *A. cibarius*, and *A. skirrowii* have so far been recovered from poultry carcasses. Although arcobacters are common on chicken carcasses, in contrast to *Campylobacter* spp., they are rarely isolated from the intestinal contents of these birds probably because birds have a relatively high body temperature ($\geq 41\ ^{\circ}C$). However, *Arcobacter* spp. have been isolated from the cloacal swabs taken from various types of poultry, including chickens, turkeys, ducks, and geese. Eggs are not regarded as a source of arcobacters.

In contrast to the situation with campylobacters, contamination of poultry carcasses with arcobacters is considered to occur from the processing and slaughterhouse environment rather than the intestinal tract of the birds. Arcobacters are able to survive and multiply in the warm and wet environment of processing plants, and form biofilm on processing lines and surfaces. They have also been detected in piggery effluent, and effluent-irrigated soils, and in farm environments including surface water. Whether or not arcobacters have been detected in animals and from different farms could be affected by a number of factors, including the isolation/identification methods used and sample size. *Arcobacter* spp. are also found in various aqueous environments, including drinking water reservoirs, canal waters, river or surface water, ground water, and sewage. *Arcobacter butzleri* is sensitive to chlorine, so consumption of so nonchlorinated drinking water or contact with contaminated water sources may be a source of infection with these bacteria. *Arcobacter* infection may be more common in developing countries with poor water supplies.

Isolation, Identification, and Detection of *Arcobacter* spp.

Isolation

Arcobacter spp. were initially isolated from bovine fetuses using a semisolid *Leptospira* medium (Ellinghause–McClullough–Johnson–Harris) containing 5-fluorouracil. There is still no standard isolation method, but a number of isolation media and protocols have been devised in order to detect the three most common arcobacters (*A. butzleri*, *A. cryaerophilus*, and *A. skirrowii*) in various types of sample. These methods generally exploit the ability of arcobacters to grow at lower temperatures ($\leq 30\ ^{\circ}C$) and in aerobic conditions in order to avoid isolating camplyobacters. Prolonging incubation of plating media up to 7 days aids detection of less robust species such as *A. cryaerophilus* and *A. skirrowi*, for which inclusion of hydrogen in the incubation atmosphere may be helpful. Basal media originally developed for other bacterial pathogens such as cefsulodin–irgasan–novobiocin agar base, and modified campylobacter cefoperazone deoxycholate agar base have been used in combination with different selective agents, for example, cefoperazone, amphotericin B, and teicoplainin (CAT) and 5-fluorouracil with some success for the isolation of arcobacters. They can also be recovered from various types of samples by using different isolation strategies that depend on their high motility and small size. In this context, arcobacters can be recovered by dispensing a small volume of a suspension of the samples, either before or after enrichment, onto a 0.45 or 0.65 μm pore size membrane filter laid on a plate of selective and/or nonselective agar medium for about 30 min before removing the filter and spreading the liquid remaining evenly over the agar. This allows the highly motile arcobacters and campylobacters to penetrate though the filter, whereas leaving other bacteria behind, and also allows detection of strains of *Arcobacter* more sensitive than most to selective agents. For instance, Atabay and Corry in 1997 were able to recover three species of *Arcobacter*, *A. butzleri*, *A. cryaerophilus*, and *A. skirrowii*, the latter two for the first time, from chicken carcasses using blood agar and this isolation protocol after enrichment. At present, a commercially marketed arcobacter enrichment broth (Oxoid), which incorporates peptone, yeast extract, and sodium chloride and CAT supplement as selective agents, is available. A number of other media and protocols have been devised to recover arcobacters by using different combinations of antibacterial agents and/or incorporating indicators such as color change and swarming on a semisolid medium. For instance, Johnson and Murano in 1999 developed an enrichment and plating medium (JM) to isolate *A. butzleri*, *A. cryaerophilus*, and *A. nitrofigilis* from poultry. They used cefoperazone, thioglycollate, bile salts, and pyruvate in both media. In addition the liquid broth contained 5-fluorouracil., and activated charcoal in enrichment broth and sheep blood in plating agar were used as detoxifying agents. A deep red color around colonies, possibly due to the thioglycollate, was observed with JM plating medium. Houf *et al.* in 2001 described a new liquid and a similar solid medium, both incorporating amphotericin B, cefoperazone, 5-fluorouracil, novobiocin, and trimethoprim as selective agents, with Oxoid arcobacter enrichment broth as basal medium. Their media were less effective for isolating *A. skirrowii* than *A. butzleri* and *A. cryaerophilus* from poultry

samples. The review articles written by Philips in 2001 and Corry *et al.* in 2003 provide more detailed information regarding the isolation media and methods used for arcobacters.

Identification

Arcobacters are not easy to culture, and they are unable to ferment or utilize carbohydrates, so that only a few biochemical tests are available for characterization and speciation, so identification of *Arcobacter* species using phenotypic tests is difficult. In addition, their close resemblance to campylobacters can lead to misidentification in routine laboratories. Catalase activity, indoxyl acetate hydrolysis, cadmium chloride susceptibility, nitrate reduction, α-hemolysis, growth on MacConkey agar and in the presence of 3.5% NaCl and 1% glycine are the most useful tests to differentiate *Arcobacter* spp. phenotypically. Whole-cell protein profiling using sodium dodecyl sulfate polyacryamide gel electrophoresis or whole-cell fatty acid profiling has also been used successfully. In addition, there is a phenotypic identification scheme based on the probabilistic identification of campylobacters and arcobacters, but these are difficult to perform for a routine laboratory and aberrant results can be encountered. Therefore, several nucleic acid-based methods such as multiplex-polymerase chain reaction (PCR), and real-time PCR, 16S rDNA sequencing and microarray techniques can be utilized accurately and definitively for the differentiation of *Arcobacter* to the species level. The primers and oligonucleotide probes used for the identification and detection of arcobacters are mainly based on a part of the 16S rRNA and/or 23S rRNA genes. A species-specific multiplex-PCR assay is widely used, which involves five primers targeting for simultaneous detection and identification of *A. butzleri*, *A. cryaerophilus*, and *A. skirrowii*. Because this m-PCR enables differentiation of only three species of arcobacters, a new 16S rDNA-restriction fragment length polymorphism method has been developed to differentiate the six species of *Arcobacter* of interest in human and/or animal health.

Molecular Detection

Although cultural methods are a well-established way of isolating pure cultures of living bacteria, the methods available for *Arcobacter* spp. are slow and labor-intensive, and not as sensitive as those used, for example, for *Salmonella* spp. Therefore, PCR-based detection–identification of *Arcobacter* spp. is often used after enrichment culture, instead of or as well as selective plating. The current PCR protocols generally target 16S or 23S rDNA sequences.

Two TaqMan-based real-time PCR methods have recently been developed using primer-probe sets developed for the *rpoB/C* and 23S rDNA sequences of *A. butzleri* and *A. cryaerophilus*, respectively. These have been used for detecting arcobacters from environmental samples, without the need for prior enrichment, with a detection sensitivity of 5–7 CFU per reaction. Use of several different real-time PCR assays with different genomic DNA targets will enable the development of better detection–identification schemes for a wider range of *Arcobacter* spp.

Control/Preventive Measures for *Arcobacter* spp.

As previously mentioned, *Arcobacter* spp. are similar to *Campylobacter* spp. except that (like *Salmonella*) they are able to multiply at lower temperatures and in air, and thus might multiply in domestic and catering kitchens, or after cross-contamination onto ready-to-eat foods. Campylobacters sometimes cause outbreaks of infection from contaminated water or milk, and a significant proportion of human cases are linked to contaminated raw poultry. Although arcobacters are common in untreated water and on raw poultry meat, human infection from these sources does not seem to be very common and the authors have very little information concerning any other sources of human infection with *Arcobacter* spp. Consumers probably do not need to be educated specifically with respect to the risks of infection with arcobacters, as they are a relatively rare infection compared to *Campylobacter* and *Salmonella*, and precautions to avoid these two more common infections should be effective against arcobacters.

In general, *Arcobacter* spp. are susceptible to various treatments including heating and food preservatives such as nisin, and lactic and citric acids, although they are somewhat more resistant than *Campylobacter* spp. to freezing, desiccation, irradiation, and disinfectants. Therefore, they do not seem likely either to survive well, or multiply in chilled processed foods. However, some field isolates of *Arcobacter* are resistant to antibiotics commonly used for the treatment of bacterial diseases in humans and animals (e.g., chloramphenicol, vancomycin, methicillin, sulfamethoxazole-trimethoprim, erythromycin, ciprofloxacin, and ampicillin) so *Arcobacter* infections might be difficult to treat.

Bacteria in biofilms or attached to surfaces are well known to be more difficult to inactivate using disinfection or heat than planktonic bacteria. The ability of *Arcobacter* spp. to colonize food processing environments indicates that they are more likely than campylobacters to contaminate foods from this source. For this reason, and their tendency to be resistant to antibiotics, they should not be ignored as possible emerging pathogens.

Research Needs

Reliable, standardized, and optimal methods are required to recover all species of *Arcobacter* including sublethally damaged strains from food, environmental, and clinical samples in addition to faster and more reliable detection methods. Currently few countries monitor human clinical samples for arcobacters, and their significance as human pathogens is not known. More information is needed concerning their antimicrobial resistance mechanisms. Studies concerning their mechanisms of pathogenicity are also needed. In addition, the ecology and epidemiology of *Arcobacter* spp. needs to be elucidated in more detail.

See also: Foodborne Diseases: Overview of Biological Hazards and Foodborne Diseases. Safety of Food and Beverages: Poultry and Eggs

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BACTERIA

Bacillus anthracis

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Glossary

Ascites Excess fluid in the space between the tissues lining the abdomen and abdominal organs (the peritoneal cavity).
Cyanosis Blue or purple coloration of the skin or mucous membranes due to the tissues near the skin surface being low on oxygen.
Dysphagia Difficulty in swallowing.
Dyspnea Difficulty in breathing caused by interactions among multiple physiological, psychological, social, and environmental factors, and may induce secondary physiological and behavioral responses.
Edema A condition of abnormally large fluid volume in the circulatory system or in tissues between the body's cells (interstitial spaces).
Hematemesis Vomiting of blood.
Obligate pathogen An organism that must cause disease in order to be transmitted from one host to another.
Stridor Abnormal, high-pitched, musical breathing sound caused by a blockage in the throat or voice box (larynx).

Background

The bacterium *Bacillus anthracis* is the causative agent of the rapidly lethal zoonotic disease anthrax. Written accounts of anthrax as a scourge of man and livestock date back to the first and second millennium BC. Because of its agricultural impact, *B. anthracis* was the subject of seminal nineteenth-century microbiological studies, enabling Koch to establish his postulates in 1877 by proving *B. anthracis* (named by Cohn in 1875) was the cause of anthrax. Subsequent researchers, such as Greenfield and Pasteur, in the early 1880s demonstrated the feasibility of using attenuated live vaccines to protect livestock, establishing history's second bacterial vaccine. At the turn of twentieth century, Metchnikoff employed *B. anthracis* to characterize the ability of macrophages to kill microbes and helped establish the field of immunology.

Characteristics of Organism

Bacillus anthracis is the only obligate pathogen within the genus *Bacillus*, which is comprised of gram-positive, aerobic or facultatively anaerobic, spore-forming, and rod-shaped bacteria. The ability to form spores accounts for its reported longevity and resistance to physical and chemical disinfectants (Figure 1). Fortunately, spores are susceptible to agents, such as formaldehyde, which have historically been used to decontaminate animal hides.

To initiate infection, spores must undergo germination, a process where they transform from an inactive spore into a biologically active bacterium. The factors that trigger germination are not well characterized, but are influenced by temperature, pH, moisture, and the presence of oxygen and carbon dioxide. *In vitro* studies have demonstrated that *B. anthracis* spores will germinate at 8–45 °C, pH 5–9, relative humidity >95%, and with adequate nutrition. Optimum germination conditions for the Vollum strain of *B. anthracis* have been shown to be 22 °C in the presence of the amino acid L-alanine. Although spores germinate poorly in serum, the process is considerably more efficient within professional phagocytic cells, such as alveolar macrophages. Indeed, studies suggest that exposure to antibacterial free radicals generated within the phagolysosome triggers germination.

The *Bacillus* genus includes the genetically closely related '*Bacillus cereus* group' and comprises *B. cereus*, *B. anthracis*, *Bacillus thuringiensis*, *Bacillus mycoides*, *Bacillus pseudomycoides*, and *Bacillus weihenstephanensis*. *B. anthracis* can be clearly distinguished from the other members at the genetic level by using a range of deoxyribonucleic acid (DNA)-based approaches, including multilocus sequence typing. Although these techniques enable rapid assessment of relationships between large numbers of isolates, it is now economically feasible to determine the complete genetic sequence of a bacterium. Analysis of the genome of *B. anthracis Ames* revealed a 5.23-megabase chromosome with considerable sequence homology to *B. cereus* and *B. thuringiensis*, suggesting a common insect pathogen ancestor, which acquired additional plasmid-borne virulence factors.

The phenotypic diversity within the *B. cereus* group is often mediated by plasmid-encoded factors; and in the case of *B. anthracis*, these consist of two plasmids, pXO1 (182 kb) and pXO2 (96 kb), both encoding major virulence factors. The ability of these virulence plasmids to be transferred to other members of the *B. cereus* group has been reported. An example of this is *B. cereus* G9241, which was isolated from individuals presenting with an infection clinically indistinguishable from inhalational anthrax. Genetic analysis revealed the presence of a

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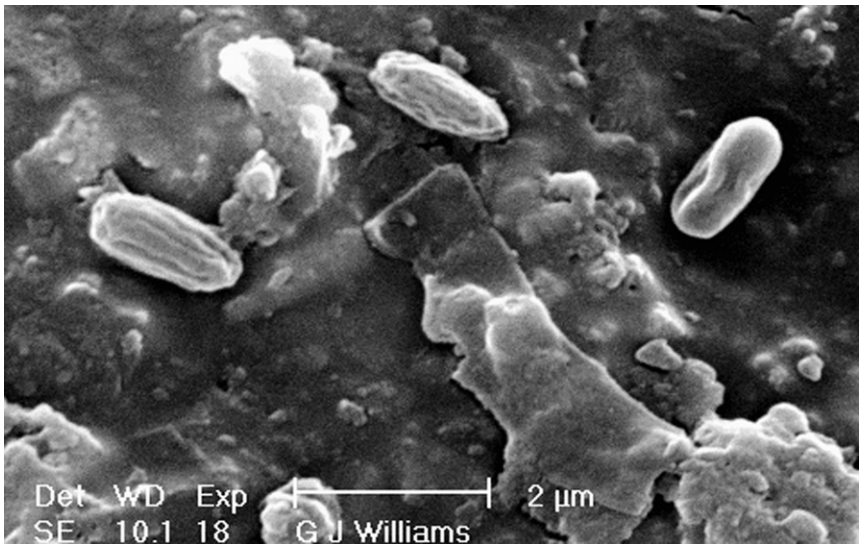

Figure 1 Scanning electron microscopy image of *B. anthracis* spores on a metal surface.

homolog to pXO1 and a second plasmid, which although genetically distinct from pXO2, encoded a phenotypically similar antiphagocytic capsule. Given the central role of virulence plasmids in pathogenicity and their ability to move between closely related strains, we should take care in dismissing all clinical isolates of *B. cereus* as environmental contaminants.

Clinical Manifestations

Although primarily a disease of herbivores, particularly human food animals, the organism can infect humans, frequently with fatal consequences if left untreated. In herbivores, the disease usually follows a hyperacute course in which the signs of illness are absent until shortly before death. At death, the blood of the animal exudes from the nose, mouth, or anus and contains $>10^8$ bacilli per milliliter. It is thought that the release of bacteria from infected animals in the form of resistant spores plays an important role in the infective cycle; the spores contaminate the soil, healthy animals that graze on contaminated land are exposed to the spores and subsequently develop an infection, which leads to the death of the animal and the release of more bacteria.

The institution of mass animal vaccination programs and the maintenance of stringent veterinary control measures have drastically reduced the incidence of the disease in the first-world countries. In other parts of the world, where vaccination is not routinely available, the organism remains a significant cause of animal mortality and human disease.

The sudden death of a food animal in UK would be routinely investigated by veterinary authorities, and, if due to anthrax, the animal and its products would be destroyed. In countries with less well-developed public health systems, the meat of an infected animal may be considered too valuable to 'waste' and subsequently the flesh is likely to be consumed or sold. In Zambia, custom dictates that an animal that dies from unknown causes cannot be disposed of, but must be butchered and shared among relatives and friends to be eaten. Efforts to advise local communities on the dangers of such behavior encounter resistance due to the economic loss caused by burying or burning.

Although most human cases arise as a consequence of contact with contaminated animal products, it should be noted that infections can also occur as a result of illicit activity (i.e., contaminated heroin) and intentional exposure such as the 2001 US anthrax bioterrorist mail attacks. Although both events are extremely rare, the potential use of *B. anthracis* as a biological weapon is a cause for considerable concern.

The disease presents in four forms in humans: cutaneous, injectional, inhalational, and gastrointestinal with meningitis developing as a possible complication. The injected, gastrointestinal tract and inhalational forms are regarded as being most frequently fatal as they can go unrecognized until it is too late to instigate effective treatment.

Of all the forms, cutaneous infection accounts for most human cases ($>95\%$). It is generally believed that *B. anthracis* is noninvasive and thus requires a break in the skin to gain access to the body. Workers who carry contaminated hides or carcasses on their shoulders are liable to infection on the back of their necks and handlers of other food materials or products tend to be infected on the hands, arms, or wrists.

Entry of infecting spores occurs via a lesion in the skin resulting in the formation of a small pimple within 3–5 days, the center of which ulcerates to form a dry, black, firmly adherent scab, surrounded by a ring of vesicles, the typical anthrax eschar. Despite its deadly appearance, there is little pain; pain and pus develop only if there is a secondary infection. Lesions vary greatly in size from approximately 2 cm to several centimeters across and are accompanied by pronounced edema, which can become life threatening if located on the face or neck. In uncomplicated cases, the eschar begins to resolve in approximately 10 days after the appearance of the initial papule; resolution takes 2–6 weeks, regardless of treatment, leaving little trace. Complications arise when the organism spreads to the bloodstream, resulting in an overwhelming infection in approximately 20% of untreated cutaneous cases.

A new clinical form of anthrax (injectional) has been reported in drug users with cases occurring in Norway and UK

due to the injection of contaminated heroin. This novel route of infection presents considerable diagnostic challenges as it results in a clinical picture that differs markedly from that encountered during more traditional infections. In 2009, an outbreak of anthrax occurred amongst heroin users in Scotland, which resulted in 31 cases. The route of transmission in most of these cases was assumed to be through the use of contaminated needles, although smoking and snorting the drug may also have acted as a route of transmission.

The early stages of infection are characterized by a severe, painless, soft tissue focus at the site of injection, which leads to abscess formation. Examination of surgically removed material reveals the presence of capillary bleeding, superficial necrosis, extensive edema, but not fasciolysis and necrotic material along the length of the needle track. Systemic symptoms include sepsis, organ dysfunctions, and shock ultimately leading to death.

In contrast, inhalation anthrax is caused by aerosolized spores produced either during processing of contaminated animal products, such as hides, wool, and hair, or as a consequence of a bioterrorist attack. The illness has an incubation period of 1–6 days, during which nonspecific symptoms of fever, sweats, fatigue, dyspnea, nonproductive cough, and nausea can occur. These symptoms persist for 2 or 3 days; and in some cases, there is a short period of clinical improvement. This is followed by the sudden onset of increasing respiratory distress with dyspnea, stridor, cyanosis, increased chest pain, and sweating. Respiratory distress is typically followed by rapid onset of shock and death within 24–36 h. The recent bioterrorist attack in USA in 2001 resulted in 22 cases of laboratory-confirmed anthrax, half of which were inhalational and resulted in 5 fatalities.

Gastrointestinal anthrax is extremely rare in industrialized countries and occurs mainly in Africa, the Middle East and Central and South Asia. Where the disease is infrequent or rare in livestock, it is hardly seen in humans. Most cases of intestinal anthrax result from eating insufficiently cooked meat from anthrax-infected animals.

Historically, it is estimated that there is one cutaneous case for every 10 infected carcasses butchered and one enteric outbreak for every 30–60 animals eaten. Owing to the rareness of the conditions, there are no figures for the number of organisms that need to be ingested to cause disease. Although usually fatal, there are serological and epidemiological data to suggest that low-grade infection may occur.

There are two clinical forms of gastrointestinal anthrax, which may occur following ingestion of contaminated food or drink:

- Intestinal anthrax: The symptoms include nausea, vomiting, fever, abdominal pain, hematemesis, bloody diarrhea, and massive ascites. Toxemia and shock develop and results in death.
- Oropharyngeal anthrax: The main clinical features are sore throat, dysphagia, fever, regional lymphadenopathy in the neck, and toxemia. Even with treatment, the mortality is approximately 50%.

It is extremely important that effective treatment is started early as the prognosis is often death. Suspicion of the case being anthrax depends greatly on awareness and alertness on the part of the physician to the patient's history and the likelihood that he/she had consumed contaminated food and drink.

Pathogenesis and Virulence Factors

The organism adopts a range of strategies to evade and subvert the immune defenses of the infected individual. The pathogen gains entry to the host as a spore, either as the consequence of some form of trauma or by ingestion or inhalation. Once internalized, the spore is taken up by phagocytic cells, such as macrophages, and germination is triggered by a yet-to-be-determined process. Subsequent growth and replication within the macrophage serves two functions; it neutralizes a key component of the innate immune system and provides a safe environment for the bacteria to increase in numbers. The infected macrophage ultimately lyses to release biologically active bacteria, which go on to establish extracellular infection. The organism is aided in this process by two major virulence factors, a tripartite AB toxin and an antiphagocytic capsule. The toxin encoded on plasmid pXO1 accounts for most of the organism's pathology, whereas the antiphagocytic acid capsule encoded by pXO2 aids the replicating organism in evading uptake by immune effector cells. The loss of either of these plasmids leads to significant attenuation.

Plasmid pXO1 encodes the genes for calmodulin-sensitive adenyl cyclase (*cya*), lethal factor (*lef*), and protective antigen (*pagA*) and the combined products of these genes result in the production of anthrax toxin (ATx). ATx is comprised of three nontoxic proteins; lethal factor (LF, 90 kDa), edema factor (EF, 89 kDa), and protective antigen (PA, 83 kDa), which combine on eukaryotic host cell surfaces to make noncovalent, toxic complexes. ATx follows the AB model with the A moiety composed of two alternative catalytic subunits LF and EF, whereas the B moiety, PA, translocates EF or LF into the cytosol. A schematic representation of ATx endocytosis and cytoplasmic delivery is shown in **Figure 2**.

PA is so named due to its role as the key protective immunogen in the current human vaccines. PA binds to one of at least two identified ubiquitous cell surface receptors; ATx receptor I/tumor endothelial marker 8 (ANTXR1/TEM8) or ATx receptor 2/capillary morphogenesis protein 2 (ANTXR2/CMG2), and to a coreceptor identified as the low-density lipoprotein receptor-related protein 6. On binding, PA is cleaved into two fragments by a cell surface furin-like protease, producing a 20 kDa peptide (PA_{20}) cleaved from the *N*-terminus and the 63 kDa A moiety binding site (PA_{63}). Spontaneous generation of the membrane-inserting ring-shaped heptameric $(PA_{63})_7$ or octameric $(PA_{63})_8$ prepore complex creates binding sites for up to three (heptamer) or four (octamer) molecules of LF and/or EF. Once the prepore has bound LF or EF, creating anthrax lethal toxin (LeTx) or anthrax edema toxin (EdTx), respectively, the complex is endocytosed. The acidic pH of the early endosome triggers major conformational changes, resulting in the formation of the transmembrane structure of the pore. The complex is transported through the early endosome in intraluminal vesicles and delivered to the late endosome. At the low pH of the late endosome ($\sim$pH 5–6), the intraluminal vesicles undergo back

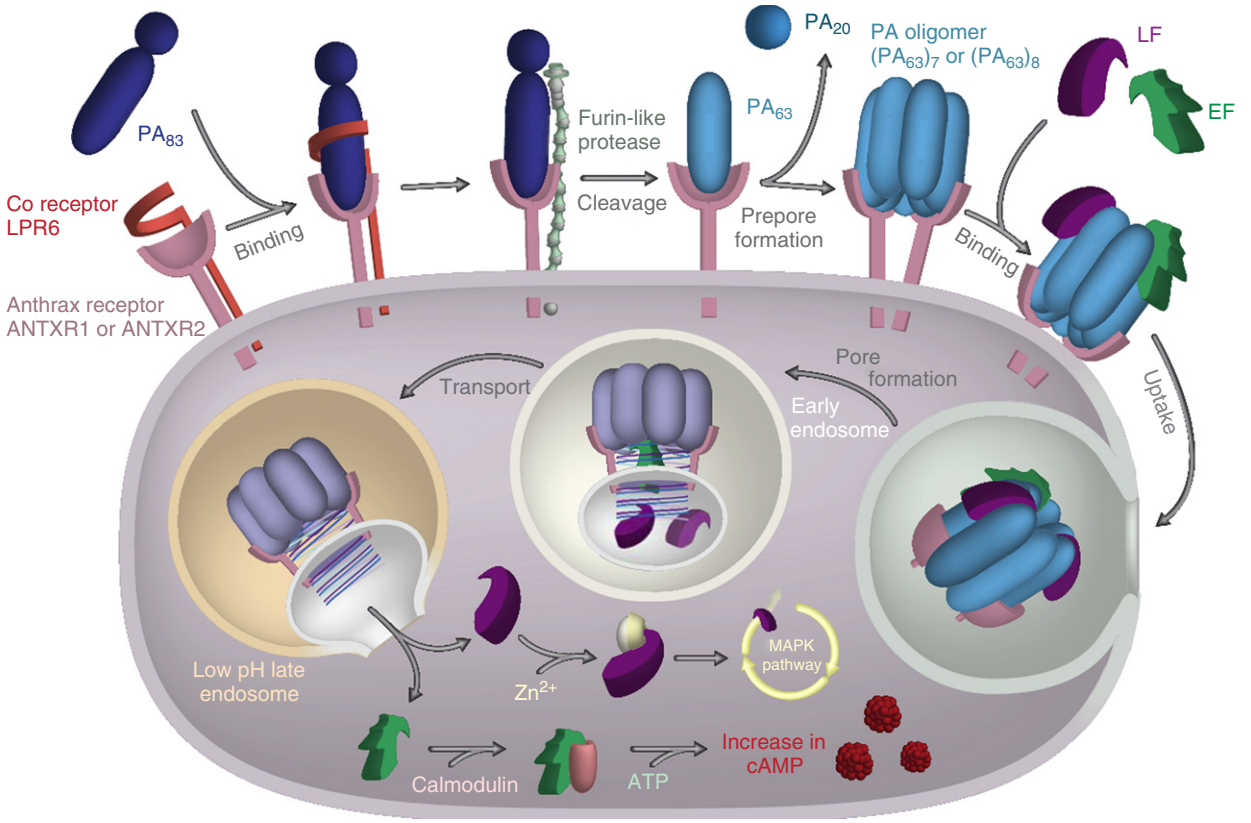

Figure 2 A schematic representation of ATx endocytosis and cytoplasmic delivery.

fusion with the endosomal membrane resulting in translocation of LF and EF, in an unfolded state, through the pore into the cell cytosol, where they catalyze reactions that disrupt the host cell.

LeTx, the combination of LF and PA, is considered to be the central effector of death. LF contains a thermolysin-like active site and zinc-binding consensus motif HExxH, which acts as a Zn^{2+} metalloprotease, specifically cleaving most isoforms of mitogen-activated protein kinase (MAPK) kinases (MEKs). MEKs are functional components of three-component phosphorylation cascades and these signals lead to the upstream activation of MAPKs. The MAPK cascade is essential for full induction of the oxidative burst and proinflammatory cytokine expression, and its disruption by LeTx neutralizes macrophage activation favoring bacterial escape from lymph nodes during the initial phase of infection.

The combination of EF and PA results in EdTx causing edema through elevation of cellular cyclic adenosine monophosphate (cAMP) concentrations in affected tissues. EF is an inactive adenyl cyclase when transported into the target cell by PA. Once located in the cytosol, the binding of calmodulin (a eukaryotic calcium-binding protein) induces conformational transition, which activates the EF adenyl cyclase catalytic site. The activity of EF leads to overproduction of cAMP from adenosine triphosphate (ATP), which in turn leads to various disruptions of cytokine secretion and an increase in ANTXR expression that culminates in an increased rate of toxin internalization. The resulting effects of EdTx are the same as those caused by cholera toxin with the affected cells secreting large amounts of fluid.

The toxins are employed as a means to impair host defense and immunity in the establishment of infection. Almost all cells of the immune system utilize MAPK pathways for proper function and phagocytic cells of the innate immune system, macrophages and neutrophils, are the first line of defense against microbial infection before the induction of the adaptive immune response. LeTx is able to induce the proinflammatory programmed cell death pathway in macrophages and dendritic cells, block neutrophil chemotaxis, and inhibit superoxide and nitric oxide production, thus suppressing the immune response and crippling host defenses. Death resulting from administration of LeTx in murine studies is associated with shock, vascular collapse, and generalized hypoxia, exhibiting some of the symptoms and pathology seen in human inhalational anthrax patients.

Like LeTx, a major role for EdTx appears to be disabling of host defense by impairing the function of phagocytic cells. However, in contrast to LeTx, the overall contribution to the pathological process is ill defined. Increased intracellular cAMP induced by EF or other means inhibits neutrophil chemotaxis, phagocytosis, superoxide production, and microbiocidal activity. Interestingly, intravenous administration of EdTx in murine studies have demonstrated that death occurs at lower doses and with a more rapid onset that with LeTx. EdTx causes widespread tissue damage and multiorgan failure accompanied by hemorrhage and hypotension, features that are also observed in

human patients. These observations support the idea that the two toxins synergistically impair cellular function.

Although toxins play a major role in the virulence of *B. anthracis,* to be fully pathogenic, the infecting strain must also produce a capsule. The structure is composed of a polypeptide (γ-polyglutamic acid (γ-PGA)), which inhibits phagocytosis and opsonization of the bacilli by virtue of its negative charge. The genes controlling capsule synthesis, *capA*, *capB*, and *capC* are organized in an operon (*capBCADE*) located on the plasmid, pXO2. In a murine model of pulmonary anthrax, encapsulation of the bacilli was essential for dissemination from the lungs and for persistence and survival within the host. Virulence appears to be associated with the antiphagocytic properties of the capsule, and degradation of the capsule by γ-PGA depolymerase enhances both *in vitro* macrophage phagocytosis and neutrophil killing. Capsule expression is, in part, subject to regulation by serum, temperature, CO_2, and bicarbonate via an, as yet, unclear mechanism involving the *atxA* and *acpA* genes. These regulators also control the level of expression of the ATx genes.

Bacillus anthracis also expresses a number of other plasmid and chromosome encoded factors that contribute to the overall pathogenesis of the organism. Differential expression of any or all of these 'minor factors' could account for the difference in virulence between wild-type strains. Candidate virulence factors include chromosomally encoded extracellular proteases; phospholipases, such as cereolysin; and S-layer proteins. Interestingly PlcR, a global transcription regulator of a virulence regulon thought to play a major role in insect virulence, is defective in *B. anthracis*, possibly due to the acquisition of pXO1.

Epidemiology

Bacillus anthracis is an organism that is widely distributed around the globe and the mechanism of its continued existence in the ecosystem has been a topic of debate for decades. Currently, it is considered to be an obligate pathogen whose environmental presence is dependent on the continued infection of susceptible animals. During infection episodes, the spore form of the organism is able to persist in the soil in a dormant state for many years.

Although cases of the disease are rare in western countries, relatively high incidence of the disease occurs in sub-Saharan Africa, the Indian subcontinent and Indonesia, certain provinces of China, parts of Turkey, and various countries of the former USSR. The disease was largely eradicated from the western world by the implementation of mass animal vaccination programs, maintenance of stringent veterinary control measures, and an increase in the use of man-made alternatives to animal products. Nevertheless, specialized leather and woolen industries continue to depend on hides and wool from particular species or breeds raised in countries where anthrax is still endemic.

Anthrax epidemics occurring in livestock herds are of the point source type with animals acquiring infection as a consequence of grazing on spore-contaminated soil. Animal-to-animal transmission appears to be a rare event and when it does occur, it is usually a result of contact with contaminated bodily fluid from an infected carcass or dispersion mediated by necrophilic blow flies or hemophagic biting flies. Outbreaks have been linked to environmental changes, particularly flooding, which may result in the redistribution and concentration of anthrax spores in particular areas.

Analytical Methods

Given the scarcity of anthrax in the developed world, it is unlikely that many routine diagnostic laboratories would have the experience, or access to the materials required, to identify the organism correctly. The main problem is the differentiation of *B. anthracis* from the phenotypically similar *B. cereus* or *B. thuringiensis* group, which may also be present in many of the samples examined for anthrax.

Direct detection of the organism in the field is relatively simple in animals that have died suddenly of the disease. At death, the blood of an animal generally contains $>10^8$ bacilli per milliliter. Blood films are dried, fixed immediately by heat or immersion for 1 min in absolute alcohol, and stained with polychrome methylene blue, which after 20 s is washed off. When the slide is dry, it is examined for characteristic deep blue, square-ended bacilli surrounded by a well-demarcated pink capsule (McFadyean's reaction). It should be noted that in some animal species, such as pigs, the terminal bacteremia is limited and the bacilli are unlikely to be seen in McFadyean-stained blood smears.

If available, antibody and DNA-based assays can also be used; these target unique signatures, such as major virulence factors (toxins and capsule), and are considerably more sensitive than staining. Indeed, although a plethora of rapid assays have been developed, which claim to be able to directly detect and confirm the presence of *B. anthracis* in clinical and environmental samples, there has as yet been no independent validation of the veracity of these claims.

The antigen-based methods, which have been developed, focus mainly on detecting the presence of PA. One such immunochromatographic assay based on a specific monoclonal capture antibody can detect as little as 25 ng ml^{-1} of PA and can be performed in a few minutes without the need for special reagents. It has thus been proposed that this test could be used in addition to staining to screen animal blood and tissue and confirm the presence, or absence, of the organism. DNA-based detection based on processes, such as the polymerase chain reaction (PCR) methodologies, has also been used successfully to detect the presence of *B. anthracis* in animal-derived and environmental samples.

The somewhat surprising experience of the 2001 anthrax postal attacks was that traditional culture, although relatively time consuming compared with rapid PCR or antibody-based assays, was more sensitive, required less technical training, and was able to detect viable organisms unlike the other assays.

Indeed, unless there is an index of suspicion, it is unlikely that animal products would be routinely cultured for the presence of *B. anthracis.* In cases where contamination with *B. anthracis* is suspected, the isolation protocol shown in **Figure 3** is conducted. The sensitivity limit of this technique is approximately 5 spores per gram of starting material. It should be borne in mind that the number of bacteria isolated very

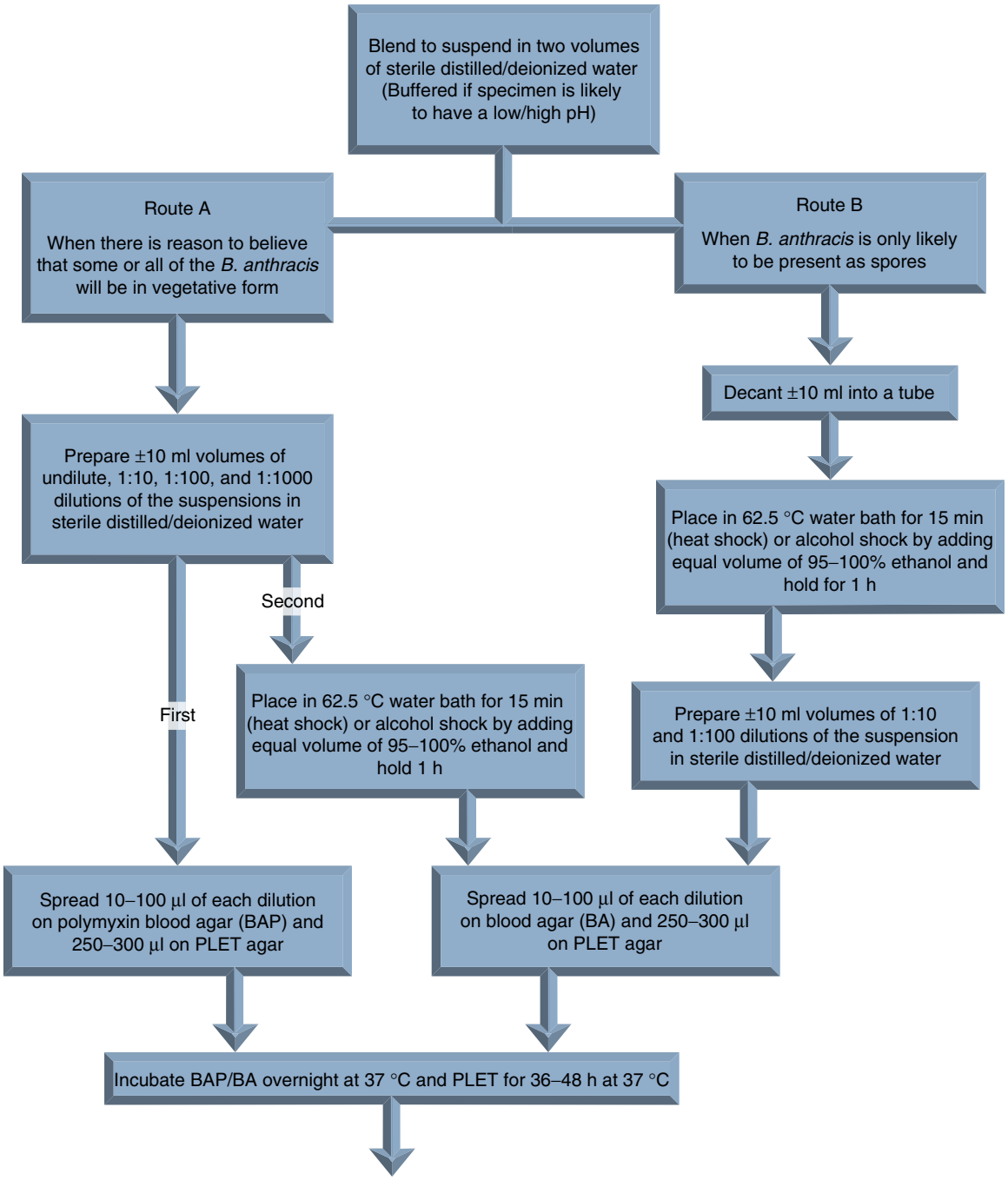

Figure 3 WHO protocol for the isolation of anthrax.

much depends on the distribution of the organism within the sample.

The polymyxin B–lysozyme–ethylenediaminetetraacetic acid (EDTA)–thallous acetate agar (PLET agar) described in the method is a semiselective medium for *B. anthracis*, which contains polymyxin (30 000 units l^{-1}), lysozyme (300 000 units l^{-1}), EDTA (0.3 g l^{-1}), and thallous acetate (0.04 g l^{-1}). The bacterium grows well on a variety of culture media and produces characteristic colonies when grown on sheep blood agar, which appear rough, gray, and nonhemolytic when incubated aerobically and often show a classic 'medusa edge' (Figure 4). When incubated in the presence of 5% CO_2, fully virulent isolates of *B. anthracis* produce mucoid, wet-looking colonies as a consequence of capsule formation. Thus, culture from clinical samples is relatively simple given the ability of the organism to grow on routine laboratory media and the likely absence of other members of the closely related *B. cereus* group, which are ubiquitous in the environment and can be misidentified as *B. anthracis* based on these morphological criteria.

Once colonies have been isolated, further testing is required to confirm their identity. Many saprobic species of

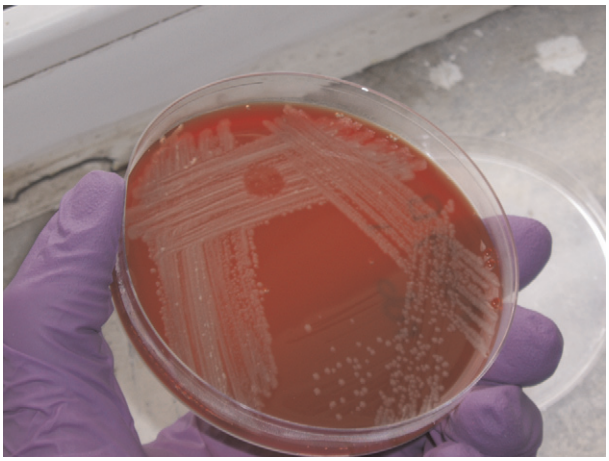

Figure 4 An environmental isolate of *B. anthracis* cultured on a blood agar plate. The zone of inhibition is due to lysis by diagnostic γ-phage.

Table 1 Detection and identification methods for *B. anthracis*

Direct tests
Microscopy (McFadyean's stain)
Antigen detection
PCR
Preliminary tests
Lack of motility
Lack of hemolysis
Sensitivity to diagnostic γ-phage
Sensitivity to penicillin
Commercial biochemical kits: API 50CHB, Biolog
Confirmatory tests (specialist labs)
Virulence in animals – guinea pig
Capsule formation – McFadyean's stain
Toxin detection – immunoassays
Virulence gene detection – PCR

aerobic spore-forming bacilli are hard to distinguish from *B. anthracis* except on the basis of pathogenicity. The most commonly encountered are *B. cereus*, or *B. thuringiensis*, or *B. mycoides*; *Bacillus subtilis*; and *Bacillus licheniformis*.

The preliminary tests shown in Table 1 allow the presumptive identification of an isolate as *B. anthracis*. Commercially available biochemical screening systems such as API 50CHB (bioMerieux, France) and Biolog (Biolog Inc., Hayward, CA, USA) have been developed, which provide a simple, first-line, one-step screen test for the presumptive identification of *B. anthracis*.

The tests described above are called presumptive tests as other strains of bacilli can give similar reactions to *B. anthracis*. The demonstration of virulence constitutes the principle point of difference between typical stains of *B. anthracis* and those of other anthrax-like organisms. Traditionally, the guinea pig has been the model used to demonstrate virulence. The animal is injected with the sample, and if it dies, the cause of death is confirmed by isolation of *B. anthracis* from blood. Although this traditional technique is sensitive, it is likely to be replaced by more sensitive *in vitro* tests.

Virulent isolates of *B. anthracis* produce both a capsule and exotoxins. Detection of capsule formation is relatively simple. Capsule-forming organisms when grown on medium containing bicarbonate and in the presence of CO_2 produce colonies that are raised and mucoid in appearance, whereas noncapsule forming organisms produce flat, dull colonies. In addition, the presence of the capsule can be confirmed by McFadyean stain.

Detection of active toxin production is not as straightforward and requires either an animal system, a tissue culture assay using toxin-sensitive cell lines, or an immunological technique, such as an enzyme-linked immunosorbent assay. An alternative approach is to employ PCR to detect the presence of the genes encoding these virulence factors.

Control and Prevention

Most countries have regulations concerning the handling and disposal of infected food animals and their products. The World Health Organization has produced detailed comprehensive and practical guidelines on anthrax detailing the best practices on all aspects of the disease. In many areas where anthrax is endemic, particularly Africa, the problem is not the lack of regulations but rather the will and the means to enforce them in the face of local customs.

In essence, the control of anthrax requires the well-supervised disposal of infected animals carcasses, the application of biocides to reduce spore numbers below an infectious dose, immediate vaccination, and/or prophylactic treatment of other members of the affected herd or at-risk individuals. Although the recommendations in most countries is that anthrax carcasses should be buried or burnt, the legacy of contaminated land from past burials capable of infecting animals many years later shows that incineration is the only truly satisfactory option. Mobile incinerators are available but complete destruction of a bovine carcass can take more than 24 h and is relatively costly. Some countries prefer rendering, although the problem of limiting environmental contamination of land and equipment during transportation to the rendering plant has to be addressed.

The decontamination of anthrax spore-contaminated soil is a vital step in breaking the infection cycle as direct animal-to-animal transmission is extremely rare. To date, decontamination modalities for anthrax spores have centered on the application of toxic biocides (formaldehyde; chlorine releasing agents, such chlorine dioxide; and hydrogen peroxide), or γ-radiation. Although effective, these approaches suffer from the fact that they damage the environment and are extremely expensive to deploy when treating large areas. For this reason, mass livestock vaccination is usually instigated once cases of anthrax have been identified. Although effective, the duration of protection is thought to be approximately a year, thus necessitating the need for repeated immunization. Consideration is being given to the development of suitable oral vaccines for this purpose, although numerous obstacles must be overcome before satisfying concerns over safety, environmental contamination, and efficacy.

Importance to the Food Industry

The number of reported cases of foodborne illness involving *B. anthracis* is extremely small compared with other traditional

foodborne pathogens. To date, there has never been a documented case in USA. In countries with well-developed veterinary and public health systems, infected animals will be identified and removed from the food chain. In countries where such systems are not in place, there is potential for contaminated animals and their products to be processed and consumed. A survey of animals in a slaughterhouse in eastern Nigeria revealed that 5% of cattle and 3.3% of sheep tested positive for anthrax. These infected animals not only pose a risk to the people consuming the meat, but are also an occupational risk to workers exposed to the carcasses. In the same survey, it was found that 13% of butchers/skinners had acquired cutaneous anthrax, whereas a retrospective survey of human anthrax cases in Eastern Turkey between 1990 and 2007 found that 95.2% of 426 infections had a history of contact with animal products. Indeed, slaughterhouse waste in the form of offal for animal feed, and slurry discharged into the environment, represents a further source of potential infection. A study of uncut anthrax-contaminated slaughterhouse waste showed that viable anthrax could still be recovered after the offal had been heat treated for 30 min at 130 °C. The use of bone charcoal by the food industry in the production of sugar products presents an avenue for anthrax contamination. The bones are normally obtained from areas of the world in which anthrax is endemic and on occasions *B. anthracis* has been isolated. For this reason, the bones must be sterilized, usually by γ-irradiation, before use.

Owing to the scarcity of the disease, there are few published records of human infection. As one would expect, the cases that have been reported originate mainly from Africa, the Middle East, and Central and South Asia. Figures for human anthrax in China showed that of 593 recorded cases, 384 were linked to the dismembering and processing of infected animals and 192 cases were due to the consumption of contaminated meat. In neighboring Korea, sporadic outbreaks of human anthrax have been reported. From 1992 to 1995, three outbreaks occurred, a total of 43 cases, all linked to the consumption of contaminated beef or bovine brain and liver. An outbreak in India was centered on an infected sheep; of the five individuals who skinned and cut up its meat for human consumption, four developed fatal anthrax meningitis. Another person who wrapped the meat in a cloth and carried it home on his head developed a malignant pustule on his forehead and went on to develop meningitis. It is noteworthy that a large number of people who cooked or ate the cooked meat of the dead sheep remained well.

Research Needs

Anthrax is primarily a disease of food animals in economically disadvantaged regions of the world. History has shown that the incidence of the disease can be controlled by the introduction and adherence to effective veterinary and public measures. Research is needed to develop practices, which will achieve these aims at minimal cost to these communities.

See also: Bacteria: *Bacillus cereus* and Other Pathogenic *Bacillus* Species. Characteristics of Foodborne Hazard and Diseases: Cost of Foodborne Diseases; International Classification of Diseases. Foodborne Diseases: Overview of Biological Hazards and Foodborne Diseases. Safety of Food and Beverages: Meat and Meat Products

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Bacillus cereus and Other Pathogenic *Bacillus* Species

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Glossary

Emetic toxin Compound that results in vomiting.
Enterotoxin Toxin (protein) that causes diarrhea.
Hepatocytes Cells of the main tissue of the liver.
Necrotic enteritis Inflammation of the intestine, with cell death.
Non-ribosomal synthesis Synthesis of peptides from enzymes and not through normal ribosomal protein synthesis.
Polymerase chain reaction (PCR) A genetic tool to detect specific genes.

Introduction

There are several *Bacillus* species that have been responsible for foodborne illness (Table 1), although the only one that is frequently involved is *Bacillus cereus*. Six species are closely related and grouped together in the *B. cereus* group, among them is *Bacillus anthracis*. All these species can probably cause foodborne illness and will, in most cases, not be distinguished in routine food laboratories, apart from *B. anthracis*, which is usually not hemolytic and is sensitive to penicillin. It has also been suggested that these species are so closely related that they should be considered as one species. In this article, the *B. cereus* group (apart from *B. anthracis*) will mostly be handled as one species, although *Bacillus thuringiensis* and *Bacillus weihenstephanensis* will partly be dealt with separately.

The other *Bacillus* species that might cause foodborne illness are all isolated from soil and foods and include mainly species in the *Bacillus subtilis*-group (*B. subtilis*, *Bacillus mojavensis*, *Bacillus licheniformis*, and *Bacillus pumilus*). However, in contrast to the members of the *B. cereus* group, only a small number of isolates of these species have the ability to produce toxins that can result in foodborne illness (Table 1).

As an agent of foodborne illness, *B. cereus* is of growing concern although it does not usually cause the type of disease leading to news headlines. However, outbreaks of both the emetic and the diarrheal type of foodborne illness have caused deaths during recent years. As non-reportable diseases, the true number of cases and outbreaks is unknown, but large outbreaks have been reported for several countries. The fact that it is usually a relatively mild and short lasting disease (less than 24 h) may at least partly account for the high underreporting in official statistics.

Bacillus cereus is a Gram-positive, spore-forming, motile, aerobic rod that also grows well anaerobically. It is a common soil saprophyte and is easily spread to many types of foods, especially of plant origin, but is also frequently isolated from meat, eggs, and dairy products. *Bacillus cereus* and other members of the *B. cereus* group can cause two different types of foodborne illness: the diarrheal type and the emetic type. The diarrheal type of foodborne illness is probably caused by enterotoxins, produced by *B. cereus* during vegetative growth in the small intestine. At least three different enterotoxins are suspected to be involved. In contrast, the emetic toxin is preformed during the growth of emetic *B. cereus* strains in the food. For both types of foodborne illness, the food involved has usually been heat-treated, and surviving spores are the source of the foodborne illness. *Bacillus cereus* is not a competitive microorganism, but grows well after cooking and cooling (less than 42–50 °C). The heat treatment will cause spore germination, and in the absence of competing flora, *B. cereus* grows well. Invasion by psychrotolerant strains from the *B. cereus* group in the dairy industry has led to increasing surveillance of *B. cereus* in recent years.

The closely related *B. thuringiensis* can produce enterotoxins and has been shown to cause foodborne illness symptoms when given to human volunteers. Furthermore, foodborne illness outbreaks involving *B. thuringiensis* have been reported. The extensive use of this organism as a protective agent against insect attacks on crops may contribute to the increasing problems with organisms of the *B. cereus* group observed in the food industry. Standard confirmation procedures for *B. cereus* does not differentiate between the two species, if at all possible from heat-treated food products, because *B. thuringiensis* will frequently loose the insecticidal plasmids when grown above 30 °C. Thus, investigation of the true number of foodborne illness cases caused by commercially used *B. thuringiensis* is difficult. To ensure safe spraying with *B. thuringiensis*, the strain in use should be unable to produce foodborne disease toxins. Already, The Health and Consumer Protection Directorate-General (European Commission) has accepted that only nontoxin producing *Bacillus* spp. should be allowed for use in animal nutrition. *Bacillus weihenstephanensis* is the psychrotolerant species within the *B. cereus* group, although a few *B. cereus* strains able to grow at temperatures as

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Table 1 The different *Bacillus* species that have been involved in foodborne illness. The first six species belong to the *B. cereus* group and are very closely related

Species	*Illness*
B. cereus	Frequently involved in foodborne illness
B. anthracis	Has also caused foodborne illness (see Characteristics of *Bacillus cereus* Disease)
B. thuringiensis	Involved in foodborne illness
B. mycoides	Might have been involved in foodborne illness
B. weihenstephanensis	Probably not, but might have been involved in foodborne illness
B. pseudomycoides	Might have been involved in foodborne illness
B. licheniformis	Involved in foodborne illness
B. pumilus	Involved in foodborne illness
B. subtilis	Involved in foodborne illness
B. brevis	Involved in foodborne illness

low as 4 °C have been described. *Bacillus weihenstephanensis* strains seem to be non- or low-enterotoxin producing at 37 °C, and hence should be less likely to cause diarrheal disease. Recently, a few *B. weihenstephanensis* strains have been found able to produce emetic toxin.

Taxonomy of the *Bacillus cereus* Group

The aerobic endospore-forming bacteria are traditionally placed in the genus *Bacillus*. Over the past three decades, this genus has expanded to accommodate more than 260 species. Analysis of 16S ribosomal RNA sequences from numerous *Bacillus* species indicated that the genus *Bacillus* should be divided into at least five genera or rRNA groups. The species that are treated in this text (Table 1) still belong to the genus *Bacillus* in the suggested division.

Bacillus anthracis, *B. cereus*, *Bacillus mycoides*, *B. thuringiensis*, and more recently *Bacillus pseudomycoides* and *B. weihenstephanensis* comprise the *B. cereus* group. These bacteria have highly similar 16S and 23S rRNA sequences, indicating that they have recently diverged from a relatively common evolutionary line. Extensive genomic studies of *B. cereus* and *B. thuringiensis* have shown that there is no taxonomic basis for separate species status. Nevertheless, the name *B. thuringiensis* is retained for those strains that synthesize a crystalline inclusion (Cry protein) or δ-endotoxin that may be highly toxic to insects. The *cry* genes are usually located on plasmids, and loss of the relevant plasmid(s) makes the bacterium indistinguishable from *B. cereus*. It is now clear that all species of the *B. cereus* group, including *B. thuringiensis*, carry enterotoxin genes.

Foodborne Outbreaks Caused by the *Bacillus cereus* Group

Bacillus cereus is well recognized as a foodborne disease agent. Outbreaks can be divided into two types according to their symptoms. The diarrheal type is the most frequent in Europe and USA whereas the emetic type appears more prevalent in Japan. Typical foods implicated are stews, puddings, sauces, pasta, and rice dishes. When expressed as a proportion of all reported foodborne illnesses, outbreaks ascribed to *B. cereus* seem to be concentrated in Scandinavia and Canada (10–47% of total have been reported) and are less frequent in Central Europe, UK, USA, and the Far East (1–5% of total). Although these differences might partly be due to different consumer habits, they are also not comparable due to dissimilar reporting practices. Thus, in the Netherlands, in 1991, *B. cereus* was responsible for 27% of outbreaks in which the causative agent was identified. However, the incidence was only 2.8% of the total, because the majority of cases of foodborne illness were of unknown etiology. In addition, when the number of foodborne illness cases ascribed to *B. cereus* is expressed on a per head of population basis, many of the large regional differences in incidence disappear. In recent years, *B. cereus* have been reported from foodborne outbreaks and isolated from foods in South America (Brazil and Argentina), Africa (Kenya and Botswana), and Asia (Korea and Thailand). However, official statistics are not available for these continents. Examples of foods involved in outbreaks in different countries are given in Table 2.

Characteristics of *Bacillus cereus* Disease

The emetic toxin, cereulide, causes vomiting, whereas diarrhea is the main symptom of the second type of disease, caused by enterotoxins. In a very small number of cases, both types of symptoms are recorded, probably due to ingestion of preformed emetic toxin together with live *B. cereus* cells/spores, which may produce enterotoxins in the small intestine after a few hours. The question of whether or not the enterotoxin(s) can cause intoxication by preformation in foods has caused some debate. On review of the literature, it seems clear that the incubation time is a little too long for enterotoxins (more than 6 h; average 12 h), and model experiments have shown degradation of the enterotoxins on the way to the ileum. Although the enterotoxin(s) can be preformed, the number of *B. cereus* cells in the food would need to be at least two orders of magnitude higher than that necessary for causing foodborne illness, and such products would no longer be acceptable to the consumer for sensory reasons. The characteristics of *B. cereus* foodborne illness are given in Table 3.

In recent years, foodborne illness due to ingestion of *B. anthracis*-infected meat has been reported from three

Table 2 Examples of the variety of foods involved in *Bacillus cereus* foodborne illness

Type of food	*Country*	*Number of people involved*	*Type of syndrome*[a]
Barbecued chicken	Many countries	–	E, D
Cooked noodles	Spain	13	D
Cream cake	Norway	5	D
Eclair (pastry)	Thailand	>400	E (D)
Fish soup	Norway	20	D
Lobster pâté	UK	–	D
Meat loaf	USA	–	D
Meat with rice	Denmark	>200	D
Milk	Many countries	–	E, D
Pea soup	The Netherlands	–	D
Pasta salad	Belgium	4	E
Sausages	Ireland, China	–	D
School lunch	Japan	1877	E
Scrambled egg	Norway	12	D
Several rice dishes	Many countries	–	E, D
Stew	Norway	152	D
Turkey	UK, USA	–	D
Vegetable purée	France	44	D
Vanilla sauce	Norway	>200	D
Wheat flour dessert	Bulgaria	–	D

[a]E, emetic syndrome; D, diarrheal syndrome.

Table 3 Characteristics of the two types of disease caused by *Bacillus cereus*

	Diarrheal syndrome	*Emetic syndrome*
Infective dose	10^5–10^8 (total)	10^5–10^8 (cells per gram food to produce enough emetic toxin)
Toxin produced	In the small intestine of the host	Preformed in foods
Type of toxin	Protein(s)	Cyclic peptide
Incubation period	8–16 h (occasionally >24 h)	0.5–6 h
Duration of illness	12–24 h (occasionally several days)	6–24 h
Symptoms	Abdominal pain, watery diarrhea (in rare occasions with bloody diarrhea) sometimes with nausea	Nausea, vomiting, and malaise (sometimes followed by diarrhea, due to additional enterotoxin production?)
Foods most frequently implicated	Meat products, soups, vegetables, puddings/sauces, and milk/milk products	Fried and cooked rice, pasta, pastry, and noodles

Table 4 Toxins involved in foodborne illness produced by *Bacillus cereus*

Toxin	*Type/size*	*Food poisoning*
Hemolysin BL (Hbl)	Protein, three components transcribed from one operon: HblC (L_2-component), HblD (L_1-component), and HblA (B-component). The proteins are between 37 and 46 kDa.	Probably
Non-hemolytic enterotoxin (Nhe)	Protein, three components transcribed from one operon: NheA, NheB, and NheC. The proteins are between 36 and 41 kDa.	Yes
Cytotoxin K (CytK)	Protein, one component, 34 kDa	Yes, three deaths
Emetic toxin (cereulide)	Cyclic peptide, 1.2 kDa	Yes, two deaths

continents (Asia, Africa, and America). Patients without anthrax symptoms developed diarrhea (sometimes bloody) with fever and rashes. However, the enterotoxins known from *B. cereus* are unlikely to have caused these symptoms, because their genes are not transcribed in *B. anthracis*. From the total genome sequence of *B. anthracis*, enterotoxin genes (*nhe*) are known to be present, but the positive regulator for enterotoxin production (see Table 4) is nonfunctional.

Infectious Dose of *Bacillus cereus* Disease

Counts ranging from 10^4 to 10^9 g^{-1} (or ml) *B. cereus* have been reported in the incriminated foods after foodborne illness, giving putative total infective doses ranging from approximately 5×10^4 to 5×10^{11} cfu. The variation in the infective dose is partly due to the differences in the amount of enterotoxin produced by different strains (at least two orders

of magnitude), and partly due to the difference in infective dose between vegetative cells and spores, as the spores survive the passage through stomach acidity. Thus, any food containing more than 10^3 *B. cereus* g^{-1} cannot be considered completely safe for consumption.

Virulence Factors/Mechanisms of Pathogenicity

Very different types of toxins cause the two types of *B. cereus* foodborne illness. The emetic toxin, causing emesis (vomiting), is a ring-formed small peptide, whereas several different enterotoxins are involved in the diarrheal disease (Table 4).

The Emetic Toxin

The emetic toxin causes emesis in some primates and also has cell toxic activities. Its structure remained mysterious for many years, as the only detection system involved living primates. The discovery that the toxin could be detected by its vacuolation activity on HEp-2 cells led to its isolation and structure determination. The emetic toxin was named cereulide, and consists of a ring structure of three repeats of four amino- and oxy-acids: [D-*O*-Leu-D-Ala-L-*O*-Val-L-Val]3. This ring structure (dodecadepsipeptide) has a molecular mass of 1.2 kDa, and is, chemically, closely related to the potassium ionophore valinomycin. It has been shown in model animals that cereulide stimulates the vagus afferent through binding to the 5-HT3 receptor. The emetic toxin is resistant to heat, pH (2–12), proteolysis, and is not antigenic. Cereulide is produced by a nonribosomal peptide synthetase, encoded by the 24 kb cereulide synthethase (*ces*) gene cluster which is located on a megaplasmid related to pXO1.

Cereulide was responsible for the death of a 17-year-old Swiss boy some years ago, due to fulminant liver failure. A large amount of *B. cereus* emetic toxin was found in the residue from the pan used to reheat the food (pasta) and in the boy's liver and bile. In 2003, a 9-year-old Belgian girl died after eating a contaminated pasta salad, and there have also been other cases of serious outcomes of cereulide intoxication. In a recent study, mice were injected with synthetic cereulide, and the development of histopathological changes was examined. At high cereulide doses, massive degeneration of hepatocytes occurred. The serum values of hepatic enzymes were highest on days 2–3 after the inoculation of cereulide, and rapidly decreased thereafter. General recovery from the pathological changes and regeneration of hepatocytes were observed after 4 weeks.

Enterotoxins

As shown in Table 4, three different enterotoxins believed to be involved in foodborne illness have been characterized to date. The three-component hemolysin (Hbl; consisting of three proteins: B, L_1, and L_2) with enterotoxin activity was the first to be fully characterized. Convincing evidence has shown that all three components are necessary for maximal enterotoxin activity. This toxin also has dermonecrotic and vascular permeability activities, and causes fluid accumulation in ligated rabbit ileal loops. Hbl has been suggested to be a primary virulence factor in *B. cereus* diarrhea. A model for the action of Hbl suggests that the components of Hbl bind to target cells independently, subsequently forming a membrane-attacking complex causing cell lysis by a colloid osmotic mechanism. Substantial heterogeneity has been observed in the components of Hbl, and strains can produce different combinations of variants of each component.

A 'non-hemolytic' three-component enterotoxin (Nhe) was more recently characterized. The three components of this toxin are different from those of Hbl, although there are similarities. Nhe enterotoxin was first purified from a *B. cereus* strain isolated in a large foodborne illness outbreak in Norway in 1995. Binary combinations of the components of this enterotoxin possess some biological activity, but not nearly as high as when all the components are present. The three components act optimally in a ratio of 10:10:1 (NheA:NheB:NheC), and make a huge pore in the epithelial cells, resulting in diarrhea.

All tested *B. cereus*/*B. thuringiensis* strains carry genes for Nhe, and approximately 60% do for Hbl. Presently, the respective importance of the two toxins in foodborne illness is unknown, but several foodborne illness isolates are not Hbl-producing. Furthermore, it has been shown that the majority of the toxicity on epithelial cells is due to Nhe, and not to Hbl.

There is significant sequence identity between the three proteins of Nhe, and between the Nhe and Hbl proteins. In spite of the similarities between the components of Hbl and Nhe, they cannot substitute for each other to give biologically active complexes.

The more recently described cytotoxin K (CytK) is similar to the β-toxin of *Clostridium perfringens* (and other related toxins) and caused the symptoms in a severe outbreak of *B. cereus* foodborne illness in France in 1998. In this outbreak, several people developed bloody diarrhea, and three died. It can reasonably be characterized as an outbreak of *B. cereus* necrotic enteritis, although it was not as severe as *C. perfringens* type-C foodborne necrotic enteritis.

Other Possible Virulence Factors

The *B. cereus* spore is more hydrophobic than any other *Bacillus* spp. spores, which makes it adhesive to several types of surfaces. Additionally, the *B. cereus* spore carries appendages or pili that might be involved in adhesion (Figure 1) and may lead to clustering together of spores. Therefore, *B. cereus* spores are not easily removed by cleaning, and they are difficult targets for disinfection. In addition to enabling spores to survive sanitation (and thus be available for contamination of foods), these properties may also aid spore adherence to epithelial cells. Experiments have shown that spores from a *B. cereus* outbreak strain can indeed adhere to Caco-2 cells in culture, and that adherence is linked to hydrophobicity and possibly to the appendages. A longer incubation period observed in this disease case would be expected, as the spore would first have to germinate.

Commercial Methods for Detection of the *Bacillus cereus* Toxins

Two commercially available immunoassays detect one component each of *B. cereus* enterotoxins Nhe and Hbl. Neither assay is quantitative. The Oxoid Bacillus cereus RPLA enterotoxin detection kit (Oxoid Ltd., Basingstoke, UK) detects the

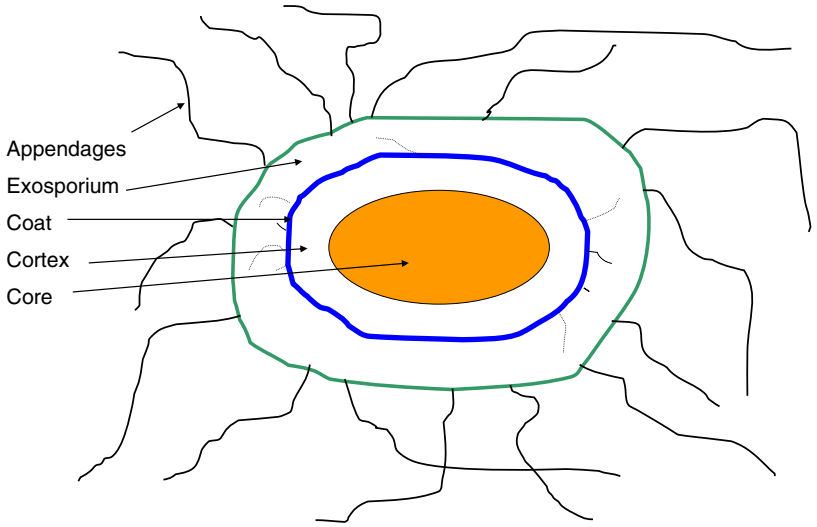

Figure 1 The *Bacillus cereus* spore with the different layers and appendages.

Table 5 Characteristics of the illness caused by the three *Bacillus* species other than *B. cereus* causing food poisoning

	Bacillus subtilis	Bacillus licheniformis	Bacillus pumilus
Foods involved	● Meat dishes with elements of vegetables ● Seafood with rice ● Bread and pastry products ● Sandwiches ● Pizzas	● Meat dishes with elements of vegetables ● Bread and pastry products ● Chicken	● Meat products ● Sandwiches ● Canned tomato juice ● Rice
Infective dose	$>10^5$	$>10^6$	$>10^6$
Incubation period	10 min–14 h	2–14 h	15 min–11 h
Duration	2–8 h	6–24 h	
Symptoms:			
● Vomiting	80%	54%	Yes
● Diarrhea	49%	92%	Yes (not all)
● Abdominal pain/cramps	27%	46%	Yes
● Other symptoms	Nausea, headaches, and flushing/sweating	Not reported	Headache, dizziness, chills, and elevated heart rate

HblC (L_2) component, whereas the Tecra Bacillus diarrheal enterotoxin visual immunoassay kit (Bioenterprises pty, Australia) mainly detects the NheA protein. Although only one toxin component is detected, a positive result from *B. cereus* culture supernatant in either assay indicates that the strain is enterotoxin positive. If the supernatant is also cytotoxic on cultured epithelial cells, the strain can be regarded as enterotoxin positive. For the third cytotoxin, CytK, there is no commercial detection method available. Unfortunately, this is also the case for detection of the emetic toxin. However, a sensitive screening assay on boar sperm has been developed for the emetic toxin, in addition to specific liquid chromatography–mass spectroscopy (LC–MS) methods.

Foodborne Disease Potential of Other *Bacillus* Species

Since the early 1970s, a number of reports have implicated *Bacillus* species other than *B. cereus* as the cause of foodborne disease, mainly involving species in the *B. subtilis* group (*B. subtilis*, *B. pumilus*, and *B. licheniformis*). Common symptoms for these foodborne illness incidents are vomiting and diarrhea and in some cases abdominal pain/cramps. Additional symptoms are headache, flushing, sweating, and dizziness. The incubation period of the incidents range from acute to 14 h, and the duration of the disease is reported from 1.5 to 24 h depending on the species involved. The implicated food vehicles in most cases are meat pasties or pies with vegetables, rice dishes, and pasta. The infective dose variation for these species is not clear, although it must be quite high (usually more than 10^6) (Table 5). Foodborne illness incidents where *B. brevis* (now *Brevibacillus brevis*) was detected in large numbers ,either alone or with *B. cereus*, from the implicated foods have also been reported. This organism was also, in one incident, isolated from the feces of a patient. The main symptoms in these episodes were vomiting and diarrhea, and the incubation times ranged from 1 to 9.5 h. In 2006, a small foodborne illness outbreak

implicating *B. pumilus* as the causative agent was reported. The incident involved three people who suffered from acute symptoms including dizziness, headache, chills, and back pain after a meal in a Chinese restaurant. Stomach cramps and diarrhea, which lasted for several days, developed later. The implicated food vehicle in this case was cooked, left-over, and reheated rice.

In the reported cases described above, no well-known foodborne pathogens were detected in the implicated foods, whereas large numbers of the suspected *Bacillus* species (more than 10^5–10^9 cfu g^{-1}) were isolated in almost pure culture in each case. The variation in symptoms and course of disease (length of the incubation time, duration) in these incidents suggests the involvement of more than one type of toxin or that the same toxin can be preformed in food and later act in the small intestine. Enterotoxins similar to those of *B. cereus* have been detected from several of the other *Bacillus* species (Table 1) using immunological- and 'polymerase chain reaction (PCR)'-based methods. However, it seems that these results were based on incorrect species identification and that the strains involved are atypical *B. cereus* strains rather than other *Bacillus* species (Table 5).

Putative Virulence Factors

Although *Bacillus* spp. other than *B. cereus* have been incriminated as foodborne illness agents, the link between toxin production in such strains and foodborne illness has not been fully established, although various toxins have been found. A sperm-toxic compound produced by *B. licheniformis* strains isolated from baby milk powder involved in the fatality of an infant has been isolated, characterized, and later identified as lichenysin A. In the incident in the Chinese restaurant described above, *B. pumilus* producing the boar sperm-toxic compound pumilacidin was isolated in nearly pure culture from the implicated food. Recently, a novel toxin produced by strains of *B. subtilis* and *B. mojavensis* isolated from cases of foodborne illness was partly characterized. This toxin, amyloisin, is non-ribosomally synthesized, heat-stable, and exhibits similar toxic properties as cereulide.

Lichenysin A and pumilacidin belong to a family of lipopeptides produced by certain *Bacillus* species. These lipopeptides are small, structurally diverse cyclic lactones, and are characterized by their high resistance to extreme heat, pH, and enzymatic challenges. The chief representative and best studied member of this group is surfactin which is produced by certain strains of *B. subtilis*. Surfactin exhibits an amazing array of different properties including strong biosurfactant, antiviral, and antimycoplasma activity. The unusual structural complexity of surfactin, especially its three-dimensional structure, probably accounts for its biological and physiochemical properties. It is produced nonribosomally by a synthetase enzyme complex, and consists of a heptapeptide part linked to the carboxyl and hydroxyl groups of a ß-hydroxy fatty acid. Because surfactin production is widely distributed among *B. subtilis*, *B. mojavensis*, *B. pumilus*, *B. licheniformis*, and *B. amyloliquefaciens* strains, a diverse collection of surfactin isoforms has been described under different names including lichenysin and pumilacidin. Lipopeptides of the surfactin family are highly bioactive and have been reported to induce voltage-independent, selective cationic channels in lipid bilayer membranes and to lyse red blood cells and other types of eukaryotic cells presumably by interacting with the cell membrane. Plasma membrane interactions such as leakage, and at higher concentrations complete membrane destruction, have been shown for surfactin as well as highly selective cell-membrane binding, depending on membrane lipid composition. The fatty acid portion of surfactin has high affinities to cholesterol and phospholipids and is found anchored in the lipid bilayer. Although both cereulide from *B. cereus* and surfactin inhibit boar sperm motility, the mechanism of interaction differs. Cereulide causes motility loss through destruction of oxidative phosphorylation in the mitochondria, while surfactin impairs the motility through destruction of the cell membrane, with subsequent mitochondrial depolarization.

Although the acute toxicity of members of the surfactin family to mammals is considered to be low, as lactones, these cyclic compounds are potentially highly bioactive. Their resistance toward heat, enzymatic degradation, and pH alterations, enables survival through food processing and passage through the gastrointestinal tract without reduction of biological activity. This might explain the intoxication symptoms reported in foodborne illness by lipopeptide-producing strains of non-cereus *Bacillus* species. An explanation for diarrhea (in some cases) may be provided by the cyclic AMP phosphodiesterase inhibitory activity reported for *B. subtilis* lipopeptides. Increased cyclic AMP levels in intestinal cells as a result of exposure to cyclic lipopeptides may lead to leakage of Cl^- and secretory diarrhea. The ability of cyclic lipopeptide-producing *Bacillus* species to establish in the intestine and to cause membrane damage of the intestinal cells (resulting in diarrhea) is yet to be proven. Whether intake of food contaminated with preformed lipopeptides of the surfactin family might lead to an intoxication of the consumer is also uncertain.

Little attention has been drawn to members of the *B. subtilis* group and their role in foodborne disease. As a result of this lack of focus on *Bacillus* species other than *B. cereus* as potential agents of foodborne illness, there has been a tendency to ignore incidents where small amounts of *B. cereus* but high amounts of other *Bacillus* species have been detected in the implicated food vehicle. Before the toxicity of *in vitro* toxic compounds produced by *Bacillus* species are tested to a greater extent in animal model feeding trials, our knowledge of the involvement of cyclic lipopeptides in non-*B. cereus* foodborne illness is limited. Until this knowledge is established, one should be cautious about ruling out *Bacillus* species outside the *B. cereus* group as possible agents of foodborne disease.

Prevention and Control of *Bacillus* Foodborne Illness

Control of *Bacillus* spp. is relatively easy in principle, though proving troublesome in the dairy industry and in the growing market of lightly processed precooked long-life products. Both product types are difficult to be kept completely free from spores of *Bacillus* spp. Growth characteristics of the four most

Table 6 Some factors determining the growth of the four most common foodborne illness *Bacillus* species

Species	*pH-range*	*Temperature range*	*Other factors*
Bacillus cereus	4.3–9.3	4–50 °C	● Growth at aw to 0.92 ● Inhibited by 0.2% sorbic acid (pH less than 5.0) ● Growth in 7% NaCl
Bacillus subtilis	5.0–8.5	10–50 °C	● Growth in 7% NaCl ● Growth with only glucose and ammonium
Bacillus licheniformis	5-?	>15–55 °C	● Growth in 7% NaCl
Bacillus pumilus	5-?	10–45 °C	● Growth in 7% NaCl

common foodborne illness *Bacillus* spp. are shown in Table 6. If foods are kept at temperatures between 6 and 60 °C for too long, spore germination and outgrowth of these species can occur. Rapid cooling and proper reheating of cooked food are essential if the food is not consumed immediately. Long-term storage must be at temperatures below 8 °C (or preferably between 4 and 6 °C to prevent growth of *B. cereus*). Low pH foods (pH less than 4.3) can be considered safe from growth of the foodborne illness *Bacillus* species.

Typical Foods

Bacillus spp. spores are commonly isolated from spices, cereals, and dried foods. For example, during the period 1960–1968, *B. cereus* was the third most common cause of foodborne illness in Hungary, and meat dishes were frequently involved, perhaps due to a preference for well-spiced meat dishes in Hungary. Several cases of *B. cereus* foodborne illness, involving many people, due to meat-containing dishes have also been reported from Norway recently.

The emetic *B. cereus* food poisoning has often been connected with consumption of rice in Chinese restaurants. The predominance of episodes involving this type of restaurants is linked to the common practice of bulk cooking of boiled rice. Portions of boiled rice are stored, usually at room temperature, overnight and *B. cereus* can multiply. The same problem may arise when other starch-rich foods (pasta, pizza) are stored for extended time at room temperature, as emetic strains are more often found in such foods.

Presently a main problem with *B. cereus* seems to be in the dairy industry, where the number of *B. cereus* cells/spores in the product is important in determining the keeping quality of milk. A defect known as bitty cream, where the creamy layer of pasteurized milk aggregates, is caused by the lecithinase activity of *B. cereus*. Furthermore, *B. cereus* is responsible for sweet curdling (without pH reduction) both in homogenized and non-homogenized low-pasteurized milk. It seems impossible to completely avoid the presence of *B. cereus* in milk, as it contaminates raw milk already at the farm, the principal source probably being soiling of the cow udders (soil has been shown to contain 10^5–10^6 spores g^{-1}). Thorough cleaning of the udder and the teats is therefore essential. Milk transport and storage at the dairy plant may result in further contamination of the raw milk from *B. cereus* spores present (adherent) in tanks or pipelines. Although vegetative bacteria

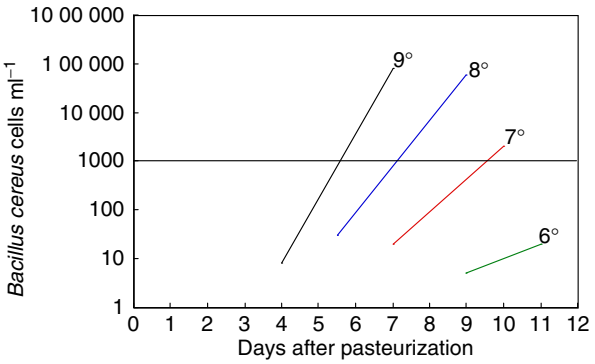

Figure 2 Growth rate of *Bacillus cereus* cells in milk stored at 9, 8, 7, and 6 °C.

are killed in the pasteurization process, the spores survive, and the pasteurization might activate some spores (heat activation), resulting in germination and possible vegetative growth.

To handle *B. cereus* problems in milk and milk products, control of the presence and outgrowth of spores, from farmer to packaging, is necessary. The temperature of storage is the most important factor in keeping *B. cereus* numbers low. Figure 2 shows the number of days required for milk to contain more than 10^3 *B. cereus* ml^{-1} at different temperatures. An increase of just 2 °C during storage, from 6 to 8 °C, increases *B. cereus* growth rates tremendously. At the dairy the milk is, in general, kept at 4 °C, and this assures a good keeping quality. However, during distribution, maintaining low temperatures can prove more challenging, and, therefore, temperatures up to 8 °C and above can occur. Furthermore, the consumer often exposes milk to higher temperatures for longer periods, for instance, at the breakfast table. As for the risk of foodborne disease, the dominating *B. cereus* group member in cooled milk products is *B. weihenstephanensis* (or cold-tolerant strains of the other members), and laboratory experiments have shown that those are usually low in enterotoxin production at human body temperature. Infant formula dried milk products should be free of pathogenic *Bacillus* species, as suggested by European Food Safety Authority (EFSA).

The strongly adhesive nature of *B. cereus* spores to different hydrophobic surfaces (such as glass and stainless steel) is a

major reason for its presence in food plants and kitchen environments. Three spore characteristics are responsible for the strong adhesion: high relative hydrophobicity, low surface charge, and surface morphology (appendages). At present the only way to overcome a spore problem, when present, is through the use of hypochlorite (0.2% at pH 7–8) or UVC light, because neither low – nor high – pH cleaning is sufficient to control the problem.

See also: Foodborne Diseases: Overview of Biological Hazards and Foodborne Diseases

Further Reading

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BACTERIA

Brucella

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Glossary

Anthropozoonotic A disease that can be transmitted from animals to humans.

Pasteurization Process of heating food/milk to below the boiling temperature (71.7 °C (161 °F) in dairy industry settings), for a specific period of time, usually 15–20s, in order to eliminate any bacterial aggregates without irreversibly affecting the synthesis and characteristics of the product. Named after Louis Pasteur.

Background

Brucellosis is a disease attributed to bacteria of the genus *Brucella*, and is not only one of the most prevalent zoonotic infections and foodborne infections worldwide, but also a disease predating humanity and recognized since antiquity in humans: lesions similar to spondylitis caused by *Brucella* have been demonstrated in primate, prehuman, skeletons as well as in skeletons from Pompeii (dating back almost 20 centuries). The disease is also known as Malta fever, underlining its historical correlation with the island of Malta, where Sir David Bruce, a British army physician, first identified the zoonotic nature of the disease in the late 1800s and isolated the causative agent with the assistance of Themistocles Zammitt. Initially termed *Micrococcus melitensis*, and identified in goats and goat milk, the pathogen was subsequently renamed *Brucella*, as suggested by Alice Evans, a leading brucellosis researcher and Bruce's wife. *Brucella* species were recognized in later years in cows, swine, dogs, and wildlife (see subsequent article).

Characteristics

Historical knowledge refers to six *Brucella* species, of which four are able to induce human disease. Although initial molecular studies have suggested that *Brucella* is a monospecific genus, this theory has been now disputed both for scientific (less homology than projected) and practical reasons (each species largely correlates with a particular animal host). In recent years, more than six novel *Brucella* species have been characterized, expanding the ecology of the bacterium significantly. Table 1 lists currently characterized *Brucella* species, their typical animal hosts, as well as their potential for inducing human disease. There are numerous biovars for the major species, with varying epidemiology and varying molecular homology, although there is limited evidence regarding their probable varying virulence for humans.

Brucella belongs to the a2 proteobacteria, its closest relatives being predominantly plant pathogens such as *Agrobacterium* and *Ochrobacter* species, and in a more distant relationship, *Bartonella* species. *Brucella* is a small Gram-negative coccobacillus that grows slowly and, in traditional culturing methods, may require specific growth media. The recent sequencing of the complete genome of *Brucella melitensis*, *Brucella abortus*, and *Brucella suis* has allowed for a continuing effort to understand and analyze metabolism, survival, growth, and virulence characteristics: yet, little is typical to what is established for other Gram-negative pathogens, and knowledge of individual species' characteristics is constantly accumulating, making it difficult to adequately summarize this knowledge at a specific time-point.

Brucella species can grow and survive for protracted periods in the environment: survival in water for weeks has been demonstrated as well as prolonged (months) survival in soil for the novel species *Brucella microti*. In animals, the pathogen exhibits a predilection for the reproductive tract, responding to high concentrations of erythritol. Direct contact with infected raw animal tissues is a typical means of disease transmission to humans.

Foodborne brucellosis is less commonly attributed to meat consumption, and more often is the result of ingestion of unpasteurized dairy products, including milk, fresh cheese, cream/ice cream, and yoghurt. Survival of *Brucella* in dairy products is not related to fat content. Hard cheese is less effective in transmitting brucellosis, due to propionic as well as lactic fermentation taking place. Acidification processes necessary for the production of sour dairy products and, to a degree, yoghurt may also inhibit *Brucella* survival and growth. Pasteurization of milk remains the most effective means of prevention of foodborne brucellosis. Infected meat incriminated in human brucellosis is typically undercooked and is in the form of local delicacies (particularly liver/kidneys/spleen). Refrigeration does not inhibit *Brucella* survival, on the contrary, it lengthens it. Adequate cooking, however, is an efficient means of prevention of disease arising from food of animal origin.

As already stated, *Brucella* is not a typical Gram-negative pathogen, lacking virulence factors that are recognized in other Gram-negative bacteria: its lipopolysaccharide induces a vastly inferior immune response, compared with *Escherichia*

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Table 1 *Brucella* species, their animal hosts, and their anthropozoonotic potential

Species	*Animal hosts*	*Anthropozoonotic potential*
B. melitensis	Sheep, goats, camels	Commonest cause of brucellosis worldwide
B. abortus	Cattle, buffalo, elk, yaks, camels	Major cause of brucellosis in specific countries
B. suis	Domestic pigs, wild boar, reindeer, caribou, rodents	Typically in isolated communities with a tradition in swine raising
B. canis	Canines	Increasingly recognized as human pathogen, particularly in Latin America
B. ceti	Dolphins, whales, porpoises	Isolated case reports of severe human disease
B. pinnipedialis	Seals	
B. ovis	Sheep	Not reported as human pathogen
B. neotomae	Rodents	Not reported as human pathogen
B. microti	Foxes, rodents	Not reported as human pathogen, except in experimental cases where a high degree of virulence was observed
B. inopinata	Unknown	Isolated from a single human case

coli, for example, and there are no exotoxins or endotoxins implicated in the initiation of human disease. Certain outer membrane proteins have been demonstrated as playing an important role in the intracellular survival and proliferation of *Brucella*; at present though, practically only the presence of a type-IV secretion system has been systematically acknowledged as of major importance for infection initiation.

The requisite bacterial load for transmission of *Brucella* to humans has not been adequately studied, and animal models may not allow for extrapolation due to the differences in disease pathophysiology and host–pathogen interaction observed in different animal models and for different *Brucella* species. Loads as low as of 10–100 bacteria are known to induce disease in humans, yet this may refer to airborne transmission and not to foodborne disease. It is certainly difficult to assess the bacterial load necessary for invasion through the gastrointestinal tract because the food products usually implicated, typically, carry large loads of the pathogen; moreover, the protracted incubation period of human disease usually precludes availability of the incriminated food product for evaluation of its bacterial load. There is also limited knowledge about the potential mechanisms of direct inoculation of *Brucella* through skin abrasions.

Clinical Manifestation (Disease)

The incubation period in human disease ranges from 2 to 8 weeks, the first symptoms typically observed in the fourth to fifth week after exposure. There have been no descriptions of differences in incubation period related to the means of transmission (direct contact vs. airborne exposure vs. foodborne disease).

Although characterizations such as acute, subacute, and chronic forms of the disease are often met in the literature, the definitions of these syndromes are vague, and the vast frame of clinical presentations often makes such categorizations useless. Brucellosis can be present as: (1) an acute disease with high-spiking fevers and bacteremia; (2) in a protracted form with low-grade fever, excessive malodorous perspiration (pathognomonic for the clinicians acquainted with it), malaise, myalgias and arthralgias, and evidence of reticuloendothelial system-related immune response (in the form of lymphadenopathy or hepatosplenomegaly); or (3) by manifestation of a focal complication – practically any organ and tissue of the human body can serve as the focus of brucellosis, but this is commonly observed in the osteoarticular system and the male genitourinary system (requiring a high index of suspicion by relevant specialists as orthopedics and urologists who may be the first to examine the patient). However, brucellosis has been systematically implicated in hematologic, cardiac, neurologic, respiratory, dermatologic, and even ophthalmologic complications. Complications range from benign laboratory abnormalities that rapidly regress on initiation of treatment (e.g., mild increases in liver aminontransferases with no clinical significance), to life-threatening situations as endocarditis or central nervous system involvement, and chronically debilitating, difficult-to-treat situations as spondylitis. An increased risk for abortion in pregnant females has been recorded in various patient series from the Middle East.

Eradication of *Brucella* postinfection remains one of the most scientifically puzzling and constantly resurfacing medical subjects of debate. Chronic disease can be focal, or in the form of repeated relapses without an apparent focal site of pathogen persistence, or can be vaguely described as a chronic fatigue syndrome. Brucellosis specialists are also familiar with a subgroup of chronic patients complaining of depressive symptoms in the absence of any objective diagnosis of brucellosis relapse/persistence: such behavioral and depressive symptoms have been demonstrated in chronic brucellosis since the 1940s, yet there has been limited attention to the subject in modern research. Recent advances in molecular diagnostics have allowed demonstration of bacterial load persistence in apparently healthy, successfully treated brucellosis patients, even years after the initial disease episode. The significance of such a carrier state has not been elucidated. It also remains unclear whether human-to-human transmission actually takes place apart from the occasional reports of sexual transmission. Transmission of *Brucella* through the fecal–oral route is not an issue though, in contrast to the typical foodborne pathogens arising from raw foods of animal origin.

Misdiagnosis is common when the disease appears (through patient importation by migration and international travel, or through infected food product international trade) in nonendemic areas. Misdiagnosis carries a significant risk for

a variety of reasons: (1) recognition of human disease and possible means of exposure allows for early identification of other exposed individuals (typically belonging to the same household) and early or even preemptive diagnosis and treatment initiation; (2) informing the laboratory personnel of a possible diagnosis of brucellosis allows for implementation of appropriate precautions (owing to its volatility, brucellosis remains the commonest laboratory-acquired infection worldwide, usually affecting more than one microbiologist/technician, either as an aerosol-spread infection or through multiple personnel dealing with the same culture and thus being exposed); (3) early diagnosis allows for avoidance of severe complications such as spondylitis and paravertebral abscess formation; and (4) on a purely theoretical level, because *Brucella* is a Category B potential biological weapon, it may allow for early identification of any epidemiologically unusual cluster of cases and thus early initiation of prophylactic measures for the remainder of the potentially exposed population (it should be noted here though, apart from the already stated theoretical-only risk of such a scenario, that exposure in such a case would not be through the food chain but airborne).

Epidemiology

An estimated 500 000 new cases of human brucellosis are recorded annually worldwide; one must take into account that these numbers are vastly underestimating the actual disease prevalence, because underreporting remains a major public health problem even in endemic parts of the developed world.

Novel hyperendemic areas have emerged in the past two decades, typically subject to major socioeconomic and political alterations. The disease remains prevalent in the Middle East and North Africa, in parts of Central America, and in the Indian subcontinent, although absolute data for the latter are missing (Figure 1). In the majority of the former Soviet republics of Central Asia, brucellosis has evolved into a public health emergency, with annual incidence rates exceeding 100 or even 200 per million of population. Another geographic zone where the disease has rapidly emerged/re-emerged in recent years is the Balkan Peninsular, where political regime alterations, re-designing of borders, the birth of novel countries with limited infrastructure in public health and veterinary surveillance, war, massive involuntary population movements, and loosening of border controls have all assisted in an explosion in disease incidence. This has reached a plateau in the Former Yugoslav Republic of Macedonia, is only now recognized in terms of extent in Albania, and is rapidly expanding in Bosnia-Herzegovina. This, in conjunction with the steady annual incidence rates of Greece and the hyperendemic situation observed in certain areas of Turkey, create a zone where exclusively national control efforts are doomed to be ineffective. In Western Europe, effective control through mass vaccination programs has resulted in virtual eradication of the disease in France, significant decreases in incidence in Spain and Portugal, and localization of the disease only in Sicily in Italy. In Central and Northern Europe, as well as in Canada and the USA, the disease is almost exclusively observed in association with international travel or immigration.

Populations at risk include farmers and their families, abattoir workers, veterinarians, and laboratory personnel as described above. There have been numerous reports of travel-associated clusters of brucellosis, in cases when consumption of local delicacies that may include fresh cheese or undercooked meat products, has led to delayed presentation of disease, long after return to the nonendemic homeland.

Table 2 lists some recent outbreaks of human brucellosis of potential foodborne nature.

A newly identified population at risk that is increasingly attracting interest is hunters. These may be exposed to *Brucella* through consumption of undercooked bush meat or during

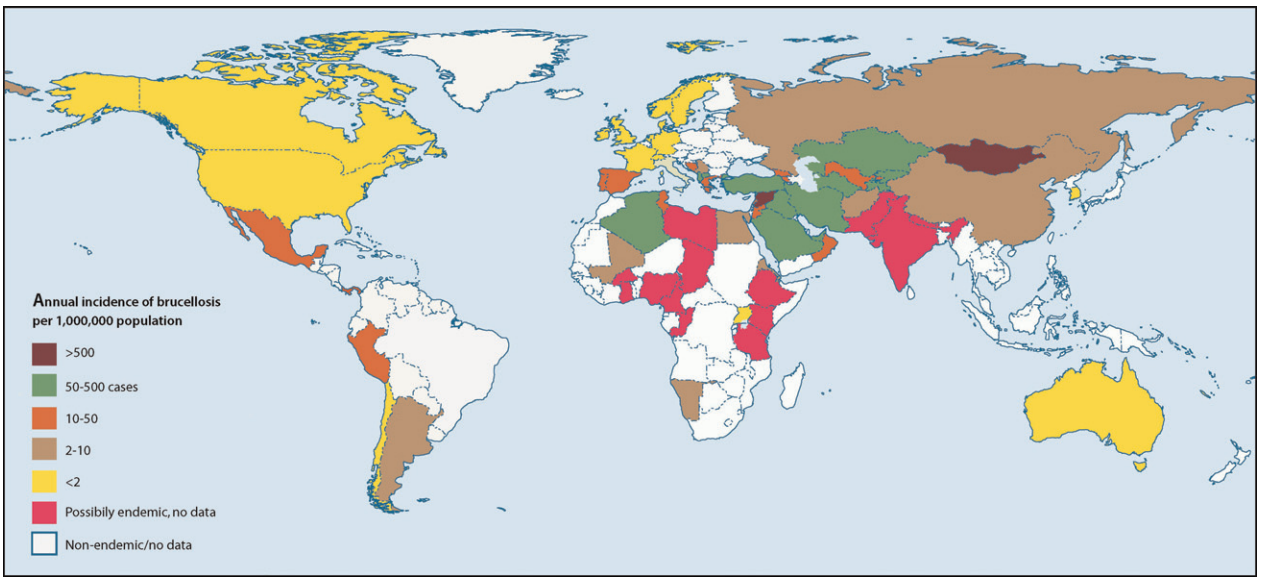

Figure 1 Global endemicity of brucellosis.

Table 2 Recent outbreaks of foodborne human brucellosis

Country, year	*Dairy/meat product implicated*	*Comments*
Thassos, Greece, 2008	Unpasteurized dairy products from infected sheep	More than 55 cases, the vast majority of which consumed unpasteurized dairy products
Elche, Spain, 2006	Unpasteurized goat milk	Observed in nine Moroccan immigrants
Treviso, Italy, 2005	Pecorino cheese from Sicily	Five cases observed in a nonendemic area
Messina, Italy, 2003	Ricotta cheese	29 cases, infected during a traditional religious ceremony which included consumption of local delicacies
Cordoba, Spain, 2002	Unpasteurized raw goat cheese	11 cases, all related to products from a particular farm

the process of skinning and meat preparation through direct contact. The recognition of brucellosis in wildlife, in animals as diverse as wild hare and elk, and the association of the disease with bush meat consumption indicate that wildlife may serve as a major reservoir of the disease. A typical example here is the demonstration of a direct relationship between elk brucellosis and bovine brucellosis in the Yellowstone area in the USA.

As Table 1 demonstrates, domesticated animals remain the principal reservoir of human pathogens. Although all anthropozoonotic species of *Brucella* can be foodborne, *B. melitensis* followed by *B. abortus* are reported as the main causes of *Brucella*-related foodborne disease cases or outbreaks worldwide. Other species may be implicated in cases associated with local food-consumption habits such as *B. suis*, mostly reported from Polynesia through pork products. However, *B. abortus*, *B. suis*, and *Brucella canis* demonstrate specific epidemiologic characteristics, which may still be understudied.

As already discussed, brucellosis is a household disease, and an index case should always prompt evaluation of the remainder of the family or other individuals exposed in common. Outbreaks are usually associated with social events which include consumption of local dairy products. On a wider scale, disease re-emergence is always associated with: weakened of the public health and veterinary surveillance infrastructure; an absence of health literacy on the subject for the population at risk; and socioeconomic factors. Elimination of infection in a herd through animal slaughtering imposes a significant economic burden for animal owners, who are often prone not to disclose the presence of the pathogen in their herd.

Analytical Methods for Organisms

The diagnosis of brucellosis should theoretically be based on the isolation of *Brucella* cultures though this is technically demanding for a variety of reasons: (1) a positive culture may take up to 4–6 weeks until detection, although the novel automated systems usually shorten this period to less than a week; (2) A proper process demands biosafety level-III practices which are not widely available (this is the reason, along with the pathogen's high volatility, that *Brucella* is so easily transmitted to laboratory workers); (3) proper process demands specialized culture media (as e.g., the Castaneda broth) and performance of subcultures; and (4) bacteremia in brucellosis, particularly in long-standing cases, is universally present – cultures of bone marrow samples have long been considered as more sensitive, but are always inconvenient for the patient. Cultures, however, allow for characterization of the incriminating pathogen to species and biovar level.

Serology remains the most widely used diagnostic approach. Serum agglutination tests give a positive result in the majority of novel cases, although, seropositivity may be observed for protracted periods irrespective of clinical response to treatment, and thus serology is not useful for follow-up. A more rapid serologic test, Rose Bengal, is widely employed worldwide, but its efficacy in diagnosis is not universally accepted and may be subject to technical inconveniencies.

Enzyme-linked immunosorbent assays (ELISAs) may be superior to classic serology in diagnosis and follow-up of disease evolution, but have been understudied in long-term follow-up. Molecular techniques have been developed in recent years and have been applied, albeit not widely, not only in diagnosing human disease and patient follow-up but also in the food industry, for an accurate detection of product infection.

Polymerase chain reaction (PCR) is an extremely sensitive diagnostic test, and real-time PCR can additionally inform on the bacterial burden of a specific product or a patient. Numerous independent researchers have systematically, in recent years, demonstrated that a positive bacterial burden can be traced by real-time PCR in apparently healthy subjects who had been successfully treated for brucellosis years, or even decades, before. This observation raises questions not only about the ability to actually eradicate the pathogen (which may survive in a latent form in a manner all too similar to tuberculosis), but also minimizes the utility of molecular techniques in the follow-up of patients. There have been contradictory results regarding the relationship between bacterial burden and potential for disease relapse in the literature.

Control and Preventive Measures

As is typical of zoonoses, brucellosis control is a multitask issue. Elimination of human disease (given that there are no effective vaccines) is largely based on elimination of animal disease, although the latter is subject to adequate veterinary practices. Veterinary surveillance for the disease and systematic animal vaccination products have assisted in minimizing brucellosis incidence in many parts of the developed world. In areas of the developing world, where inadequate veterinary infrastructure or economic issues may preclude the implementation of a widespread vaccination program, a 'test and

slaughter' policy may be more effective in minimizing animal disease incidence and spread, as well as human cases. However, the illegal animal trade has expanded in recent years and has been incriminated in the emergence and re-emergence of brucellosis in countries that were brucellosis-free or had a very low disease incidence in the past, the typical example being the Balkan Peninsula. Illegally traded animals are not subject to surveillance and vaccination, but their presence is primarily a political (regional and international) and economic issue. An adequate and sustained veterinary infrastructure allows for disease control in areas that are not neighboring endemic regions, and this is a possible explanation of the observed success in eliminating disease in Spain and France but not in Greece, the latter bordering with three of the top-20 hyperendemic countries for brucellosis worldwide. A similar explanation stands for the small, but sustained, disease incidence observed in the USA, almost exclusively in states neighboring the hyperendemic Mexico (through illegal importation of infected dairy products or illegal entry of infected humans).

Surveillance at food-chain level, however, has always been successful, particularly in the developed world, but is related to the need of industry for product reliability and the direct correlation between product quality/safety and financial returns. In the case of milk, pasteurization, or at least protracted heating of milk at 80–85 °C before consumption or further processing, is a safe approach. Fresh cheese ingested 4–6 months after production, carries small risk for brucellosis transmission. Thorough cooking of meat, even from an infected or unknown infection-status animal further minimizes the risk of foodborne brucellosis. Hygiene measures directed at herd owners, shepherds, abattoir workers, and laboratory workers relate to other modes of brucellosis transmission (direct, airborne), and should always be based on preexisting suspicion of disease. Regulatory measures relate to strict control of illegal animal movements.

Increasing awareness about the disease should be targeted at various population groups. These include not only at-risk professionals, such as the ones described in Epidemiology, but also individuals with underlying medical conditions, such as liver disease, preexisting valvular heart disease, and those with prosthetic devices who may be at risk of contracting serious disease complications. Pregnant women may exhibit increased abortion risk after contracting brucellosis (in addition to the pregnancy-related difficulties related to the administration of effective antibiotic regimens). International travelers to endemic areas should be aware of the risks of ingesting unpasteurized or undercooked local products of unknown or doubtful synthesis and origin (the traditional delicacies that are based on unpasteurized milk/milk products or raw/undercooked meat products are too numerous to mention and differ geographically); a traveler should always ensure that testing local foods does not expose him/her to any such products. Finally, it is important that policymakers understand the close proximity of disease incidence, both in animals and humans to specific socioeconomic parameters, and should thus focus on reversing these parameters.

See also: Foodborne Diseases: Foodborne Diseases in Travelers; Overview of Biological Hazards and Foodborne Diseases; Prevalence of Foodborne Diseases in South East and Central Asia

Further Reading

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Vivian Wyatt website. Brucellosis.

Campylobacter

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Glossary

Bacteremia Presence of bacteria in the blood.
Broiler chickens (broilers) A male or female chicken reared and kept primarily for meat production.
Microaerobic atmosphere One which has a lower oxygen concentration than air.
Prodrome Initial phase of a disease in which the first symptoms appear; these may differ from the classic disease symptoms.
Thermophilic Requiring heat.
Vertical transmission Transmission of infection from mother to offspring.

Background

Campylobacter spp. were well described in veterinary medicine from the early twentieth century. However, it was only in the 1970s that the importance of *Campylobacter* spp. as enteric pathogens in humans was widely recognized. With increasing laboratory capacity and testing in the 1970s and 1980s, *Campylobacter* spp. quickly became recognized as one of the leading causes of bacterial enteritis in humans worldwide.

As reported incidence rates increased during the last decades of the twentieth century, there was evidence that the data were reflecting a true upward trend over and above the apparent increase that would be expected to occur with improved detection and surveillance methods.

In the twenty-first century, *Campylobacter* infection is the most commonly reported cause of bacterial gastroenteritis in many industrialized nations, and is an important cause of bacterial gastroenteritis in young children living in developing countries. It is likely that the true incidence far exceeds the number of reported cases.

Although some countries have recently noted a decline in *Campylobacter* disease incidence, there is concern that the human immunodeficiency virus (HIV) epidemic may cause a significant increase in the disease burden of *Campylobacter* in the developing world; concurrent HIV infection is known to increase both the incidence risk and severity of *Campylobacter* gastroenteritis.

The considerable disease burden of *Campylobacter* gastroenteritis is not the only public health concern related to these organisms. Recent research has highlighted the important role played by *Campylobacter* infection in the etiology of Guillain–Barré syndrome (GBS) (a serious neurological condition) and in other postinfectious conditions such as reactive arthritis.

Campylobacter infection is of particular relevance to food production given the widespread contamination of the food chain by *Campylobacter* and the many food and waterborne routes by which humans may be infected.

A number of approaches to disease prevention and control are outlined in a later section of this article, but it is clear that controlling *Campylobacter* infection is a complex and challenging task.

Characteristics of the Organisms

Campylobacter spp. belong to the family Campylobacteriaceae. They are Gram-negative rods which may be spiral-shaped, S-shaped, or curved (Figure 1). Most are motile, exhibiting a characteristic corkscrew-like motion. Motility is conferred by flagella, usually in the form of a single flagellum at one or both ends of the cell, although nonmotile species or species with multiple flagella have been described.

The genus *Campylobacter* has 18 species, but only some of these species have relevance to human health. The two species most frequently associated with human disease are *Campylobacter jejuni* and *Campylobacter coli* which together account for more than 95% of human infections; related species such as *Campylobacter laridis* and *Campylobacter upsaliensis* are more rarely reported in humans. In developed countries, C. *jejuni* is estimated to cause at least 80–90% of *Campylobacter* infections. For this reason, where *Campylobacter* organisms are discussed in relation to human health, the convention has arisen that the term *Campylobacter* is understood to refer to *C. jejuni* or *C. coli* unless otherwise indicated. This convention has been followed in the current text.

With ever-improving subtyping techniques, the variability of *Campylobacter* organisms has become increasingly apparent (see the Section on Analytical Methods). This is an important aspect of *Campylobacter* control as characterization of strains may facilitate source attribution and identification of epidemiologically-related cases during an outbreak.

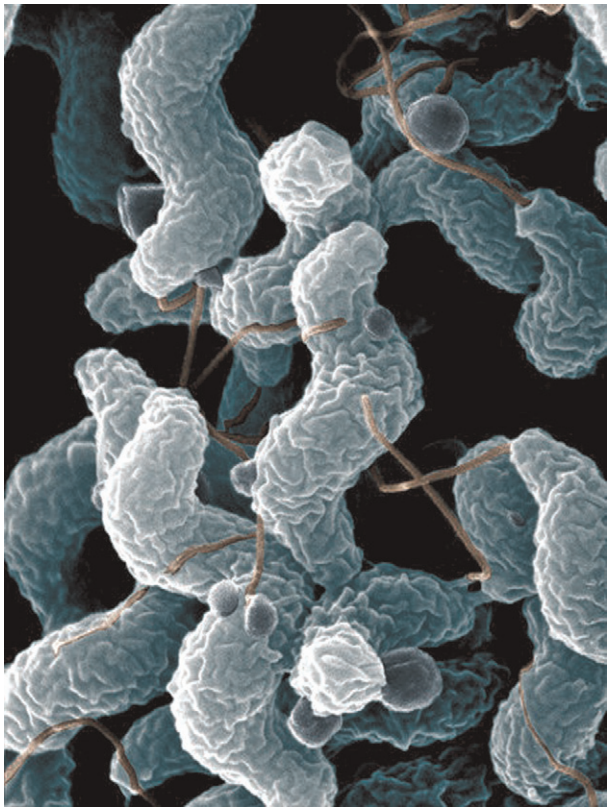

Figure 1 *Campylobacter* bacteria are the number-one cause of food-related gastrointestinal illness in the US. To learn more about this pathogen, ARS scientists are sequencing multiple *Campylobacter* genomes. This scanning electron microscope image shows the characteristic spiral, or corkscrew, shape of *Campylobacter jejuni* cells and related structures. Photo by De Wood; digital colorization by Chris Pooley. *Source*: Agricultural Research Service (ARS) is the US Department of Agriculture's chief scientific research agency.

Campylobacter spp. are notable for being exacting in their growth requirements when cultured under laboratory conditions. Most species require a microaerobic atmosphere with an oxygen concentration of approximately 5%. *C. jejuni* and *C. coli* are considered to be thermophilic: they grow optimally at 42 °C and do not grow at temperatures below 30 °C.

Tolerance of the organism to heating and freezing is of great interest because of the implications this may have for control of foodborne infection. There have been numerous studies reporting *Campylobacter* survival at different temperatures; however, they show considerable variation in their findings, possibly as a result of varying temperature tolerance between strains. Despite this variability, there is some evidence that *Campylobacter* survives better under refrigeration temperatures than at room temperature. Freezing causes a significant reduction in organism counts, and the organism is rapidly inactivated at normal cooking temperatures.

The intestinal mucosa of most warm-blooded species provides appropriate conditions for growth and replication of *Campylobacter*, with poultry and other avian species thought to be the primary hosts. However, it is interesting that such a fastidious organism can also be widely distributed in the environment, where conditions are unlikely to be suitable for growth. It is now known that *Campylobacter* spp. are able to enter a viable but nonculturable (VBNC) state under adverse conditions. A detailed understanding of the survival mechanisms of *Campylobacter* in the environment may be extremely useful for disease control.

Clinical Manifestation

Clinical disease caused by *Campylobacter* is referred to as campylobacteriosis. When considering this diagnosis, it is worth remembering that the following description of the clinical manifestations of campylobacteriosis is derived from experience of laboratory-confirmed cases. In the community, where the majority of affected persons do not seek medical help, campylobacteriosis may present a different clinical picture.

Typically, symptoms of campylobacteriosis appear 2–5 days after infection with a range of 1–7 days or even longer. The mean incubation period of 3 days is longer than usual for intestinal infections, which is an important consideration when tracing exposures.

Most patients experience a rapid onset of cramping abdominal pain and diarrhea, but approximately 30% have an influenza-like prodrome with fever, headache, and myalgia, and this is believed to be associated with a more severe disease course. Nausea is common but vomiting less so. Diarrhea can be mild or may be profuse enough to cause dehydration. Approximately 15% of patients have frank blood in the stool. The abdominal pain may become continuous and severe and thus mimic other intraabdominal pathology such as appendicitis, Crohn's disease, or ulcerative colitis. Pain without diarrhea is a known variant but is uncommon.

Generally, the diarrhea starts to improve after 3–4 days, but the abdominal pain will usually persist for longer than this. Symptoms usually lasts 3–6 days, but relapses are fairly common. Patients with underlying immune deficiency such as HIV infection may have a chronic or recurrent course. However, campylobacteriosis is generally a self-limiting disease and only approximately 5–10% of cases for medical attention require hospitalization.

Fatal outcomes are rare and are usually associated with extremes of age, concurrent disease (such as malignancy), or immune deficiency. *Campylobacter*-associated bacteremia carries a high risk of death, variously reported as 4–10% in the medical literature. Deaths from delayed complications of campylobacteriosis such as myocarditis and GBS may not be attributed to the correct underlying cause, so mortality risk from this infection has probably been underestimated. For example, a cohort study conducted in Sweden found a threefold increase in the risk of death within the first month following the infection.

Following the resolution of symptoms, patients may excrete *Campylobacter* in feces for several weeks unless treated with antibiotics. However, secondary infections from this source are not common.

Both acute and postinfectious complications of campylobacteriosis have been described.

Acute Complications

Appendicitis

As previously mentioned, the abdominal pain associated with campylobacteriosis may be severe enough to mimic appendicitis. However, true appendicitis is also a known complication of this infection.

Colitis

Colitis and proctitis are common findings in campylobacteriosis patients who undergo sigmoidoscopy and rectal biopsy. It can be difficult to distinguish this from acute nonspecific inflammatory bowel disease.

Bacteremia

This is probably underreported due to the practical difficulties in culturing the organism from blood samples. It is a serious complication in patients with severe immune deficiency, because mortality risk is considerably higher.

Peritonitis

This does not appear to be a risk in otherwise healthy persons, but several cases of peritonitis have been described in patients undergoing peritoneal dialysis for renal disease.

Hepatitis

Laboratory findings suggest that a mild, subclinical degree of hepatitis is common; however clinical hepatitis is rare.

Fetal Death

Campylobacter spp. infection is a well-known cause of fetal death in animals. Although some cases have been described in humans, this complication is extremely rare.

Postinfectious Phenomena

Guillain–Barré Syndrome

GBS is an autoimmune disorder of the peripheral nervous system. *Campylobacter* infection is now recognized as the most frequent identifiable antecedent infection in GBS cases, with onset usually 1–3 weeks after the infective episode.

A number of variants have been described, but in general the condition is characterized by weakness which is usually symmetrical and which evolves over several days. It can affect any age group.

Patients typically experience an ascending paralysis with loss of movement, and approximately a third require ventilatory support during the acute phase. The condition is generally self-limiting, but symptoms may take up to 2 years to resolve and some patients are left with residual symptoms.

Treatment is mainly supportive, with the addition of intravenous immunoglobulin or plasma exchange. Reports of mortality risk vary widely, but in a setting which allows for optimal support, mortality can be as low as 5%.

Reactive Arthritis

Patients with campylobacteriosis commonly report joint pain, and reactive arthritis develops in approximately 1–5% of cases. This typically occurs approximately 10 days after the acute infection and involves one or two peripheral joints. It may be severe, but complete recovery is believed to be the usual outcome. However, this is a somewhat under-researched complication, and it is possible that long-term sequelae are more prevalent than is currently recognized.

Postinfectious Irritable Bowel Syndrome

There is an increased risk of persistent diarrhea after *Campylobacter* infection.

Myocarditis

The mechanism for heart muscle involvement is unknown. Although rare, this may be serious when it occurs.

Pathogenesis

Campylobacteriosis is a zoonosis, that is, a disease transmitted from animals to humans.

It is generally accepted that *Campylobacter* spp. do not replicate outside of animal hosts; therefore, the bacterial load will tend to diminish in the environment (at varying rates) until another suitable host is found. Multiple animal hosts or reservoirs for *Campylobacter* have been identified including birds (likely the primary natural hosts of *Campylobacter*), ruminants, pigs, rodents, and other native fauna. Insects have also been implicated as vectors for the transmission of *Campylobacter*.

Campylobacter colonization is generally asymptomatic in animals, and disease occurs only when the organism is ingested by humans, a frequent occurrence because of the widespread distribution of *Campylobacter* in the environment and the food chain.

The basic mode of *Campylobacter* transmission is by fecal–oral spread. This may occur by a relatively direct transmission route (e.g., direct contact with animal feces) or by an indirect route (e.g., through consumption of fecally contaminated food or water).

Originally, information about infectious dose levels was derived from a human volunteer study in which a dose of 800 CFU was sufficient to cause infection. However, recent work suggests that the probability of infection for naïve individuals is high even for doses of <10 CFU; the organism demonstrates a high infectivity when the number of organisms ingested is likely to be extremely low.

After ingestion, the organisms are likely to be killed by the acidic environment of the stomach, but food can act as a buffer, allowing increased survival. The use of proton pump inhibitors for gastric acid suppression increases the risk of infection.

Once the organism has reached the intestine, it actively penetrates the mucus layer and adheres to colonic epithelial cells before penetrating the mucosa. This may or may not result in gastroenteritis. Asymptomatic colonization may ensue, in which the organism survives and replicates in the intestine but does not cause disease. This is common in developing countries, where persons may experience repeated exposure to *Campylobacter*, but it is less common in the developed world where such exposures are not as frequent.

Experience with other bacterial causes of gastroenteritis suggests that the pathogenesis of campylobacteriosis is likely to be toxin related. However, to date only one *Campylobacter* toxin has been identified. It is known as cytolethal distending toxin and is produced by a variety of *Campylobacter* species. The organism is able to invade epithelial cells and induce a cytokine response, thus precipitating an inflammatory cascade and hence the typical symptoms of campylobacteriosis.

Postinfectious Phenomena

GBS is thought to arise by molecular mimicry. Ganglioside-like structures in *Campylobacter* lipo-oligosaccharides induce an immune response which cross-reacts with the ganglioside components of peripheral nerves, causing weakness and loss of function.

Immune Response

Immune deficiency (such as hypo or agammaglobulinemia, or acquired immunodeficiency syndrome (AIDS)) may lead to increased incidence, more severe disease, and a higher likelihood of chronic carriage and recurrence.

Although the role of protective immunity in campylobacteriosis is far from clear, the experience of developing countries and of persons with a high occupational exposure to *Campylobacter* suggests that chronic exposure may induce protective immunity, although this is likely to be of a short duration only.

Virulence Factors

Despite the importance of *Campylobacter* as a cause of gastrointestinal infection, surprisingly little is known about the mechanisms of *Campylobacter* virulence compared to other enteric bacteria. Research is hampered by the fact that *Campylobacter* does not usually cause diarrhea in animals; therefore it is difficult to study in animal models.

In the absence of an easily available animal model, virulence is generally assessed using *in vitro* studies investigating pathogenesis mechanisms such as adhesion, invasion, protein secretion, and toxin production at the molecular level. The results should be cautiously applied to human disease *in vivo*, but a lot of useful information has been gained from *in vitro* research.

In recent years there has been considerable progress in understanding the role of flagella in *Campylobacter* virulence. The obvious function of the flagellum is to confer motility: In mutant strains with defective flagella, the organism is unable to colonize the intestinal epithelium. However, it has now become clear that there is more to flagellar function than motility, and that *Campylobacter* organisms also utilize their flagella to export virulence proteins.

Interestingly, *Campylobacter* seems to share few of the known virulence factors of other enteric bacteria; one exception is the bacterial capsule. Capsular polysaccharides are found on the bacterial cell surface of many organisms including *Campylobacter*; they appear to influence *Campylobacter* virulence by a number of mechanisms, particularly adhesion and invasiveness.

Campylobacter appears to differ from other enteric organisms with regard to posttranslational glycosylation. *Campylobacter* has been shown to have two separate systems, known as *N*-linked and *O*-linked glycosylation, which determine virulence by influencing adhesion and invasion.

Campylobacter strains exhibit marked genetic variability and this is reflected in the wide variation in virulence between the strains. This variability may also facilitate adaptive processes such as antibiotic resistance.

Epidemiology

Incidence

Campylobacter is the most commonly reported cause of bacterial gastroenteritis in the developed world, and the global disease burden of campylobacteriosis is considerable.

The Centers for Disease Control and Prevention (CDC) report that the annual incidence in the USA is approximately 13 cases per 100 000 population, with an estimated 2.4 million persons affected each year. They estimate that there are approximately 124 fatal campylobacteriosis cases every year, and that approximately 1 in 1000 diagnosed infections lead to GBS. The healthcare costs of GBS are high because of the frequent need for ventilation or other intensive care-based supportive measures.

In 2007, the European Food Safety Authority estimated that the European Union campylobacteriosis incidence was 45.2 cases per 100 000 population (200 507 confirmed cases).

The highest reported incidence of campylobacteriosis in the developed world is seen in New Zealand, where the national notification rates reached approximately 380 cases per 100 000 population in 2006. The incidence has declined in subsequent years but remains high.

Estimating disease incidence in the developing world is more difficult because of the lack of routine surveillance data. However, estimates of 90 cases per 100 000 population have been reported in the literature, derived from case–control studies.

Surveillance data from the industrialized nations showed a clear trend of increasing incidence in the last decades of the twentieth century, which appeared to be over and above what would be normally expected from improved detection and reporting alone. The reason for this increase is unknown.

More recently, there has been a trend of decreasing incidence in some countries. This decline may be a result of prevention measures related to poultry meat which have been instituted in several countries; however, reductions have also been seen in countries without such measures (see the Section on Control/Preventive Measures).

There has also been concern that global climate change may lead to an increased incidence of campylobacteriosis. However, the relationship between climate and disease incidence is not fully understood, and it is thus difficult to model changes in disease risk with climate change. It is not necessarily the case that disease incidence would increase with climate change. For example, if ultraviolet levels were to increase significantly, this would tend to decrease *Campylobacter* survival in the environment.

Origins and Distribution of Disease

There are a number of potential sources of *Campylobacter* infection (**Figure 2**). They include:

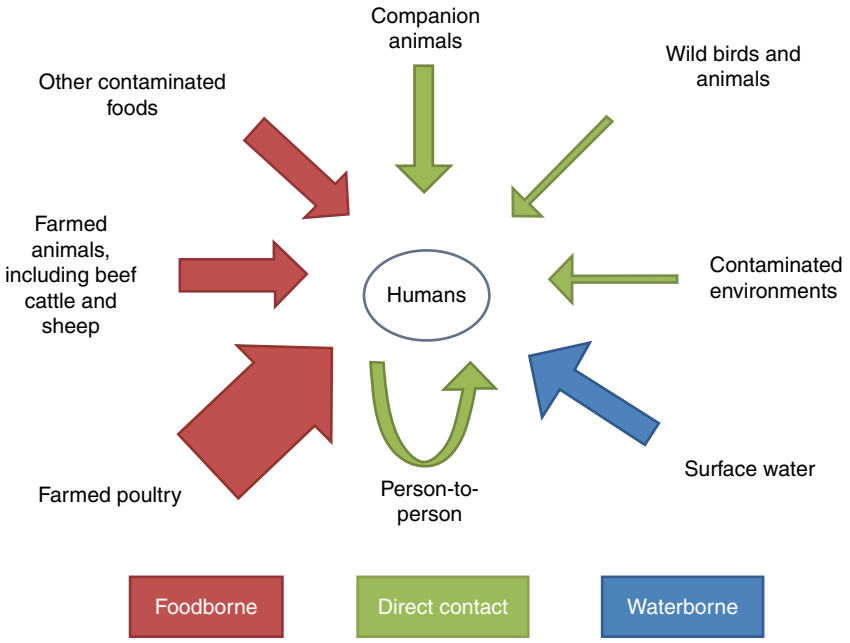

Figure 2 Most important routes for human infection by *Campylobacter jejuni*.

- Contaminated food – including consumption of undercooked food and through cross-contamination;
- Contaminated water – via drinking or recreational exposure;
- Contaminated environments – such as fecally contaminated pasture;
- Direct contact with infected animals – occupationally or domestically;
- Contact with infected humans – person-to-person transmission.

Contaminated food is thought to be the dominant source of infection in industrialized nations. Many specific foods have been identified as contributing, based on case–control studies of sporadic disease and investigations of outbreaks. However, poultry is considered the most important single source. Infection occurs after ingestion of undercooked poultry meat or by contamination of other foods with raw chicken during food transport, storage, and preparation.

Infection may occur in a variety of settings. Contact with animals may occur in the home (domestic pets) or on farms; and rural environments may also facilitate transmission via contaminated pasture, soil, or water. Foreign travel, particularly to developing countries, is also an important setting for populations in industrialized nations.

In developing nations the main drivers of infection are likely to be living conditions with close proximity to animals, and contaminated water supplies.

Campylobacter outbreaks are relatively rare compared to sporadic cases. Outbreaks tend to be small; the exceptions are outbreaks resulting from milk or waterborne infection which may affect a large number of people. Such an outbreak was reported in May 2000 in Walkerton, Canada, when municipal water supplies became contaminated and a large number of campylobacteriosis cases were reported as a result. Ingestion of unpasteurized milk on farms open to the public is a well-described source of outbreaks. Investigations continue to identify unusual modes of transmission such as a *Campylobacter* outbreak in Canada traced to the ingestion of mud by participants in a bicycle race.

Contamination of food in a retail or restaurant setting may cause small outbreaks, and a number of foods have been implicated including poultry, fresh produce, fish, and shellfish. A common mechanism of infection in restaurants is cross-contamination from raw poultry to foods waiting to be served.

Person-to-person transmission appears to be rare, and this perhaps explains the generally limited nature of *Campylobacter* outbreaks.

Factors Determining the Disease Risk in Individuals

A number of characteristics have been identified which may place individuals at a greater risk of infection.

Age

Most populations in industrialized nations show a bimodal age distribution with the highest incidence seen in young children and young adults.

It has been suggested that the peak in campylobacteriosis notifications amongst preschool children may relate to the relatively naïve immunological systems of children and infants. Other possible explanations include more frequent exposure to *Campylobacter* reservoirs (such as domestic pets), increased environmental exposure through playing outdoors, or poorer levels of hygiene. Another possibility is that higher levels of parental concern surrounding diarrheal illness in children may lead to a lower threshold for medical assessment and testing, resulting in a higher diagnosis rate among children in this age group.

In a number of epidemiological studies of *Campylobacter* prevalence, young children in rural areas have been shown to

be at a greater risk of the disease than other demographic groups (see the Section on Rurality).

The reasons for the peak in the 20–29 year age group remain unclear but may relate to factors such as increased contact with young children and their feces, particularly through nappy changing; increased occupational exposures to *Campylobacter* reservoirs; high-risk food consumption patterns (including increased consumption of food from commercial food premises); and less robust food hygiene practices.

In developing nations the age distribution of disease is somewhat different: The majority of symptomatic infections are seen in very young children, and adult disease is relatively rare.

Sex

Men have a higher rate of reported disease than women, and this has been shown to be a consistent finding across most populations and age groups.

A number of reasons for the male preponderance have been proposed, including possible underlying immunological factors (particularly as this male excess is seen in younger age groups where behavioral explanations are less likely); increased male exposure through high-risk occupations (such as farming and meat processing); differences in food and water consumption patterns (including quantity consumed and food preferences); and food hygiene practices.

Differences in healthcare-seeking behavior (and therefore, probability of diagnosis) have also been proposed as a possible reason for the reported differences between male and female campylobacteriosis rates.

Ethnicity

A number of epidemiological studies have identified variations in disease incidence between different ethnic groups living in the same geographical area. These differences may reflect a surveillance artifact with certain ethnic groups having differing healthcare seeking behavior and access to health services. Other possible explanations include differences in food preparation and providers, and travel to higher-risk countries. It is also possible that immunological factors play a part in determining variations in disease risk between well-defined ethnic groups, but to date no such factors have been identified.

Seasonality

In industrialized countries campylobacteriosis is often reported to have a higher incidence in spring and summer, whereas seasonality is generally not reported in developing countries.

The drivers behind the seasonality of campylobacteriosis remain unclear. It has been suggested that agricultural practices may play a role in campylobacteriosis seasonality, leading to increased fecal contamination of the environment during spring months. Other proposed mechanisms include differences in human behaviors (e.g., more outdoor activities possibly leading to increased *Campylobacter* exposure and greater use of barbecuing with associated consumption of undercooked meat such as chicken) and seasonal changes in human immunity.

In their analysis of *Campylobacter* seasonality in England and Wales, Louis *et al*. found a strong association with temperature, with children under the age of five experiencing the highest risk during seasonal peaks.

Campylobacter is a common contaminant of surface water. A recent study demonstrated an association between a seasonal peak of campylobacteriosis resulting from a common strain and contamination of surface water with that strain, where the association did not appear to be mediated by poultry consumption. This finding suggests that seasonality may be linked to the ability of certain strains to survive in the environment.

Overall, it is likely that the mechanisms by which seasonal variation may occur are multifactorial.

Rurality

The relationship between rurality and disease risk is not straightforward. In some populations the risk is higher in rural areas compared to urban areas, with rural children considered to have the highest risk; in others, the risk is higher for urban dwellers.

Source attribution studies from New Zealand have identified a difference in origin of infection between rural and urban areas: Rural cases are more likely to be of ruminant origin, whereas urban cases are more strongly associated with poultry. This suggests that the risks may be different for rural and urban populations: For instance, contact with animals (either directly or *via* contaminated environments) may be a significant risk in rural areas, whereas foodborne disease is likely to be relatively more important in urban areas.

Measuring Disease Burden, Incidence, and Prevalence

A degree of caution should be employed when assessing the impact of *Campylobacter* infection at a population level. Most infections are self-limiting and do not require medical attention: of those gastroenteritis cases that do, only a proportion will be investigated in such a way as to establish a proven *Campylobacter* diagnosis (**Figure 3**). In the UK, for example, a large population based study found there were cases of laboratory-confirmed *Campylobacter* infection in the community for every case recorded by the disease surveillance system, with most cases never being presented for medical attention.

Variation in disease risk is very difficult to measure because surveillance practices, self-reporting of disease, and access to

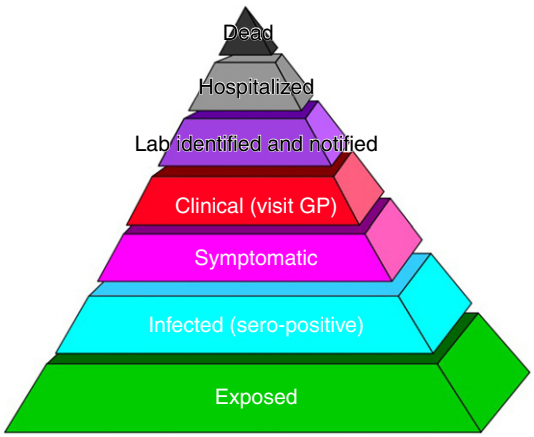

Figure 3 The pyramid or iceberg of infectious disease.

healthcare vary between regions, population groups, and individuals, and this is likely to exaggerate or mask true differences in risk. For example, campylobacteriosis as a result of foreign travel may be over-represented as a risk factor because people are more likely to seek medical advice for gastroenteritis if they have recently traveled abroad.

Analytical Methods

A range of analytical methods are used to investigate campylobacteriosis within the food safety area. These methods are concerned with: confirming illness in cases; identifying and quantifying organisms in potential sources and reservoirs; typing organisms to establish relationships between cases and with sources; investigating and identifying the sources of outbreaks; and investigating and quantifying the sources of sporadic disease. They use a mix of laboratory, epidemiological, and environmental investigation methods.

Laboratory Identification of *Campylobacter* spp. and Strains

Traditional Methods

Traditional methods of identification include direct detection in stool samples, and culturing of the organism followed by phenotypic tests. Gram staining has high sensitivity and specificity during the acute phase of the disease, and the organism may be detected in blood and stool cultures, or in food samples.

Colonies which have a typical Gram stain appearance and are oxidase positive may be considered provisionally to be *Campylobacter* species, and the hippurate hydrolyis test can be used to identify *C. jejuni*.

However, culturing the organism has a number of limitations, most notably in the time taken to obtain results. It usually takes at least 48 h to identify a positive result, but the time lag may be as much as five days if only a few organisms are present and an enrichment culture is required. This delay may seriously hamper the investigation and control of outbreaks. Culture methods may also introduce a bias against detecting rarer *Campylobacter* species, leading to an underestimation of their relative importance.

Serological approaches are also effective for identifying *Campylobacter* with latex-agglutination and enzyme-linked immunosorbent (ELISA) methods having high specificity and sensitivity.

Molecular Methods

The complete genome sequence has been determined for a number of *Campylobacter* strains, and this has enabled the use of a large array of molecular-based methods for investigating and characterizing the organism.

Molecular techniques offer more precision than phenotypic methods in identifying specific strains, and are thus extremely useful in linking cases and sources of infection in outbreak investigations, and in understanding the pathogenesis and determinants of virulence of campylobacteriosis. Although molecular diagnostic techniques are not yet generally used in routine *Campylobacter* identification, this may change in the near future.

One molecular method that has proved useful for investigating the *Campylobacter* organism is pulsed-field gel electrophoresis (PFGE). In this method an enzyme is used to digest the genome into fragments of different sizes, which can then be separated and visualized using gel electrophoresis.

Multilocus sequence typing (MLST) is another useful technique in *Campylobacter* research. MLST is a genotyping method in which bacterial isolates are characterized by indexing the variation present in several genes, thus allowing gene lineages to be identified. Comparisons can then be made between human isolates and isolates obtained from other sources such as food or water.

A number of polymerase chain reaction (PCR) assays have been described for species-specific identification of *Campylobacter*. In this method, specific genetic locations are targeted and amplified. PCR has shown particular promise in studies testing food products for *Campylobacter* contamination.

In an extensive review of molecular typing methods and their use in the investigation of Gram-negative foodborne pathogens, Foley *et al*. noted that no particular method is superior: instead, the choice of method will depend on the requirements of the investigation. For example, PCR-based methods can give rapid results, but PFGE results may be more consistent across multiple laboratories in a widespread outbreak. Limited resources may also be a consideration, as methods differ in their requirements for expensive equipment and highly trained personnel.

Although molecular techniques are exciting to contemplate, offering the possibility of resolving many of the unanswered questions in *Campylobacter* research, it is worth noting that no method is infallibly precise.

The methods described above may produce varying results when used to differentiate between strains; sometimes the best approach is to use a combination of methods. Moreover, because *Campylobacter* exhibits high strain variability, it may not be clear whether two similar, yet nonidentical, strains are epidemiologically related or should be considered to be separate entities. Different statistical modeling approaches may lead to different conclusions in this regard.

It is thus important to be aware that molecular methods are not intended for use in isolation, but rather in association with, and complementary to, epidemiological data. Only in this way can a full and accurate picture be obtained.

Investigating Campylobacteriosis

Common-Source Outbreaks

Outbreak investigation uses two main types of epidemiological investigations, with the choice largely determined by the type of outbreak. Outbreaks affecting a well-defined group, such as a wedding party, are usually investigated using a retrospective cohort study. Dispersed outbreaks, such as might occur from a widely distributed food or drink, are usually investigated using a case–control study. Such studies have some important limitations when investigating campylobacteriosis outbreaks. Given the relatively long incubation period, and the wider range of potential sources that such studies often need to investigate, they are prone to recall bias. They are also limited to investigating immediate sources of infection, and cannot usually identify reservoirs of infection.

Although it is generally accepted that *Campylobacter* outbreaks are far less common than sporadic cases, the increasing use of molecular techniques may change this perception by demonstrating links between cases which previously would have been regarded as discrete entities.

Conversely, molecular methods can also show that a case which appears to be linked to an outbreak is in fact a separate occurrence. This ability to exclude unrelated cases is very useful in epidemiological studies, as inclusion of unrelated cases in an outbreak investigation may obscure a true association.

Source Attribution

A key challenge in *Campylobacter* investigation is source attribution, that is, the ability to identify and quantify the contribution of different sources to the observed burden of disease. These studies are usually concerned with the total burden of disease, which for campylobacteriosis is largely sporadic infections and small clusters. A variety of approaches are available, including microbiological and epidemiological approaches, intervention studies, and expert elicitation.

This is where molecular techniques are particularly useful because they allow investigators to differentiate between organisms at strain level, and hence to link cases to likely sources of infection.

A number of studies have been published worldwide, demonstrating the use of molecular techniques in source attribution. For example, Frost *et al*. reviewed reported outbreaks of campylobacteriosis in the UK from 1995 to 1999, and found that foodborne transmission accounted for 70% of the outbreaks, with implicated food types including poultry, red meat, fish and shellfish, salads, fruits and vegetables, milk and milk products. Animal contact and person-to-person transmission were thought to account for 18% of the cases, and waterborne transmission in rural areas accounted for 8%.

Although some variation in results can be seen between regions and between studies, there is a consistent finding that foodborne infections form the majority of reported campylobacteriosis infections in industrialized nations, and that within these, poultry is the type of food most commonly implicated in the disease.

These findings are particularly important in determining appropriate control measures for the prevention of *Campylobacter* infection.

Control/Preventive Measures

The major components to *Campylobacter* control are:

- Treatment and management of infected individuals;
- Control of outbreaks;
- Prevention of *Campylobacter* infection;
- Surveillance to support prevention and control measures.

Treatment of *Campylobacter* Infection

The World Health Organization (WHO) advises that apart from supportive measures such as electrolyte replacement and rehydration, treatment is not generally indicated for campylobacteriosis. Antimicrobial treatment (erythromycin, tetracycline, or quinolones) may be indicated in severe cases or to eliminate the carrier state. Severe cases or cases with complications (e.g., GBS) may require admission to an intensive care unit for appropriate support.

Antibiotic resistance among *Campylobacters* is of great concern. The routine use of fluoroquinolones in commercial poultry flocks led to an increase in the prevalence of human *Campylobacter* isolates testing positive for ciprofloxacin resistance. In 2005, the Food and Drug Administration (FDA) in the US withdrew approval for these agents for use in poultry.

Control of Outbreaks

An important public health function is the rapid identification and control of common-source outbreaks, particularly if they are continuing or likely to recur. In the case of campylobacteriosis, this activity is relatively less important than for some enteric infections because the bulk of disease does not occur as part of recognizable outbreaks. As noted, there is a range of analytical methods available to support such investigations.

Prevention of *Campylobacter* Infection

Preventing *Campylobacter* infection is probably the most important and challenging component of global efforts to reduce the public health impact of this infection. Because the organism is widespread in the environment and in food sources, exposure is common. The infective dose is low. Consequently, a successful control program many need multiple components to reduce the risk of infection to acceptably low levels.

It is useful to consider approaches to *Campylobacter* prevention in terms of the important sources and settings of the infection. They are:

1. Contaminated food
2. Contaminated water
3. Contaminated environments, particularly in rural settings
4. Direct contact with infected animals
5. Direct contact with infected people
6. Foreign travel

Each source of infection and its relevant control measures will be considered separately, and all have a place in the prevention of *Campylobacter* infection. However, because foodborne routes of transmission account for the majority of reported *Campylobacter* infections, public health strategies for reducing infection risk have prioritized foodborne transmission in the control of *Campylobacter* infection.

There is a strong focus internationally on preventing the transmission of infection by contaminated poultry products, because analytical studies have consistently indicated that the majority of human *Campylobacter* infections are from poultry products. Furthermore, poultry contamination levels are known to be high. In their 2008 meta-analysis of *Campylobacter* contamination of retail poultry, Suzuki *et al*. found the prevalence of contamination to be 58% when averaged across the results of 72 studies worldwide. Consequently, the

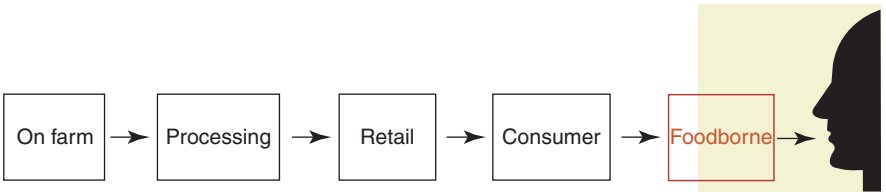

Figure 4 Control of foodborne *Campylobacter* infections: Points in the food production chain where interventions can operate.

following discussion provides most specific details on control of *Campylobacter* in chicken (Figure 4).

1. Control of foodborne *Campylobacter* infection

It is useful to consider each stage of food production in turn. They are:

a. On-farm/primary production
b. Processing
c. Retail and distribution
d. Consumer

a. On-farm/primary production: Primary prevention strategies include experimental concepts such as the selection of genetically resistant animals, as well as practical measures such as preventing contamination of the environment on farms and during transport of animals (frequently referred to as biosecurity measures). Other proposed measures include prevention of vertical transmission, eliminating *Campylobacter* from drinking water given to animals, additives to feed and water that may prevent colonization, and biological methods including vaccines. Of particular importance is the cleaning and disinfection of chicken houses before re-stocking, cleaning of transportation crates, and routine surveillance of flocks. Poultry meat arising from contaminated flocks may be designated for freezing only, a strategy that has proved effective in Iceland. This approach may also provide a financial incentive for farmers to keep their flocks free of *Campylobacter*, as frozen meat is less desirable to the consumer and therefore attracts a lower retail price.

   In future, primary prevention is likely to be an extremely effective strategy in the control of human disease; however, elimination of *Campylobacter* is expensive and difficult to achieve with currently available technology. As *Campylobacter* colonization does not appear to have an impact on the health and welfare of animals, there may be little perceived benefit of primary prevention for food producers.

b. Processing: This stage includes slaughter of broiler chickens, which involves a high risk of cross-contamination during scalding, plucking, and evisceration procedures. The risk may be reduced by the use of disinfectants such as acidified sodium chlorite.

   In general, it is more cost-effective to control contamination of the finished food product. Milk production is a good example of this: it is difficult to prevent raw milk from becoming contaminated with *Campylobacter*, but pasteurizing milk before distribution is an effective method of inactivating the organism.

   Various methods can be employed in the treatment of contaminated food. *Campylobacter* is sensitive to heat and can be eliminated by pasteurization or normal cooking temperatures. Freezing will reduce organism numbers, albeit to a limited extent, and other physical measures such as acidification (as occurs in pickling), salt, and irradiation can be effective. The organism's limited ability to survive in air at room temperatures reduces the risk from cooked food.

c. Retail and distribution: Control measures at retail level include the use of leak-free packaging and monitoring of *Campylobacter* contamination levels in retail poultry, as well as regulation of restaurants and commercial food premises.

d. Consumer: Measures available to the consumer include care with food preparation so as to avoid contamination of fresh food with raw meat, and cooking all meat thoroughly. This information can be conveyed to consumers as a targeted education campaign. However, the evidence base supporting the effectiveness of consumer education is very limited. Changing consumer behavior is difficult. There are also technical limitations. Kitchen contamination studies show that preparation of meals with raw chicken results in widespread cross contamination of hands, plates, chopping boards, utensils, and ready-to-eat food.

2. Contaminated water

Measures to decrease the risks from contaminated water include:

- Provision of a clean treated municipal water supply in urban areas
- Support for local water treatment methods for rural areas
- Public education about drinking water from safe sources
- Effective sewage treatment and disposal

3. Animals: The CDC advice regarding *Campylobacter* and contact with domesticated animals stresses the importance of handwashing. Persons who are immunocompromized are advised to be particularly cautious of contact with animals, especially if the animals have diarrhea. This advice is available to the public on the CDC website.

4. Contaminated environments: The epidemiological evidence indicates that children living in rural areas are particularly at risk from contaminated environments. This exposure source is difficult to control, although caregiver awareness of this risk (with associated hygiene measures) may help minimize *Campylobacter* infection in this setting.

5. Direct contact with infected people: In any healthcare setting where campylobacteriosis patients are treated, it is essential to ensure adequate handwashing and enteric precautions to avoid further transmission of the infection. Similar control measures are also necessary in settings such

as childcare centers where there is a higher risk of contact with contaminated feces.
6. Travelers: The WHO has published food safety advice for travelers which is available on their website. Contamination of drinking water is likely to be of particular concern to travelers in developing countries.

Surveillance

Surveillance is critically important to support both prevention and control measures for campylobacteriosis. It has two main purposes. In common with other infectious diseases

1. Control-focused surveillance – provides information to support control measures, notably the identification of outbreaks. This is the reason why campylobacteriosis is a notifiable disease in many countries and also frequently under surveillance by laboratories.
2. Strategy-focused surveillance – provides information to support prevention strategies. This includes a wider set of aims, notably:

- Monitoring the occurrence and distribution of disease, including epidemiological, clinical, and microbiological features – for example, to describe disease burden and the impact of interventions.
- Monitoring the occurrence and distribution of hazards, risk factors and determinants – for example, contamination levels in fresh poultry.
- Monitoring coverage and effectiveness of interventions – for example, the extent to which food retailers are complying with food safety standards.

Surveillance systems established for control-focused surveillance generally also support strategy-focused aims. This is particularly the case for notifiable disease surveillance where a well-functioning surveillance system should be able to alert health authorities in good time when a common-source *Campylobacter* outbreak has occurred locally. Data gathered by the system can be very helpful in identifying and eliminating the source as quickly as possible. These data are also useful for describing incidence trends at the local, regional, and national levels.

The USA, for example, has several highly developed systems in place for *Campylobacter* surveillance, and data compiled by these systems have contributed to scientific knowledge about campylobacteriosis as well as facilitating disease control.

The CDC has been operating a national *Campylobacter* surveillance program since 1982. National surveillance is conducted through the Public Health Laboratory Information System (PHLIS). In response to an increasing need for detailed information on foodborne diseases, an active surveillance system was instituted in five sites in 1996 and subsequently expanded to ten sites. This is known as the Foodborne Diseases Active Surveillance Network, or FoodNet.

FoodNet monitors the incidence and trends of human *Campylobacter* infection and conducts studies to identify risk factors for infection. A key feature of FoodNet is its close links with other agencies. FoodNet is a collaborative project of the CDC, 10 Emerging Infections Program (EIP) sites, the US Department of Agriculture (USDA), and the FDA. The USDA conducts research on prevention in poultry, and the FDA has developed a model for safety in the food industry (the Food Code). This is regularly updated and is used as the basis for food safety controls in the USA. In addition to these systems for monitoring campylobacteriosis, the National Antimicrobial Resistance Monitoring System (NARMS) conducts surveillance on antimicrobial resistance of *Campylobacter*. Data acquired from these surveillance systems are regularly analyzed and the results have added considerable knowledge about *Campylobacter* epidemiology and control.

Many of the interventions outlined above have been in use for some years in various countries, and the key question is: Are they succeeding in their aim of reducing *Campylobacter* infections? A number of countries, including the USA, the UK, Iceland, Denmark, Norway, and Sweden have reported a decline in campylobacteriosis rates following the introduction of control measures in poultry production. However, one of the most dramatic declines of recent years occurred in New Zealand during the years 2007–2008.

Campylobacteriosis first became notifiable in New Zealand in 1980. Over time, notifications and hospitalizations for campylobacteriosis were seen to increase, and this was found at least in part to reflect a true increase rather than a notification artifact. By 2006, campylobacteriosis notifications in New Zealand exceeded 380 per 100 000 population. This exceptionally high incidence was investigated using standard epidemiological methods and source attribution modeling, and the results strongly implicated poultry as the major source of infection.

Accordingly, a number of interventions were introduced, with the aim of reducing the risk of poultry-associated foodborne *Campylobacter* infections in New Zealand. These interventions included implementation of an updated industry Code of Practice for primary processing of poultry with mandatory targets; monitoring and reporting of contamination levels during production; leak-proof retail packaging; consumer education; and enhanced human campylobacteriosis surveillance and source attribution research.

Using the period 2002–2006 as the baseline for comparison, campylobacteriosis notifications and hospitalizations showed a decrease of greater than 50% in 2007–2008, coinciding with the introduction of the above interventions. Analysis of the surveillance data indicated that the decline was unlikely to have occurred by chance, with the interventions being the most likely explanation for the decline. This conclusion was also supported by source attribution modeling work undertaken over the same period, with a reduction seen in the proportion of human cases attributable to poultry, as compared to other sources. Although no single intervention could be identified as being responsible for the reduction in notifications, key informants identified two particularly important individual measures: the monitoring and reporting of *Campylobacter* enumeration on carcasses at the end of primary processing; and mandatory *Campylobacter* performance targets for contamination levels at the end of primary processing.

Research Needs

Pathogenesis Research

One very important research need is for a better understanding of *Campylobacter* pathogenesis, including the relationship of immunological and virulence factors to disease in humans and animals. For example, it would be helpful to be able to predict which campylobacteriosis patients are most at risk of serious complications. A detailed understanding of disease and resistance mechanisms would greatly facilitate the search for new, effective methods of prevention and control.

Research in this field has been considerably hampered by the lack of readily available animal models. Mouse models are used for studying immunity and vaccine responses in a number of diseases. However, mice are not naturally colonized by *Campylobacter* and do not usually develop diarrhea, necessitating cautious extrapolation from mouse studies to studies of human disease.

Chicken models are helpful in studying colonization and *in vivo* survival of the organism, but provide very limited information on the mechanisms of gastroenteritis, as chickens are frequently colonized by *Campylobacter* yet rarely develop symptomatic disease.

However, studying *Campylobacter* infection in chickens is a fruitful line of research in its own right, as successful strategies for reducing infection in the chicken population are likely to reduce the risk of foodborne infection in humans as well.

In the absence of good animal models, useful information has been derived from studies using human volunteers; but recent research has highlighted the importance of campylobacteriosis as a cause of GBS, and this raises considerable ethical concerns about exposing volunteers to the risk of such a serious complication. Work continues on developing new animal and tissue models.

Vaccines

There is much research interest in the possibility of vaccination both for humans and poultry.

Methods under investigation for human vaccines include whole-cell vaccines (using live attenuated organisms or killed organisms); subunit vaccines; and live attenuated *Salmonella* strains expressing *Campylobacter* proteins.

There is concern about the potential of whole-cell vaccines to cause GBS by inducing cross-reactivity with ganglioside cells. This in turn indicates an urgent research need for clear information about the mechanisms of GBS induced by *Campylobacter* infection, in order to avoid the complication of vaccine-induced paralysis.

There is also considerable research interest in developing a *Campylobacter* vaccine for chickens, as a way of decreasing the prevalence of contamination of poultry flocks.

Laboratory Methods

There is an ongoing need for sensitive, rapid, and cost-effective *Campylobacter* detection methods for food testing and surveillance purposes. The ability to test quantitatively is important as this allows food industry standards to be set and maintained, and quantitative testing can demonstrate specific steps in food production where the risk of contamination is unacceptably high.

There is also a need for robust methods of strain differentiation in order to continue source attribution work. As current control methods become effective, the relative contribution of the various sources of infection may change. There is also likely to be a geographical variation in relative contribution, for example, between rural and urban populations, and a clear understanding of this variation will inform the development of control strategies appropriate to specific circumstances and environments.

Other *Campylobacter* Species

As detection methods become more sensitive, it is possible that further *Campylobacter* species will be implicated as pathogens in the human disease. It will be important for clinical diagnosis and effective surveillance to take this evolving knowledge into account.

Novel Control Strategies for Poultry

Several novel control strategies are being investigated, with the goal of eliminating or reducing *Campylobacter* contamination of poultry flocks. They include:

- Competitive exclusion: The principle of competitive exclusion is that if the chicken gut is colonized with harmless bacteria at an early age, there will be less opportunity for colonization with harmful organisms. This has already been shown to be effective in limiting colonization with *Salmonella* spp., and work is now in progress to develop competitive exclusion products suitable for *Campylobacter*.
- Bacteriocin treatment: Bacteriocins are proteins produced by bacteria. They are of interest because they have an antimicrobial action against other bacteria, yet offer several advantages over standard antimicrobial therapy. Bacteriocin treatment for *Campylobacter* is still at an experimental stage, but appears promising.
- Bacteriophages: Bacteriophages (also known as phages) are a promising area of *Campylobacter* research. These are described as natural predators of bacteria, and as such may have useful antimicrobial properties. A number of *Campylobacter*-associated phages have been identified and work is in progress to establish their potential to control *Campylobacter* contamination of poultry flocks.
- Vaccination as mentioned above.

Climate Change

Given the high current global burden of campylobacteriosis, it will be important to monitor the impact of climate change on the epidemiology of *Campylobacter* infection.

See also: Foodborne Diseases: Prevalence of Foodborne Diseases in Europe; Prevalence of Foodborne Diseases in North America.

Public Health Measures: Foodborne Disease Outbreak Investigation; Modern Approach to Food Safety Management: An Overview; Surveillance of Foodborne Diseases. Safety of Food and Beverages: Milk and Dairy Products; Water (Bottled Water, Drinking Water) and Ice

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WHO.

Clostridium botulinum

MW Peck, Institute of Food Research, Norwich Research Park, Colney, UK

Glossary

Botulinum neurotoxin Most potent toxins known (30 ng is sufficient to cause illness and possibly death), formed by *Clostridium botulinum* and some strains of *C. baratii* and *C. butyricum*, seven neurotoxin types (types A–G).

Botulism A severe and often fatal neuroparalytic intoxication caused by a botulinum neurotoxin.

Clostridium A diverse genus of Gram-positive rod-shaped endospore-forming anaerobic bacteria.

Clostridium botulinum A heterogeneous species containing four phylogenetically and physiologically distinct bacteria that share the common feature of forming the botulinum neurotoxin.

Background

A disease frequently reported in Central Europe in the eighteenth and nineteenth centuries involved muscle paralysis, breathing difficulties, and a high fatality rate. This disease was often associated with consumption of blood sausage, and came to be known as 'botulism,' after the Latin word *'botulus'* meaning sausage. A major study by the Chief Medical Officer of Wurttemburg (Germany) in 1793 of 19 cases (six fatal) of muscle paralysis and breathing difficulties reported that the illness may have been due to consumption of nightshade leaves or berries, although consumption of locally produced blood sausage *blunzen* (pig stomach filled with blood and other ingredients, briefly boiled, preserved by smoking) was also considered. In July 1802, however, a warning notice was issued about consumption of harmful smoked blood sausages. Following this outbreak, there was an increase in the number of reported cases of sausage poisoning, prompting a study by Justinius Kerner. He described more than 200 cases, made suggestions for its prevention and treatment, and proposed that anaerobic conditions are required for toxin formation, and that the toxin was a highly potent biological neurotoxin.

In December 1895, more than 100 years after the outbreak in Wurttemburg, Emile van Ermengem investigated an outbreak of botulism in Ellezelles (Belgium). This outbreak affected 23 musicians (three fatally) who consumed homemade raw salted ham at a funeral wake. Importantly, van Ermengem isolated an obligately anaerobic spore-forming bacterium from cultures of ham. He called the isolated bacterium '*Bacillus botulinus*,' and although now lost, the physiological properties are consistent with those of nonproteolytic *Clostridium botulinum*. He also demonstrated that portions of macerated ham, filtered extracts, and bacterial culture filtrates caused flaccid paralysis and were toxic to various animal species. Thus, it was conclusively demonstrated that botulism was not an infection but an intoxication caused by a toxin formed by '*B. botulinus*.' At this time there was an increased use of canning and bottling processes both commercially and in the home to extend the shelf-life of foods. In 1904, Landmann investigated a large botulism outbreak in Darmstadt (Germany) that affected 21 people (11 fatally) that was associated with canned white beans. An obligately anaerobic spore-forming bacterium was again isolated, but the neurotoxin type was different from that formed by the strain isolated by van Ermengem, and could be distinguished by differential neutralization with specific antisera. The bacterium, also now lost, was probably proteolytic *C. botulinum*. The use of canning and bottling processes increased over the next few decades, and resulted in many hundreds of botulism cases as ineffective heat treatments were used. For example, the first reported outbreak of foodborne botulism in the UK (in 1922) affected eight people (all fatally), and was associated with consumption of a picnic lunch of sandwiches containing (underprocessed) wild duck paste at Loch Maree (Scotland). Between 1920 and 1930 the number of botulism cases fell following the identification and successful application of a standard minimum heat treatment (known as the botulinum cook) for canned and bottled foods. Since this time, occasional, but often severe, outbreaks of foodborne botulism have been reported in many countries either when known control measures have not been implemented, or when unsafe processes have been followed.

Characteristics of the Bacterium and Neurotoxins

Characteristics of the Six Bacteria that Form Botulinum Neurotoxins

Clostridium is a phylogenetically diverse genus of Gram-positive rod-shaped endospore-forming anaerobic bacteria.

This genus includes more than 100 species, many of which are pathogenic, including *C. botulinum*, *Clostridium tetani*, *Clostridium difficile*, and *Clostridium perfringens*. All bacteria that form the botulinum neurotoxin are members of the genus *Clostridium*. The species known as *C. botulinum* is defined on the basis of just one physiological property, which is the ability to form botulinum neurotoxin, rather than any close phylogenetic relationship. This approach is taken in order to recognize and emphasize the importance of neurotoxin formation. *C. botulinum* is in fact a heterogeneous species that can be separated into four distinct phylogenetic groups (*C. botulinum* Groups I–IV), with the distinction among the groups strong enough to merit separation into four different species. For each of the four groups, a phylogenetically equivalent, but non-neurotoxigenic, organism has been described. For example, strains of *C. botulinum* Group I are very closely related to *Clostridium sporogenes*, but only distantly related to strains of other *C. botulinum* groups. A relatively recent discovery has been that some strains of *Cbutyricum baratii* and *Cbutyricum butyricum* also possess the ability to form botulinum neurotoxin, and except for this ability they are indistinguishable from other more typical strains. The different physiological properties of the six botulinum neurotoxin-forming clostridia reflect their different genetic backgrounds.

Proteolytic C. botulinum *(*C. botulinum *Group I)*

Proteolytic *C. botulinum* is frequently associated with foodborne botulism, and also with wound and infant/intestinal botulism. Strains possess one or two neurotoxin genes of type A, B, or F (Table 1). Some of these strains form two active neurotoxins, with the major and minor neurotoxin types designated by an uppercase and lowercase letter. For example, type Ab strains form a larger quantity of type A neurotoxin than type B neurotoxin. Other strains form only one active neurotoxin; some have a single neurotoxin gene, whereas some have two neurotoxin genes, one of which does not encode an active neurotoxin. For example, type A(B) strains possess a type A and a type B neurotoxin genes, but only form active type A neurotoxin. The neurotoxin gene(s) is (are) located on the chromosome or on a large plasmid, and have evolved independently of the remainder of the genetic complement. Proteolytic *C. botulinum* is a mesophilic bacterium with a minimum growth temperature of 10–12 °C, although some

Table 1 Characteristics of the six physiologically and phylogenetically distinct clostridia that form the botulinum neurotoxin[a]

Neurotoxigenic clostridia	*Proteolytic* C. botulinum *(*C. botulinum *Group I)*	*Nonproteolytic* C. botulinum *(*C. botulinum *Group II)*	C. botulinum *Group III*	C. argentinense *(*C. botulinum *Group IV)*	C. baratii	C. butyricum
Neurotoxins formed	A, B, F[b]	B, E, F	C, D	G	F	E
Location neurotoxin gene	Chromosome/ plasmid	Chromosome/ plasmid	Bacteriophage	Plasmid		
Non-neurotoxigenic equivalent clostridia	*C. sporogenes*	No species name given	*C. novyi*	*C. subterminale*	All typical *C. baratii* strains	All typical *C. butyricum* strains
Minimum growth temperature (°C)	10–12	2.5–3.0	15		10–15 °C	12 °C
Optimum growth temperature (°C)	37–42	25–30	40	37	30–45 °C	30–37 °C
Minimum pH for growth	4.6	5.0	5.1			4.8
NaCl concentration preventing growth (%)	10	5		6.5		
Minimum water activity for growth, humectant: NaCl/ glycerol	0.94/0.93	0.97/0.94				
Spore heat resistance[c]	$D_{121\,°C}$=0.21 min	$D_{82.2\,°C}$=2.4/ 231 min[d]	$D_{104\,°C}$=0.9 min	$D_{104\,°C}$=1.1 min		$D_{100\,°C}$<0.1 min
Ferment glucose	+[e]	+	+	−	+	+
Ferment fructose	+/−	+	+/−	−	+	+
Ferment maltose	+/−	+	+/−	−	+	+
Ferment mannose	−	+	+	−	+	+
Ferment sucrose	−	+	−	−	+	+
Ferment trehalose	−	+	−	−	−	+

[a]Modified from Lund and Peck (2000).

Where values are absent, they are not readily available in literature.

[b]Two neurotoxins are formed by dual-toxin strains.

[c]Spore heat resistance determined in phosphate buffer, pH 7.0.

[d]*D*-value without/with lysozyme during recovery.

[e]+ all strains positive, +/− some strains are positive with others negative, − all strains negative.

strains grow poorly below 15 °C. The optimum growth temperature is in the range of 37–42 °C, and the maximum is approximately 48 °C. Growth and neurotoxin do not occur at pH < 4.6, or at ≥ 10% NaCl (Table 1). Spores of proteolytic *C. botulinum* are of high heat resistance and the principal concern for the safe production of low acid canned foods. The botulinum cook (121 °C for 3 min) is used by the canning industry as the standard minimum heat treatment for low acid canned foods. Quantifying the effect of various combinations of preservative factors on growth and neurotoxin formation and on thermal death is important to ensure the safety of foods with respect to proteolytic *C. botulinum*. Software packages, such as ComBase, are freely available that contain this information (www.combase.cc), and predictive models have been developed (www.combase.cc/predictor).

The genomes of several strains of proteolytic *C. botulinum* have been sequenced. All have a low %G + C content (28%) and are of a similar size (3.8–4.3 Mb). The genome of the type A strain ATCC 3502 (Hall 174) was the first to be sequenced, and comprised a circular chromosome (3.9 Mb, 3650 coding sequences (CDSs)), and a small plasmid (16.3 kb, 19 CDSs). The genomes of more recently sequenced strains of proteolytic *C. botulinum* show synteny with the ATCC 3502 genome. Based on whole genome analysis, it is estimated that for 61 strains of proteolytic *C. botulinum* and *C. sporogenes*, the core gene set comprises 2155 of the CDSs (approximately 63%) of ATCC 3502. These observations provide one line of evidence for the close genetic relationship between strains of proteolytic *C. botulinum* and *C. sporogenes*, and for a relatively stable genome. From analysis of the ATCC 3502 genome and various physiological tests, it seems that proteolytic *C. botulinum* is a bacterium well adapted to a saprophytic lifestyle, which relies on the highly potent neurotoxin to kill a wide range of species, and then a variety of extracellular enzymes to obtain nutrients. This bacterium is highly proteolytic, and also secretes enzymes dedicated to the catabolism of carbohydrates (including starch and chitin) and lipids. Five putative chitinases are widespread amongst strains of proteolytic *C. botulinum* and *C. sporogenes*, suggesting that chitin is an important source of carbon and nitrogen.

Nonproteolytic C. botulinum *(C. botulinum Group II)*

Nonproteolytic *C. botulinum* is primarily associated with foodborne botulism, with only a single case reported involving infant and wound botulism. Strains form a single neurotoxin of type B, E, or F (Table 1), and possess a single neurotoxin gene that is located on the chromosome or on a plasmid. Nonproteolytic *C. botulinum* is a psychrotrophic saccharolytic bacterium that ferments a wide range of sugars (Table 1). Nonproteolytic *C. botulinum*, unlike proteolytic *C. botulinum*, is not highly proteolytic. The minimum temperature for growth and neurotoxin formation is 2.5 °C–3.0 °C, although some strains grow poorly below 5 °C. The optimum growth temperature is approximately 25 °C–30 °C, and the maximum approximately 40 °C. Nonproteolytic *C. botulinum* does not multiply or form neurotoxin formation at pH < 5.0, or at ≥ 5% NaCl (Table 1). Spores of nonproteolytic *C. botulinum* are less heat resistant than those of proteolytic *C. botulinum*, and heat resistance is not related to the type of neurotoxin formed. Measured spore heat resistance can be substantially increased by the presence of lysozyme in the recovery medium. The highest reported *D*-value for spores heated in phosphate buffer at 82.2 °C (185 °F) is 2.4 min with recovery in the absence of lysozyme, and 231 min with recovery in the presence of lysozyme (Table 1). These heat treatments give sublethally injured spores that are unable to germinate. Lysozyme can circumvent the sublethal damage, and induce germination by hydrolyzing peptidoglycan in the cortex, enabling growth. This effect of lysozyme may be significant for food safety, as it is naturally present in many foods and is relatively heat-stable. Care also needs to be taken when using lysozyme as a food preservative. Quantifying the effect of various combinations of preservative factors on growth and neurotoxin formation, and on thermal death is important to ensure food safety, and software packages and predictive models are freely available (e.g., ComBase (www.combase.cc; www.combase.cc/predictor)).

At present, the genomes of only two strains of nonproteolytic *C. botulinum* have been sequenced. The genomes of these two strains are marginally smaller (3.7–3.8 Mb) than those of strains of proteolytic *C. botulinum*, and the %G + C is slightly lower (27%). However, although the genomes of the two nonproteolytic *C. botulinum* strains are similar to each other and show synteny, they are very different from the genomes of proteolytic *C. botulinum* strains and there is no synteny. This finding supports earlier tests that demonstrated the wide phylogenetic and evolutionary distance between proteolytic *C. botulinum* and nonproteolytic *C. botulinum*.

C. botulinum *Group III*

Strains of *C. botulinum* Group III are not associated with botulism in humans, but are responsible for botulism in birds (avian botulism) and animals (e.g., horses, cows, sheep, farmed foxes, and mink). *C. botulinum* Group III is a mesophilic saccharolytic bacterium, with strains forming either type C or type D neurotoxin. Strains have a minimum growth temperature of approximately 15 °C, and an optimal growth temperature of 40 °C (Table 1). Spores are of an intermediate heat resistance. The genome sequence of one *C. botulinum* Group III strain has been completed and other draft genomes are available. The small chromosome (2.8 Mb, %G + C = 28%) is highly conserved amongst isolates and closely related to that of *Clostridium novyi*, but not that of other *C. botulinum Groups*. The neurotoxin genes are located on a bacteriophage. Strains that lose this bacteriophage no longer form neurotoxin, and reinfection with the bacteriophage re-establishes neurotoxin formation. The bacteriophage can be easily lost in laboratory cultivation; thus maintenance of strains forming type C or type D neurotoxin is demanding. A bacteriophage containing a type C neurotoxin gene was found to contain linear double-stranded DNA of 186 kb with low %G + C content (26%), and its sequence has been published.

Clostridium argentinense *(C. botulinum Group IV)*

Strains of *C. argentinense* form type G neurotoxin. This bacterium has not been associated with botulism in humans, animals, or birds, although laboratory tests have shown that type G neurotoxin causes a typical flaccid paralysis in a range of animals. *C. argentinense* is a mesophilic proteolytic bacterium, with an optimum growth temperature of 37 °C. The

spores are of an intermediate heat resistance. *C. argentinense* was originally isolated from soils in Argentina, and is the least studied of the six botulinum neurotoxin-forming organisms. The *C. argentinense* genome has a low %G + C (28–30%), and is presently being sequenced. The type G neurotoxin gene is located on a single large plasmid of 123 kb. This plasmid and its ability to form neurotoxin are very easily lost in culture, and the resulting non-neurotoxigenic isolates are indistinguishable from *Clostridium subterminale*.

C. baratii *type F*

Strains of neurotoxigenic *C. baratii* type F have been associated with at least one case of foodborne botulism, and several cases of infant botulism and adult intestinal botulism. *C. baratii* type F is a mesophilic saccharolytic bacterium with a minimum growth temperature of 10–15 °C, and an optimum growth temperature of 30–45 °C (Table 1).

Typical strains of *C. baratii* that do not form neurotoxin and strains of neurotoxigenic *C. baratii* type F are closely related, but are phylogenetically separated from other botulinum neurotoxin-forming clostridia.

C. butyricum *Type E*

Outbreaks of foodborne botulism involving neurotoxigenic *C. butyricum* type E have been reported in Italy, China, and India, although human cases are more commonly associated with infant botulism and adult intestinal botulism. *C. butyricum* type E is a mesophilic saccharolytic bacterium, with a minimum growth temperature of 12 °C, and an optimum growth temperature of 30–37 °C (Table 1). Spores of neurotoxigenic strains of *C. butyricum* type E are of intermediate heat resistance. Neurotoxigenic strains of *C. butyricum* type E are closely related to the more commonly isolated nonpathogenic strains of *C. butyricum* that do not form neurotoxin, rather than other botulinum neurotoxin-forming clostridia.

Characteristics of the Seven Botulinum Neurotoxins and Their Encoding Genes

Properties of the Neurotoxins and Neurotoxin Complex

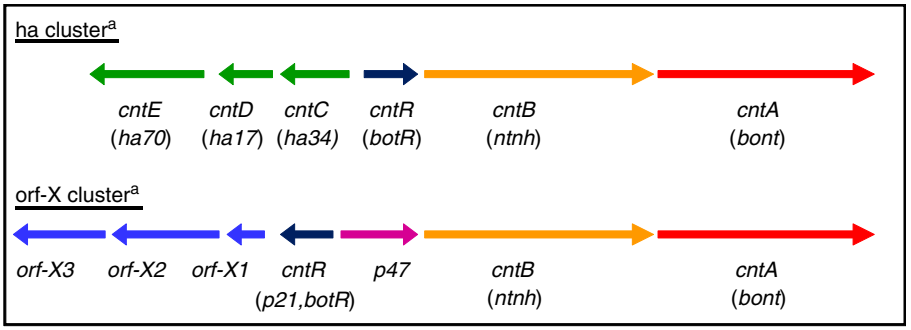
The botulinum neurotoxins are the most toxic substance known, being several orders of magnitude more potent than ricin or cyanide. The specific toxicity of botulinum neurotoxin complexes ranges from 3×10^7–10^8 MLD_{50} (mouse minimum lethal doses)/mg protein, and from cases of foodborne botulism and various animal experiments it has been estimated that symptoms of foodborne botulism or even death might be caused by consuming as little as 3000 MLD_{50} or 30–100 ng of neurotoxin (depending on the size of the neurotoxin complex). In a 2006 outbreak of foodborne botulism in Canada/USA involving temperature abused carrot juice (that contained 7×10^5 MLD_{50}/ml of neurotoxin), it appeared that consumption of only 5 µl of carrot juice, or a few micrograms, would have been sufficient to cause botulism. In reality, much larger quantities were consumed, resulting in severe cases of botulism. The botulinum neurotoxins are relatively sensitive to heating at 80 °C or higher. For example, heating in buffer at 85 °C for 5 min reduced the concentration of active neurotoxin by a factor of 10 000, although some foods were protective.

There are seven major types of botulinum neurotoxin (types A–G) and more than twenty subtypes (including five subtypes of type A neurotoxin (termed A1–A5) and five subtypes of type B neurotoxin). The seven major types of botulinum neurotoxins were originally distinguished using polyclonal antibodies raised against purified neurotoxins (as was the case for the neurotoxins formed by the strains isolated by van Ermengem and Landmann). The classification is supported by comparison of neurotoxin gene sequences and studies of the site of action of the neurotoxins. Subtypes of each botulinum neurotoxin serotype can be distinguished using monoclonal antibodies and by comparison of gene sequences.

The botulinum neurotoxins are synthesized as single-chain proteins, and converted to the more toxic dichain molecule by an extracellular bacterial protease (or an added protease such as trypsin). The dichain molecule consists of a heavy chain of approximately 100 kDa and a light chain of approximately 50 kDa, linked by a disulfide bond. The neurotoxins form complexes of various sizes from 300 to 900 kDa (depending on the neurotoxin type) with other proteins (e.g., haemagglutinin and nontoxic nonhaemagglutinin) that protect the neurotoxin and facilitate its absorption into the body. The neurotoxins act in a four-step process. Firstly, the C-terminal domain of the heavy chain binds to receptors on the surface of the presynaptic membrane of the motor neurone, and secondly the bound neurotoxin is internalized into the newly formed vesicle by receptor-mediated endocytosis. Thirdly, a conformational change in the neurotoxin, caused by acidification of the vesicle lumen, exposes surface hydrophobic domains, enabling the N-terminal translocation domain of the heavy chain to form an oligomeric structure with permeable channels in the vesicle membrane through which the now unfolded light chain can pass into the motor neurone cytosol. Once in the neutral pH of the cytosol, the light chain refolds. Fourthly, reduction of the interchain disulfide bond leads to the release of the light chain in the motor neurone cytosol. The neurotoxin light chains possess endopeptidase activity, and cleave proteins in the neurotransmitter (acetylcholine)-containing synaptic vesicle docking/fusion complex (SNARE proteins). Each light chain demonstrates stringent substrate specificity and mostly acts at a single site. The light chain of type A, C, and E neurotoxins targets the synaptosome-associated protein of 25 kDa (SNAP-25), the light chain of type B, D, F, and G neurotoxins targets the vesicle-associated membrane protein (VAMP), and the type C light chain also cuts syntaxin. Cleavage of just one of the SNARE proteins reduces the stability of the SNARE complex, preventing binding of acetylcholine-containing synaptic vesicles and therefore neurotransmitter release, leading to flaccid muscle paralysis.

Properties of the Gene Cluster Encoding the Neurotoxin and Neurotoxin Complex

The genes encoding the neurotoxins and associated proteins (such as haemagglutinins and nontoxic nonhaemagglutinin) are located together in two major neurotoxin cluster types, the



[a] ha cluster includes neurotoxin genes; A1[b], A5, B, C[c], D[c], G[d]; orf-X cluster includes neurotoxin genes; A1[b], A2, A3, A4, E[e], F[e,f]. *cntA* encodes neurotoxin; *cntB* encodes nontoxic nonhaemagglutinin; *cntC*, *cntD* and *cntE* encode haemagglutinins; *cntR* encodes neurotoxin cluster regulator; *p47*, *orf-X1*, *orf-X2* and *orf-X3* encode proteins of unknown function
[b] Most type A1 genes in ha cluster in single toxin gene strains; all type A1 genes in orf-X cluster in dual toxin gene strains
[c] *cntR* is downstream of *cntE*
[d] *cntC* is absent from type G neurotoxin cluster
[e] *cntR* absent from type E neurotoxin cluster, and some type F neurotoxin clusters
[f] Presence of *orf-X1*, *orf-X2* and *orf-X3* not confirmed in *C. baratii* type F neurotoxin cluster

Figure 1 Generalized view of two major neurotoxin gene cluster arrangements. Reproduced from Peck MW, Stringer SC, and Carter AT (2011) *Clostridium botulinum* in the post–genomic era. *Food Microbiology* 28(2): 183–191.

'ha cluster' and the 'orf-X cluster' (Figure 1). These clusters are present either on the chromosome or plasmid (proteolytic *C. botulinum* and nonproteolytic *C. botulinum*), a bacteriophage (*C. botulinum* Group III), or a plasmid (*C. argentinense*). Most strains contain a single neurotoxin gene and one neurotoxin gene cluster, although some strains of proteolytic *C. botulinum* possess two neurotoxin genes and two neurotoxin gene clusters. Recently strains of proteolytic *C. botulinum* have been described that unusually contained two neurotoxin genes (full type A5 and truncated type B neurotoxin gene) in a single neurotoxin gene cluster. The designation of genes within the neurotoxin cluster has been inconsistent, and several schemes have been used. Here the suffix *cnt* for clostridial neurotoxin is used for all genes of known function located within the botulinum neurotoxin clusters. All botulinum neurotoxin genes are designated *cntA*, with the type A neurotoxin gene designated *cntA/A* and the type B neurotoxin gene *cntA/B*, etc. (also sometimes known as *bont/a* and *bont/b*, respectively). The ha cluster contains two transcriptional units, with the neurotoxin (*cntA*) and nontoxic nonhaemagglutinin (*cntB*, also known as *ntnh*) encoding genes, which are cotranscribed. The haemagglutinin encoding genes (*cntC*, *cntD*, *cntE*, also known as *ha*34, *ha*17, and *ha*70, respectively) are in a second transcriptional unit and are transcribed in the opposite direction (Figure 1). A gene encoding a sigma 70 factor (*cntR*, also known as *botR*) that is responsible for the positive regulation of genes in the neurotoxin gene cluster is present between these two transcriptional units. Neurotoxin genes of types A1 (most single neurotoxin gene strains), A5, B, C, D, and G are present in the ha cluster (Figure 1). In the case of type C and D neurotoxins gene clusters, *cntR* is located downstream of *cntE*, whereas the type G neurotoxin gene appears to be associated with only two haemagglutinin encoding genes.

The orf-X cluster comprises genes encoding the neurotoxin (*cntA*), nontoxic nonhaemagglutinin (*cntB*), sigma 70 factor (*cntR*, also known as *p21* or *botR*), a group of three open reading frames (*orf-X1*, *orf-X2*, and *orf-X3*), and a single CDS (*p47*) of unknown function (Figure 1). The three haemagglutinin genes are absent from the orf-X cluster. It seems that *cntA*, *cntB*, and *p47* are cotranscribed, as are *orf-X1*, *orf-X2*, and *orf-X3*, both from conserved neurotoxin gene cluster promoters. The functions of the proteins encoded by *p47*, *orf-X1*, *orf-X2*, and *orf-X3*, and their role (if any) in the neurotoxin complex remain to be established. The type A1, A2, A3, A4, E, and F neurotoxin genes are located in an orf-X cluster (Figure 1). The type A1 neurotoxin gene in single neurotoxin gene strains is more commonly found in the ha cluster than the orf-X cluster, whereas in all dual neurotoxin gene strains the type A1 neurotoxin gene is in an orf-X cluster. No *cntR* homolog has been identified in clusters containing a type E neurotoxin gene. The sequencing of further genomes will undoubtedly provide further information on neurotoxin gene clusters.

The neurotoxin genes and neurotoxin gene clusters appear to have evolved independently of each other, and of the bacterium, and are likely to have arisen from a series of recombination events. Transfer of plasmids and bacteriophages containing neurotoxin genes has undoubtedly contributed to horizontal gene movement. Putative mobile genetic elements located close to the neurotoxin gene cluster may also have played a role, and the center of the *cntB* gene (encoding nontoxic nonhaemagglutinin) is reported to be a hot spot for recombination events.

Neurotoxin formation by proteolytic *C. botulinum* and nonproteolytic *C. botulinum* is primarily associated with late exponential and early stationary phase, and the quantity of neurotoxin formed is dependent on the strain and growth conditions. It is not, however, fully understood how neurotoxin formation is regulated. The sigma 70 factor (*cntR*) is involved in the positive regulation of the genes in the

neurotoxin gene cluster transcriptional units. Each cluster type carries its own distinguishable *cntR* gene, and although *cntR* is not present in clusters that carry a type E neurotoxin gene, neurotoxin formation is tightly regulated, indicating the presence of a presently unidentified positive regulator. Evidence is beginning to emerge that neurotoxin formation, and therefore also *cntR*, is regulated by a quorum sensing mechanism. Neurotoxin release from the cell is not dependent on autolysis, and the absence of any potential signal peptide at the N-terminus raises the possibility that neurotoxins may be secreted through a nonsignal peptide mediated secretion system (e.g., holin, flagella export apparatus).

Clinical Manifestation of Botulism

Botulism is a severe neuroparalytic disease, caused by the botulinum neurotoxin, which affects animals, birds, and humans. There are three major types of human botulism: foodborne botulism, infant/intestinal (adult) botulism, and wound botulism. Foodborne botulism is caused by ingestion of food containing preformed botulinum neurotoxin, following growth of proteolytic *C. botulinum* or nonproteolytic *C. botulinum* (or more rarely *C. baratii* type F or *C. butyricum* type E) in the food. The first symptoms can be observed within 2 h, although 12–36 h is more common, but it can be as long as 8 days. Nausea and vomiting may precede other symptoms and are not caused by the neurotoxin but by other substances produced during microbial growth in the food. The first symptom of botulism is often blurred vision, followed by difficulty swallowing and speaking, paralysis of face muscles, a descending bilateral flaccid paralysis, and generalized muscle weakness. In severe cases, this may be followed by flaccid paralysis of the respiratory or cardiac muscles, and can result in death if not treated. It is likely that cases of foodborne botulism are not recognized or misdiagnosed, sometimes being confused with the Guillain–Barré syndrome, Myasthenia gravis, and the effects of other foodborne pathogens. With the use of equine antitoxin and strong supportive therapy (including mechanical ventilation), the fatality rate for adults suffering from botulism has been reduced to less than 10% of cases. In many severe cases, however, complete recovery can be very slow, taking months or even years. The use of human botulism immune globulin for treatment of infant botulism has led to a significant reduction in the time spent on mechanical ventilation, in intensive care, and in hospital.

Some strains of *C. botulinum* form as much as 1000 human lethal doses of neurotoxin per gram. Thus, consuming only a few micrograms of food in which proteolytic *C. botulinum* and nonproteolytic *C. botulinum* (or more rarely *C. baratii* type F or *C. butyricum* type E) has grown could result in foodborne botulism. Consumption of as little as 30 ng of neurotoxin is sufficient to cause illness and even death. Because the quantity of neurotoxin consumed is so small, the body is unlikely to raise its own antibodies to the neurotoxin, and several individuals have contracted foodborne botulism more than once. Further details of the epidemiology of foodborne botulism are given in the next section.

Infant and intestinal (adult) botulism are both infections of the gastrointestinal tract. The first clinical cases of infant botulism were described in 1976. Between 1976 and 2006, 2419 cases were reported in the USA (where it is now the most frequently reported type of botulism), 366 cases in Argentina, and 158 cases in other countries. Only a few dozen cases of intestinal (adult) botulism have been described world-wide. Infant botulism affects those less than 12 months of age (infants between 2 and 26 weeks are most susceptible), and intestinal (adult) botulism affects adults with a suppressed normal intestinal flora (e.g., by antibiotic treatment). The consumption of as few as 10–100 spores of proteolytic *C. botulinum* (or more rarely *C. baratii* type F, nonproteolytic *C. botulinum*, or *C. butyricum* type E) can lead to colonization of the large intestine and the formation of neurotoxin *in vivo*. Colonization and multiplication by these neurotoxigenic clostridia in the gastrointestinal tract is prevented by a normal adult intestinal flora. Two major sources of spores have been identified for infant botulism: the environment (e.g., soil, dust) and honey. This association with honey has led to the recommendation in several countries that jars of honey should carry a warning indicating that the product should not be consumed by infants less than 12 months of age. Typical symptoms include extended constipation and flaccid paralysis. The fatality rate is approximately 1% of cases. Infant botulism has been proposed as one possible cause of sudden infant death syndrome.

Wound botulism is an infection involving growth and neurotoxin formation (almost invariably by proteolytic *C. botulinum*) in a wound in the body. Wound botulism was first described in 1943, and for approximately half a century was an uncommon disease generally associated with gross trauma. Many countries have recently reported their first cases of wound botulism or a significant increase in the number of reports, associated with drug abuse. Spores either originally present in heroin or on contaminated needles enter the body via subcutaneous or intramuscular injection. The spores then germinate, leading to growth and neurotoxin formation in a wound or abscess that provides the necessary anaerobic environment. Wound botulism has also been associated with inhalation of cocaine (and spores), and subsequent growth and neurotoxin formation in the sinus. Other types of human botulism are extremely rare, and include botulism following injection of licensed and unlicensed botulinum neurotoxin intended for therapeutic purposes, and the inadvertent inhalation of aerosolized neurotoxin by veterinary workers. Additionally botulinum neurotoxin has attracted the attention of some governments and terrorist groups as a potential bioterrorism weapon. For example, between 1990 and 1995, aerosols of botulinum neurotoxin were dispersed in Japan by the Aum Shinrikyo cult in a series of unsuccessful attacks.

Epidemiology of Foodborne Botulism

Overview

Despite the severity of foodborne botulism the true incidence of this disease is under-reported, with the extent of under-reporting varying from country to country. In 1999/2000, more than 2500 cases of foodborne botulism were reported in Europe, with a high incidence reported in Russia, Belarus, Azerbaijan, Armenia, Poland, Uzbekistan, Georgia, and Turkey

Table 2 Reported foodborne botulism in European countries in 1999/2000

Country	*Number of reported cases*
Russia	887
Belarus	344
Azerbaijan	181
Armenia	176
Poland	169
Uzbekistan	131
Georgia	122
Turkey	114
Kyrgyzstan	94
France	60
Lithuania	36
Portugal	33
Moldova	32
Germany	30
Italy	21
Bulgaria	18
Croatia	16
Czech Republic	7
Latvia	7
Slovenia	7
Norway	5

Note: Modified from Peck (2006). Only includes countries reporting five or more cases. May also include cases of other types of botulism.

Table 3 Reported foodborne botulism in different countries

Country	*Period*	*Number of cases*	
		Total	*Average per year*
Argentina	1980–2004	84	3
Belgium	1982–2000	32	2
Canada	1971–2005	439	13
China	1958–1983	4377	168
Denmark	1984–2000	18	1
France	1971–2003	1286	39
Georgia	1980–2002	879	40
Germany	1983–2000	376	22
Italy	1979–2000	750	34
Japan	1951–1998	530	11
Poland	1971–2000	9219	307
Romania	2003–2008	210	35
Spain	1971–1998	277	10
Sweden	1969–2000	13	1
UK	1971–2006	38	1
USA	1971–2008	1029	27

Note: Modified from Lund and Peck (2000) from various sources. May also include cases of other types of botulism.

(Table 2). The Republics of Armenia, Belarus, and Georgia have amongst the highest nationally reported rates of foodborne botulism per head of population. In recent years, between 20 and 40 cases of foodborne botulism have been recorded annually in France, Georgia, Germany, Italy, and USA, with a higher incidence in China and Poland (Table 3). In view of the severity of the foodborne botulism hazard, improperly processed foods are often recalled if faulty processing is known or strongly suspected.

To understand the causes of botulism outbreaks and to prevent any recurrence, it is highly desirable to identify the type of neurotoxin formed, and essential to establish which of the neurotoxigenic clostridia is responsible. For example, if type B or type F neurotoxin is detected, but the organism not isolated, it is unclear whether proteolytic *C. botulinum* or nonproteolytic *C. botulinum* is responsible. Outbreaks of foodborne botulism have involved foods prepared in the home and commercially, often when known control measures have not been implemented. Outbreaks of foodborne botulism are most frequently associated with proteolytic *C. botulinum* or nonproteolytic *C. botulinum*, very rarely with neurotoxigenic strains of *C. baratii* type F or *C. butyricum* type E, but not with *C. botulinum* Group III or *C. argentinense*. Because proteolytic *C. botulinum* and nonproteolytic *C. botulinum* are physiologically distinct and present different hazard scenarios, it is appropriate to consider the epidemiology of foodborne botulism for each separately.

Foodborne Botulism Outbreaks Associated with Proteolytic *C. botulinum*

Most outbreaks are concerned with strains that form type A or type B neurotoxin. The original Landmann strain was probably proteolytic *C. botulinum* type A. One very large botulism outbreak in Algeria in 1998 was associated with commercially produced Cashir sausage, in which proteolytic *C. botulinum* type A had grown and formed neurotoxin. There were 340 cases and 37 deaths reported. Three factors have contributed to a majority of foodborne botulism outbreaks associated with proteolytic *C. botulinum*. Further details of recent outbreaks are given in Table 4.

Inadequate Heat Processing of Canned or Bottled Foods

Low acid canned (or bottled) foods rely on the effective delivery of the botulinum cook to ensure safety, and outbreaks of foodborne botulism have occurred when it has not been fully delivered. Proteolytic *C. botulinum* has been responsible for most outbreaks of botulism involving such foods (Table 4), a reflection of high spore heat resistance. The failure to deliver the botulinum cook to home canned bamboo shoots led to a very large botulism outbreak in Thailand in March 2006. Spores of proteolytic *C. botulinum* type A survived the inadequate heat treatment, and led to growth and neurotoxin formation during subsequent ambient storage. Consumption of the bamboo shoots at a local religious rite resulted in 209 cases of botulism, 42 of which required mechanical ventilation. It is likely that there would have been significant mortality if the Thai authorities had not rapidly mobilized such a large number of mechanical ventilators. Proteolytic *C. botulinum* type A had been earlier associated with a 1998 botulism outbreak involving home canned bamboo shoots in Thailand. In June 2007, spores of proteolytic *C. botulinum* type A survived the inadequate thermal process applied to cans of hot dog chili sauce at a commercial canning facility in Augusta (Georgia, USA). Subsequent consumption of the hot dog chili sauce led to four cases of foodborne botulism, all of whom required mechanical ventilation. This outbreak was associated

Table 4 Examples of outbreaks of foodborne botulism

Country (year)	*Product*	*Bacterium*	*Toxin type*	*Cases (deaths)*	*Factors contributing to botulism outbreak*
Madagascar (1982)	Commercial pork sausage	Nonproteolytic *C. botulinum*	E	60 (30)	Inadequate preservation
USA (1985)	Uneviscerated salted, air-dried fish (*kapchunka*)	Nonproteolytic *C. botulinum*	E	2 (2)	Poorly controlled salting, lack of refrigeration
Canada (1985)	Commercial garlic-in-oil	Proteolytic *C. botulinum*	B	36	Temperature abuse
USA/Israel (1987)	Commercial uneviscerated salted, air-dried fish (*kapchunka*)	Nonproteolytic *C. botulinum*	E	8 (1)	Poorly controlled salting, lack of refrigeration
Canada (1987)	Bottled mushrooms	Proteolytic *C. botulinum*	A	11	Underprocessing and inadequate acidification
UK (1989)	Commercial hazelnut yoghurt	Proteolytic *C. botulinum*	B	27 (1)	Underprocessing of hazelnut conserve
Japan (1989)	Herring, Flounder and Dace *Izushi* (3 outbreaks)	Nonproteolytic *C. botulinum*	E	6	Poor fermentation
Japan (1991)	Dace and Ayu *Izushi* (2 outbreaks)	Nonproteolytic *C. botulinum*	E	2	Poor fermentation
Egypt (1991)	Commercial uneviscerated salted fish (*faseikh*)	Nonproteolytic *C. botulinum*	E	>91 (18)	Putrefaction of fish before salting
USA (1992)	Commercial uneviscerated salted fish (*moloha*)	Nonproteolytic *C. botulinum*	E	8	Insufficient salt
USA (1993)	Restaurant, commercial process cheese sauce	Proteolytic *C. botulinum*	A	8 (1)	Contaminated after opening, then temperature abused
Italy (1993)	Commercial canned roasted egg plant in oil	Proteolytic *C. botulinum*	B	7	Insufficient heat treatment; improper acidification
Sweden (1994)	Vacuum-packed hot-smoked rainbow trout	Nonproteolytic *C. botulinum*	E	?	Temperature abuse (?)
USA (1994)	Restaurant; potato dip (*skordalia*) and egg plant dip (*meligianoslata*)	Proteolytic *C. botulinum*	A	30	Baked potatoes held at room temperature
USA (1994)	Commercial clam chowder	Proteolytic *C. botulinum*	A	2	Temperature abuse at home
USA (1994)	Commercial black bean dip	Proteolytic *C. botulinum*	A	1	Temperature abuse at home
Georgia (1994)	Fish consumed at wedding	Nonproteolytic *C. botulinum* (?)	E?	173	?
Japan (1995)	Salmon, Gizzard Shad, and Dace *Izushi* (3 outbreaks)	Nonproteolytic *C. botulinum*	E	10	Poor fermentation
Canada (1995)	'Fermented' seal or walrus (4 outbreaks)	Nonproteolytic *C. botulinum*	E	9	Unsafe process
Italy (1996)	Commercial mascarpone cheese	Proteolytic *C. botulinum*	A	8 (1)	Temperature abuse
Japan (1997)	Char and Dace *Izushi* (2 outbreaks)	Nonproteolytic *C. botulinum*	E	4	Poor fermentation
Germany (1997)	Commercial hot-smoked vacuum-packed fish (*Raucherfisch*)	Nonproteolytic *C. botulinum*	E	2	Suspected temperature abuse
Argentina (1997)	Home cured ham	Nonproteolytic *C. botulinum*	E	6	?
Germany (1997)	Home smoked vacuum-packed fish (*Lachsforellen*)	Nonproteolytic *C. botulinum*	E	4	Temperature abuse
Italy (1997)	Home-made pesto/oil	Proteolytic *C. botulinum*	B	3	Unsafe process
Germany (1997)	Home-prepared beans	Proteolytic *C. botulinum*	A	1	Poor preparation
Iran (1997)	Traditional cheese preserved in oil	Proteolytic *C. botulinum*	A	27 (1)	Unsafe process
Germany (1998)	Commercial smoked vacuum-packed fish	Nonproteolytic *C. botulinum*	E	4	Temperature abuse (?)

(*Continued*)

Table 4 Continued

Country (year)	*Product*	*Bacterium*	*Toxin type*	*Cases (deaths)*	*Factors contributing to botulism outbreak*
France (1998)	Commercial frozen vacuum-packed seafood (2 outbreaks)	Nonproteolytic *C. botulinum*	E	2	Temperature abuse (?)
Thailand (1998)	Home canned bamboo shoots	Proteolytic *C. botulinum*	A	13 (2)	Inadequate processing (?)
Argentina (1998)	Commercial chilled meat roll (*matambre*)	Proteolytic *C. botulinum*	A	9	Temperature abuse
UK (1998)	Home bottled mushrooms in oil (imported from Italy)	Proteolytic *C. botulinum*	B	2 (1)	Unsafe process
Japan (1998)	Bottled olives in brine	Not known[a]	B	18	?
Algeria (1998)	Commercial cashir (Maghrebian halal) sausage	Proteolytic *C. botulinum*	A	340 (37)	?
Croatia (1998)	Ham	Not known[a]	B	20	?
Finland (1999)	Whitefish eggs	Nonproteolytic *C. botulinum*	E	1	Temperature abuse
France (1999)	Gray mullet	Nonproteolytic *C. botulinum*	E	1	Temperature abuse (?)
France (1999)	Commercial chilled fish soup	Proteolytic *C. botulinum*	A	1	Temperature abuse at home
Japan (1999)	Commercial boil in bag curry	Proteolytic *C. botulinum*	A	1	Temperature abuse
Azerbaijan (1999)	Fish consumed in restaurant	Not known	?	90 (4)	?
Morocco (1999)	Commercial mortadella sausage	Not known[a]	B	78 (20)	?
France (2000)	Home-made asparagus soup	Proteolytic *C. botulinum*	B	9	Inadequate processing (?)
Australia (2001)	Reheated chicken	Nonproteolytic *C. botulinum*	E	1	Poor temperature control
USA (2001)	Home-made fermented beaver tail and paw	Nonproteolytic *C. botulinum*	E	3	Temperature abuse
Canada (2001)	Home-made fermented salmon roe (2 outbreaks)	Nonproteolytic *C. botulinum*	E	4	Unsafe process
USA (2001)	Commercial frozen chili sauce from salvage store	Proteolytic *C. botulinum*	A	24	Temperature abuse at salvage store
USA (2002)	Home-made *muktuk* (from beluga whale)	Nonproteolytic *C. botulinum*	E	12	Unsafe process
South Africa (2002)	Commercial tinned pilchards	Proteolytic *C. botulinum*	A	2 (2)	Secondary contamination after corrosion of tin
Canada (2002)	Restaurant, baked potato in aluminum foil	Proteolytic *C. botulinum*	A	1	Baked potato held at room temperature (?)
Germany (2003)	Home-salted air-dried fish	Nonproteolytic *C. botulinum*	E	3	Temperature abuse (?)
Germany (2004)	Commercial vacuum-packed smoked salmon	Nonproteolytic *C. botulinum*	E	1	Consumed after 'use-by date'
Italy (2004)	Restaurant preserved green olives in saline	Not known[a]	B	24	?
USA (2004/5)	Home-made pruno (2 outbreaks)	Proteolytic *C. botulinum*	A	5	Unsafe process
USA (2005)	Home-salted uneviscerated fish	Nonproteolytic *C. botulinum*	E	5	Insufficient salt
Turkey (2005)	Home-made *suzme* (condensed) yoghurt	Proteolytic *C. botulinum*	A	10 (2)	Unsafe process
Russia (2005)	Canned food	Not known	?	15 (1)	Inadequate processing (?)
Russia (2005)	Home canned cucumbers	Not known	?	16	Inadequate processing (?)
Kazakhstan (2005)	Home dried fish	Not known	?	25 (1)	?
Iran (2006)	Traditional soup (*ashmast*)	Nonproteolytic *C. botulinum*	E	11	?
Finland (2006)	Commercial vacuum-packed smoked whitefish	Nonproteolytic *C. botulinum*	E	1	Temperature abuse (?)
USA (2006)	Home fermented tofu	Proteolytic	A	2	Unsafe process

(*Continued*)

Table 4 Continued

Country (year)	*Product*	*Bacterium*	*Toxin type*	*Cases (deaths)*	*Factors contributing to botulism outbreak*
Thailand (2006)	Home canned bamboo shoots	*C. botulinum* Proteolytic	A	209	Inadequate processing
Canada/USA (2006)	Commercial refrigerated carrot juice	*C. botulinum* Proteolytic	A	6 (1)	Temperature abuse
Taiwan (2006)	Fermented goat meat (*cinkrugan*)	*C. botulinum* Not known[a]	B	5	?
USA (2007)	Commercial hot dog chili sauce	*C. botulinum* Proteolytic	A	4	Inadequate processing
Australia (2007)	Commercial nacho meal	*C. botulinum* Proteolytic	A	1	Inadequate processing (?)
China (2007)	Commercial sausage	*C. botulinum* Proteolytic	A	66	Temperature abuse
Turkey (2008)	Unprocessed black olives	*C. botulinum* Not known[a]	B	8	Poor preparation (?)
USA (2008)	Home canned green beans/ carrots	*C. botulinum* Proteolytic	A	4	Inadequate processing (?)
France (2008)	Commercial chicken enchiladas	*C. botulinum* Proteolytic	A	2	Temperature abuse
Rwanda (2009)	Prepared vegetables	*C. botulinum* Not known[a]	B	64 (2)	Poor preparation (?)
France (2009)	Commercial vacuum-packed hot-smoked whitefish	Nonproteolytic *C. botulinum*	E	3	Temperature abuse (?)
Russia (2010)	Commercial dried fish	Not known	?	5	Poor preparation (?)

[a]Not known whether proteolytic *C. botulinum* type B or nonproteolytic *C. botulinum* type B.
Source: Updated from Lund and Peck (2000) and Peck (2006) from various sources.

with a massive product recall involving tens of millions of cans.

Temperature/Time Abuse of Products Intended to be Stored Chilled

Proteolytic *C. botulinum* has a minimum growth temperature of 10–12 °C, and the only barrier to controlling neurotoxin formation by this bacterium in many chilled foods appears to be refrigerated storage. Temperature abuse of commercial chilled carrot juice permitted growth and neurotoxin formation by proteolytic *C. botulinum* type A, resulting in a very severe botulism outbreak in Canada (Toronto) and USA (Florida and Georgia) in 2006. It is probable that the six people affected all consumed massive quantities of neurotoxin, because as little as 5 µl of carrot juice may have constituted a lethal dose (see Properties of the Neurotoxins and Neurotoxin Complex). All six required mechanical ventilation, one of whom died, and two were still dependent on mechanical ventilation 1 year after the initial intoxication. Temperature abuse of commercial chicken enchiladas in France in August 2008 allowed growth and neurotoxin formation by proteolytic *C. botulinum* type A, resulting in a small botulism outbreak (Table 4). Both people affected required mechanical ventilation, and probably consumed large quantities of neurotoxin. It has been estimated that 10 mg of food may have constituted a lethal dose.

Addition of Ingredients Containing Preformed Botulinum Neurotoxin to a Correctly Refrigerated Product

In 1989, 27 people were intoxicated with botulinum neurotoxin (one fatally) in the largest botulism outbreak in the UK. The outbreak involved commercially produced hazelnut yoghurt. An inadequate heat treatment was delivered to the hazelnut conserve permitting the survival of spores of proteolytic *C. botulinum* type B, and after subsequent ambient storage neurotoxin was formed. Hazelnut conserve containing type B neurotoxin was then added to natural yoghurt. In 1994, a large botulism outbreak in the USA was associated with consumption of *skordalia* in a restaurant. Thirty people were affected, four of whom required mechanical ventilation. Foil-wrapped potatoes were baked in an oven, and then held at ambient temperature. Spores of proteolytic *C. botulinum* type A survived the heat treatment, and then multiplied and formed neurotoxin during ambient storage. Potatoes contaminated with neurotoxin were then added to natural yoghurt to give *skordalia* (Table 4).

Foodborne Botulism Outbreaks Associated with Nonproteolytic *C. botulinum*

Reported outbreaks of foodborne botulism associated with nonproteolytic *C. botulinum* most frequently involve strains forming type E neurotoxin (Table 4). Strains forming type B neurotoxin are, however, also very important, and many outbreaks in Europe associated with type B neurotoxin undoubtedly involve strains of nonproteolytic *C. botulinum* type B. It is likely that the original van Ermengem strain was nonproteolytic *C. botulinum* type B. Three types of foods are commonly associated with foodborne botulism outbreaks and nonproteolytic *C. botulinum* (Table 4).

Salted Fish

Inadequate salting, particularly of fish, has been associated with a number of outbreaks of foodborne botulism. The

consumption of commercially produced uneviscerated salted fish (*faseikh*) led to a very large outbreak of foodborne botulism in Egypt in 1991 that affected more than 91 people (18 fatally). The fish, from fish ponds, was stored in a cool room for 1 day to allow putrefaction before salting, enabling growth and neurotoxin formation by nonproteolytic *C. botulinum* type E before the salting process was complete. Other outbreaks involving inadequate salting of fish and a lack of appropriate refrigeration have been reported in the USA and Israel (Table 4).

Vacuum and Smoked Fish

Botulism outbreaks in several European countries have been associated with vacuum-packed fish. Temperature and/or time abuse has been suspected in a number of these outbreaks. For example, three botulism cases were associated with suspected temperature abuse of vacuum-packed hot-smoked whitefish in France in September 2009.

Fermented Foods

A number of outbreaks of botulism in Japan have been caused by consumption of home-prepared *izushi* or a similar fermented fish product (Table 4). *Izushi* is prepared by soaking fleshy pieces of fish in water for a few days, with occasional changes of water to remove blood, and then packing the fish tightly in a tub with diced vegetables, cooked rice, vinegar, salt, and spices and allowing to ferment for several weeks. The product is consumed without cooking. Neurotoxin formation by nonproteolytic *C. botulinum* type E may occur during soaking or early fermentation, but not once the fermentation has taken the pH below pH 5.0. Home-made foods prepared by the peoples of Alaska and north Canada (e.g., fermented seal flipper, fermented beaver tail and paw, and fermented salmon roe and *muktuk*) are responsible for outbreaks of foodborne botulism on an annual basis. These foods are generally fermented before consumption, and if this fermentation takes place at a temperature of 3 °C or higher then growth and neurotoxin formation by nonproteolytic *C. botulinum* may occur. Further details of recent outbreaks are given in Table 4.

Analytical Methods for *C. botulinum* and Its Neurotoxins

Methods for the Isolation of Proteolytic *C. botulinum* and Nonproteolytic *C. botulinum*

The extreme potency of the botulinum neurotoxins and the hazardous nature of the six botulinum neurotoxin-forming clostridia require that all practical work is restricted to containment laboratories that afford a high level of worker protection. When enumerating or isolating proteolytic *C. botulinum* or nonproteolytic *C. botulinum* from foods or the environment it is essential to determine the efficiency of the method(s) used, by demonstrating that known strains can be recovered following addition in low numbers to test samples. Because proteolytic *C. botulinum* and nonproteolytic *C. botulinum* are physiologically distinct, a single enrichment procedure cannot be relied upon for both organisms. A heat treatment or ethanol treatment, before enrichment, can be used to eliminate or reduce the number of competing vegetative bacteria. A heat treatment at 75–80 °C for 10–15 min should be used during isolation of proteolytic *C. botulinum*, whereas a lower heat treatment (e.g., 60 °C for 30 min) is more suitable when culturing from spores of nonproteolytic *C. botulinum*. There may also be merit in testing untreated samples to isolate vegetative bacteria. The enrichment media should be fully anaerobic, and the redox dye resazurin is a useful indicator. A temperature of 35 °C is used for enrichment cultures of proteolytic *C. botulinum*, whereas a lower incubation temperature (e.g., 12 °C or 26 °C) has been used for nonproteolytic *C. botulinum*. Lysozyme may be added to the enrichment medium to increase recovery of heated spores, whereas addition of trypsin may inactivate bacteriocins that may be formed by closely related clostridia. An extended period of incubation (7 day or longer) should be allowed for growth and neurotoxin formation. A portion of the enriched culture can be used to test for botulinum neurotoxin or the encoding gene, and a portion plated onto a selective (e.g., *C. botulinum* Isolation (CBI) agar, Botulinum Selective Medium (BSM)) or nonselective plating medium (e.g., egg-yolk agar). Egg-yolk is included in these plating media as colonies of proteolytic *C. botulinum* and nonproteolytic *C. botulinum* have a typical appearance associated with their lipase activity.

Molecular Methods for the Detection and Characterization of Proteolytic *C. botulinum* and Nonproteolytic *C. botulinum*

Polymerase chain reaction (PCR)-based tests have been developed that detect botulinum neurotoxin genes, including multiplex PCR methods for simultaneous detection of type A, B, E, and F neurotoxin genes in a single reaction. Detection in these tests is achieved by gel electrophoretic confirmation of product size. There is merit in confirming the specificity of a positive PCR result, for example by sequencing of the PCR product. Real time PCR methods have also been developed. PCR-based tests have been widely used in the investigation of botulism outbreaks, and in the testing of enrichment media during surveys for the presence of proteolytic *C. botulinum* and nonproteolytic *C. botulinum* in food, clinical, and environmental samples.

Various typing methods have been used for the molecular characterization of strains of proteolytic *C. botulinum* and nonproteolytic *C. botulinum*. These include ribotyping, pulsed-field gel electrophoresis (PFGE), amplified fragment length polymorphism (AFLP), multilocus sequence typing (MLST), DNA sequencing, and comparative genomic indexing. Ribotyping is widely used for molecular typing of unknown bacteria, and can distinguish strains of proteolytic *C. botulinum* and nonproteolytic *C. botulinum*. PFGE has a higher discriminatory power than ribotyping, and involves the digestion of genomic DNA with rare-cutting restriction enzymes, followed by separation by electrophoresis. PFGE has been used to investigate the diversity of strains of proteolytic *C. botulinum* and nonproteolytic *C. botulinum*. AFLP involves digestion of genomic DNA with a pair of restriction enzymes, ligation with restriction site-specific adapters, and PCR amplification of a subset of the fragments. AFLP clearly differentiates between

proteolytic *C. botulinum* and nonproteolytic *C. botulinum*, and is suitable for typing at the strain level. MLST has been shown to discriminate strains of proteolytic *C. botulinum*. Whole genome sequencing is now becoming affordable and rapid, and provides excellent discrimination of strains. Comparative genomic indexing using a DNA microarray based on the genome sequence of proteolytic *C. botulinum* is an effective tool to distinguish strains of proteolytic *C. botulinum*, but not those of nonproteolytic *C. botulinum* as they are too distinct. A separate DNA microarray based on the genome of strain(s) of nonproteolytic *C. botulinum* would be needed for this purpose. Unlike other typing methods, these two methods provide extra information on the genome content of the tested strains.

Methods for the Detection and Quantification of Botulinum Neurotoxins

For a great many years, the gold standard method for detection and identification of botulinum neurotoxins has involved intraperitoneal injection into mice. Specificity is achieved by looking for typical symptoms of botulism and the use of specific antisera. Advantages of the mouse test are that it is extremely sensitive (5–10 pg of neurotoxin), measures the biological activity of the neurotoxin, is repeatable and reproducible, and should detect previously undescribed neurotoxins. The major disadvantage of the mouse test is the ethical issue concerning the use of animals; other limitations are the need to wait several days before a sample can be judged negative, the need for highly skilled personnel, and the cost. Alternative animal methods have been described, some of which are nonlethal.

Immunochemical methods have been widely used for the detection and quantification of botulinum neurotoxins, including enzyme-linked immunosorbent assays (ELISA), affinity immunochromatography columns (AICC), and lateral flow immunoassays (LFIA). These methods are cheaper, quicker, and much easier to use than the mouse test, and in some cases have the same sensitivity and specificity. Limitations of the various immunochemical methods may include a failure to detect undescribed neurotoxins, a different response to neurotoxin subtypes (that differ antigenically), response to biologically inactive neurotoxin, crossreactivity, and some tests require complex and expensive amplification systems.

Advantage has been taken of the highly specific endopeptidase activity of the botulinum neurotoxin light chains to develop a series of *in vitro* assays. The first assays used an immunochemical approach to detect cleavage of SNAP-25 or VAMP by botulinum neurotoxins, and some are as sensitive as the mouse test. Other assays employ a synthetic peptide (to mimic SNAP-25 or VAMP), labeled with quenched fluorophores, as the substrate. These peptides are cleaved by the endopeptidase activity of the botulinum neurotoxins, releasing fluorescence. Although these tests measure the biological activity of the light chain (but not the heavy chain) and are not affected by variations in antigenicity of neurotoxin subtype, interference by other proteases can be a limitation requiring the incorporation of an additional step to capture the neurotoxin before measurement of endopeptidase activity.

Control and Prevention of Foodborne Botulinum

Overview

In order to set processes to control the foodborne botulism hazard, it is necessary to have information on the extent of contamination of the environment and foods with spores of proteolytic *C. botulinum* and nonproteolytic *C. botulinum*. Figure 2 summarizes data from 83 independent surveys of incidence of *C. botulinum* spores in soils and sediments across the globe. Many of the original surveys may have well been subjected to various limitations; for example, small sample size, use of heat treatments that may have destroyed spores of nonproteolytic *C. botulinum*, and a failure to distinguish between proteolytic *C. botulinum* and nonproteolytic *C. botulinum*. The overall picture, however, is that proteolytic *C. botulinum* and nonproteolytic *C. botulinum* are ubiquitous in the environment, although generally present at a low spore concentration. From the summary presented in Figure 2, the most frequently reported spore concentration is in the range of 11–100 spores per kg. Occasionally, however, higher concentrations of spores will be found, and very occasionally much higher concentrations will be present. In view of the severity of foodborne botulism, the approach taken is to assume that a high concentration of spores will be present, and to deliver an appropriate control process to the food.

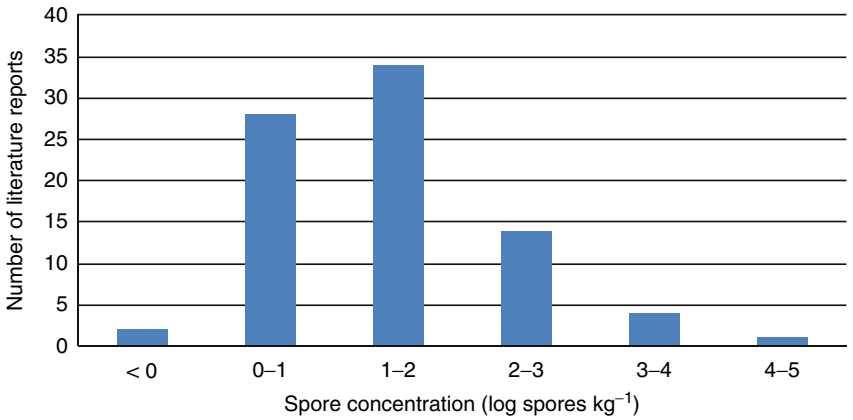


Figure 2 Summary of literature reports on the incidence of *C. botulinum* spores in the environment.

Quantitative microbiological risk assessments have been developed for a number of products. Because proteolytic *C. botulinum* and nonproteolytic *C. botulinum* are physiologically distinct it is necessary to apply different control processes (either single factors or combinations of factors) to prevent growth and neurotoxin formation by these two pathogens. It is estimated that in the USA, each case of foodborne botulism associated with a commercial product costs $30 million, at least three orders of magnitude greater than that for a case of listeriosis or salmonellosis.

Control of Proteolytic *C. botulinum* in Food Processing Operations

Proteolytic *C. botulinum* produces spores of high heat resistance and is the principal concern for the safe production of low acid canned foods. A heat treatment at 121.1 °C for 3 min has been adopted as the minimum standard for a botulinum cook for canned foods. This heat treatment (the botulinum cook) is intended to deliver a safety factor of at least 10^{12} (12-D (12-decimal) process) with respect to spores of proteolytic *C. botulinum*, and its use has ensured the safe production of low acid canned foods over many decades. Botulism outbreaks have occurred only when the full heat treatment has not been appropriately delivered or there has been postprocess contamination. Other controlling factors include storage at less than 10–12 °C, pH < 4.6, or by ≥ 10% NaCl. The use of other factors and combinations of factors to control or prevent growth of proteolytic *C. botulinum* has been described, and predictive models have been developed.

Control of Nonproteolytic *C. botulinum* in Food Processing Operations

Spores of nonproteolytic *C. botulinum* are much less heat resistant than those of proteolytic *C. botulinum*, and are therefore readily killed by the botulinum cook given to low acid canned foods. Various single and combinations of control factors have been identified to prevent growth and neurotoxin formation by nonproteolytic *C. botulinum*, and predictive models have been developed. Sales of minimally heated refrigerated foods (variously known as refrigerated processed foods of extended durability (REPFEDs), cook–chill foods and ready meals) are increasing by approximately 10% per annum in many countries. These foods receive a minimal heat treatment (typical maximum of 70–95 °C) and are not therefore sterile, and are stored under refrigeration (typically ≤ 8 °C). They can be packed under a modified low oxygen atmosphere or vacuum, but even when packed in air the foods themselves are likely to be highly reduced (enabling growth and neurotoxin formation). For some foods, intrinsic/extrinsic factors may also contribute to product safety. The UK Advisory Committee on the Microbiological Safety of Food (ACMSF) has made recommendations on the safe production of vacuum and modified atmosphere packed chilled foods with respect to *C. botulinum* and the associated foodborne botulism hazard. It is recommended that the heat treatments or combination processes deliver a safety factor of 10^6 (a 6-D process) with regard to spores of nonproteolytic *C. botulinum*. The recommended safety factors are as follows:

1. storage at < 3.0 °C;
2. storage at ≤ 8 °C and a shelf-life of ≤ 10 day;
3. storage at chill temperature (specified as ≤ 8 °C in England and Wales) combined with heat treatment of 90 °C for 10 min or equivalent lethality (e.g., 80 °C for 129 min, 85 °C for 36 min);
4. storage at chill temperature combined with ≤ pH 5.0 throughout the food;
5. storage at chill temperature combined with a salt concentration ≥ 3.5% throughout the food;
6. storage at chill temperature combined with ≤ a_w 0.97 throughout the food;
7. storage at chill temperature combined with a combinations of heat treatment and other preservative factors, which can be shown consistently to prevent growth and neurotoxin production by *C. botulinum*.

Emerging Issues: The Continued Control of Proteolytic *C. botulinum* and Nonproteolytic *C. botulinum* in Food Processing Operations

In view of the severity of foodborne botulism, vigilance is needed to ensure that proteolytic *C. botulinum* and nonproteolytic *C. botulinum* do not become emerging pathogens. The correct application of known control measures in the home and in the commercial setting is essential for the prevention of botulism, and there have been many botulism outbreaks across the globe where such control measures have not been effectively applied. One example is the home and commercial canning of foods, where a failure to apply the botulinum cook has led to botulism outbreaks in many countries. There is, therefore, a need for improved training and education of food handlers to ensure that known control measures are effectively implemented. It is also important that proteolytic *C. botulinum* and nonproteolytic *C. botulinum* are controlled as new foods (e.g., minimally heated refrigerated foods) are developed and new food processes are applied (e.g., high hydrostatic pressure). Climate change may provide new niches and opportunities for the multiplication of proteolytic *C. botulinum* and nonproteolytic *C. botulinum* in the environment, and present new challenges with respect to controlling growth and neurotoxin formation in food. The increased movement of people, and raw materials and foods (through trade globalization) may also bring an increased risk. There is also the need to be alert to the potential transfer, either naturally or deliberately, of botulinum neurotoxin genes to other bacteria, and to the deliberate introduction of botulinum neurotoxin or neurotoxigenic clostridia into the food chain through a bioterrorism act.

See also: Bacteria: *Bacillus cereus* and Other Pathogenic *Bacillus* Species; *Clostridium perfringens*. Characteristics of Foodborne Hazard and Diseases: Cost of Foodborne Diseases. Foodborne Diseases: Overview of Biological Hazards and Foodborne Diseases

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BACTERIA

Clostridium perfringens

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Glossary

Claudins Major constituents of tight junctions. There are 24 different claudins currently recognized. These ~22 kDa proteins have four transmembrane domains and two extracellular loops. They polymerize into strands and are critical for tight junction structure and function.

***D* value** Time a bacterial culture or spores must be held at a given temperature to reduce viability by one log.

Multilocus sequence typing (MLST) analysis A molecular biology technique for characterizing the relatedness of different strains of a bacterial species. It involves comparing the DNA sequences of internal sequences of multiple housekeeping genes and then using that information to differentiate strains based upon their allelic variations.

Sigma factor Protein that binds to RNA polymerase that provides promotor specificity.

Small acid soluble protein Produced by sporulating cells of both *Clostridium* and *Bacillus* spp. These small proteins bind to, and protect, DNA in spores. They are important contributors to the resistance properties of bacterial endospores. They are also degraded during germination, where they can provide amino acids and energy for germinating cells. *Clostridium perfringens* produces at least four different small acid soluble proteins.

Tight junction Also known as the zonula occludens, this is a region where membranes of two adjacent mammalian epithelial or endothelial cells interact to form a seal. Tight junctions are comprised of protein strands and possess both fence and gate properties. They are 'fences' that prevent nonregulated passage of fluids between cells and maintain polarity differences between the apical and basolateral cell surface. However, they also have 'gate' functions that regulate the paracellular permeability between two adjacent cells.

Historical Background

Clostridium perfringens was first associated with food poisoning in 1945. However, its well-deserved prominence as a major foodborne pathogen was firmly acquired following the classic report by Hobbs in 1953 describing *C. perfringens* (then *Clostridium welchii*) type A food poisoning. Another key development in understanding *C. perfringens* type A food poisoning occurred in the early 1970s, when the *C. perfringens* enterotoxin (CPE) responsible for causing the gastrointestinal (GI) symptoms of this food poisoning was identified, purified, and characterized. Since that time, extensive studies have provided insights into the action and genetics of CPE, as well as factors contributing to the resistance properties of *C. perfringens* type A food poisoning isolates.

Characteristics

Clostridium perfringens is a rod-shaped, encapsulated, endospore-forming, gram-positive bacterium. This bacterium is classified as an anaerobe because it does not produce colonies on agar plates exposed to air; however, compared to many other anaerobes, *C. perfringens* is relatively aerotolerant. It grows when provided a low oxidation–reduction potential (E_h) environment, which can be influenced by environmental factors such as pH. *Clostridium perfringens* also produces reducing molecules such as ferredoxin that modify the environmental E_h to create favorable growth conditions. The E_h of common foods such as raw meats, poultry, seafoods, or their derivative products like gravy or stews is sufficient to support *C. perfringens* growth.

Clostridium perfringens has an extremely short generation time, with some food poisoning strains (as described later) capable of doubling in ~10 min at the optimum growth temperature of 43 °C. This rapid growth can contribute to foodborne illness by facilitating the presence of large numbers of vegetative cells in precooked food products following spore germination. In addition, some food poisoning strains can grow slowly at temperatures >50 °C, which confirms the importance of rigid adherence to temperature control guidelines (discussed later) for food preparation and holding.

The virulence of *C. perfringens* is largely dependent upon toxin production, with 17 different *C. perfringens* toxins reported in literature. However, individual isolates of this bacterium never produce all these toxins, forming the basis for a classification scheme used to assign *C. perfringens* isolates into one of five types (A–E) based upon their production of five typing toxins (Table 1). As implied by its name, *C. perfringens* type A food poisoning almost always involves type A isolates.

However, it is important to appreciate that only a small proportion of type A isolates, i.e., the <1–5% of isolates producing CPE, can cause *C. perfringens* type A food poisoning. Although rare in developed countries (and thus only briefly discussed in this article), beta toxin-producing *C. perfringens* type C isolates can cause a foodborne disease named enteritis necroticans, also known as Pigbel.

Amongst *cpe*-positive type A isolates, the *cpe* gene encoding the enterotoxin can be either chromosomal or carried on large plasmids. Importantly, ~75–80% of *C. perfringens* type A food poisoning isolates in the USA and Europe carry a chromosomal *cpe* gene. Type A plasmid *cpe* isolates may be somewhat more important for food poisoning in Japan compared to the USA or Europe, perhaps due to differences in food preparation and consumption.

The association between type A chromosomal *cpe* isolates and food poisoning, particularly in the USA and Europe, does not involve these isolates producing a CPE variant or a greater amount of CPE compared to plasmid *cpe* isolates. Instead, this association likely involves, in part, the spores and vegetative cells of most *C. perfringens* type A chromosomal *cpe* food poisoning isolates possessing considerably greater resistance against food environment stresses such as heat, cold, osmotic pressure, pH, and chemical preservations, for example, nitrites (Table 2). For example, the spores of type A chromosomal *cpe* food poisoning isolates possess, on average, approximately 60-fold-higher *D* values at 100 °C compared to spores made by either type A plasmid *cpe* isolates or *cpe*-negative *C. perfringens* strains (Table 2). These vegetative cell and spore resistance properties of chromosomal *cpe* isolates should favor their survival in foods, so they can later cause food poisoning.

Small acid soluble proteins (Ssps) contribute to spore resistance properties by binding to, and thus protecting, DNA inside bacterial spores. The strong spore resistance properties exhibited by most type A chromosomal *cpe* food poisoning isolates are attributable, in part, to the ability of these strains to express a variant of a small acid soluble protein named Ssp4 (Table 3). *Clostridium perfringens* type A food poisoning strains produce a variant Ssp4 with an Asp at residue 36, whereas this Ssp4 residue is a Gly in *C. perfringens* strains producing sensitive spores. At least in part, the Asp variant Ssp4 is more protective for the environmentally resistant spores made by most food poisoning strains because it binds more tightly to spore DNA compared to the Gly Ssp4 variant made by those *C. perfringens* isolates producing environmentally sensitive spores. Additional factors are also likely to contribute to the exceptional spore resistance properties of chromosomal *cpe* isolates. For example, the spore core is smaller in type A chromosomal *cpe* isolates compared to other *C. perfringens* isolates. Differences in concentrations of DPA and metal ions, density of the spore core, and protoplast-to-sporoplast ratios may also contribute to the unusual spore heat resistance properties of chromosomal *cpe* isolates.

Other factors besides stress resistance probably also contribute to the strong association between type A chromosomal *cpe* isolates and food poisoning. For example, between 25 and 43 °C, chromosomal *cpe* isolates grow faster than either type A plasmid *cpe* isolates or type A *cpe*-negative isolates. Their faster growth, which (as mentioned) includes a generation time of only ~10 min at 43 °C, likely allows type A chromosomal *cpe* strains to more quickly reach pathogenic levels in foods compared to type A plasmid *cpe* isolates. In addition, relative to other *C. perfringens* isolates, type A chromosomal *cpe* isolates can grow at higher maximum (53.3 °C ± 0.7 °C) and lower minimum temperatures (12 °C ± 1.9 °C), which may facilitate the ability of the chromosomal *cpe* isolates to multiply in temperature-abused foods. However, no *C. perfringens* isolates grow at 6 °C or less, supporting the importance of storing food at proper refrigeration temperatures in order to prevent *C. perfringens* food poisoning outbreaks (further discussion later).

These growth differences suggest there are considerable variations between the genetic backgrounds of type A chromosomal *cpe* isolates versus other *C. perfringens* isolates, including type A isolates carrying a plasmid-borne *cpe* gene. This conclusion is supported by multilocus sequence typing (MLST)

Table 1 Toxin type classification of *C. perfringens* isolates

Type/Toxin	*Alpha*	*Beta*	*Epsilon*	*Iota*
Type A	+	−	−	−
Type B	+	+	+	−
Type C	+	+	−	−
Type D	+	−	+	−
Type E	+	−	−	+

Table 2 Compared to plasmid *cpe* isolates, chromosomal *cpe* food poisoning isolates of *C. perfringens* exhibit greater heat, cold, nitrites, and osmotic pressure resistance

Treatment	*Vegetative cells (*D *value)*		*Spores (*D *value)*	
	Chromosomal cpe *isolates*	*Plasmid* cpe *isolates*[d]	*Chromosomal* cpe *isolates*	*Plasmid* cpe *isolates*
Heat[a]	14.9 min	6.2 min	61.1 min	0.9 min
Cold (4 °C)	12.6 day	1.6 day	0.3 log reduction[b]	1.2 log reduction[b]
(−20 °C)	1.6 day	0.5 day	0.8 log reduction[c]	1.5 log reduction[c]
NaCl	2.8 day	1.4 day	0.2 log reduction[b]	1.1 log reduction[b]
$NaNO_2$	2.2 day	1.0 day	0.9 log reduction[c]	3.5 log reduction[c]

[a]The temperature of heat treatment to vegetative cells is 55 °C and to spores is 100 °C.
[b]The time of this treatment is 6 months, at which time the experiment was terminated before reaching a one log reduction required to calculate a *D* value.
[c]The time of this treatment is 3 months, at which time the experiment was terminated before reaching a one log reduction required to calculate a *D* value.
[d]*cpe*-negative *C. perfringens* strains exhibit similar resistance characteristics as plasmid *cpe* isolates (not shown).

Table 3 Ssp4 is important for the stronger spore resistance properties of *C. perfringens* chromosomal *cpe* food poisoning isolates compared to plasmid *cpe* isolates

Stains	*Heat treatment (100 °C)*	*Nitrous acid treatment*	*Cold treatment*	
	D *value (min)*	*Log reduction after 60 min*	*Log reduction after 6 months*	
			4 °C	*– 20 °C*
F4969WT	0.5	4.0	0.88	1.23
F4969::*ssp4*[a]	0.5	4.6	2.02	3.12
F4969::*ssp4*(pCS)[b]	14.0	3.1	1.07	1.61
F4969::*ssp4*(pCF)[c]	0.5	3.8	1.30	1.81
F4969::*ssp4*(pJIR751)[d]	0.5	4.9	1.82	2.70
SM101WT	59.1	1.1	0.35	0.58
SM101::ssp4	8.4	4.0	0.82	1.91
SM101::*ssp4*(pCS)	9.1	1.1	0.40	0.65
SM101::*ssp4*(pCF)	44.7	3.2	0.50	1.22
SM101::*ssp4*(pJIR751)	9.3	3.8	1.03	1.86

[a]The designation '::*ssp4*' indicates a null mutant unable to produce Ssp4. SM101 is a chromosomal *cpe* food poisoning isolate and F4969 is a plasmid *cpe* isolate.
[b]pCS is a complementing plasmid encoding the Asp36 Ssp4 variant of SM101.
[c]pCF is a complementing plasmid encoding the Gly36 variant of F4969.
[d]pJIR751 is the empty shuttle vector (no *ssp4* insert).

approaches, indicating that, regardless of their geographic origin, date of isolation, or isolation source, type A chromosomal *cpe* isolates show considerable genetic relatedness to one another. However, compared to other *C. perfringens* isolates, chromosomal *cpe* isolates possess a distinct genetic background. The chromosomal *cpe* gene of type A isolates is associated with a putative transposon. Therefore, it is possible that type A chromosomal *cpe* food poisoning isolates have resulted from integration of a transposon (carrying the *cpe* gene) onto the chromosome of a unique *C. perfringens* progenitor cell whose vegetative cells and spores already possessed numerous favorable traits for growth and survival in the food environment.

Epidemiology

Clostridium perfringens has a widespread distribution in soil, feces, and the GI tract of humans and other animals, which provides this bacterium with ample opportunities to contaminate food. However, the specific reservoir remains uncertain for the type A chromosomal *cpe* isolates, which most commonly cause food poisoning. These bacteria are not usually found in soil or on home kitchen surfaces and are only infrequently present in healthy human carriers and sewage. However, they can be present in raw meats and seafood by the time of retail purchase (discussion later).

Recent statistics from the US Centers for Disease Control indicate that *C. perfringens* currently ranks as the second most common cause of bacterial foodborne disease in the USA. Estimates indicate that one million Americans become ill with *C. perfringens* type A food poisoning each year, resulting in approximately 10 deaths (typically in the elderly) and economic losses exceeding $200 million dollars. This food poisoning is also the second most commonly identified bacterial foodborne illness in the UK and Australia. Further supporting its worldwide importance, *C. perfringens* has been responsible for ~20% of all foodborne disease outbreaks in Finland and for at least 4000 cases of Japanese foodborne illness each year.

Typically, *C. perfringens* type A food poisoning outbreaks are large (average 50–100 people) and occur in institutionalized settings. *Clostridium perfringens* type A food poisoning occurs throughout the year, but is somewhat more common during the summer. It typically results from temperature abuse during food holding, storage, or cooking. Although rare outbreaks involving only vegetables have been reported, the most common food vehicles include meats, particularly beef and poultry, or meat-containing foods such as stews and gravies.

Surveys have detected *C. perfringens* in 70% of Japanese raw retail meats and in 30–80% of American raw retail meats or seafood. Of more direct relevance for understanding the epidemiology of *C. perfringens* type A food poisoning, ~0.5% or ~3% of Japanese raw meats or American raw meats/seafood, respectively, are reportedly contaminated with *cpe*-positive type A *C. perfringens* strains at the time of retail purchase. Those potentially enterotoxigenic strains are most commonly present in turkey, chicken, pork/pork products, beef, and fish, all of which are known vehicles for *C. perfringens* type A food poisoning. CPE-producing type A strains have also been found in spices, which thus represent another potential source of food contamination.

The strong association between *C. perfringens* type A food poisoning and meats or meat products could be attributable, in part, to *C. perfringens* requiring several presynthesized amino acids for growth that may be supplied by meats. Another possible explanation for the association between meats and *C. perfringens* type A food poisoning is the common presence of this bacterium in the intestinal tract of food animals, which might foster meat contamination at the time of slaughter.

Clinical Manifestation (Disease)

As shown in Figure 1, *C. perfringens* type A food poisoning typically begins with the ingestion of foods contaminated with

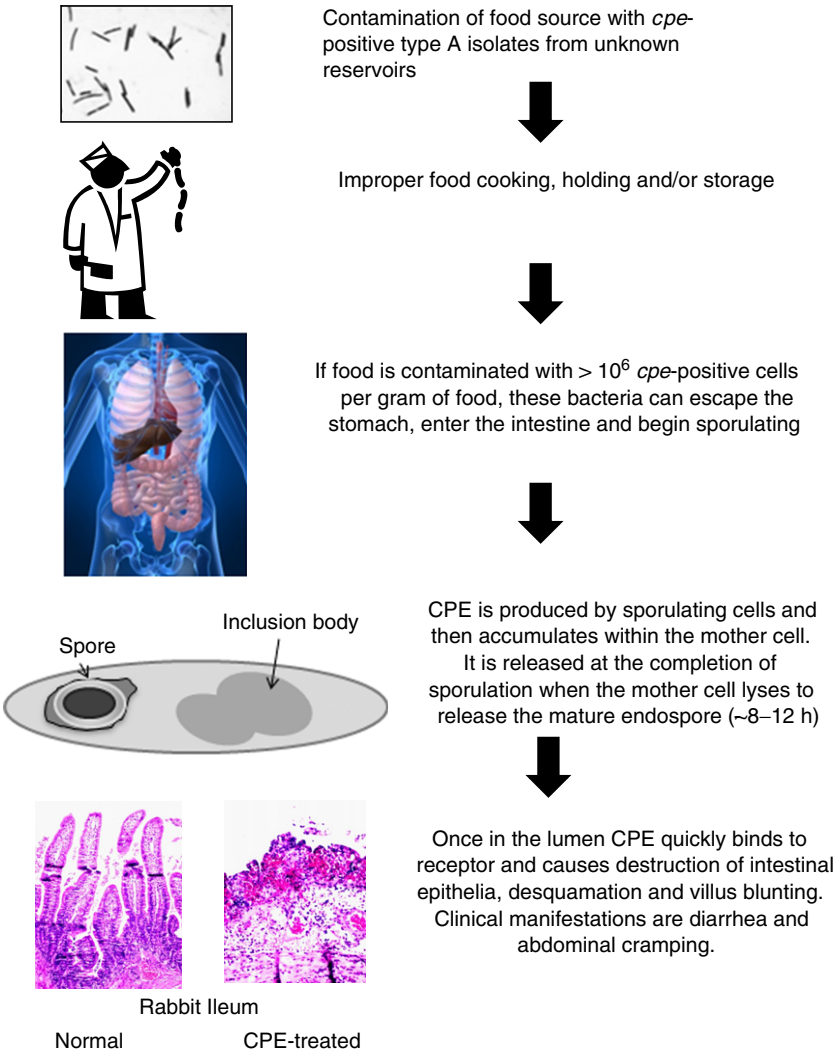

Figure 1 An overview of *Clostridium perfringens* type A food poisoning.

large numbers of vegetative cells of a type A chromosomal *cpe* strain. The infectious dose requires ingestion of $>10^6$–10^7 vegetative cells of a *cpe*-positive isolate/gram of contaminated food.

Following ingestion of a contaminated food, many vegetative cells are destroyed by exposure to gastric acid. However, when the contamination burden is sufficiently high (see preceding paragraph), some vegetative cells survive to escape the stomach. Once present in the small intestine, these vegetative cells initially multiply. During this *in vivo* multiplication, the short doubling times of the chromosomal *cpe* isolates likely contribute to the intestinal presence of large numbers of these bacteria.

After stimulation by various factors, probably including stomach acids and intestinal bile salts, the vegetative cells begin sporulating in the small intestine. It is during this *in vivo* sporulation that *cpe*-positive *C. perfringens* strains produce CPE. Production of CPE is regulated by the master sporulation regulator Spo0A, along with several alternative sigma factors. One of these alternative sigma factors, SigF, apparently directs the production of two other sigma factors named SigK and SigE. Interaction of SigE and SigK with RNA polymerase then drives CPE expression from three SigE- or SigK-dependent promoters located upstream of the *cpe* gene. The presence of multiple *cpe* promoters likely helps to explain why some *cpe*-positive strains produce very high levels of the enterotoxin, i.e., CPE can represent up to 20% of total protein inside some sporulating *C. perfringens* cells. After expression, CPE is not secreted but instead accumulates inside the mother cell. It is eventually released into the intestinal lumen when the mother cell lyses to release the mature endospore.

Once present in the intestinal lumen, CPE rapidly binds to receptors (discussed later) and damages the intestinal epithelium. Figure 1 shows an example of CPE-induced desquamation of the intestinal epithelium in animal models. This damage is apparently required to obtain the fluid and electrolyte losses that present clinically as diarrhea during *C. perfringens* food poisoning. Typically, symptoms of

C. perfringens type A food poisoning are relatively mild and self-resolving and include, besides diarrhea, abdominal cramps. These symptoms usually develop within 8–22 h of ingestion of contaminated foods. This incubation period roughly corresponds to the time needed for completion of *in vivo* sporulation and subsequent lysis of the mother cell to release CPE into the lumen. Illness is then usually resolved within 24 h. There are unusual reports of some patients having less severe symptoms lasting for up to 2 weeks. Fatalities are not common in the young and healthy but can occur in the elderly and physically debilitated due to secondary complications from dehydration. *Clostridium perfringens* type A food poisoning cannot be readily spread person-to-person because the doses required to initiate disease are so large.

It is sometimes incorrectly claimed that *C. perfringens* type A food poisoning is an intoxication. However, there is only one documented case involving disease following ingestion of preformed CPE toxin, in contrast to the many intoxication cases involving foodborne botulism. Furthermore, although ingestion of purified CPE by human volunteers can reproduce all the effects caused by the natural disease, those people had to consume very large amounts of toxin plus sodium bicarbonate to neutralize stomach acidity.

As mentioned, *C. perfringens* type A food poisoning cases usually resolve with little to no long-term complications. In fact, due to the relatively mild symptoms typical of the disease, it is believed that most cases of this disease go undiagnosed. Many Americans and Brazilians were shown to have detectable levels of serum IgG against CPE, supporting a high prevalence for this food poisoning. However, there is no evidence to suggest that previous exposure provides any substantial protection against future disease.

Although cases of *C. perfringens* type A food poisoning are rarely fatal in younger or middle-aged healthy individuals, in November 2001 an unusually severe outbreak of this food poisoning occurred in an Oklahoma residential care facility for the mentally ill. Seven residents of this institution, with an average age of 50 years old, became ill within 18 h after consuming a Thanksgiving meal. All of these people displayed classical *C. perfringens* type A food poisoning symptoms, including diarrhea and abdominal cramping, with some also experiencing vomiting and fever. Three of the ill people developed necrotizing entertitis, which proved fatal in two patients. Until this *C. perfringens* type A food poisoning outbreak in Oklahoma, necrotizing enteritis (or enteritis necroticans) caused by *C. perfringens* had only been associated with beta toxin-producing *C. perfringens* type C isolates. For comparison, foodborne enteritis necroticans caused by type C strain is much more severe than typical cases of type A food poisoning, with symptoms including abdominal pain, bloody diarrhea, vomiting, and obstruction of intestinal mucosa. Patients with severe cases of enteritis necroticans require prompt surgery or the illness can be fatal.

In the Oklahoma mental institution outbreak, several laboratory findings support the involvement of *C. perfringens* type A food poisoning. First, multiplex PCR identified *cpe*-positive type A isolates obtained from the cultured stools of the infected patients. Second, Western blotting confirmed the expression of CPE. Finally, immunohistochemistry demonstrated CPE binding to colonic epithelium in one of the 2 patients who died (the second patient's epithelium was completely destroyed).

In addition to involving necrotizing enteritis caused by *cpe*-positive type A isolates, the other unusual feature of the Oklahoma food poisoning outbreak was that the deceased patients were relatively young and physically healthy. However, the psychiatric drugs administered to these patients appeared to be a contributing factor to disease severity. Side effects of those drugs include constipation and fecal impaction, which interfere with the traditional course of *C. perfringens* type A food poisoning, where diarrhea flushes CPE from the intestine. Thus CPE, and possibly other toxins made by *cpe*-positive strains of *C. perfringens*, remained in contact with the intestinal mucosa for much longer than usual, causing greater damage that produced necrosis of the colonic tissue.

Recently, i.e., May 2010, another *C. prefringen*s food poisoning outbreak occurred at a Louisiana mental hospital. This outbreak involved 40 patients, leading to three fatalities. Chicken salad contaminated with *C. perfringens* was suggested as the potential food vehicle. Interestingly, the severity of this Louisiana mental hospital outbreak is reminiscent of the 2001 Oklahoma mental hospital outbreak, suggesting an emerging linkage between severe *C. perfringens* type A food poisoning outbreaks and mental institutions.

CPE Causes the Symptoms of *C. Perfringens* Type A Food Poisoning

Compelling evidence indicates that CPE is responsible for the symptoms of *C. perfringens* type A food poisoning. For example, when promptly tested, CPE can be detected in the feces of nearly all *C. perfringens* type A food poisoning victims, although this toxin is not found in the feces of healthy people. Also, the levels of CPE present in feces of food poisoning victims are often similar to CPE concentrations known to cause GI disease in animal models. Third, *cpe*-positive type A strains of *C. perfringens* are substantially more pathogenic than CPE-negative type A strains in animal models. Finally, molecular Koch's postulates approaches showed that CPE expression is essential for a type A chromosomal *cpe* food poisoning isolate to cause GI effects in rabbit ileal loops.

CPE Protein

CPE, a single polypeptide of ~35 kDa, has a relatively unique 319 amino acid sequence, sharing only a limited region of homology with a nontoxic protein produced by *Clostridium botulinum*. The C-terminal half of CPE possesses receptor-binding ability and shares some structural resemblance to the receptor-binding regions of the Cry family of insecticidal toxins produced by *Bacillus thuringiensis*. The N-terminal half of CPE is important for cytotoxicity because it possesses regions involved in complex formation (see CPE Mechanism of Action) and toxin insertion into membranes. The extreme N-terminal sequences of CPE may be removed by intestinal proteases during disease, which may activate CPE toxicity by 2- to 3-fold. Recent structural biology studies identified CPE as a member of the aerolysin pore-forming toxin family.

CPE Mechanism of Action

The preferred animal model to study CPE action is the rabbit, although CPE affects all mammalian species thus far examined. The rabbit small intestine, and in particular the ileum, is very sensitive to the enterotoxin, resulting in fluid and electrolyte losses. In the ileum, CPE rapidly induces histopathological damage with epithelial desquamation and villus shortening. This intestinal damage appears to be necessary to obtain intestinal fluid and electrolyte loss. It may also alter paracellular permeability to further increase the fluid secretion that presents clinically as diarrhea. Electron microscopy studies of rabbit intestinal epithelial cells have shown that CPE specifically causes rapid and extensive damage to intestinal brush border membranes. *In vitro*, high CPE doses cause cell death via oncosis, opening the possibility that inflammatory cytokines may also contribute to intestinal disease in some cases of *C. perfringens* type A food poisoning.

CPE action at the cellular level is overviewed in Figure 2. This process begins when the enterotoxin binds to certain claudins, which are a 24 member family of ~22 kDa proteins found in mammalian tight junctions. At physiologically relevant concentrations, CPE has been reported to bind only to claudins 3, 4, 6, 8, and 14. This binding involves interactions between a loop in the C-terminal half of CPE and residues present in the second extracellular loop of claudin receptors. Recent studies implicate an asparagine residue in the second extracellular loop of claudin receptors as being particularly important for CPE binding.

Binding of CPE to claudin receptors initially results in the formation of an SDS-sensitive small (~90 kDa) CPE-containing complex that localizes on the membrane surface. However, formation of this ~90 kDa complex is not sufficient for cytotoxicity. For example, at 4 °C CPE forms this small complex in cultured cells although no cytotoxicity develops.

At physiological temperatures formation of the small CPE complex is only transitory. This complex is thought to rapidly oligomerize with other small complexes to form a larger (~450 kDa) CPE-containing complex. This ~450 kDa large complex is referred to as CPE hexamer-1 or CH-1 because it contains six CPE molecules. The size of CH-1 also reflects the presence of both claudin receptors and claudins incapable of functioning as CPE receptors. The association of nonreceptor claudins with CH-1 is likely attributable to natural claudin:-claudin interactions that occur in plasma membranes. This natural clustering of claudins, including claudin receptors, in plasma membranes may explain why CPE does not need to use lipid rafts for oligomerization.

CH-1 is believed to initially assemble as a prepore on the plasma membrane surface of enterocytes before it inserts into the membrane to form an active pore. The presence of the active CH-1 pore then results in membrane permeability alterations that allow an influx of small molecules, such as ions, into the cytoplasm of host cells. The influx of calcium then triggers cell death via apoptosis (at low CPE doses) or oncosis (at high CPE doses). These alterations also cause morphologic alterations to the cell that disrupt tight junctions and allow CPE access to the basolateral side of the cell, where even more claudin receptors are present than on the apical cell surface. Besides resulting in formation of additional amounts of CH-1 complex, exposure of the basolateral

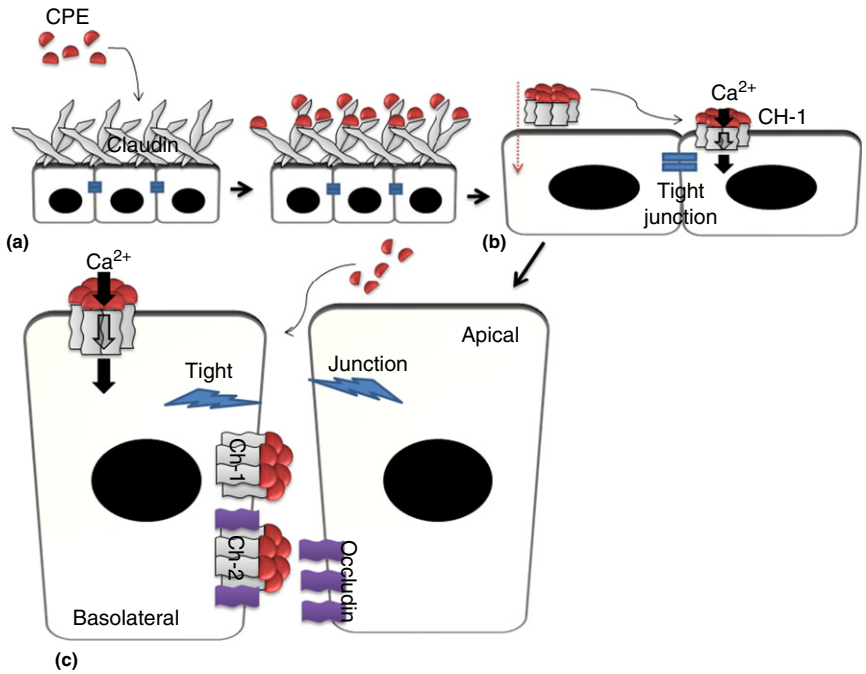

Figure 2 CPE mode of action. (a) CPE binds to claudin receptors on the apical surface of epithelial cells, forming a small ~90 kDa complex. (b) The small complex rapidly oligermerizes to form the ~450 kDA CPE hexamer-1 or CH-1. This hexamer inserts into the cell membrane, forming a pore through which small ions gain access to the cell. (c) This pore causes cell permeability changes leading to cell death. Additionally the tight junctions are disrupted, so free CPE can gain access to the basolateral side, where it can bind more claudin, forming small complex to form more CH-1. A second large CPE complex of ~600 kDa, referred to as the CH-2, now also forms and contains occludin.

cell surface also allows CH-1 access to another tight junction protein named occludin. This results in formation of a second large complex of ~600 kDa, which is termed CH-2 or CPE hexamer 2. Besides containing CPE and claudins, CH-2 also contains occludin. A consequence of CH-1 and CH-2 formation is the internalization of claudins and occludin, which might cause paracellular permeability alterations that contribute to diarrhea.

In summary, CPE is a unique bacterial toxin in many respects. Besides possessing a relatively unique primary sequence, this pore-forming toxin does not require lipid rafts for oligomerization. CPE is also an unusual toxin by remaining closely associated with claudin receptors throughout the pore formation process. Beyond pore formation, CPE may possess a second action, i.e., disruption of tight junctions, which could also contribute to disease.

Analytical Methods for *C. Perfringens* and CPE

Clostridium perfringens type A food poisoning outbreaks have traditionally been diagnosed by demonstrating the presence of large numbers of *C. perfringens* in feces of food poisoning victims or in food vehicles. However, this approach has significant limitations because, for example, feces from some healthy elderly people already contain very high numbers of (*cpe*-negative) *C. perfringens*. A more meaningful diagnostic approach is to demonstrate the specific presence of large numbers of *cpe*-positive *C. perfringens* cells or spores in food vehicles or the feces of food poisoning victims. Because *cpe*-positive strains can also cause other GI diseases, such as antibiotic-associated diarrhea, it is important to consider positive detection of *cpe*-positive isolates in feces within the full epidemiologic context, i.e., is the fecal specimen from a point source foodborne disease outbreak? If so, do the symptoms, incubation time, and duration of the outbreak correspond to those of a typical *C. perfringens* type A food poisoning outbreak? Are there also *cpe*-positive isolates present in the suspected food vehicle? An alternative approach for identifying a *C. perfringens* type A food poisoning outbreak is to demonstrate the presence of CPE in feces of food poisoning victims using commercially available serologic testing kits. This method is most reliable when feces are collected soon after the onset of diarrhea, the time when fecal CPE levels are maximal. However, as stated above for the detection of *cpe*-positive cells in feces of food poisoning victims, positive detection of CPE should also be considered within an epidemiologic context.

Beyond these basic diagnostic approaches, many tools are now available for more detailed epidemiologic studies of *C. perfringens* type A food poisoning. Selective plating methods are available to isolate *C. perfringens* from food, slaughterhouses, soil, sewage, aquatic environments, domestic or commercial kitchens, or human feces. However samples usually should first be enriched for the presence of vegetative cells and spores before plate counts being made for enumeration. An example of isolation and identification of *C. perfringens* type A from food sources has been described by Wen and McClane. A suspected food source is homogenized and aliquots are added to 10 ml tubes of sterile FTG medium. To enrich for samples containing *C. perfringens* spores, one of these tubes is heat shocked at 72 °C for 20 min before a subsequent incubation at 37 °C for 18–24 h. A second tube is grown for the same time period at 37 °C to enrich for *C. perfringens* vegetative cells. Enrichment cultures are then streaked onto TSC plates with egg yolk. Those plates are then anaerobically incubated for ~18 h at 37 °C. Colonies are presumptively *C. perfringens* if they show lecithinase activity on the egg yolk agar. The identity of these colonies can be confirmed using standard methods outlined by the FDA. This approach also provides isolated colonies to perform multiplex PCR to identify toxin type and *cpe* carriage. When *cpe*-positive isolates are identified, a duplex PCR can also be performed to determine whether the *cpe* gene is chromosomal or plasmid. The isolates can be inoculated into sporulation media and, if sporulation is achieved, CPE production can be demonstrated by Western immunoblotting. These assays are described more in detail in the following paragraphs.

The levels of *C. perfringens* per gram of food can be approximated by determining the most probable number (MPN). A 10 g aliquot of a food suspension is diluted in 10 fold increments and those dilutions are each inoculated into 3 tubes containing differential reinforced clostridial broth medium (DRCM), followed by overnight incubations at 37 °C. Cultures that form a black precipitate in the DRCM are considered presumptively positive for *C. perfringens* and results are used to calculate the MPN, as described by Wen and McClane.

A multiplex PCR assay can assign *C. perfringens* isolates to one of five types, A–E, and determine whether an isolate is *cpe*-positive. DNA template is prepared from an isolated colony by suspending it in PBS. After microcentrifugation, the bacteria are resuspended in PCR-quality water and then lysed by microwave. The resulting supernatant is used as the template. PCR analysis is carried out using primers that amplify sequences found in genes encoding alpha toxin (*cpa*), beta toxin (*cpb*), beta-2 toxin (*cpb2*), epsilon toxin (*etx*), CPE (*cpe*), or the A component of iota toxin (*iap*). PCR products are electrophoresed on 2% agarose and visualized by ethidium bromide staining.

To determine whether a *cpe*-positive *C. perfringens* type A isolate carries a chromosomal *cpe* locus or a plasmid *cpe*, a duplex PCR assay can be used, as described by Wen and McClane. For this assay, *cpe*-positive isolates are inoculated into TGY medium and then grown overnight at 37 °C. Genomic DNA is extracted from that culture, as described above, and subjected to PCR using primers that amplify a 2.1 kb product when the *cpe* gene is chromosomal, but a 3.3 kb product from type A isolates carrying most *cpe* plasmids.

CPE Western immunoblotting can be used to determine if a *cpe*-positive isolate can express the enterotoxin. FTG cultures of the *cpe*-positive isolate are inoculated into Duncan-Strong sporulation medium and then incubated at 37 °C for ~8 h or until spores are present. Cultures are then sonicated until >95% of the cells are lysed. The lysate is then subjected to electrophoresis and detection of CPE expression by Western Blot. A limitation of this approach is that not all *cpe*-positive isolates form spores when grown *in vitro*, as required for CPE expression.

Table 4 Examples of some recent *C. perfringens* type A food poisoning outbreaks

Outbreak location	*Food vehicle*	*Persons ill*	*Food handling error*
#1, UK	Chicken curry	73	Inadequate temperature and time control during food preparation and storage
#2, Japan	Braised chop suey (contains pork, seafood, and vegetables)	81	Inadequate temperature control during holding
#3, UK	Chicken and lamb	93	Inadequate temperature control during food handling
#4, USA	Turkey	~60	Inadequate temperature and time control during cooking food storage
#5, USA	Casserole (contained noodles, ground beef, ground turkey, and vegetables)	>100	Inadequate temperature and time control during cooking food storage

Control and Preventative Measures

Clostridium perfringens has ample opportunity to contaminate food because it is widely distributed throughout the natural environment, being found in 75% of soil samples (at levels ranging from 10^3 to 10^4 CFU g^{-1}), in sewage, and (at numbers up to 10^6 CFU g^{-1}) in the GI tract of some healthy livestock and humans. However, as mentioned earlier, the reservoir is not clear for the <5% of all *C. perfringens* type A isolates that possess a chromosomal *cpe* gene, which are the strains causing most cases of food poisoning. Nevertheless, as mentioned, by the time of retail purchase, approximately 0.5–3% of raw retail meats are already contaminated with type A chromosomal *cpe* isolates. Because it is not yet known when food poisoning strains enter the food supply from their (still unidentified) reservoir, it is currently difficult to rationally interfere with the presence of these bacteria in foods.

At present the best practical approach for controlling this food poisoning is provided by epidemiologic observations indicating that temperature abuse during the cooking, cooling, or handling of foods is the major contributor to *C. perfringens* type A food poisoning (Table 4). Temperature abuse is a particularly important factor considering the already-described impressive resistance properties possessed by the cells and spores of most type A food poisoning isolates. Thus, the best way to prevent *C. perfringens* type A food poisoning is rigid adherence to cooking regimens, holding of foods at >60 °C for serving, and rapid cooling of cooked meats for storage. To facilitate rapid cooling, foods should be distributed in small portions in shallow containers. It is also essential that refrigerators be functioning below 6 °C, because food poisoning isolates can grow at lower temperatures than other *C. perfringens* strains.

No vaccine has yet been developed to prevent this food poisoning due to its typical self-resolving nature. However, the noncytotoxic C-terminal fragment of the enterotoxin (C-CPE) has been shown to elicit antibodies capable of neutralizing native CPE. Therefore, C-CPE represents a potential vaccine candidate if ever needed.

See also: Characteristics of Foodborne Hazard and Diseases: Cost of Foodborne Diseases. Foodborne Diseases: Overview of Biological Hazards and Foodborne Diseases. Public Health Measures: Foodborne Disease Outbreak Investigation; Surveillance of Foodborne Diseases

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Relevant Websites

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CDC National Center for Zoonotic, Vector-borne and Enteric Infections: Foodborne illness.

http://www.fda.gov/Food/FoodSafety/FoodborneIllness/FoodborneIllnessFoodbornePathogensNaturalToxins/BadBugBook/default.htm
US Food and Drug Administration *Bad Bug Book*.

Toxigenic Corynebacteria

AA Zasada, National Institute of Public Health – National Institute of Hygiene, Warsaw, Poland

Glossary

Internalization Transfer of molecules from the outside to the inside of a cell usually by phagocytosis.
Invasive infection/disease An infection/disease with the presence of bacteria in normally sterile anatomical sites.
Nontoxigenic (in this article) A strain of *Corynebacterium pseudotuberculosis*, *Corynebacterium ulcerans*, or *Corynebacterium diphtheriae* that does not produce diphtheria toxin.
Toxigenic (in this article) A strain of *C. pseudotuberculosis*, *C. ulcerans*, or *C. diphtheriae* producing diphtheria toxin.
Translocation Transfer of molecules through a membrane, for example a membrane of endosome.

Characteristics of the Genus

The genus *Corynebacterium* has been described as Gram positive (some strains appear Gram variable); straight-to-slightly curved rods with tapered ends; pleomorphic; asporogenous, nonmotile; nonacid fast; forms metachromatic granules; facultatively anaerobic, although some organisms are aerobic; catalase positive; and chemoorganotrophic.

Currently, the genus *Corynebacterium* comprises more than 80 recognized species. Only three of them are known to possess the potential to produce a lethal exotoxin, called diphtheria toxin. These are *Corynebacterium diphtheriae*, *Corynebacterium ulcerans*, and *Corynebacterium pseudotuberculosis*. The three species are closely related. The relationship is based on a similar morphology, cell wall composition, as well as 16S ribosomal ribonucleic acid (rRNA) gene sequences. All the three species differ from other corynebacteria by the presence of large amounts of fatty acids. Colonies of all the three species form gray–brown halos on Tinsdale medium, which identifies the production of cystinase, and are pyrazinamidase negative.

Biochemically, *C. ulcerans* and *C. pseudotuberculosis* can be distinguished from *C. diphtheriae* by urease production and the positive reverse CAMP test (the reverse CAMP test is a reaction whereby hemolysis by the *β*-hemolysin of staphylococci is inhibited through the production of phospholipase C or D by some organisms) (Table 1). Moreover, *C. ulcerans* ferments glycogen, starch, and trehalose.

On the basis of colonial morphology and biochemical properties, four biotypes of *C. diphtheriae* were identified: *gravis*, *mitis*, *intermedius*, and *belfanti*. On the Hoyle's tellurite agar, biotypes *mitis* and *belfanti* produce gray/black opaque colonies, 1.5–2.0 mm in diameter with an entire edge and glossy smooth surface. Variation in size is common. Biotype *gravis* produces dull, gray/black opaque colonies, 1.5–2.0 mm in diameter with a matt surface, friable, tending to break into small segments when touched with a straight wire. Colonies of biotype *intermedius* are small, gray/black, discrete, translucent, 0.5–1.0 mm in diameter with shiny surface. On blood agar, colonies of biotypes *mitis*, *intermedius*, and *belfanti* exhibit a small zone of *β*-hemolysis, whereas biotype *gravis* is usually nonhemolytic. Specific biochemical characteristics are presented in Table 1.

The Diphtheria Toxin

The diphtheria toxin is regarded as the main virulence factor of *C. diphtheriae*, *C. ulcerans*, and *C. pseudotuberculosis*. It is encoded by the *tox* gene. The gene is carried on a family of closely related corynebacteriophages of which the *β*-phage is the most extensively studied. The *β*-like phages are capable of integrating into the *Corynebacterium* chromosome during the lysogenic phase of the infective cycle. The integration can occur at two specific sites: *attB1* and *attB2*. The expression of *tox* in lysogens is regulated by the DtxR protein in response to the Fe^{2+} level. DtxR is a global regulator, chromosomally encoded by the *dtxR* gene. This protein regulates the expression of multiple genes involved in iron metabolism, protection against oxidative stress, and pathogenesis. In the absence of Fe^{2+}, apo-DtxR exists as an inactive monomer that is in weak equilibrium with a dimeric form. Once activated by Fe^{2+}, DtxR forms stable dimers, and two pairs of dimers have been shown to bind to almost opposite faces of the *tox* operator sequence. DtxR is composed of two major structural domains linked by a flexible tether containing a proline-rich region. The *N*-terminal domain contains the ancillary and primary metal ion-binding sites, a canonical helix-turn-helix deoxyribonucleic acid (DNA)-recognition motif, and an extensive hydrophobic surface necessary for the formation of stable dimers. After the DtxR dimers bind to the *tox* promoter, the transcription of *tox* promoter is repressed. When iron is in

Table 1 Characteristics differentiating the potentially toxigenic species of the genus *Corynebacterium*

Species	*Reverse CAMP*	*Alkaline phosphatase*	*Rhamnose*	*Sucrose*	*Trehalose*	*Salicin*	*Starch*	*Dextrin*	*Glycogen*	*Nitrate*	*Urease*
C. diphtheria var. *gravis*	−	−	+	−	−	+	+	+	+	+	−
C. diphtheria var. *mitis*	−	−	+	−	−	+	−	+	−	+	−
C. diphtheria var. *intermedius*	−	−	+	−	−	+	−	−	−	+	−
C. diphtheria var. *belfanti*	−	−	+	−	−	+	−	+	−	−	−
C. ulcerans	+	+	+	−	+	+	+	+	+	−	+
C. pseudotuberculosis	+	−	−	+	−	−	+	−	−	−	+

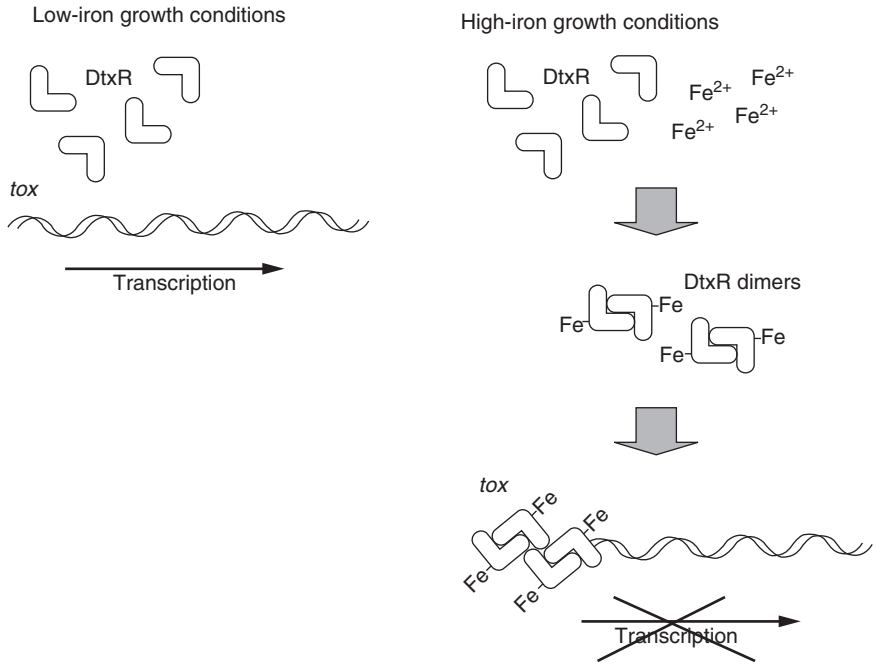

Figure 1 Model of regulation of diphtheria toxin expression. Description in the text.

limiting concentration, the uncomplexed form of DtxR is unable to bind DNA, leading to the induction of diphtheria toxin (Figure 1). Diphtheria toxin is composed of three domains: the catalytic domain, the transmembrane domain, and the receptor-binding domain. The catalytic domain is called fragment A, whereas the transmembrane- and the receptor-binding domains together are called fragment B. Fragment A with fragment B is connected by a disulfide bond. The catalytic domain is an *N*-terminal adenosine diphosphate (ADP)-ribosyltransferase. The transmembrane domain facilitates the delivery of the catalytic domain across the eukaryotic cell membrane. The receptor-binding domain binds to the receptor at the surface of the eukaryotic cell. The intoxication of a single eukaryotic cell is schematically presented in Figure 2. In the first step, the toxin binds to its cell surface receptor through its receptor-binding domain (fragment B). The complex is then internalized by receptor-mediated endocytosis. Both fragments A and B of the toxin unfold in response to low pH in the endosome, leading to the exposure of hydrophobic domains and an increased tendency to interact with the membrane lipids. In the next steps, the transmembrane domain is inserted into the membrane and the fragment A of the toxin is translocated across the membrane to the cytosol. The disulfide bond connecting fragment A and B is reduced. The final step is the translocation of the remainder of the fragment A to the cytosol, where it refolds. The catalytic domain (fragment A) of the toxin stops protein synthesis by inactivating elongation factor 2 via ADP ribosylation from NAD. The result is death of the cell.

Other Virulence Factors

Other Virulence Factors of *C. diphtheriae*

Toxigenic corynebacteria are well investigated with respect to toxin production, whereas little is known about the other virulence factors crucial for colonization of the host, invasion, and avoidance of the host immune system. Microbial factors that distinguish epidemic from endemic strains have not been

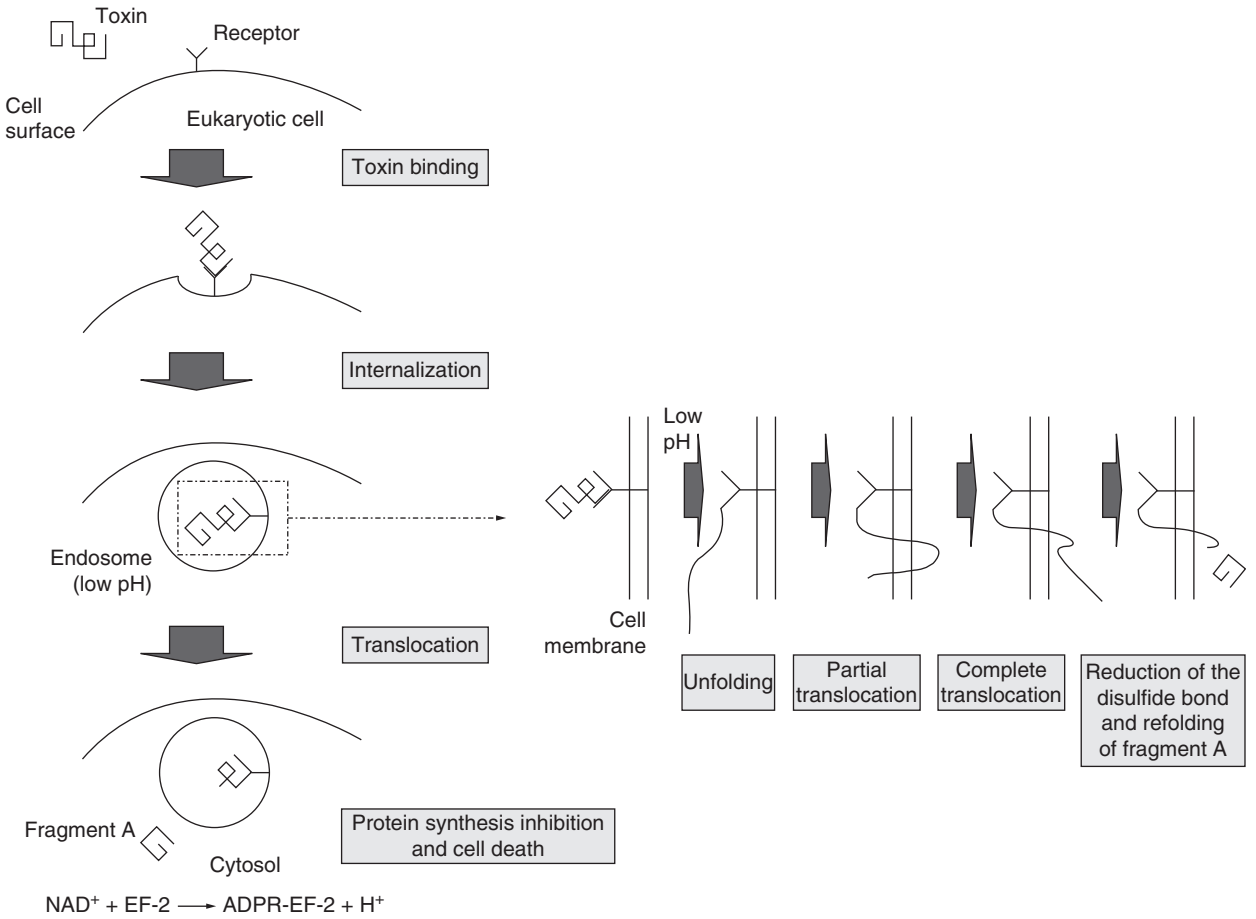

Figure 2 Model of diphtheria toxin action. Description in the text.

identified. The fact that specific epidemic clones are responsible for severe outbreaks of diphtheria with thousands of deaths in industrialized countries supports the argument that bacterial virulence factors, other than toxin, are important for their potential to cause human disease. The increasing frequency of cases of invasive disease, such as endocarditis, bacteremia, pneumonia, osteomyelitis, spleen abscesses, and septic arthritis, caused by nontoxigenic clones of *C. diphtheriae* also points the importance of other microbial factors.

In a few studies, mechanisms of adhesion, invasion, and intracellular survival of *C. diphtheriae* have been investigated. It has been shown that *C. diphtheriae* strains are not only able to adhere to laryngeal HEp-2 or pharyngeal Detroit 562 cells but also enter these cells and survive after the internalization. Although host cell receptors and invasion-associated proteins of the pathogen are still unknown, bacterial adhesion factors have been at least partially characterized recently on the molecular level. *Corynebacterium diphtheriae* is able to assemble at least three distinct types of pili on its surface. Mutant analysis have shown that the SpaA-type pilus is sufficient for adhesion to pharynx cells, shaft proteins are not crucial for pathogen–host interaction, and adherence to pharyngeal cells is greatly diminished when minor pili proteins SpaB and SpaC are lacking. However, the existence of additional proteins besides pili subunits involved in adhesion to larynx, pharynx, and lung epithelial cells have been demonstrated because a total loss of attachment to pharyngeal cells due to mutagenesis of pili- and sortase-encoding genes could not be observed and attachment to lung and larynx cells was less affected by the mutation. Moreover, the potential importance of hemagglutinins, exposed sugar residues, hydrophobins, and *trans*-sialidase enzymes in adhesion have also been revealed. It can be stated that adhesion is a multifactorial mechanism. Furthermore, three distinct patterns of adherence to HEp-2 cells have been described: an aggregative, a localized, and a diffuse form. These observations prove the existence of several adhesion factors and different receptors on the host cell surface. The involvement of different *C. diphtheriae* proteins to adherence to distinct cell types is further reported by the work on adhesion to human erythrocytes, showing that nonfimbrial surface proteins 67p and 72p are involved in this process. Besides different mechanisms of adhesion, growth-dependent effects have also been observed, for example, an effect of iron supply on hemagglutination and lectin-binding properties of *C. diphtheriae*.

Corynebacterium diphtheriae strains are able to produce siderophores and other iron-uptake mechanisms that allow them to express virulence factors, such as toxins and enzymes. Published results also imply regulation of adherence and

slime production as part of a global response to iron-limited environment conditions that includes derepression of genes for the synthesis of cytotoxin and siderophores and transport of the Fe(III)–siderophore complexes.

Interestingly, all genes encoding pili and adhesins as well as iron-uptake systems of *C. diphtheriae* are located in pathogenicity islands. Great heterogeneity in genome organization and in pathogenicity of *C. diphtheriae* have been shown. For example, comparison of genomes of two *C. diphtheriae* strains: NCTC 13129 and C7(−) revealed that 11 of 13 pathogenicity islands identified in the NCTC 13129 strain were absent in the C7(−) strain.

Other Virulence Factors of *C. ulcerans* and *C. pseudotuberculosis*

Corynebacterium ulcerans and *C. pseudotuberculosis* produce phospholipase D (PLD), which is unique among corynebacteria. PLD is a prominent virulence factor of these two species. It promotes the hydrolysis of ester bonds in sphingomyelin in mammalian cell membranes, possibly contributing to the spread of the bacteria from the initial site of infection to the secondary sites within the host. Moreover, it provokes dermonecrotic lesions; and at higher doses, it is lethal to a number of different species of laboratory and domestic animals.

The surface lipids of *C. pseudotuberculosis* are also regarded as a virulence factor. The toxicity of the extracted lipid material has been demonstrated by the induction of hemorrhagic necrosis following intradermal injection in guinea pigs. Moreover, it has been observed that *C. pseudotuberculosis* was resistant to killing and digestion by caprine macrophages due to its lipid coat. Recently, the complete genome sequence of human isolate of *C. pseudotuberculosis* has been published, thus enabling to deduce its more potential virulence factors. The *fagCBA–fagD* genes encoding an iron-uptake system and gene clusters associated with pathways for siderophore biosynthesis have been identified. SpaABC and SpaDEF pili from *C. pseudotuberculosis* showing similarity to subunits of adhesive pili from *C. diphtheriae* can also be regarded as potential virulence factors. Moreover, the genome sequence has revealed four genes encoding secreted proteases. Extracellular proteases may exhibit a wide range of pathogenic potentials when interacting with the defense mechanisms and tissue components of the host. Another candidate virulence factor of *C. pseudotuberculosis* is the extracellular neuraminidase NanH. Neuraminidases, or sialidases, belong to a class of glycosyl hydrolases that catalyze the removal of terminal sialic acid residues from a variety of glycoconjugates and can contribute to the recognition of sialic acids exposed on host cell surfaces. Sialidases located on the bacterial cell surface can be used for the decoration of sugar moiety acceptors with sialic acid to enable the invasion of hosts under certain conditions. Another potential virulence factor of *C. pseudotuberculosis* is represented by the *nor* gene encoding nitric oxide reductase. This enzyme is generally involved in the detoxification of nitric oxide and consequently necessary for the long-term persistence of pathogens in macrophages. Further work should be done to compare the existence of deduced virulence factors genes in other *C. pseudotuberculosis* human and animal isolates in order to evaluate the heterogeneity of the species.

Clinical Manifestation

The clinical features of illnesses caused by toxigenic *C. ulcerans* and *C. pseudotuberculosis* are similar to diphtheria caused by toxigenic *C. diphtheriae*. Depending on the anatomic site that is affected by the disease, there could be respiratory or cutaneous diphtheria. In rare instances, other sites, such as the eye, ear, and vulva, can also be affected. Symptoms of respiratory diphtheria occur usually after an incubation period of 1–10 days. The onset is relatively slow and characterized by moderate fever and a mild exudative pharyngitis. In severe cases, pseudomembranes gradually form in the throat, recognizable by their typical asymmetric, grayish-white appearance, strong attachment to the underlying tissue, and bleeding following attempts to remove or dislodge them. Such pseudomembranes may extend into the nasal cavity and the larynx causing obstruction of the airways. Laryngeal diphtheria, which sometimes occurs even without pharyngeal involvement, is a medical emergency that often requires tracheostomy. Enlarged anterior cervical lymph nodes and edema of soft tissues giving a "bull neck" appearance are also observed.

Cutaneous diphtheria usually appears on exposed parts. The lesions start as vesicles and quickly form small, clearly demarcated, and sometimes multiple ulcers. The lesions are usually difficult to treat and may take months or even years to heal.

Diphtheria toxin absorbed from the mucosal or cutaneous lesions may account for toxic damage to organs, such as the myocardium, kidneys, and nervous system. The following clinical conditions are associated with an increasing risk of toxin-induced systemic disease: (1) the catarrhal form (erythema of pharynx and no membranes), (2) the follicular form (patches of exudates over pharynx and the tonsils), (3) the spreading form (membranes covering the tonsils and posterior pharynx), and (4) the combined form (more than one anatomical site involved, e.g., throat and skin).

Epidemiology of Infections

After the introduction of vaccination against the diphtheria toxin in 1940s, the infections caused by toxigenic corynebacteria have seemed to be well controlled in developed countries. However, infections recorded during the past several years point at *C. ulcerans* and *C. diphtheriae* as reemerging human pathogens.

C. ulcerans Infections

The epidemiology of human infections caused by *C. ulcerans* is not well known. The infections are usually acquired through contact with animals (see Section Occurrence in the Environment and Food) or by eating or drinking unpasteurized dairy products. However, such risk factors have not been identified for some cases of classical diphtheria caused by *C. ulcerans*, which suggest that there may be other routes of infections. Person-to-person transmission of *C. ulcerans* is thought to occur only rarely.

Infections caused by toxigenic strains of *C. ulcerans* are increasingly reported from developed countries. The infections

seem to replace infections caused due to toxigenic *C. diphtheriae*. In UK between 2000 and 2009, 43 isolates of toxigenic corynebacteria were identified, 27 of which (63%) were *C. ulcerans*. In France between 2002–08, 12 (63%) *C. ulcerans* were isolated from 19 cases of toxigenic corynebacteria infections in humans. In USA, 2 (29%) isolates of toxigenic *C. ulcerans* strains were isolated from 7 toxigenic *Corynebacterium* infections during the period 1999–2005; but before 1999, the last reported case of diphtheria-like illness caused by *C. ulcerans* in USA occurred in 1996. Other examples are 3 (33%) isolates of toxigenic *C. ulcerans* of 9 cases of toxigenic corynebacteria infections in Canada from 1999 to 2003 and 1 (33%) isolate of toxigenic *C. ulcerans* of 3 cases of toxigenic *Corynebacterium* infections in Italy from 1999 to 2001. Also in Japan, 2 cases of human infection with toxigenic *C. ulcerans* were identified in 2001 and 2002.

It seems that women are the risk groups acquiring *C. ulcerans* infections, especially those older than 50 years. They are probably more exposed to domestic animals and, as shown in studies conducted in Europe, women have been less protected against diphtheria than men older than 40 years.

C. pseudotuberculosis Infections

Very little information about *C. pseudotuberculosis* have been published. Fewer than 30 human cases have been described in the literature so far. Cases are most often linked to contact with goats and sheep. Toxigenic strains usually cause illness similar to diphtheria. Among 25 European countries participating in Diphtheria Surveillance Network, only three cases of *C. pseudotuberculosis* human infections were recorded during the period 2000–10, two cases in France in 2004 and 2005 and one case in UK in 2008. Infections due to toxigenic strains have been similar to diphtheria. Most human cases of nontoxigenic *C pseudotuberculosis* infections have had lymphadenitis, apart from one case of eosinophilic pneumonia and one case of eye infection.

C. diphtheriae Infections

Infections caused by *C. diphtheriae* are usually acquired through contact with infected persons and objects that have been touched by them. In 1990, a diphtheria epidemic due to *C. diphtheriae* occurred in the Newly Independent States (NIS) of the former Union of Soviet Socialist Republics (USSR). The epidemic began in the Russian Federation in 1990 and affected all the NIS countries by the end of 1994. At the peak of the epidemic in 1995, 50 425 cases were reported in the NIS, compared with 24 cases in other countries. After mass immunization campaigns, the incidence of diphtheria in the Russian Federation and in the NIS began to decrease. Between 1990 and 2001, more than 160 000 cases were reported in the region. In 2002, 1189 cases were reported from the World Health Organization (WHO) European region, 95% of the cases were from the Russian Federation and the NIS. In 2003, a total of 896 cases were reported from the WHO European region and 99% (892) were from the Russian Federation and the NIS; the 4 remaining cases were reported from Turkey (1 case) and UK (3 cases). According to the WHO data, in 2007, 4190 diphtherial cases (due to *C. diphtheriae*) were reported worldwide. Across the European Union, 47 diphtheria cases (due to *C. diphtheriae* and *C. ulcerans*) were reported in 2008. There were 29 cases in Latvia, 6 cases in UK, 5 cases in France, 2 cases in Lithuania, and 1 case in Sweden. The most affected age groups were 5–14 years and 45–64 years. The number of cases was higher among females than among males.

Countries with endemic diphtheria are Algeria, Angola, Egypt, Niger, Nigeria, Sudan, and other sub-Saharan countries; Bolivia; Brazil; Colombia; Dominican Republic; Ecuador; Haiti; Paraguay; Afghanistan; Bangladesh; Bhutan; Burma (Myanmar); Cambodia; China; India; Indonesia; Laos; Malaysia; Mongolia; Nepal; Pakistan; Papua New Guinea; Philippines; Thailand; Vietnam; Iran; Iraq; Saudi Arabia; Syria; Turkey; Yemen; Albania; Russia; and countries of the former USSR.

It is worth underlying that invasive diseases caused by *C. diphtheriae* have been described increasingly worldwide stressing the importance of this species as a serious bloodborne pathogen. Between 1893 and 1995, only 58 cases of bacteremic infections due to *C. diphtheriae* were described. During 1990s, the situation had been changed. During the period 1999–2003, 13 cases of *C. diphtheriae* invasive infections were noticed in Canada. In Switzerland, 14 isolates of *C. diphtheriae* were obtained from invasive infections in 1990–96. In Poland, the first *C. diphtheriae* invasive infection was recorded in 2004; and in the period 2005–07, seven additional cases were recorded. Isolation of *C. diphtheriae* from invasive infections also increased in Brazil during the past years. The invasive diseases are caused by both toxigenic and nontoxigenic *C. diphtheriae* strains. Predisposing factors for the development of the invasive disease are homelessness, injection drug use, alcohol abuse, diabetes mellitus, and homosexuality. More often males are affected than females.

Occurrence in the Environment and Food

Corynebacterium species are a widely distributed group of bacteria, typically found in the environment in soil and on the skin and mucous membranes of human beings and animals. Corynebacteria have also been documented to survive for long periods of time on objects that have been touched by infected individuals.

Corynebacterium ulcerans is a commensal in animals and has been isolated from a wide host of domestic and wild animals (e.g., dogs, cats, horses, goats, cows, pigs, camels, monkeys, squirrels, and otters). The animals may serve as reservoirs for human infection. Moreover, the bacterium causes mastitis in cattle and goats. In mastitis infection, the bacteria are present in milk. A significant portion of human *C. ulcerans* infections has been associated with the consumption of raw dairy products. Also handling of infected animals and their products could be a source of infections. Recently, contact with companion animals, such as dogs and cats, has been recognized as a way of infection.

Corynebacterium pseudotuberculosis is a rare zoonosis. Similar to *C. ulcerans*, raw dairy products are the source of infection as well as handling of infected animals and their products (e.g., butchery). Sheep, followed by goats, are the most

commonly affected animal hosts; but horses, cattle, and deer may also be infected. *Corynebacterium pseudotuberculosis* has also been isolated from milk in mastitis cases.

Corynebacterium diphtheriae is traditionally considered as a nonzoonotic pathogen. However, recent reports of horses as carriers of *C. diphtheriae* highlight the possible emergence of a new route of human infection. *Corynebacterium diphtheriae* has also been isolated from a cat. In older publications, isolation of *C. diphtheriae* from other animals has been reported as well. The species is infrequent cause of bovine mastitis and has been associated with dermatitis with pyrexia in cattle. It is thought to be transferred to cows from infected dairy workers. Moreover, *C. diphtheriae* has been documented in other domestic animals, including equids and canids. The dairy products obtained from infected animals and handling of infected animals could be a source of infection for humans.

Identification and Toxigenicity Testing

A selective tellurite medium, such as Hoyle's agar, and Tinsdale agar are very useful in the first step of identification of potentially toxigenic *Corynebacterium* species (see Section Characteristics of the Genus). Identification of suspected isolates usually relies on Gram staining and biochemical reactions. ApiCoryne system (BioMerieux) is broadly used for biochemical identification of corynebacteria. However, the system does not identify *C. diphtheriae* var. *intermedius* and groups together *C. diphtheriae* var. *mitis* and *C. diphtheriae* var. *belfanti*. Biotypes *mitis* and *belfanti* can be easily distinguished manually on the basis of results of nitrate reduction (included in the ApiCoryne system). But additional tests need to be done for biotype *intermedius* identification.

Molecular methods such as 16S rRNA gene sequence analysis or *rpoB* gene sequence analysis are also used. Nevertheless, *rpoB* sequencing does not differentiate *C. ulcerans* and *C. pseudotuberculosis*. Polymerase chain reaction (PCR) targeting the *pld* gene is very useful for the identification *C. pseudotuberculosis*. Recently, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry has been proposed as a tool for rapid diagnosis of potentially toxigenic *Corynebacterium* species.

Classical and modified Elek tests are commonly used to demonstrate the toxigenicity of *C. diphtheriae*, *C. ulcerans*, and *C. pseudotuberculosis* strains. Moreover, a rapid immunochromatographic method for detection of diphtheria toxin has been developed. But the test is not available commercially. Owing to the problems with diphtheria antitoxin accessibility, some laboratories are using cell culture for detection of diphtheria toxin production. But the test gives a delayed answer to the diagnosis. As faster methods, conventional PCR were described for detection of fragment A and B of the *tox* gene. But positive results in the tests do not confirm whether the strain is producing toxin. Real-time PCR test was also developed, which provide more rapid results than conventional PCR for *C. diphtheriae*. However, it was observed that real-time PCR analysis of *C. ulcerans* isolates could produce atypical amplification of fragment A and no amplification of fragment B, although conventional PCR is useful to detect the presence of subunits A and B of the *tox* gene in *C. ulcerans*. There is no available information concerning the usefulness of real-time PCR for *tox* gene detection in *C. pseudotuberculosis*.

Antimicrobial Susceptibility

Owing to the high fatality rate of potentially toxigenic corynebacteria infections, appropriate antimicrobial treatment is crucial (apart from diphtheria antitoxin treatment). Most of the isolates are susceptible to a broad range of antimicrobial agents. Penicillin and erythromycin are the drugs of choice for treatment of *C. ulcerans*, *C. diphtheriae*, and *C. pseudotuberculosis* infections. Nevertheless, *C. diphtheriae* isolates resistant to β-lactams and erythromycin, intermediate to cefotaxime, as well as multidrug-resistant strains have been described. *Corynebacterium ulcerans* strains resistant to erythromycin and clindamycin have also been isolated. *Corynebacterium pseudotuberculosis* isolates resistant to penicillin, streptomycin, and neomycin have been reported. Moreover, in the experiments where *C. pseudotuberculosis* was growing as a biofilm, the bacterium was highly resistant to all the drugs tested. In case of *C. pseudotuberculosis* infections, the antibiotic therapy alone is usually not successful and should be combined with a surgical intervention. This may be because *C. pseudotuberculosis* is a facultative intracellular pathogen multiplying in macrophages. Cell death and release of bacteria lead to necrotic lesions and to the formation of a thick collagen capsule that protects the bacterium from antibiotics.

Typing Methods

There have been several typing schemes described based on the traditional methods, such as serotyping, phage typing, and bacteriocin typing. The methods are regarded as historical and performed only in a few laboratories in the world. Currently, molecular typing methods are broadly used. Ribotyping has been described as a gold standard for potentially toxigenic corynebacteria. The international nomenclature for *C. diphtheriae* ribotypes has been established and a database of all recognized ribotypes has been built. Eighty-six ribotypes have been described. However, the method is time consuming and labor intensive. To the best knowledge of the author, only three national centers of diphtheria in Europe are currently able to perform ribotyping. Recently, the multilocus sequence typing (MLST) method for *C. diphtheria* typing has been developed together with a database of all recognized sequence types. Until now, 208 sequence types have been described. The method is increasingly used in more and more laboratories and is replacing ribotyping. Also many other molecular typing methods have been described as useful for typing of potentially toxigenic corynebacteria, for example, pulsed-field gel electrophoresis (PFGE), random amplification of polymorphic DNA (RAPD), multilocus enzyme electrophoresis (MLEE), amplified fragment length polymorphisms (AFLP), and enterobacterial repetitive intergenic consensus PCR (ERIC-PCR). In 2005, a microarray-based method called spoligotyping (spacer oligonucleotide typing) was developed as a new genotyping tool with high discriminatory power. The method allows fast and efficient discrimination within the

C. diphtheriae epidemic clonal group. Currently, studies on development of multiple-locus variable-number tandem repeat analysis (MLVA) for *C. diphtheriae* typing are conducted.

Control and Preventive Measures

Infections due to toxigenic and nontoxigenic *C. diphtheriae*, *C. ulcerans*, and *C. pseudotuberculosis* can be prevented by washing hands, especially after contact with animals; avoiding the consumption of raw dairy products; pasteurization of milk; and education of domestic animals owners, persons handling the animals, and dairy workers.

Moreover, infections due to toxigenic strains of *C. diphtheriae*, *C. ulcerans*, and *C. pseudotuberculosis* can be prevented by the use of antidiphtheria vaccine. Antibiotic treatment should be administered to diphtheria patients, contacts, as well as asymptomatic carriers to eliminate the bacteria and prevent the spread of infection. Patients with diphtheria should be nursed in strict isolation until bacteriological clearance has been demonstrated by negative cultures of nasopharyngeal and throat swabs. All objects in direct contact with the patient and objects soiled by discharges from the patient should be disinfected while the patient is in isolation.

See also: Characteristics of Foodborne Hazard and Diseases: Pathogenesis and Virulence. Institutions Involved in Food Safety: Trust in Animals and Food Safety (TAFS) Forum. Safety of Food and Beverages: Milk and Dairy Products

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Coxiella burnetii

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Glossary

16S rRNA 16S ribosomal ribonucleic acid.
Axenic medium Pure culture using artificial cultivation medium.
Containment level 3 Biosafety level three.
HEL Human embryonic lung.
IS*1111* Insertion sequence *1111*.
LCV Large cell variant.
SCV Small cell variant.
Shell vial Cell line supported on glass coverslip.

Background

History

Edward Derrick described a febrile illness among abattoir workers in Brisbane, Australia during 1937, that became known as Query (Q) fever. Shortly after the causative organism now known as *Coxiella burnetii*, was recovered independently by both Burnet and Freeman in Australia and Davis and Cox in USA. Despite significant ensuing research effort, our understanding of this pathogen; its interactions with host species; and pathophysiological mechanisms remain to be fully disclosed.

Characteristics of Pathogen

This obligate intracellular pathogen is grown in various cell culture lines, but was originally cultivated in embryonated eggs. It is a microaerophile with Gram-negative cocco-bacillary cell wall structure (although it cannot be stained using the Gram-staining method, but can be visualized using methods such as Gimenez). The cells are typically pleomorphic ranging from 0.3 to 1.5 μm in length and 0.2–0.4 μm in width. Two antigenic forms of this microbe exist, phase I that is the highly virulent variant associated with acute infection, and the avirulent phase II associated with prolonged *in vitro* cultivation. Its highly infectious nature has led to its requirement for handling under containment level 3. Its significant association with livestock has called into question the role of *C. burnetii* as a potential food safety pathogen.

Systematics of *Coxiella*

Its physiological, lifestyle, and morphological resemblance to the rickettsiales led to its initial mistaken inclusion among this family within the alphaproteobacteria. Application of molecular sequenced-based studies such as 16S rRNA gene analysis, revealed its similarity to *Legionella*, and resulted in its subsequent reclassification. The Coxiellaceae family forms a monophyletic group among the gammaproteobacteria. The phylogenetic proximity to the Legionellales within the gammaproteobacteria has been further confirmed through whole genomic comparisons of available complete sequences. Some have postulated that *C. burnetii* has evolved from a free-living environmental bacterium, possibly related to members of the halophilic gammaproteobacterium. Indeed, this could account for its possession of features such as introns and inteins, likely to have arisen from horizontal gene transfer, now restricted through its current obligate intracellular lifestyle.

Molecular investigations have furthermore disclosed variability among *C. burnetii* with eight different genomic groups within the species. Whether these relate to host correlations or clinical presentations remains speculative. Further subtyping can be achieved through application of high-resolution typing methods such as multispacer typing or variable number tandem repeat typing (see Section Molecular Detection and Typing of *C. burnetii*). These methods are particularly useful for the identification of related strains in the case of suspected outbreaks of infection.

Natural Resistance of *Coxiella*

The SCV of *Coxiella* exhibits remarkable resistance where it has been reported to withstand heat, desiccation, UV light and a variety of different disinfectants. Indeed, it has been reported to have acid resistance up to pH of 4.5. Furthermore, its temperature resistance with survival at 70 °C for 15 min poses particular problems with regard to food hygiene. Similarly, the survival over prolonged periods at 4 °C raises food hygiene concerns. This is compounded by the preference of *Coxiella* to survive and indeed multiply in eggs, again raising concerns regarding food safety issues.

Laboratory Diagnostic Methods

Isolation of C. burnetii

Cultivation of *C. burnetii* requires use of conventional cell culture methods, inoculation of the yolk sac of embryonated

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eggs, or intraperitoneal inoculation of laboratory animals such as mice, hamsters, or guinea pigs and subsequent recovery from infected spleens. Probably the most frequently used method is the "shell vial" tissue culture with cell lines such as human embryonic lung (HEL) fibroblasts or buffalo green monkey cells. The sample is inoculated into these cells by centrifugation and cells examined for periods of up to 3 weeks for growth of *Coxiella*. Where samples are potentially contaminated, inclusion of selective antimicrobials such as gentamicin (50–100 $\mu g\,ml^{-1}$), penicillin (50–100 $\mu g\,ml^{-1}$), or streptomycin (100–200 $\mu g\,ml^{-1}$) can assist isolation. Some have used tick or insect cell lines particularly when attempting to isolate *C. burnetii* from arthropods. Tissue cultures are assessed using staining with Gimenez, Macchiavello, Stamp, or Giemsa methods to reveal the typical morphological appearance of *C. burnetii*.

Recently, growth of *C. burnetii* has been reported in axenic medium, however, it remains uncertain whether this would be suitable for primary isolation.

Serodiagnosis of Q Fever

Conventional serological methods have been used for serological diagnosis of Q fever in both humans and animals. Complement fixation tests have been superseded by the use of either indirect immunofluorescence tests or enzyme-linked immunosorbent assays. These can be used to detect specific IgM, IgG, or IgA antibodies as appropriate. For IgM, titers more than 1:50 are usually considered positive, whereas for IgG, the cutoff is often set at 1:200 for acute infection (see Table 1). The two phase variants of *C. burnetii* should both be assessed, with acute infection associated with elevated titers against the phase II variant, whereas those with chronic infection will produce raised titers against both phase I and phase II variants. The phase variants of *C. burnetii* are associated with a change in the lipopolysaccharide O-chain, with phase I representing virulent cells, whereas phase II is associated with the nonreversible loss of branched-chain sugars virenose and dihydrohydroxystreptose, resulting in a nonpathogenic variant. This has been likened to the smooth (phase I) and rough (phase II) variants seen among other Gram-negative bacteria. The structure of the phase I *C. burnetii* variant is pivotal for its toll-like receptor 4-mediated uptake by phagocytic cells.

On a cautionary note, some infected animals have been reported not to seroconvert. Similarly, seroconversion has been reported following consumption of infected milk after effective pasteurization. Slight differences in serological reactivity have been noted depending on the infecting strain used as antigen and variability noted among commercially available assays. Serology is, however, good for population studies to give evidence of disease activity. Similarly, on an individual level, it can provide useful information regarding acute or chronic infection.

Table 1 Diagnostic serological titers

Stage	*Immunoglobulin class*	*Phase I antigen*	*Phase II antigen*
Acute	IgM	<1:50	>1:50
	IgG	<1:200	>1:200
	IgA	<1:50	>1:25[a]
Chronic	IgM[c]	>1:50[b]	<1:50
	IgG	>1:800	>1:200
	IgA	>1:25[a]	>1:25[a]

[a]Elevated IgA is not observed in all cases.
[b]Not elevated in all cases.
[c]Low levels of IgM can persist in chronic Q fever.

Molecular Detection and Typing of C. burnetii

Polymerase chain reaction (PCR) provides probably the best methods for detection of *C. burnetii*. Not only does this eliminate the need to cultivate, but can also be used to type these organisms, sometimes directly in clinical material if sufficient numbers of organisms are present (see Table 2). Various primers sets have been used, with the most frequently used being the IS*1111* insertion sequence. This is a multicopy target (ranging from 7 to 110 copies depending on isolates characterized to date) is present in all isolates of *C. burnetii*, thus enhancing sensitivity over single-copy targets. The main disadvantage of this method is its lack of detecting viability,

Table 2 Advantages and disadvantages of different laboratory diagnostic methods

Detection method	*Advantages*	*Disadvantages*
Microscopy	Quick and cheap	Poor sensitivity and nonspecific (*Chlamydophila* and brucellae may resemble *Coxiella*)
Isolation	Demonstrates viability and provides isolate for further typing	Time consuming (may take 3 weeks), expensive, requires tissue culture facilities at containment level 3, and low yield
Serology	Cheap and good for high-throughput testing	Imprecise, may require repeat testing during acute infection, need to test phase I and phase II antigens, and seroconversion can follow exposure to nonviable organisms
PCR	Quick, can also be used to type organisms detected, good during acute infection, and can be used to detect organisms in foods	Will not demonstrate viability, appropriate samples need to be tested (blood is not often positive), PCR inhibitors, and expensive
Animal Inoculation	Provides viable isolates and good when contaminated samples are used	Time consuming, expensive, infection risks, and requires legislative justification

thus when used for testing milk or foods, it may still detect nonviable organisms killed by effective processing.

Molecular typing of *C. burnetii* has been achieved through use of various high-resolution typing methods. Use of multiple noncoding intragenic spacers has been of particular value for differentiating different clonal groups of Coxiellae, with several new groups being recently added to our expanding knowledge of strain diversity. Alternatively, variable tandem repeat typing has been shown to also be highly discriminatory. These methods being PCR-based, can be applied to clinical samples or produce where suitably large numbers of *C. burnetii* are present. Post-genomic sequencing has also permitted design of single nucleotide polymorphism (SNP)-based typing, alleviating the need to undertake sequence analysis.

Of the above methods, only PCR-based approaches have potential for detection in food as the others are unlikely to offer suitable sensitivity. The food matrix might be refractory to amplification, thus DNA should be extracted before testing. Another dilemma results from the lack of information on viability, thus complicating interpretation and assessment of infection risk.

Epidemiology

Natural Maintenance Reservoirs

Transmission routes for *C. burnetii*. The organism, being an obligate intracellular pathogen, requires a suitable host cell environment in which it can multiply. It was thought that the natural reservoir for *C. burnetii* was livestock, however, over recent years, the organism has been detected in numerous diverse sources ranging from fresh-water amoeba, various arthropods including ticks (both hard and soft ticks), domestic pets, exotic zoo animals, reptiles, amphibians, birds, and rodents. Infection in the majority of these species appears to be silent. It may be that natural ecological niches for this organism remain silent, with only accidental "spill over" hosts, such as humans, manifesting clinical consequences. Indeed, even humans infected do not uniformly succumb to clinical disease, with an estimated 60% of infection believed to remain asymptomatic. Despite these diverse natural reservoirs, human infection is significantly correlated with livestock infection, thus behaving as a zoonotic disease.

Once infected, mammals (including man) can chronically excrete *Coxiella* in bodily secretions such as milk, feces, urine, and vaginal secretions. In livestock this shedding of *C. burnetii* can be protracted lasting several months. Attempts to reduce shedding through treatment of livestock appear not to succeed in reducing either the shedding or the duration of shedding. Conversely, vaccination of livestock with phase I cells, has been reported to reduce shedding and to offer a protective effect when used before exposure, however, vaccine availability is currently limited to certain countries (see Section Vaccination).

The organism may produce both large cell and small cell variants (LCV and SCV, respectively). It is the SCV that has been likened to an endospore, which demonstrates particular resistance to adverse conditions (see Section Natural Resistance of *Coxiella*), enabling prolonged extracellular environmental persistence or survival within food. It is this SCV that is typically taken up by the new host. In contrast, the more fragile, but metabolically active LCV appears within the phagolysosomes of infected host cells. It is within this intracellular niche of an acidic parasitophorous vacuole, that *C. burnetii* multiplies.

Clinical Manifestation

Human Q Fever

Infection results in a flu-like illness with fever in approximately 40% of individuals, often resolving without complications (Figure 1). Disease manifestations show a spectrum of severity, with some developing atypical pneumonia or hepatitis, or less frequently myocarditis, pericarditis, meningitis or encephalitis, potentially requiring hospitalization (approximately 5%, Figure 1, Table 3). More seriously, infection may result in potentially life-threatening endocarditis in approximately 1% of individuals, particularly among those with preexisting cardiac defects (Figure 1). Furthermore, the increased incidence in adverse outcomes following acquisition of infection during pregnancy is a worrying concern. Infection has been associated with placentitis, premature birth, fetal death and spontaneous abortion. Numbers studied to date have been small, thus further studies are needed to fully assess the risks of infection during pregnancy.

Less well established is the potential for infection to result in a protracted "chronic fatigue" syndrome. Long-term clinical follow-up of several notable outbreaks have been associated with increased incidence of fatigue-like complications. These studies have been restricted in size and consequently their ability to conclusively establish the cause and effect, thus this potential consequence is still hotly debated. Many obligate intracellular bacterial pathogens have a propensity for persistence, some showing reactivation at stages later in life. Thus, it would not be surprising if latent infection might follow primary infection.

Livestock Infections

In livestock, when symptomatic, infection is primarily associated with late stage abortion particularly among small ruminants. High bacterial numbers are found in placental and fetal membranes. Abortion, stillbirth, reduced fertility, and endometritis have all been linked with *C. burnetii* infection. Infected cattle often remain asymptomatic, but may show abortion, reduced fertility, or metritis. Transmission is likely to be particularly high among animals confined in close proximity under poor conditions such as those in Figure 2.

A recent major outbreak in the Netherlands (2007–10) was correlated with intensive goat farming practices in close proximity with highly populated human areas. Although some abortion has been noted among goats, the infection has been largely silent, with airborne transmission to humans the probable route of spread. Intervention attempts including vaccination and culling of pregnant goats finally abated this outbreak.

Epidemiology of Disease

Coxiella burnetii is considered ubiquitous affecting all countries with the exception of New Zealand. Incidence data are patchy

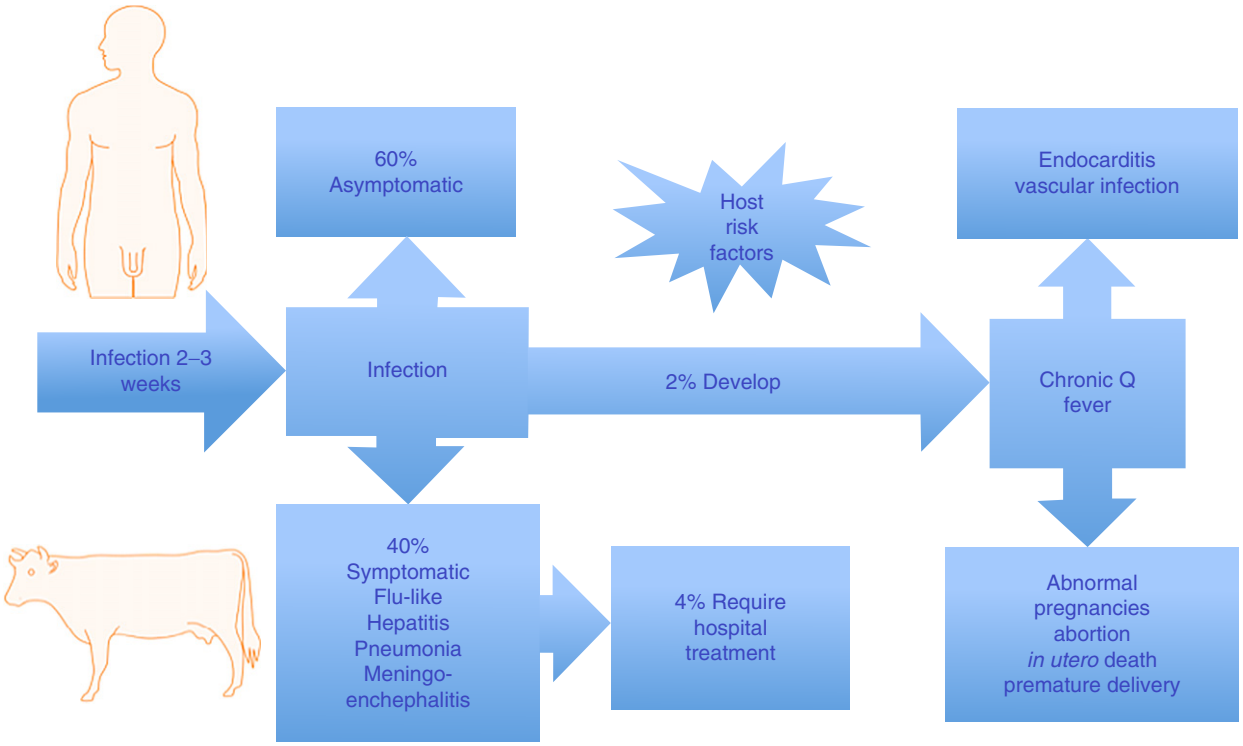

Figure 1 Postinfection natural history of *C. burnetii* infection in humans. Adapted from Tissot-Dupont H and Raoult D (2007) Clinical aspects, diagnosis, and treatment of Q fever. In: Raoult D and Parola P (eds.) *Rickettsial Diseases*, pp. 291–301. London: Informa Healthcare.

Table 3 Clinical signs of Q fever seen in humans

Acute Q fever
Fever and systemic signs
- Abrupt onset of high fever with or without flu-like illness
- Incubation 14–39 day (average 20 day)

Respiratory symptoms
- Cough, shortness of breath, chest pain, pneumonia, or no respiratory signs

Cardiovascular symptoms
- Chest pain
- Myocarditis – can be fatal

Neurological signs
- Stiff neck and headache
- Meningoencephalitis or encephalitis, meningitis and myelitis, and peripheral neuropathy

Chronic Q fever
Endocarditis
- "Culture negative" endocarditis. Can occur months to years after acute infection
- Accompanied by fatigue, fever, dyspnea, and rash

Various physical findings dependent on clinical syndrome (aneurisms, osteomyelitis, chronic hepatitis, and vasculitis)

Figure 2 Animal husbandry practices can facilitate *C. burnetii* transmission. Reproduced from Cutler SJ, Paiba GA, Howells J, and Morgan KL (2002) Q fever – a forgotten disease? *Lancet Infectious Diseases* 2: 717–718.

reflecting local research interests rather than results of systematic surveillance. Incidence reports from Australia have ranged from 3 to 4.99/100 000, whereas results from Marseille, France have suggested that this might reach as high as 50/100 000.

Intriguingly, clinical Q fever is found more often in men than women, even if an equal seroprevalence exists in both groups (in some studies approaching 2.5:1). It has been hypothesized that female sex hormones (such as estrogen), might have a protective effect with ovarectomized mice showing male levels of symptomatic disease. Epidemiological studies from around the globe have confirmed the

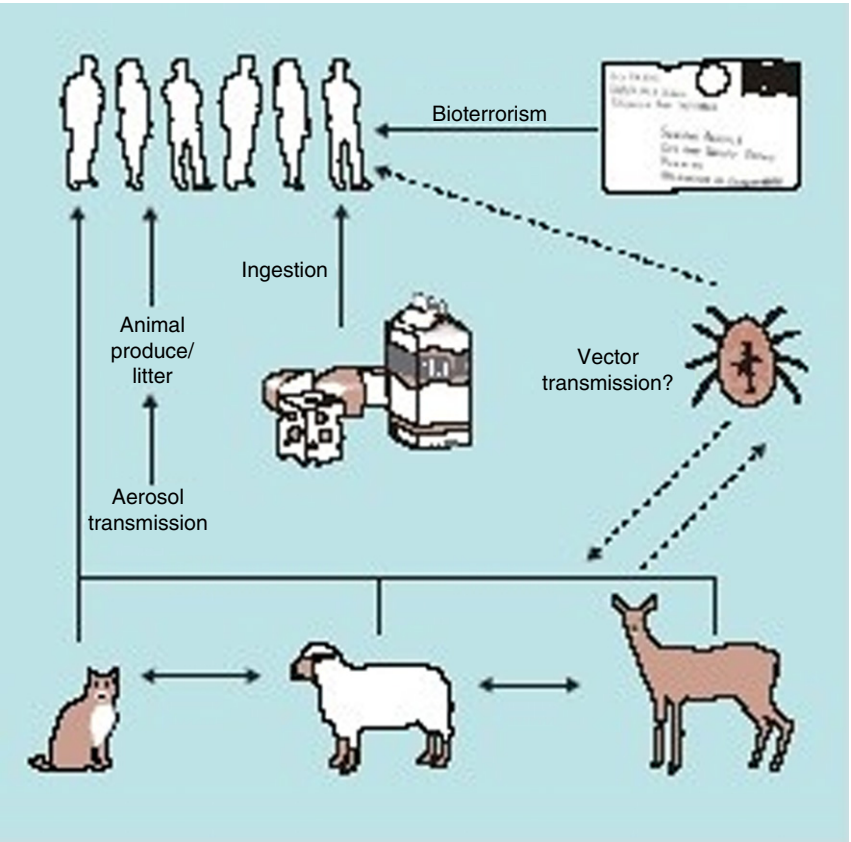

Figure 3 Transmission routes for *C. burnetii*. Reproduced from Tissot-Dupont H and Raoult D (2007) Clinical aspects, diagnosis, and treatment of Q fever. In: Raoult D and Parola P (eds.) *Rickettsial Diseases*, pp. 291–301. London: Informa Healthcare, with permission from Taylor and Francis.

predominance of clinical infection in males, and a remarkable lack of symptomatic disease among children.

The seasonality observed for acquisition of Q fever correlates with the lambing or calving seasons, thus underscoring the role of parturient livestock in human Q fever.

Transmission Routes

Probably the most important transmission route through which infection is acquired, is the respiratory route (Figure 3). Indeed, it is estimated that as few as 10 organisms are sufficient to result in human infection. Contact with infected material is again an important route for infection (Figure 3), with sporadic and clusters of infection attributed to contact with straw (probably contaminated with animal excreta). Whether this is direct physical contact, or a result of aerosolization of infected materials is difficult to conclusively prove. Human infection has been recorded as a consequence of occupational contact with livestock (abattoir workers; veterinary staff; farmers). Further cases have been linked with parturient companion animals, with liberation of infectious particles associated with delivery.

The role of tick bites appears not to be a major risk for acquisition of Q fever. Even though the Nine Mile reference isolate was isolated from a *Dermacentor andersoni* tick, no human cases have been directly attributed to tick bites. Even sexual contact has been described for transmission between an occupationally exposed worker and his wife. By far the most important exposure appears to be respiratory transmission following exposure to liberated infectious particles, often approaching 10^9 organisms per gram of placental tissue during delivery of livestock, and subsequent environmental contamination, and prolonged shedding in the milk, urine, feces and vaginal secretions of infected animals.

Potential for Foodborne Infection

The significance of foodborne infection remains a realistic possibility, however, study designs have usually been restricted to demonstration of *C. burnetii* in foodstuffs rather than causation of disease following consumption of infected foods. Consumption of contaminated milk has been demonstrated to result in seroconversion, however, clinical cases did not ensue, nor was recovery of *C. burnetii* attempted from those individuals who seroconverted. Given the expanding environmental niches being demonstrated for persistence, the plethora of potential host species and innate resistance of the SCV to many procedures used to process our foods such as desiccation, heat, biocides, and acidic conditions (pH 4–5), it is likely that exposure by this route does occur, but significance remains to be established.

Food Hygiene Implications

With regard to food hygiene, risk assessment models whereby hazard identification; exposure assessment; dose–response; and risk characterization are important steps used to assess food safety. Often added to this are steps to manage risks identified, and communication. By following these steps, it is clear that *C. burnetii* might readily be present in meats and milk destined for human consumption. Furthermore, experimental studies have highlighted the highly infectious nature of *C. burnetii*, although these studies have used respiratory exposure rather than via the gastrointestinal tract. Exposure assessment aspects are challenging to interpret regarding *C. burnetii*, in part through the clinical diversity seen following infection, and protracted clinical course that subsequently follows infection.

General foodborne infection reduction measures applied to improve food safety are likely to reduce the burden of infection. Regarding livestock, the association of *C. burnetii* with abortion episodes and its subsequent excretion over the ensuing months following delivery, highlight the potential role of dairy products as a vehicle of infection rather than meat. Indeed, this has led to initial evaluation of *C. burnetii* as an indicator organism for successful pasteurization. Studies from USA have reported 94% of bulk tank milk samples to be positive by PCR for *C. burnetii*. Such data illustrate the potential for exposure and underscore why *C. burnetii* was used as an indicator for evaluation of temperatures and times for pasteurization. Studies from the 1950s determined that 30 min at 62.8 °C or 15 s at 71.7 °C were sufficient to maximize destruction of viable *Coxiella*. These investigations formed the basis for pasteurization recommendations aimed at reducing vegetative bacterial burden to a level considered safe for human consumption. It was also recognized that even following successful pasteurization, that seroconversion might follow, thus multiple passage in guinea pigs was used to assess successful killing of test organisms. This raises an important issue regarding the use of serology as a diagnostic tool for Q fever and could account for some of the high levels of positive serology reported in the absence of clinical signs. Indeed, a study based on a farming population utilized ambulance drivers as a control group without agricultural exposure, and found a higher seroprevalence among controls than the test group of farmers.

We cannot directly disregard meat as a source for infection. Indeed, when we broaden our perspectives to include those involved in the processing of food, significant outbreaks of infection have been linked with abattoirs and meat-packing workers. A recent abattoir-associated outbreak occurred in Stirling, Scotland following spontaneous abortion of a sheep in the holding area, resulting in infection of more than 200 workers associated with the abattoir. Whether retail meat could serve as a vehicle for transmission remains to be assessed (see Figure 4).

Surveillance of bulk milk samples has disclosed an excess of 94% positive for *C. burnetii* DNA using PCR from countries such as USA. European studies have reported much lower values of 4.7% (Switzerland) and 3.5% (Turkey) of bovine and ovine milks, respectively. Prolonged shedding of *C. burnetii* has been reported in bovine milk; however, this does not appear to be the case for milks from ovine or caprine sources. The significance of oral infection remains unresolved. Studies of human volunteers drinking contaminated milk resulted in seroconversion, but not overt clinical disease. It must, however, be remembered that 60% of respiratory-exposed individuals will similarly seroconvert, but remain asymptomatic, thus the afore mentioned volunteer studies might just reflect experimental groups of insufficient size. Where milk and milk products are likely to have a more prominent role in transmission is more likely where consumption of unpasteurized milk and goat cheese is a commonplace in many parts of the world. Figure 5 illustrates a typical example of goat's cheese in Peru often made with unpasteurized milk.

Figure 4 Could retail meat markets further the dissemination of *C. burnetii*?

Figure 5 Unpasteurized goat's cheese on sale at a market in Peru.

The role of eggs and their products has also been questioned, with 4.2% of shell eggs and 17.6% of mayonnaise samples from Japan testing positive by PCR. Few studies elsewhere have addressed the role of shell eggs as a potential source for infection, but a study from Switzerland was unable to corroborate these findings.

Our knowledge of *C. burnetii* is still formative and continuing to evolve making it important to use recent data sources to avoid misinformation. Indeed, peer-reviewed data from 1997 in reputable food microbiology journals would have make you believe that *C. burnetii* was exotic to the

Netherlands and that human infection was of little significance! This was then followed by a major and protracted outbreak from 2007 to 2010 involving more than 4000 human cases, serving as a reminder of the need to remain vigilant. Such misled opinions underscore the need for multidisciplinary approaches to assess the infection risks of such pathogens within the food chain.

Control and Prevention of Disease

As with many zoonoses, control is best delivered through reduction of infection in the reservoir, in this case, among livestock. This will reduce both direct and indirect human infection (such as through the food chain). Several measures have been suggested which are detailed below.

Animal Husbandry and Hygienic Measures

Various husbandry and hygienic measures can reduce the level of infection. These include stringent vermin control measures coupled with rendering of any aborted fetuses or placental material together with incineration of bedding. Movement restrictions help to contain infection where detected. Farmyard manure poses additional risks as this is often spread on the land as fertilizer from where it can be aerosolized posing risk of infection. This should be composted for 3 months before use on the land.

Vaccination

Inactivated vaccines have been prepared from phase I cells (Coxevac, CEVA, Hungary) and used to show some protective effect in animals, however, annual vaccination is generally recommended to prevent infection among vulnerable livestock species. Vaccination of infected livestock has been suggested to show some beneficial reduction of shedding into the environment. Human vaccination (Q-VAX, CSL Ltd., Australia) has been used to protect high-risk individuals in certain countries (such as abattoir workers in Australia), but has been associated with adverse side effects, particularly among those with preexisting exposure. Consequently, serological testing should be a prerequisite before vaccination.

Conclusion

Interest in both *C. burnetii* and Q fever has been elevated in part through its inclusion as a potential biothreat agent, and following several notable large outbreaks of infection. These have served to highlight our knowledge gaps regarding the various natural reservoirs, transmission, and pathophysiological mechanisms employed by *C. burnetii*. Many unanswered questions remain, such as why disease is rarely seen during childhood; the reasons as to why infection predominates in males; the risks of infection during pregnancy; and the role of nonrespiratory transmission of *C. burnetii*? We hope that renewed research efforts into genomic analysis, improved diagnostics, and continuing efforts to produce vaccines to both prevent disease and shedding of the organism into the environment will enable us to reduce the risks of infection in years to come.

See also: Foodborne Diseases: Overview of Biological Hazards and Foodborne Diseases

Further Reading

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Shiga Toxin-Producing *Escherichia coli* and Other Pathogenic *Escherichia coli*

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Glossary

Animal reservoir Animals that directly or indirectly transmit a pathogen, although being immune to its effects.
Genomic plasticity Characteristic of the bacterial chromosomes allowing the exchange of DNA between microorganisms and causing their genomes to adapt rapidly to changes in the environmental conditions.
Pathogenicity island Bacterial DNA trait vehiculating genes involved in virulence, and containing other genes, governing the insertion and excision from an integration site in the bacterial chromosome.
Polymerase chain reaction Technology allowing the specific amplification of a DNA fragment from complex DNA preparation, increasingly used to detect DNA sequences associated with specific pathogens in foodstuffs.
Super-shedder cattle Individuals who excrete many more *E. coli* O157 than others.

Introduction

Escherichia coli is part of the microflora of the gastrointestinal tract of mammals and birds, but certain strains have been associated with diarrheal diseases in both humans and animals. Pathogenic *E. coli* have evolved the capability to colonize almost every district of the human organism and cause a wide range of diseases and symptoms. The extraintestinal pathogenic *E. coli* (ExPEC) infect the mucosal districts outside the gastrointestinal tract and are a leading cause of urinary tract infections. Some ExPEC can be isolated from the blood of children with neonatal meningitis. The *E. coli* strains involved in the induction of enteric forms are clustered in a large pathogroup termed diarrheagenic *E. coli*, which is in turn subdivided into a number of different pathotypes based on the pathogenetic mechanism of the induced disease. Diarrheagenic *E. coli* are able to cause diarrhea as a result of the acquisition by horizontal gene transfer of genetic elements coding for toxin production, adhesion to and invasion of host cells, and interference with cell metabolism. The different diarrheagenic pathotypes include: (1) enterotoxigenic *E. coli* (ETEC), which produce enterotoxins that are able to stimulate the small intestine to secrete electrolytes and water, resulting in diarrhea. ETEC are considered a leading cause of diarrhea in the low-income world and an agent most commonly involved in traveler diarrhea; (2) enteroinvasive *E. coli* , closely resembling *Shigella*, are able to invade and damage colonic mucosa cells and cause a dysenteric form of diarrhea in humans; (3) enteropathogenic *E. coli* (EPEC), which are able to adhere to the intestinal cells causing attaching and effacing (A/E) lesions and represent a major cause of profuse watery infantile diarrhea in low-income countries; (4) enteroaggregative *E. coli* (EAEC), which cause watery diarrhea by adhering to the epithelium of terminal ileum and colon in a characteristic aggregative pattern followed by a damage/secretion stage; (5) Shiga toxin (Stx)-producing *E. coli* (STEC), characterized by the production of potent cytotoxins that inhibit the protein synthesis within eukaryotic cells and can cause severe illnesses such as hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS), especially among children and the elderly.

Most of the pathogenic groups of *E. coli* have only the human reservoir and infections are diffused via the oral–fecal route. An animal origin and the foodborne transmission have been postulated for some ExPEC causing urinary tract infections (UTIs), but this matter is still under debate. On the contrary, STEC represent the only pathogenic group of *E. coli* that has a definite zoonotic origin, with ruminants, and in particular cattle, being recognized as the major reservoir for human infections. A large variety of *E. coli* serogroups has been identified in ruminants capable of producing Stx. However, only a few serogroups have been consistently associated with human disease and just a dozen of them are routinely isolated from the most severe forms of infection. As a matter of fact, approximately half of the HUS cases reported in the last 20 years in Europe were caused by STEC O157, and the vast majority of the remaining cases were due to infection caused by STEC belonging to serogroups O26, O111, O103, and O145. As a consequence, in 2007, a scientific opinion of the European Food Safety Authority (EFSA) formulated the definition of highly pathogenic STEC as *E. coli* strains able to produce Stx, to colonize the host gut by the A/E mechanism, and belonging to one of the five serogroups most associated with HUS.

STEC are by far the *E. coli* pathotype most relevant in food safety and, therefore, this article will be focused on their virulence properties, modes of transmission, and strategies and methods for their detection in food.

STEC Virulence Factors

The main STEC virulence feature is the production of Stx, potent cytotoxins, which cause inhibition of protein synthesis upon internalization by the sensitive cells. The Stx-coding genes (*stx*) are carried by bacteriophages belonging to the lambda family, which are maintained in a lysogenic state in the bacterial chromosome but retain the capability to enter the lytic cycle and disseminate from one host to the other. Stx include two main antigenically distinct types, Stx1 and Stx2, and numerous subtypes identified by differences in the deoxyribonucleic acid (DNA) sequence of the coding genes. Three subtypes of Stx1 and seven subtypes of Stx2 have been described so far, which can be found in any combination in a single STEC cell.

STEC associated with severe human illness, such as HC or HUS, possess additional virulence factors boosting their pathogenicity. Those encoded by the genes located on the pathogenicity island termed as locus of enterocyte effacement (LEE) are associated with enterocyte lesions termed 'attaching and effacing', characterized by the effacement of the brush border microvilli, followed by the intimate attachment of the bacterium to the plasma membrane. The key structure intervening the induction of the A/E lesion is a type III secretion system (T3SS). This is basically made by a needle, which is used to inject bacterial effectors inducing cytoskeleton rearrangements directly into the enterocyte. Besides the T3SS and effectors, the LEE encodes an outer membrane protein called intimin (*eae* gene), which mediates the direct binding of the bacterium to the host cell surface, and its translocated receptor, Tir (translocated intimin receptor).

Other virulence genes described in highly pathogenic STEC include *efa1/lifA*, which is located on a pathogenicity island known as OI-122 and encodes a factor involved in the colonization by inhibiting the host lymphocyte activation, and the genes carried by the pathogenicity island OI-57, probably involved in the modulation of bacterial adhesion.

A large plasmid, called pO157 in STEC of serogroup O157, is also present in most of the STEC associated with severe human disease. It encodes the enterohemolysin, a catalase peroxidase, and a serine protease. The pO157 also carries another large gene, called *toxB*, whose product has an effect comparable to that of *efa1/lifA*. Such a gene is present in the virulence plasmid of all STEC O157 and in most of the STEC belonging to serogroups O26, but it has also been described, in its entire form or as remnant, in the virulence plasmid of STEC belonging to the serogroups O103, O111, and O145.

Genomic Plasticity and STEC Evolution

E. coli is a ubiquitous species present in the intestinal tract of humans and animals as a commensal and in almost all the environmental niches, including water and soil. Its capability to survive insuch diverse environments is based on the acquisition of different abilities, such as those related with the exploitation of the available resources, such as adhesion to almost every surface, and the capability to form biofilms. As a matter of fact, an extraordinary genomic plasticity not only allows *E. coli* to easily gain DNA traits bringing new useful features but also lose genetic determinants conferring disadvantageous or obsolete characters. Such DNA exchanges are facilitated by the action of mobile genetic elements (MGEs) – DNA molecules encoding all the necessary components for the mobilization machinery and are able to accommodate part of the genomic content of the host bacterial cell – variable in size from a few to several thousand base pairs. MGEs like bacteriophages, transposons, and pathogenicity islands can excide from the bacterial chromosome upon sensing a stress signal and integrate in the genome of a different bacterial cell, ensuring their propagation in return of new features, possibly conferring advantages to the recipient cell.

The process of gaining and loosing genetic material is defined as 'horizontal gene transfer' and represents the engine for the emergence of the *E. coli* pathotypes.

As far as highly pathogenic STEC are concerned, it has been proposed that they emerged due to a stepwise mechanism, including the passage through an intermediate EPEC ancestor. An environmental *E. coli* could have first acquired the LEE locus and other MGEs encoding accessory effectors and adhesins, such as the genomic islands OI-122 and -57. Later, a plasmid carrying the *bfp* operon, encoding the fimbriae used by EPEC to trigger the intestinal adhesion, could have entered into the chimeric *E. coli*, originating the EPEC-like ancestor of STEC. Finally, the displacement of the *bfp*-carrying plasmid (EPEC adherence factor (EAF) plamid) by another one similar to the plasmid now present in STEC O157 (pO157 like), and the acquisition of an Stx-converting bacteriophage would have caused the emergence of a pathogenic STEC prototype. Such a model has been used to describe the evolution of STEC O157:H7 from an EPEC of serotype O55:H7. Evidence of contacts between pO157-like structures and the typical EAF plasmid support this theory. As a matter of fact, one of the typical features of the pO157 is the presence of the large virulence gene *toxB*. This gene is also present in STEC belonging to the pathogenic serogroups and remnants of its coding sequence are present in the EAF plasmid of the prototype EPEC strain E2348/69.

Evolution Outside the Box: The *E. coli* O104:H4 Example

Horizontal gene transfer can either cause the emergence of bacterial pathogens from harmless species, or originate new pathotypes by combining virulence genes from different pathogenic types. The *E. coli* O104:H4 strain that caused the large epidemic outbreak of HUS in Germany and other European countries in 2011 is paradigmatic of the latter scenario. The episode was one of the largest STEC outbreak that ever occurred, with more than 4000 cases, 900 of whom had HUS, and with a heavy toll of 50 deaths. Interestingly, the *E. coli* O104:H4 did not match the typical definition of pathogenic STEC. As a matter of fact, it did not belong to the list of STEC serogroups usually associated with HUS, and did not possess the *eae* or enterohemolysin-coding gene, which are markers of the LEE pathogenicity island (PAI) and pO157-like plasmids, respectively.

The whole-genome sequencing of the outbreak strain revealed a rare combination of virulence determinants, with a bacteriophage carrying the genes responsible for the production

of Stx2 inserted in the genomic backbone of another *E. coli* pathotype, the EAEC. It can be hypothesized that the emergence of such chimeric Stx-producing EAEC could have been the consequence of the biphasic 'lifestyle' of EAEC. The *E. coli* strains belonging to this pathogenic group circulate predominantly in the human host, particularly in low-income countries, and their existence is ensured by their capability to survive either in the host, where they usually cause uncomplicated diarrhea, or in the environment (out-of-the-host phase), where they are released with human feces. In turn, the environment, particularly surface waters, represents the main vehicle for spreading the infection, thus completing the epidemiological cycle.

In the human host, EAEC usually do not come into contact with the Stx phages, which have an animal reservoir and are particularly abundant in the gastrointestinal tract of ruminants and environments contaminated with ruminants' manure. It is conceivable that, in areas of the globe characterized by poor hygienic conditions, a high load of pathogenic *E. coli* of human origin, such as EAEC, can be released with insufficiently treated sewage into an environment also contaminated with animal feces. When such human *E. coli* strains are forced to coexist in the same niche with strains from animal sources, they may undergo remodeling of their genomes by the action of MGE. In the case of the epidemic *E. coli* O104:H4 strains, an EAEC belonging to this serotype may have represented a suitable recipient for an Stx2 bacteriophage.

In agreement with the model proposed, the *E. coli* O104:H4 epidemic strain seems to have originated in a low-income country, because the sprouts that caused the outbreak were produced in Germany from fenugreek seeds produced in a rural area of Egypt.

Pathogenesis of Human Infections

STEC infection is usually acquired by ingestion of contaminated food or water or by close contact with ruminants, contaminated environments, or infected persons. Illness can result from a very low infective dose, for example, <100 cells. The pathogenetic process initially involves colonization of the gut. Highly pathogenic STEC, like *E. coli* O157 and O26, adhere to the intestinal mucosa with the characteristic A/E mechanism. They later release large amounts of Stx in the intestinal lumen, which pass through the intestinal epithelium to target the endothelial cells lining the small blood vessels in the gut, kidney, brain, and other organs. The clinical manifestations include HC and HUS. The latter condition usually occurs in children below 5 years of age and is the major cause of acute renal failure in childhood. Besides the direct damage on endothelial cells, the Stx-induced activation of prothrombotic and proinflammatory cascades has a leading role in the development of HUS and central nervous system complications. Progression to HUS may depend on both the characteristics of the infecting STEC strain and host factors. Usually, STEC strains associated with HUS produce Stx2 and cause A/E lesions.

Laboratory Diagnosis of Human Infections

Laboratory diagnosis of human infections is important to perform appropriate treatment early in the course of infection, to timely detect and respond to outbreaks, and to identify emerging serotypes and monitor trends in STEC epidemiology.

For STEC isolation, colonies grown upon media suitable for *E. coli* may be tested for Stx production by immunoassays or presence of *stx* genes by polymerase chain reaction (PCR). Commercial kits are available for both purposes. STEC isolates should be forwarded to public health laboratories for confirmation and serotyping. Noncultural methods are based on the detection of free Stx in feces by using either the Vero cell cytotoxicity assay or commercial immunological assays. A serological diagnosis can also be made by detecting circulating antibodies to the lipopolysaccharide (LPS) of STEC O157 or other serogroups most involved in human infections.

Epidemiology of STEC

Human Infections

In 1982, STEC O157 was first identified as a human pathogen capable of causing foodborne disease. During the 1980s, the outbreaks caused by this new pathogen became a serious public health problem, mainly in North America and the United Kingdom. The food vehicles involved were hamburgers or other beef products, and thus this infectious disease came to be known as the 'hamburger disease' to the public. In the 1990s, STEC infections were reported throughout the industrialized world and serogroups other than O157, such as O26, O103, O111, and O145, were recognized as a frequent cause of bloody diarrhea and HUS. The last decade has been characterized by outbreaks associated with an enlarged spectrum of food vehicles, mainly of vegetal origin, or due to environmental exposures.

One of the most important features of the epidemiology of STEC infections is the occurrence of outbreaks of food- or waterborne origin in the community, often involving high numbers of cases, over long periods of time, and in large geographic areas. The German outbreak by STEC O104:H4 represents a typical, dramatic example of such outbreaks. Some of the major outbreaks that occurred in the world are reported in Table 1, according to their source. Smaller outbreaks due to secondary person-to-person transmission of STEC infection are also frequently reported in specific settings, such as schools, day care centers, and households, especially when a child is the primary case-patient.

The prompt recognition and management of outbreaks and implementation of specific strategies to control the spread of the infection in the community should be based on reliable surveillance systems, covering not only the epidemic outbreaks but also sporadic cases of infection. As a matter of fact, sporadic cases of severe illness, such as bloody diarrhea and HUS, may reveal a wider circulation of STEC strains in a community and should be considered as possible syndromic sentinel events of possible underlying clusters of infections.

HUS represents the most peculiar clinical manifestation of STEC infection in children and usually requires specialized treatment in pediatric nephrology units. Therefore, due to its high predictive value of STEC infection, it is targeted as a key event in STEC surveillance in many countries. HUS is considered a rare disease and children with HUS represent only a

Table 1 Major outbreaks of STEC infection, according to their source and setting

Year	*Number of cases*	*Country*	*STEC serogroup*	*Source/setting*
1990	174	Japan	O157	Well water
1990	633	Scotland	O157	Drinking water
1992	9[a]	Italy	O111	Unknown
1993	501	USA	O157	Hamburger (restaurant chain)
1994	>100	Scotland	O157	Contaminated pasteurized milk
1995	21[a]	Australia	O111	Fermented sausages
1996	>10 000	Japan	O157	Alfalfa sprouts
1996	120	Scotland	O157	Cross contaminated meat
1996	45	USA	O157	Unpasteurized apple juice
2000	2300	Canada	O157	Drinking water
2003/2004	25	Denmark	O157	Organic pasteurized milk
2005	135	Sweden	O157	Lettuce
2005	69	France	O157	Frozen beef burger
2006	196	USA	O157	Fresh spinach
2006	67	USA	O157	Green onions
2006	17	Norway	O103	Cured mutton sausages
2007	20	Denmark	O26	Organic fermented beef sausage
2007	11	Belgium	O145 and O26	Ice cream
2008	141	USA	O111	Eating at restaurant
2008	14	USA	O157	Raw milk
2008	99	USA	O157	Ground beef
2008	20	The Netherlands	O157	Steak tartare
2008/2009	16	USA	O157	Raw milk
2009	93	UK	O157	Petting zoo
2009	8	Germany	O157(SF)	Playground (suspected)
2011	142	Japan	O157	Rice cake
2011	3816	Germany	O104	Fenugreek sprouts

[a]Hemolytic uremic syndrome cases exclusively.

Abbreviations: SF, sorbitol-fermenting strains; STEC, shiga toxin-producing *Escherichia coli*; UK, United Kingdom; USA, United States of America.

small proportion of the overall number of cases of STEC infection in a population. However, the occurrence of a single case of HUS should alert public health authorities to the potential exposure of other people in the community to an active source of STEC infection. A wider surveillance approach could consist of extending the target population to adults and persons with bloody diarrhea, which is also strongly associated with STEC infection. Effective surveillance of STEC infection in public health should not only rely on syndromic surveillance systems, such as those based on early detection of STEC in HUS or bloody diarrhea, but also integrate molecular typing data. This would be of great benefit in terms of timeliness and sensitivity of surveillance and outbreak detection.

STEC infections are usually considered a problem in industrialized countries with respect to low-income countries, probably because of differences in the production and distribution of food. However, the entity of the problem in the latter areas could be underestimated, due to the lack of surveillance systems. In industrialized countries, STEC infections contribute significantly to the overall burden of enteric diseases, in particular for the occurrence of severe long-term sequel, which are usually associated with a high degree of disability.

In the European Union (EU), STEC were given a high priority among zoonotic pathogens since the beginning of the last decade, and STEC infections are subjected to compulsory monitoring in Member States, under the surveillance system coordinated by the European Center for Disease Prevention and Control. Moreover, as a foodborne zoonosis, prevention of STEC infections is primarily approached through harmonized control policies along the whole food production chain.

Animal Reservoirs

The presence of STEC has been reported in numerous animal species, but ruminants, and in particular cattle, represent the major reservoir of STEC, being asymptomatic shedders of these organisms, which are part of their gut microflora. Although most bovine STEC strains do not exhibit the accessory virulence factors associated with human disease and are not found in human infections, cattle are considered to be the most important source of human infections with STEC O157.

The prevalence of STEC O157 in cattle feces appears to be influenced by the age of the animals, and is higher in post-weaned calves and heifers than in younger and older animals. It also depends on the season, as increased rates of fecal shedding have been repeatedly reported in summer months. Carriage of STEC O157 is usually short, but some animals, designated as super-shedders, have been reported to shed high numbers of the organism for long periods of time, and could play a major role in the transmission of STEC O157 to human beings.

STEC, including those belonging to O157 serogroup, have also been isolated from other farm animals, such as pigs or poultry, or from domestic animals and synanthropic rodents

and birds. However, carriage by nonruminant species is rare and it is likely that these animals do not represent actual hosts but are transiently colonized by STEC after contacts with ruminant manure.

Routes of Transmission and Food Vehicles

STEC are considered typical foodborne pathogens. However, the epidemiology of STEC infections has remarkably evolved during recent years and new routes of transmission have emerged, including the direct contact with animals and a wide variety of environment-related exposures. Outbreaks associated with direct animal contact have frequently occurred during school visits to farms or petting zoos, but also among persons attending fairs, after direct contact with animals on display. Waterborne outbreaks have been less frequently described, but they often involved large number of persons. Ruminant's manure washed out from the nearby environment can cause contamination of drinking water supplies in rural settings, but outbreaks have also been associated with swimming in contaminated lakes.

Although all these routes of transmission have to be carefully considered during outbreak investigations, contaminated foodstuffs still remains the most important source of STEC infections, with a wide variety of foods that can be involved in STEC transmission.

Outbreaks have been historically associated with undercooked ground beef and raw milk, products of bovine origin that can be contaminated during slaughtering and milking operations. However, a growing number of outbreaks have been traced back to fruits and vegetables during the recent years, thus increasing the concern that plants might be more important as a vehicle for STEC infections than previously thought. Vegetables can be contaminated during either the preharvest or postharvest operations, and some of the associated outbreaks have caused large numbers of cases and heavily affected the consumers' demand for the fresh produce categories involved, with serious economic consequences for the related agriculture activities. The recent outbreak of STEC O104:H4 in Europe, associated with the consumption of contaminated sprouts, besides an extremely high burden of human casualties and health-associated costs, caused a dramatic reduction of consumer confidence in vegetable consumption, a drop of the vegetable market as a whole, and trade disputes between the EU and some non-EU countries.

STEC can move from animal reservoirs via their feces into the environment, including soil, surface, and ground water, leading to contamination of crops and entire food chain. As a matter of fact, manure, soil, and irrigation water are important sources of the STEC that contaminate plants. The interactions between vegetables and STEC may be considerably more complex than previously thought. It has been shown that *E. coli* O157 is able to colonize the exterior as well as the interior of lettuce and tomato seedlings grown in soil amended with contaminated manure, and these interactions could make it difficult to remove the bacteria from contaminated produce only by washing. Fresh produce like lettuce, tomatoes, and coleslaw are established vehicles of STEC infection, as well as unpasteurized fruit juices, which are increasingly popular among consumers. An increasing role is played by contaminated sprouts, which have caused several episodes of both STEC infections and salmonellosis. As shown in the recent outbreak of STEC O104:H4 in Germany, contaminated seeds represent the most frequent source of sprout contamination. The risk for human infections is increased by the low infectious dose (<100 organisms) of STEC, which has been demonstrated, at least for STEC O157 and O111, by microbiological testing of food consumed by persons who became ill during outbreaks sustained by strains belonging to these serogroups.

Detection of STEC in Food

STEC are characterized by a low infectious dose, thus their detection in food require the use of very sensitive methods. The detection approaches commonly used in food microbiology, based on the use of selective/differential media, are not applicable for the detection of these pathogens, due to the difficulty to discriminate STEC from the commensal *E. coli* that are frequently present in food samples.

Some of the pathogenic STEC serogroups, such as O157 and O26, display phenotypic features suitable, in principle, for their detection using cultural approaches. In fact, these strains are not able to ferment sorbitol or rhamnose, respectively, and thus can be discriminated from the majority of other *E. coli* on solid media containing these sugars. However, these biochemical features are not completely reliable. As a matter of fact, sorbitol-fermenting strains of STEC O157 have become a public health issue, with growing reports concerning severe cases of infections, and rhamnose-fermenting STEC O26 have been reported as well. Furthermore, even in the presence of these metabolic features there is no certainty that a given *E. coli* strain is indeed a STEC.

Detection of *E. Coli* O157 in Food and Feed

A cultural method, the international standard International Organization for Standardization (ISO) 16654:2001, has been developed a decade ago for the detection of *E. coli* O157 in food. It is based on the use of magnetic particles coupled with antibodies raised against the O157 LPS antigen, used to specifically capture and concentrate the bacterial cells exposing this O-antigen that are present in the preenrichment cultures of food samples. The coated beads are kept in contact with an aliquot of the preenrichment culture and later plated onto MacConkey agar plates containing sorbitol in place of lactose (SMAC), where *E. coli* O157 forms white colonies among the wealth of other *E. coli*, which grow forming red colonies. The selection of *E. coli* O157 is favored by the addition to the SMAC plates of supplements such as cefixime and K-tellurite. These compounds exert an antimicrobial action toward the majority of the bacteria possibly present in an enrichment culture from a food sample, but do not influence the growth of *E. coli* O157.

Although being very effective in identifying and isolating *E. coli* O157, the ISO 16654:2001 method has important

limitations as a tool for the routine control of foods. In particular, it is not able to detect the other STEC serogroups causing severe disease, and it doesnot allow to distinguish between the *E. coli* O157 strains that produce Stx and those that do not have this capability.

Detection of Non-O157 STEC in Food

To face the public health risks related to the exposure of EU consumers to pathogenic STEC, the European Commission entrusted the European Normalization Committee (CEN) with the task of developing an international standard aiming at the detection of all the pathogenic STEC in food and feed. This resulted in the development of a laboratory procedure destined to the detection, in these matrices, of highly pathogenic STEC matching the definition issued by EFSA. The draft document has been approved by CEN in 2008 in its first form and modified after the STEC O104:H4 outbreak occurred in Germany and France in 2011, with the aim of widening its scope by including a larger spectrum of pathogenic verocytotoxin-producing *E. coli* (VTEC). The final document has been approved and published by the ISO in November 2012 as the ISO Technical Specification 13136.

This method is based on the PCR screening of enrichment cultures of food samples for the presence of the genes *stx*1 and *stx*2, as well as of genes specifically associated with the highly pathogenic serogroups, such as the *eae* gene and those associated with the LPS antigens O157, O26, O103, O111, and O145.

PCR technology is no longer just a privilege of research laboratories but also its variant Real Time PCR, which allows following the reaction in real time and can benefit from an increasing number of companies dealing with the production of reagents for the molecular screening of foodstuffs for the presence of microbial pathogens, including STEC. ISO/Technical Specification (TS) 13136 allows a quick identification of the negative food samples by excluding those negative to the *stx* PCR and allowing their release in less than 24 h.

Samples positive for the *stx* genes can be tested for the *eae* and five serogroup-associated genes before the isolation step. For samples positive for one of the five serogroups, isolation can be facilitated by using immunomagnetic separation (IMS) techniques similar to that recommended by the ISO 16654:2001 method for the detection of *E. coli* O157. Magnetic beads coated with antibodies directed against the five pathogenic VTEC serogroups are commercially available and the information on the serogroup obtained in the PCR screening step will indicate the appropriate IMS reagent. Otherwise, colonies selected on general *E. coli* media (e.g., Tryptone Bile X-Glucuronide (TBX) or eosin–methylene blue (EMB)) will be tested for the presence of *stx* genes identified in the first screening of the enrichment culture.

Regulatory Context for STEC in Food

An epidemiology-based approach has been used in the United States (US) to develop regulations for the detection of VTEC in particular food matrices and the associated methodologies. The Centers for Disease Control and Prevention estimated that approximately 36% of the foodborne STEC illnesses occurring in the US each year are caused by *E. coli* O157:H7. This implies that the remaining 64% of the total 175 905 domestically acquired foodborne illnesses associated with STEC annually are caused by non-O157 STEC. Because of its lethality, in 1994, the Food Safety and Inspection Service (FSIS) at the United States Department of Agriculture (USDA) has considered STEC O157:H7 to be an adulterant of raw nonintact beef products and the raw intact components used to manufacture these products. Since then, these food commodities are tested for the presence of the bacterium and a recall is sought if food is found contaminated with *E. coli* O157:H7.

The characterization of the STEC strains isolated from human cases of severe disease showed that most of the confirmed non-O157 STEC illnesses are caused by STEC belonging to the following six serogroups: O26, O45, O103, O111, O121, and O145. This epidemiological picture seems to overlap to the distribution of the STEC serogroups causing severe disease in the EU with the exception of the O45 and O121 serogroups, which appear to be underrepresented in Europe.

In the US, ground beef products have been identified as the source of at least one outbreak and several sporadic cases of non-O157 STEC infection. Moreover, the six non-O157 STEC serogroups most involved in human disease have been isolated from slaughtered cattle in the US and cattle and beef products, including trims and ground beef, in several countries. In 2011, because of the increasing public health concern, FSIS announced to the public its intent to declare the six non-O157 STEC serogroups most involved in human illnesses, O26, O45, O103, O111, O121, and O145, as adulterants in nonintact raw beef products and product components. This rule has been enforced on 4 June 2012, and relies on the availability of a detection method developed by FSIS. The proposed detection and isolation approach for non-O157 STEC is equivalent to the one adopted in the EU and described by the international standard ISO TS 13136 (*see* Section Detection of Non-O157 STEC in Food). Similar to the ISO standard, the FSIS method is based on the real time PCR screening of food enrichment cultures for the presence of the STEC main virulence genes (*stx* and *eae*) followed by the screening for serogroup-associated genes. The whole FSIS methodology is described in chapter MLG 5B.00 of the FSIS *Microbiology Laboratory Guidebook*, available in the FSIS web site. The FSIS policy is based on the advice to the establishment to hold the sampled product and not release it pending negative test results. If test results are positive and the product has been released into commerce, FSIS will request that the producing establishment recall that product.

At present, specific microbiological criteria for STEC do not exist in the EU legislation on food safety. However, they are in the process of being introduced in the year 2013; among the specific rules for the enforcement are the hygiene of sprout production that will be adopted following the STEC O104:H4 outbreak. Such a microbiological criterion will be included in the amendment of the EU regulation 2073/2005 that recently passed the first stage of approval and should include the absence in 25 g of sprouts of STEC belonging to serogroups O157, O26, O103, O111, O145, and serotype O104:H4.

Conclusions

STEC represent one of the most dangerous foodborne pathogens, because of the severe clinical manifestations of the infection and capability to cause large, community-wide outbreaks. The epidemiology of STEC infections has remarkably evolved during the last 10 years. The organisms have been reported in a large variety of domestic and wild animals and new routes of transmission have emerged, like the direct contact with animals and a wide variety of environment-related exposures. Nonetheless, consumption of contaminated food still remains the most important route of transmission. Beside the classical foods of animal origin, an increasing role has been played by fruits and vegetables, which have been associated with several outbreaks. Contaminated sprouts have deserved particular attention, especially after the severe outbreak of *E. coli* O104:H4 infections that recently occurred in Germany. The huge human, economic, and political consequences of that outbreak prompted the European Commission to reconsider the safety rules for the production of sprouts intended for direct human consumption, that are currently under discussion.

In general, the strategies for the control of STEC infections must be based on the application of the general principles of food hygiene in all the steps of the food production chain. In particular, cross contamination between raw and ready to eat products must be avoided, bearing in mind that several outbreaks have originated by gross failures in this principle. The STEC O104:H4 outbreak has also confirmed that the genomic plasticity of *E. coli* represents a powerful engine for the emergence of new *E. coli* pathotypes, which can occupy new ecologic niches and enter in our food chains, representing a continuous threat to consumers' health, challenging the food safety systems worldwide.

See also: Characteristics of Foodborne Hazard and Diseases: Pathogenesis and Virulence. Food Safety Assurance Systems: Microbiological Testing, Sampling Plans, and Microbiological Criteria. Foodborne Diseases: Overview of Biological Hazards and Foodborne Diseases. Public Health Measures: Environmental Assessment in Outbreak Investigations; Surveillance of Foodborne Diseases

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Cronobacter (Enterobacter) sakazakii and Other *Cronobacter* spp.

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Glossary

Endotoxin A substance that is released when there is destruction of the bacterial cell wall. It is a constituent of the cell membrane, synonymously associated with the lipopolysaccharide.

Enterotoxin A protein produced by an organism that affects the gastrointestinal tract. An enterotoxin is a type of exotoxin that is secreted by bacteria.

Immunocompromised A condition where the immune system is weakened or impaired.

Opportunistic Able to become pathogenic when, for example, the host's resistance is impaired. An opportunistic infection is caused by an organism that is usually harmless.

Pathogenicity The ability of an organism to cause disease.

Virulence The degree in which an organism can overcome body defenses to cause disease.

Background

Cronobacter sakazakii, formerly known as *Enterobacter sakazakii*, is a foodborne pathogen that has drawn the attention of the scientific community for the last 50 years. This organism was first characterized in 1929 as a 'yellow-pigmented coliform' and was discovered to be the causative agent of septicemia in infants. In the 1960s, the emerging concern was suspected to be involved in two cases of terminal neonatal meningitis. By the 1980s, this pathogen was classified as a new species, *E. sakazakii*, and was found to severely affect infants and neonates, causing sepsis, necrotizing enterocolitis, and meningitis. *Enterobacter sakazakii*, though found in a wide range of environmental sources, has been predominantly linked to human illness via contaminated powdered infant formula (PIF). Overall, there have been a minimum of 111 reported cases of this severe infection in infants and neonates worldwide, leading to 26 deaths.

Characteristics of the Organism

Cronobacter sakazakii belongs to the genus *Cronobacter*, which is categorized in the family Enterobacteriaceae and like most species in this family, is considered to be an opportunistic pathogen. The species *sakazakii* was named after Riichi Sakazakii, a Japanese microbiologist whose research played an important role in the understanding of enteric bacteriology. At present, there are seven species within the *Cronobacter* genus that are used to classify the 15 biogroups of the formerly known *E. sakazakii*. They include *C. sakazakii*, *Cronobacter malonaticus*, *Cronobacter turicensis*, *Cronobacter muytjensii*, *Cronobacter dublinensis*, *Cronobacter condimenti*, and *Cronobacter universalis*.

Cronobacter spp. are Gram-negative organisms that are motile, peritrichously flagellated, oxidase negative, nonspore forming, nonacid-fast, and straight and rod shaped. The organism has dimensions of 0.3–1 × 1–6.0 μm. There are two types of colonies with distinct morphologies observed on trypticase soy agar (TSA) at 26–36 °C. One type of colony is dry, rubbery, and sticky due to a heteropolysaccharide that is believed to be produced. The second type of colony is smooth and nonsticky. Both colony types can produce a yellow pigment, a previously used phenotypic marker that is now not deemed to be as useful.

Cronobacter is a facultative anaerobe that can produce adenosine triphosphate (ATP) in the presence of oxygen via aerobic respiration and can change to fermentation to produce ATP in the absence of oxygen. *Cronobacter* spp. have a wide temperature range for growth, which range from 6 to 45 °C, with an optimal range of 37–43 °C. Certain species have the ability to grow at temperatures of between 4 and 47 °C. In the Enterobacteriaceae family, *Cronobacter* spp. are amongst the most thermotolerant. A study, conducted by Edelson-Mammel *et al.* (2005), demonstrated the ability of 12 *Cronobacter* strains to survive heating in rehydrated PIF at 58 °C. The *D*-values at this specific temperature ranged from 30–35 s to 591–599 s, creating two general divisions of heat tolerance in the genus. The *D*-value for the most heat resistant strain was measured at 71 °C and was found to be 0–7 s. Iversen *et al.* (2004) predicted a 21-log reduction in the cell count of the pathogen if standard pasteurization process was used, thus indicating that the probable sources of contamination would include nonsterile equipment and/or additives.

Cronobacter spp. have the ability to survive in acidic environments with pH levels as low as 3. Of the members of the Enterobacteriaceae family, *Cronobacter* spp. also appear to be well adapted to dry stress. Lin and Beuchat conducted a study that determined the effects of water activity (a_w) and temperature on *Cronobacter* spp. recovered from infant cereal over a 12-month period. Results indicated an increase in a_w or

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Table 1 Biochemical differentiation of *Cronobacter* species

Characteristics	Cronobacter *species*						
	sakazakii	malonaticus	turicensis	muytjensii	dublinensis	condimenti	universalis
Indole production	−	−	−	++	++[a]	+	−
Dulcitol	−	−	++	++	−	−	++
Lactulose	++	++	++	++	[a]	−	++
Malonate	−	++	++	++	[a]	+	++
Maltitol	++	++	++	−	[a]	−	++
Palatinose	++	++	++	+	++	−	−
Putrescine	++	+	++	++	++[a]	−	−
Melezitose	−	−	++	−	−[a]	−	++
Turanose	++	++	++	+	[a]	−	−
myo-Inositol	+	+	++	++	++	−	++
cis-Aconitate	++	++	++	+	++	−	−
trans-Aconitate	−	++	−	+	++	−	−
1-0-Methyl α-D-glucopyranoside	++	++	++	−	++	+	++
4-Aminobutyrate	++	++	++	++	++	−	−

[a]Variation within the three subspecies.

Note: ++, >90% positive; +, 20–80% positive; −, <10% positive.

storage temperature increased the rate of death of *Cronobacter* spp. in dried infant cereal. Low levels, for instance 2 CFU per g of *Cronobacter* were shown to persist and survive in infant cereals for as long as 12 months in low a_w infant cereal.

There are many phenotypic differences between *Cronobacter* spp. and species belonging to the family of *Enterobacteriaceae* that have been described. The biochemical characterizations used to differentiate the currently known and/or described *Cronobacter* spp. are shown in Table 1.

Reservoirs

Cronobacter spp. are ubiquitous organisms that have been isolated from a wide variety of foods including milk, cheese, dried foods, meats, water, vegetables, rice, bread, tea, and herbs. The most common source associated with human outbreaks appears to be contaminated PIF. Clinically, this pathogen has been isolated from cerebrospinal fluid, bone marrow, blood, intestinal tract, respiratory tract, urine, ear and eye swabs, and skin wounds. At the environmental level, they have been isolated in dust, soil, plant materials, and in household environments, such as vacuum cleaner bags. It is clear that this bacterium has an ecological niche that is very broad; however, its primary reservoir has yet to be determined. There are postulations that plant material may be the primary reservoir.

PIF is the vehicle that is predominantly linked with outbreaks of *Cronobacter* infections in infants and neonates. Possible sources of contamination include ingredients that are added to PIF without prior heat treatment, and thermally sensitive ingredients that are used in the production of PIF. Thus, it appears that raw materials are an important source and possible initial step for the entry of *Cronobacter* spp. in PIF. Other sources of contamination include utensils, equipment used to handle PIF in hospitals and kitchens, and feeding tubes.

New Classification

Before *E. sakazakii* was recognized as a new species and later renamed *C. sakazakii*, it was referred to as yellow-pigmented *Enterobacter cloacae.* Deoxyribonucleic acid (DNA)–DNA hybridization results showed that *C. sakazakii* was 53–54% related to two distinct genera: *Enterobacter* and *Citrobacter*. However, pheno- and genotypic tests determined that they were closer to *E. cloacae* than *Citrobacter freundii*, thus, the new species was included in the *Enterobacter* genus. After the pathogen was classified, 15 biogroups were further identified with suggestions that they represented multiple species of *Enterobacter*. Iversen *et al.* (2008) investigated several different strains of *E. sakazakii*, and based on fluorescent amplified fragment length polymorphism (f-AFLP) fingerprints, ribotyping, and full-length 16S ribosomal ribonucleic acid (rRNA) gene analyses, proposed a reclassification of this species. Phenotypic profiling, using 14 biochemical characteristics, permitted better differentiation of the species (Table 2). Joseph *et al.* (2011) recently reported a new species, *C. condimenti*, and reclassified *Cronobacter genomospecies* 1 as *C. universalis*, while further evaluating *Cronobacter* strains 1330 (formerly *sakazakii*), NCTC 9529, 731 (formerly *genomospecies* 1), and 96 and 1435 (formerly *turicensis*) isolated from a wide range of sources from spiced meat to leg infections.

Clinical Manifestations and Features

Cronobacter is an opportunistic pathogen that has been associated most frequently with sporadic cases of life-threatening illness; in which individuals, suffering from a compromised immune system, neonates and infants less than 28-days old and/or low-birth weight are more susceptible. There are three major clinical manifestations seen in *Cronobacter* infections: necrotizing enterocolitis (NEC), septicemia and meningitis, and cerebritis.

Table 2 Draft recommendations on PIF guidance in home or professional settings such as hospitals and daycare centers

Preparation should take place in a clean environment where counters have been cleaned and sanitized Hands should be thoroughly washed using soap and lukewarm water
Bottles, spoons, and teats/nipples should be sterilized in boiling water for 2 min and then air-dried before use or storage. Bottles and equipment should be kept covered until ready-for-use
For preterm and low-birth weight infants under 2 months of age or immunocompromised infants, water for preparing PIF should be brought to a rolling boil for 2 min before dispensing into containers of a maximum of 1 l and then cooling down to no less than 70 °C, after which the powder can be added. For all other infants, previously boiled water that has been cooled to room or body temperature (37 °C) can be used to prepare PIF, but it should be served immediately to the infant
Formula that has been mixed or prepared with boiled water, which has been cooled to 70 °C, should be further cooled down to room or body temperature before serving, to avoid scalding of the infant's mouth
It is best to prepare and serve PIF immediately after cooling to body temperature. Reducing the time from preparation to consumption will reduce the risk to infants. Formula in bottles can be cooled quickly by holding the bottle under running tap water or placing in a container of cold water as long as the cooling water is below the teat/nipple
If it is not possible to serve immediately, prepared formula in bottles or other types of containers should be refrigerated at 4 °C or lower, immediately after the powder has been added and dissolved in water. The prepared formula should be used within 24 h
Owing to the possibility of the growth of harmful bacteria at temperatures above 4 °C, stored formula should be removed from the refrigerator and rewarmed to room or body temperature, only immediately before feeding and not in advance. PIF should not be left warming for more than 15 min as rewarming for extended periods means that the PIF will be held at a temperature that is ideal for the growth of bacteria
Once feeding has started, the individual bottle should be used within the next 2 h. Any leftover formula should be discarded

Abbreviation: PIF, powdered infant formula.

Source: http://www.hc-sc.gc.ca/fn-an/nutrition/infant-nourisson/pif-ppn-recommandations-eng.php (accessed on 8 May 2013).

Several risk analyses have been conducted, with Bowen and Braden's recent results indicating the most common symptom associated with *Cronobacter* infection in neonates and infants is meningitis. Meningitis caused by *Cronobacter* is established between the fourth and fifth day after birth and can be fatal within hours to a few days. Neonatal infections have a mortality rate of 40–80%, and infants or neonates who survive the infection are often left with severe and often irreversible neurological disorders. Neurological sequelae include brain abscess, ventricle compartmentalization due to necrosis of brain tissue and liquefaction of white cerebral matter, hydrocephalus, cyst formation, and pulmonary, urinary or blood-stream infections.

The Centers for Disease Control and Prevention have estimated six new cases of *Cronobacter* infection per year, worldwide. The lack of awareness of the organism and adequate isolation methods may contribute to the under reporting. Even though the numbers of reported cases of *Cronobacter* infections are low compared to other pathogens, the outcomes of the outbreaks are severe, leading the International Commission for Microbiological Specifications for Foods to conclude that *Cronobacter* spp. are a severe hazard for restricted populations, causing life-threatening or substantial chronic sequelae.

Infants and neonates are the primary targets of the pathogen; however, immunocompromised adults, including the elderly, are also susceptible to *Cronobacter* sp. There have been at least 20 reported cases of *Cronobacter* infections in adults. The symptoms for adults included pneumonia, sepsis, foot ulcers, wound infections, osteomyelitis and splenic abscesses. Stroke patients' mouths are severely affected by *Cronobacter*. Stroke patients seem to be susceptible to Gram-negative bacilli colonization in the mouth through liquid food supplementation, such as reconstituted starch powder which at times may also contain *Cronobacter*. The generic treatment given to these stroke patients are first line antibiotics. However, *Cronobacter* can be resistant to these types of antibiotics.

Antimicrobial agents that are effective against this pathogen include acylureidopenicillins, aminoglycosides, ampicillin, antifolates, aztreonam, carbapenems, cephalosporins, chloramphenicol, nitrofurantoin, quinolones, tetracyclines, ticarcillin, and several β-lactams. *Cronobacter* spp. show resistance to oxacillin, benzylpenicillin, clindamycin, and some macrolides. If the symptoms in infants or adults are seen at an early stage of infection then antibiotics can be used to treat the patient more effectively and with minimum complications than at a later stage.

Virulence Factors and Pathogenicity

Very little is known of the virulence factors involved in the pathogenicity of *Cronobacter* spp.; however, in the past decade, much progress has been made. **Figure 1** summarizes our current model and highlights the relationship between the pathogen and the host.

The main route of entry for *Cronobacter* spp. appears to be through ingestion; therefore, factors related to contamination should be addressed. The vehicle of *Cronobacter* infections in infants and neonates is contaminated PIF. Major determinants that contribute to the presence and growth of the organism in PIF include temperature, equipment, and biofilms. PIF is normally stored at room temperature, which is approximately 23 °C. The optimal temperature for *Cronobacter* spp. is 37 °C; however, it has the ability to grow between 6 to 45 °C. PIF are nonsterile, therefore, the composition of PIF and storage temperature of reconstituted PIF can enhance the growth of the organism. Another factor that has been found in the constitution of PIF is lipopolysaccharide (LPS). LPS, a heat-stable endotoxin that has been shown to be present in PIF, appears to play a role in compromising the intestinal barrier integrity, by allowing the organism to cross the gastrointestinal (GI) tract and become bloodborne.

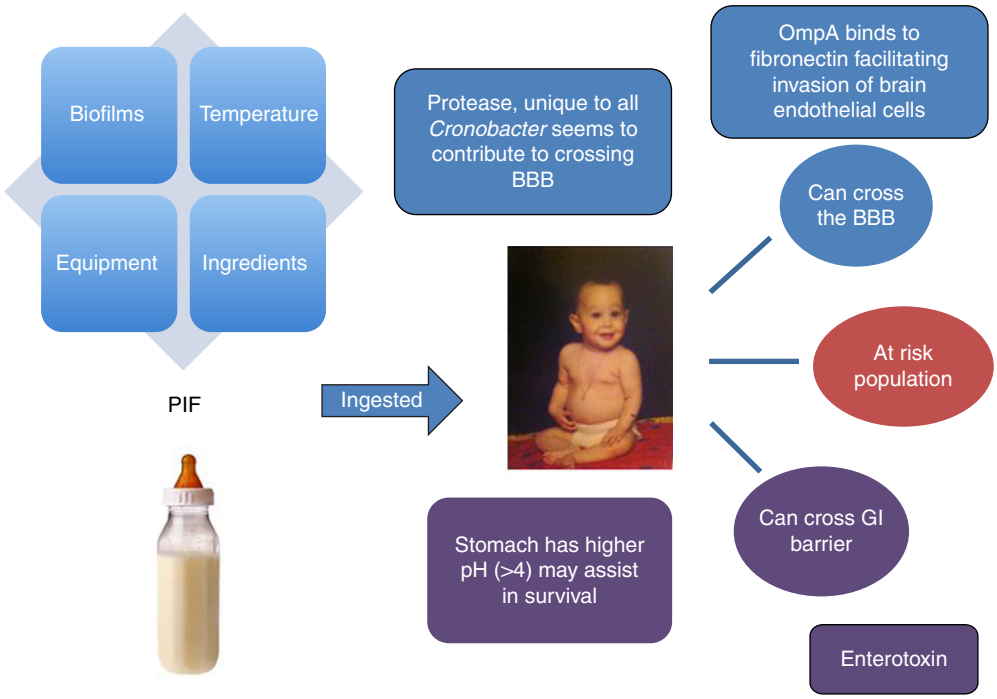

Figure 1 A current model of *Cronobacter* pathogenicity. BBB, blood–brain barrier; OmpA, outer membrane protein A.

Extrinsic contamination of reconstituted PIF can come from utensils such as blenders, brushes, and spoons. Biofilms, broadly defined as a community adhering to a surface, are an important factor in the ecology of *Cronobacter* spp.. Abiotic surfaces have been used to demonstrate *Cronobacter*'s ability to form biofilms on materials commonly associated with PIF-feeding equipment and surfaces such as glass, stainless steel, polyvinyl chloride, polycarbonate, silicone, and enteral feeding tubes.

There are three areas of focus addressing the biofilm issue in *Cronobacter*: (1) understanding the physiology and genetic basis of biofilms (i.e., identifying key genes and/or conditions), (2) determination of whether biofilm formation is a virulence factor for *Cronobacter* pathogenesis, and (3) evaluation and determination of treatments and their efficacies in reducing biofilm formation. Amalaradjou and Venkitanarayanan have recently shown that *trans*-cinnamaldehyde, an ingredient in cinnamon oil, has the ability to inhibit and inactivate *C. sakazakii* biofilms in the presence or absence of PIF on polystyrene plates, stainless steel coupons, feeding bottle coupons, and enteral feeding tube coupons at both 12 and 24 °C. Hartmann *et al.* (2010) screened a library of mutants of strain ES5 (clinical isolate of *C. sakazakii*) using the crystal violet microtiter assay and found four mutants defective in cellulose biosynthesis, three in flagellar structure, three in key metabolic functions (e.g., cell division, energy metabolism, and acid fermentation), one virulence and four unknown functions. Of the 14 mutants, two hypothetical proteins ESA_00281 and ESA_00282 proved to be important in biofilm formation structure.

One of the first efforts describing putative virulence factors was by Pagotto *et al.* (2003) where, using a suckling mouse assay, *Cronobacter* spp.' ability to produce an enterotoxin were demonstrated. Their observations were further supported by *in vitro* assays using Chinese hamster ovary (CHO), Vero, and Y-1 cells, where many *Cronobacter* strains exhibited cytopathic effects. Raghav and Aggarwal later purified and identified biochemical characteristics of the enterotoxin. The purified toxin confirmed the Pagotto *et al.* (2003) study as it demonstrated cytopathic effects in the suckling mouse assay. The toxin was reported to have a mass of 66 kDa, optimal activity at pH 6 and was heat stable, showing activity after exposure to 90 °C for 30 min. Pagotto *et al.* (2003) also described the dose–response relationships for *Cronobacter* infection using mice. Intraperitoneal injection with levels of bacteria as low as 10^5 CFU per mouse were shown to be lethal. The dose–response for the suckling mouse assay, however, may not necessarily be a good representation of the dose–response for human neonates, for which the oral infectious dose has been estimated by the World Health Organization (WHO) to range from 10^3 to 10^8 CFU.

The gastrointestinal tract is a primary target for *Cronobacter* pathogenesis and the organism's success in causing human illness is dependent on its ability to adhere to the intestinal epithelial layer. Hunter *et al.* (2008) have reported that *Cronobacter* spp. bind to enterocytes in rat pups and a cascade of host responses are triggered, which include tumor necrosis factor-alpha, nitric oxide, and interleukin-6. This was also observed when *Cronobacter* attached to the enterocytes of rat pups, where there was an increase in enterocyte apoptosis. This increase in apoptosis results in a decrease in the barrier integrity that appears to facilitate bacterial translocation. Adhesion to intestinal cells appears to be a necessary step in colonization of the intestinal tract, which induces NEC and invasion in the animal model and in IEC-6 cells, a rat

intestinal cell line. A recent study conducted by Emami *et al.* (2011) used a mouse model and Caco-2 cells to show that *C. sakazakii* damages the intestinal epithelial cells by recruiting a large amount of dendritic cells in the intestine and suppressing their maturation, which increases the transforming growth factor (TGF)-β production and results in NEC in the host.

Adhesion and invasion of the BBB and the gastrointestinal tract are crucial for infections of *Cronobacter* spp. in the host, and a better understanding of these processes would help in unraveling the mechanism(s) of pathogenesis. Mammalian tissue culture assays are a popular approach to investigate the adhesive and invasive properties. The most common cell lines used to study the adherence and invasion are HEp-2, Caco-2, and human brain microvascular endothelial cells (HBMECs). Mange *et al.* (2006) screened 50 *Cronobacter* strains and found two distinctive adherence patterns in all three cell lines tested. They were described to be either diffuse adhesion or formation of localized clusters of bacteria on the cell surface. Some strains were able to show both patterns. Mange *et al.* (2006) also demonstrated that adherence was optimal during late exponential phase of bacterial growth with a 10-fold increase in adherence cells, and also concluded that adhesion to epithelial and endothelial cells was predominantly nonfimbrial based. Townsend *et al.* (2007) studied the attachment and invasion properties of seven different *Cronobacter* strains, associated with outbreaks of different illness such as necrotizing enterocolitis, bacteremia, and meningitis, using Caco-2 cells and rat-brain capillary endothelial cells. All seven strains attached to, and invaded Caco-2 cells after 3 h. These particular isolates have the ability to replicate and adapt in macrophage cells, U937.

Bacterial translocation from the gastrointestinal barrier is critical in the pathogenesis of *Cronobacter* meningitis. Translocation from the GI tract will allow the bacteria to have access to the blood stream, which may also lead to sepsis in the host, while giving access to the BBB and eventually the central nervous system. *Cronobacter* spp. are able to overcome host defensive mechanisms, penetrate the BBB and survive in the cerebrospinal fluid. One virulence factor that has been shown to be essential in *Cronobacter* pathogenesis is OmpA. Interestingly, OmpA plays a role in the suppression of the maturation of dendritic cells. OmpA expression is also required for invasion of mammalian host cells and is necessary for *Cronobacter* resistance to blood and serum killing in newborn rats. Mittal *et al.* (2009) have shown that *Cronobacter* expression of *ompA* can successfully cross the intestinal barrier in newborn rats, multiply in the blood, and transverse the BBB; whereas *ompA* deletion mutants fail to bind to intestinal epithelial cells both *in vivo* and *in vitro*. Recent studies from Kim *et al.* (2010a) demonstrated that a protein OmpX is also required for translocation. They were the first to conduct inframe deletion mutants for OmpA and OmpX and showed that translocation does not occur in the host when both proteins are not expressed. Bioinformatic analyses of the *Cronobacter* BAA-894 genome and *E. cloacae* showed that *ompX* was found to be similar (81% identity) between the two organisms. OmpX in other bacteria, including *E. cloacae*, appears to play an important role in virulence as it assists in the invasion of the host cells and helps overcome host defenses. Kim *et al.* (2010a) have demonstrated using Caco-2 and INT-407 cells as well as rat pups that both OmpA and OmpX are required for translocation and basolateral invasion and adhesion of mammalian cells.

Singamsetty *et al.* (2008) noticed that several meningitis causing Gram-negative bacteria require the expression of OmpA to invade HBMEC. They demonstrated that OmpA was required in *Cronobacter* spp. to invade the BBB by inducing microtubule condensation and phosphoinositide (PI)3-kinase and PKC-α activation. Both Nair *et al.* (2009) and Mittal *et al.* (2009) have shown that OmpA binds to fibronectin, thereby facilitating the invasion of brain endothelial cells. Mittal *et al.* (2009) were the first to demonstrate that OmpA expression affects the onset of meningitis in newborn rats. Mortality rate was 100% when newborn rats were infected with OmpA positive strains whereas no pathological symptoms were observed when *ompA* deletion mutant strains were used.

Kothary *et al.* (2007) examined 135 different *Cronobacter* strains and identified a cell bound, zinc-containing metalloprotease encoded by a *zpx* gene. The protease was found to be active against azocasein, as well as causing the rounding of Chinese hamster ovarian cells. The *zpx* gene product may be responsible for allowing the organism to cross the BBB and may contribute to cellular destruction in neonates with NEC.

A virulence mechanism that seems to be common to species belonging to the family of Enterobacteriaceae is their active efflux. This active efflux ejects a range of xenobiotic compounds from the cell, including bile salts, antibiotics, disinfectants, and dyes.

Detection Methods

In 2002, the US Food and Drug Administration (FDA) described a modified method, based on work conducted by Muytjens *et al.* (1988) in detecting and isolating *Cronobacter* spp. from PIF. The protocol consists of several steps using multiple growth conditions in broth followed by plating on selective agar media. The first step requires enriching the PIF samples in water overnight. Second step is to further enrich the samples in Enterobacteriaceae enrichment (EE) broth for up to 24 h. Samples are plated on violet red bile glucose agar for overnight incubation and presumptive colonies purified onto TSA in which the incubation period ranges from 48 to 72 h. The resulting yellow-pigmented colonies are selected for biochemical tests using analytical profile index (API) 20E test strips. This method was lengthy, was not specific to *Cronobacter* spp., and was unable to discriminate between *Cronobacter* and other *Enterobacter* spp.

The International Organization for Standardization published a method in 2006 to isolate *Cronobacter* spp. from milk-based powdered formula. The method consists of preenriching the PIF samples in buffered peptone water at 37 °C overnight. The samples are then enriched in modified lauryl sulfate (addition of vancomycin) at 44 °C overnight. The samples are then plated on to chromogenic agar at 25 °C for a period of 48–72 h. For confirmation of *Cronobacter* spp., approved biochemical identification kits can be used. However, not all strains of *Cronobacter* have the ability to grow in the modified lauryl sulfate broth.

The FDA revised their 2002 method to incorporate both culture on chromogenic media and real-time polymerase chain reaction (PCR) for detection, which proved to be significantly better ($p < .05$) than the original method for detection of *Cronobacter* spp. The chromogenic media, developed by Iversen *et al.* (2004) was based on the work of Muytjens *et al.* on the α-glucosidase enzyme. Muytjens *et al.* identified a unique enzyme that was present in all 129 *Cronobacter* isolates tested. This enzyme appears to be unique to only *Cronobacter*, as the closely-related species *E. cloacae*, *Enterobacter aerogenes*, and *Pantoea agglomerans* were all negative for the enzyme. Media with the chromogenic substrate 5-Bromo-4-chloro-3-indolyl α-D-glucopyranoside (X-αGlc), yield *Cronobacter* colonies appearing blue–green on Druggan, Fosythe, and Iversen agar plate. The α-glucosidase present in *Cronobacter* spp. hydrolyzes X-αGlc to form a bromo–chloro indigo pigment, resulting in blue–green colonies.

In 2009, O'Brien *et al.* developed a one-step method for detection, to help address issues of lengthy and multiple enrichments. Their method consisted of a combined preenrichment/enrichment broth, *Cronobacter* enrichment broth ((CEB) formerly known as *Enterobacter sakazakii* enrichment (ESE) broth) combined with a selective-differential agar. The recovery of *Cronobacter* spp. from PIF using this protocol was significantly higher when compared with other enrichment broths. The advantage of this protocol is that it removes the need for separate preenrichment and enrichment steps, reducing the time for detection of *Cronobacter* in PIF. Al-Holy *et al.* (2011) evaluated a new enrichment broth known as Al-Holy–Rasco (AR) medium, which consists of a generic brain heart infusion broth with the addition of 1% NaCl, 15% sucrose, and 0.8 g l^{-1} sodium deoxycholate as selective ingredients. They used 10 different strains of *Cronobacter* to compare their enrichment media to EE, CEB, modified lauryl sulfate broth, and milk. AR media seem to support the growth of *Cronobacter* and suppress non-*Cronobacter* spp. better than the other media. Malorny and Wagner developed a reverse transcriptase (RT)–PCR specific for *Cronobacter* which targets 16S rRNA gene. This molecular assay is quick, shortening the process from 2 days to 2 h, and can detect *Cronobacter* at population levels of 10^3 CFU per ml in foods after enrichment. Seo and Brackett developed a more specific RT–PCR in which the principal of this technique was targeting a sequence within the macromolecular synthesis operon. The advantage of this method is that it can differentiate *Cronobacter* spp. from species belonging to the family Enterobacteriaceae. Its sensitivity of detection is approximately 100 CFU per ml in rehydrated PIF. If a 24 h enrichment step was included, then the sensitivity of the detection can be reduced to 0.6 CFU per g of PIF. Other target genes for RT–PCR detection methods for *Cronobacter* spp. have been described, which include the internal transcribed spacer sequence of the 16S–23S ribosomal DNA (rDNA), α-glucosidase gene (*gluA*), OmpA, and a zinc-containing metalloprotease. A recent PCR-based method was developed to differentiate the various species of *Cronobacter* that targets the *rpoB* gene, a β-subunit of RNA polymerase. This assay relies on single nucleotide polymorphisms in the *rpoB* gene using mismatch PCR.

Fluorescence *in situ* hybridization (FISH) has been applied in PIF for detection of low population levels of *Cronobacter* spp. The group described the use of a peptide nucleic acid probe combined with FISH to detect cells of *Cronobacter* in 10 g of PIF following an 8-h enrichment period. This was possible even when there were other bacterial populations present.

Preventive Measures

PIF is not a sterile product, and microbiological criteria to help food manufacturers minimize the risk of infections have been implemented. In 2004, the Food and Agriculture Organization of the United Nations and the WHO convened a joint meeting to discuss *Cronobacter* and other important microbial contaminants of PIF, with the aim to revise the 1979 Recommended International Code of Hygienic Infant Formula. In 2005, the document was released, and later updated in 2007. Highlights of the guidelines are based on infants that are more susceptible to infection and those that are not at risk. Infants who have a higher chance of infection are advised to consume sterile liquid infant formula, whereas infants who are not at risk have several options of PIF. Preparing PIF with water at a temperature of 70 °C is suggested as it was shown to reduce the potential for disease-causing organisms to survive. The 'hang-time,' the amount of time PIF is at room temperature in the feeding bag and accompanying lines during enteral tube feeding, has been recommended to not exceed 4 h. Reducing the time from preparation to consumption and storing the PIF at temperatures no higher than 5 °C for a maximum of 24 h have also been shown to be useful in minimizing the risk.

Health Canada has PIF guidance documents that are publicly available. These general recommendations are meant to assist individuals at home or in a professional setting, such as hospitals and daycare centers. A preliminary summary is shown in **Table 2**.

Disinfectants used to clean surfaces and reduce contamination are effective against planktonic cells. However, they appear less effective in decreasing survival of the organism when cells are part of a biofilm. Contamination can be associated with the utensils or machinery used to make PIF and *Cronobacter* spp. have been shown to form biofilms in those environments or surfaces associated with handling of PIF. Bacteriophages, viruses that infect bacteria, have been suggested to inhibit the growth of pathogenic microorganisms. Using sewage or ultraviolet irradiation of pure cultures, six bacteriophages were isolated by Kim *et al.* (2007). Two bacteriophages were demonstrated to control growth in both laboratory media and in reconstituted infant formula at 12, 24, and 37 °C. Phages at 10^7, 10^8, or 10^9 PFU per ml were used to demonstrate that higher temperatures (24 and 37 °C) and high titers (10^9) were required for contamination levels equivalent to 73 000 CFU per 100 g of PIF. Zuber *et al.* isolated 67 phages and reported that a cocktail of 5 phages were successful in preventing growth of 35 (of the 40) strains in artificially contaminated formula. Another strategy described by Hayes *et al.* (2009) was to use antimicrobial peptides produced by the fermentation of sodium caseinate using a proteolytic strain of *Lactobacillus acidophilus*. They identified two peptides derived from milk proteins, caseicins A and B, which may play a role as a bioprotective agent in dairy-based food products such as PIF.

A variety of phenolic compounds have been shown to have antimicrobial activity. A study by Amalaradjou and Venkitanarayanan (2011) demonstrated that malic, tartaric, and tannic acids have antimicrobial activity against *Cronobacter* sp. Kim *et al.* (2010b) have also shown that red muscadine juice can cause a 6-log reduction of *C. sakazakii* in commercial baby juices in approximately 2 h. Their results suggest that red muscadine could be used to prevent and/or control *C. sakazakii* in baby food formula. Recently, Yemis *et al.* (2011) investigated the antibacterial activity of vanillin, ethyl vanillin, and vanillic acid as possible preservatives in the intervention strategy for control of *Cronobacter* spp. They evaluated the effectiveness of the compounds on the growth and heat resistance. Vanillin, ethyl vanillin, and vanillic acid were shown to have bactericidal effects, prevented the growth of the organism and reduce the heat resistance in microbiological media.

When possible, mothers should be encouraged to breast feed and learn about health risks associated with infant formula. Human milk has been shown to prevent the growth of certain pathogens and protects infants and neonates from infections that can be achieved by protozoans, viruses, and bacteria such as *Staphylococcus*, Group B *Streptococcus*, *Escherichia coli*, and *Cronobacter* spp., in addition to providing maternal antibodies. Studies suggest that breastfed infants have lower chances of getting gastrointestinal, respiratory, and meningeal infections. Interestingly, work by Lenati *et al.* (2008) has shown that, *in vitro*, *Cronobacter* growth can occur in human breast milk.

Conclusion

Cronobacter spp. are important, emerging opportunistic pathogens that have the ability to survive in a variety of different environmental conditions. The number of reported cases of *Cronobacter* infections, although low compared to other foodborne diseases, primarily target high-risk populations, with a high mortality rate in infants and neonates. Our knowledge of the ecology, biology, and pathogenicity of this fascinating foodborne pathogen will no doubt continue to increase, resulting in improved detection and characterization, along with better intervention strategies, guidelines, and policies, for reducing the risk to human health.

See also: Characteristics of Foodborne Hazard and Diseases: Pathogenesis and Virulence. Food Safety Assurance Systems: Management of Biofilm Risk; Microbiological Testing, Sampling Plans, and Microbiological Criteria; Personal Hygiene and Employee Health. Institutions Involved in Food Safety: FAO/WHO Codex Alimentarius Commission (CAC); Food and Agriculture Organization of the United Nations (FAO); World Health Organization (WHO). Public Health Measures: Modern Approach to Food Safety Management: An Overview; Surveillance of Foodborne Diseases. Safety of Food and Beverages: Milk and Dairy Products

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Other Pathogenic Enterobacteriaceae – *Enterobacter* and Other Genera

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Glossary

β-Lactam antibiotics A broad class of antibiotics, consisting of all antibiotic agents that contains a β-lactam nucleus in its molecular structure. This includes penicillin derivatives (penams), cephalosporins (cephems), monobactams, and carbapenems.
Cholecystitis A painful inflammation of the gallbladder's wall.
Infection, nosocomial An infection acquired in a hospital. Specifically an infection that was not present or incubating before the patient was admitted to the hospital, but occurred within 72 h after admittance to the hospital.
Septicemia Systemic illness with toxicity due to invasion of the bloodstream by virulent bacteria coming from a local seat of infection.
Spondylodiscitis Inflammation of an intervertebral disc or disc space which may lead to disc erosion.

Enterobacter species

Background to the Genus *Enterobacter*

The genus *Enterobacter* was first proposed by Hormaeche and Edwards in 1960 when the motile and ornithine decarboxylase (ODC) positive members of the genus *Aerobacter* were separated out as *Enterobacter* in order to distinguish them from the nonmotile and ODC negative *Klebsiella* strains. Since then taxonomic complications have led to the further reclassification of several *Enterobacter* species and the transfer of the following bacteria *Enterobacter intermedius* to the genus *Kluyvera* as *Kluyvera intermedia*, Enterobacter agglomerans to the genus *Pantoea*, and *Enterobacter sakazakii* to the recently described genus *Cronobacter*.

One of the most challenging issues associated with the genus *Enterobacter* relates to those strains misidentified as *Enterobacter cloacae*. *Enterobacter asburiae*, *Enterobacter cancerogenus*, *Enterobacter dissolvens*, *Enterobacter hormaechei*, *Enterobacter kobei*, and *Enterobacter nimipressurali* are all closely related to *E. cloacae* and are part of the '*E. cloacae* complex'. The taxonomy of this *E. cloacae* complex is mainly based on the assessment of DNA–DNA hybridization of the whole genome along with associated phenotypic characteristics. All species within this complex are of clinical significance and are increasingly being implicated as the causative agent(s) of local and systemic infections in humans.

Characteristics of the Genus *Enterobacter*

Enterobacter species are widely dispersed in nature and exist in a diverse range of environments including soil, water, domestic and food processing establishments, vegetation, vertebrate and invertebrate hosts, and in the feces of humans and animals. These bacteria generally grow well, aerobically and anaerobically, at temperatures ranging between 20 and 37 °C on general laboratory media at neutral pH. Although motility has been a distinguishing characteristic used to differentiate between *Klebsiella* and *Enterobacter*, some *Enterobacter* species are nonmotile (and these include *E. asburiae*, *E. dissolvens*, *E. nimipressuralis*) whereas others demonstrate a variable motility (such as *Enterobacter gergoviae*, *E. hormaechei*, *E. intermedius*, *Enterobacter pyrinus*). Fermentation by *Enterobacter* species is more limited when compared to some *Klebsiella* species. For example, *Enterobacter* produce gas from cellobiose and ferment rhamnose, a feature that distinguishes them from most species of *Serratia*. All *Enterobacter* are nonpigmented with the exception of the newly reclassified *Cronobacter* genus, formerly known as *Enterobacter sakazakii*.

Enterobacter Linked to Food Contamination

Enterobacter species can be cultured from a wide range of foods (Table 1). *Enterobacter cloacae* is a recognized contaminant of raw milk and dairy products such as yoghurt and cheese. *Enterobacter* have been identified in pasteurized milk and cream and in dried dairy products; possibly due to postprocess contamination, as these bacteria do not survive pasteurization. Powdered infant formula (PIF) is not sterile food and may contain low levels of pathogenic microorganisms. The most frequently isolated Enterobacteriaceae species include *E. cloacae*, *Cronobacter* species, *E. pulveris*, *Enterobacter helveticus*, *Pantoea agglomerans*, and *Klebsiella pneumoniae*. To a large extent research has been carried out on *Cronobacter* species (*E. sakazakii*) in PIF, with contamination by other *Enterobacter* receiving lesser consideration. Nevertheless *Enterobacter* species have the ability to cause infections and are a known contaminant of PIF;

Table 1 A summary of bacterial genera, species, and food matrices from where these bacteria were recovered

Genus	*Species*	*Food Sources*
Enterobacter	*aerogenes* *cancerogenous* *cloacae*[a] *cowanii* *gergoviae* *helveticus* *hormaechei* *ludwigii* *oryzae* *pulveris* *radicincitans* *turicensis*	Animal feed (dried pellets), beverages, chocolate, cream, cucumber, dairy products, eggs, fish, flour, fresh frozen or powdered fruit and vegetables, grains, herbs, infant food, legume products, meat, mulberry plants, nuts, parenteral nutrition, pasta, plants, sugar beets, pork, powdered infant formula (PIF), sausage, seeds, spices, tea, water, and wild rice
Klebsiella	*granulomatis* *ornithinolytica* *oxytoca* *planticola* *pneumoniae*[a] subsp. *ozaenae* subsp. *rhinoscleromatis* subsp. *pneumoniae* *variicola*	Alfalfa, bananas, bean sprouts, cold meat, hamburgers, ice creams, infant food, milk shakes, nasogastric food, powdered milk, rice, salads, sugar cane, sweets, and maize
Pantoea	*agglomerans*[a] *anthophila* *ananatis* *calida* *citrea* *deleyi* *dispersa* *eucalypti* *gaviniae* *punctata* *stewartii* *terrea* *vagans*	Banana, beetroot, bean plant leaf, carrot juice, mango, cereal, cluster bean, corn, foxtail millet, maize, mandarin, millet, pearl millet, pineapple, plants, roses, seeds, snail vine, soil, sorghum, and water
Serratia	*entomophila* *ficaria* *fonticol* *grimesi* *liquefaciens* *odorifera* *marcescens*[a] *plymuthica* *proteamaculans* *quinivorans* *rubidaea*	Cream, fruit, meat, milk, mushrooms, salads, starchy foodstuffs, and other vegetables
Cedecea	*davisae*[a] *lapegei* *species* 3 *species* 5	Mushrooms, salads, and vegetables
Edwardsiella	*hoshinae* *ictaluri* *tarda*[a]	Fish and water
Hafnia	*alvei*	Beef, cheese, cream, freshwater fish, honey, milk, pork products, vegetables, and water
Kluyvera	*ascorbata*[a] *cochleae* *cryocrescens* *georgina*	Milk and other foods
Leclercia	*adecarboxylata*	Water and other foods

[a]Denotes type species.

therefore these bacteria pose a food safety threat to public health and the PIF manufacturing industry alike.

Clinical Manifestation of Infections Linked to *Enterobacter*

Enterobacter species are now recognized as important nosocomial pathogens responsible for bacteremia, lower respiratory tract infections, pneumonia, skin and soft tissue infections, urinary tract infection (UTI), intra-abdominal and ophthalmic infections, endocarditis, septic arthritis, and osteomyelitis. Most occurrences of *Enterobacter*-associated infections are described as hospital-acquired where these bacteria are common contaminants of hospital surfaces, medical equipment, and hospital personnel. With the exception of *E. sakazakii* (*Cronobacter* species) the former sole member of this genus to be linked epidemiologically to food-borne infections, in general, *Enterobacter* species are more commonly associated with nosocomial infections. These bacteria can affect people of all ages, but are particularly relevant to immunocompromised individuals. *Enterobacter cloacae*, along with *Enterobacter aerogenes*, *E. hormaechei*, and *E. gergoviae* have been associated with infections in neonates. Risk factors in neonates include premature birth and low birth weight, which predisposes these vulnerable individuals to developing serious conditions and sometimes fatal outcomes.

Enterobacter cloacae, followed by *E. aerogenes*, are the two most common *Enterobacter* species cultured from clinical specimens in human infections, and are responsible for more than 90% of all cases. *Enterobacter asburiae*, *E. cancerogenous*, and *Enterobacter amnigenus* have also been reported to cause bacteraemia in adults. Risk factors associated with *Enterobacter* sepsis in children include parenteral nutrition, use of antimicrobial compounds, and indwelling catheters. Low birth weight, prematurity, length of hospital stay, invasive procedure(s), and overuse of antibiotics may put infants at an increased risk of infection.

Antimicrobial Resistance Among *Enterobacter* Species

Over the years many Enterobacteriaceae have become resistant to commonly used antimicrobial compounds and antimicrobial resistance among these organisms is an emerging problem. Use of antimicrobial agents in hospitals has resulted in an increase in resistance to many β-lactam antibiotics, particularly extended-spectrum β-lactamases (ESβLs) with approximately 25% of all *Enterobacter* species now being resistant to ESβL, due to the presence of a Bush group 1 chromosomal cephalosporinase. In a recent study by Sharma *et al.* (2008), 73.68% of isolates studied were found to be ESβL producers and the *ampC*-encoding gene was identified in all isolates tested. AmpC β-lactamase producing bacterial pathogens may cause major therapeutic failure if they remain undetected. Resistance to new cephalosporins, aminopenicillins, and cephamycins is due to mutants that can express large amounts of this enzyme. This limits the choice of a suitable antimicrobial agent to treat invasive infection. Antibiotic resistance can also be plasmid mediated with five aminoglycoside-modifying enzymes being detected on the same R-plasmid.

Among the *Enterobacter* species, *E. aerogenes* has emerged as an important hospital pathogen during the past 20 years due to its ability to adapt to environmental stresses. This bacterium now accounts for more than half of all Gram-negative organisms that produce ESβLs. The overexpression of genes that regulate and encode efflux pumps, may further contribute to the resistance profile of *E. aerogenes*.

Epidemiology of *Enterobacter*

Several methods have been developed to characterize strains of *Enterobacter* including antibiogram profiling, biotyping, serotyping, plasmid profiling, ribotyping, random amplification of polymorphic DNA (RAPD), arbitrarily primed-PCR (AP-PCR), repetitive sequence-based PCR (rep-PCR), enterobacterial repetitive intergenic consensus (ERIC) PCR, amplified fragment length polymorphism (AFLP), and pulsed-field gel electrophoresis (PFGE). PFGE has been used to investigate outbreaks in neonatal intensive care units (NICUs) involving *E. cloacae*, *E. aerogenes*, *E. gergoviae* and is currently regarded as the gold standard method for molecular subtyping of food-borne pathogens.

Culture Methods used for Isolation of *Enterobacter* Species

Most *Enterobacter* strains will grow on selective media for Enterobacteriaceae, including Violet Red Bile Agar (containing glucose or lactose), Hektoen, or MacConkey agar. Some *Enterobacter* species are reported to be susceptible to antimicrobial agents commonly used in media designed for the selection of Enterobacteriaceae. These selective formulations may include antimicrobial compounds, brilliant green or crystal violet dyes, bile salts, and sodium lauryl sulfate. No selective media has been developed for the specific isolation of *Enterobacter*. To ensure the safety of PIF and also to reduce any unnecessary disposal of product, it is important to distinguish between *Cronobacter* and *Enterobacter* species. *Cronobacter* can generally be distinguished from *Enterobacter* based on the hydrolysis of the chromogenic substrate of α-glucosidase, 5-bromo, 4-chloro, 3-indolyl α-D-glucopyranoside (α-Glu), ODC activity, and the use of the 2,3-butanediol fermentation pathway (as determined by Methyl Red and Voges–Proskauer reactions). Interestingly *Enterobacter hormaechi* is an example of an organism that has been previously misidentified as *Cronobacter*. Therefore, numbers of cases of *E. hormaechi* infection in neonates may be underreported. Townsend *et al.* (2008) showed that *E. hormachei* could invade the gut and blood–brain barrier epithelial cells and these bacteria could also persist in macrophages.

Control and Preventative Measures to Limit Dissemination of *Enterobacter*

ESβL producing species such as *E. cloacae* are increasingly reported and can cause outbreaks in susceptible populations. Bacterial infections caused by antibiotic resistant organisms result in higher morbidity and mortality and increased health-economic costs compared to those infections involving susceptible bacteria. Understanding the physiology and

survival mechanisms of *Enterobacter* will help toward developing effective strategies to reduce the prevalence of these bacteria in a clinical setting. Controlling the ecology in the food production environment would be a key step in limiting the further dissemination of these bacteria through the food chain.

Klebsiella Species

Background to the Genus *Klebsiella*

The genus *Klebsiella* was named after the German pathologist, Edwin Klebs in 1885 in honor of his work. This genus has undergone extensive changes in recent years. *Klebsiella pneumoniae* and *Klebsiella oxytoca* are the two clinically and epidemiologically most important species of this genus. Organisms such as *K. pneumoniae*, *Klebsiella ozaenae*, and *Klebsiella rhinoscleromatis* are now accepted as subspecies within the *K. pneumoniae* species. *Klebsiella terrigena* has been transferred to the genus *Raoutella*. Furthermore, *Klebsiella variicola* a newly defined species in 2004 was cultured from a range of plants including banana, rice, sugar cane, and maize and from the hospital environment. *Calymmatobacterium granulomatis* produces granulomatous lesions in humans and was renamed as *Klebsiella granulomatis* based on 16S rRNA analysis and *phoE* sequencing.

Characteristics of the Genus *Klebsiella*

Klebsiella are ubiquitous in nature and can be isolated from food (Table 1), sewage, soil, plants, and these bacteria colonize the mucosal surfaces of animals and humans. *Klebsiella* are nonmotile organisms and are usually capsulated. Microscopically, they appear as straight rods of approximately 1–2 μm in length with a diameter of 0.5–0.8 μm. These bacterial cells can be arranged singly, in pairs, or short chains. Diplobacilli, very similar to pneumococci are commonly seen in the body. *Klebsiella* are facultative anaerobes but their growth under strict anaerobic conditions can be poor. Most have fimbriae but this may not be the case for some respiratory strains. These bacteria have no special growth factor requirements. Their optimum temperature for growth is 37 °C with a growth range between 4 and 43 °C. They are killed by moist heat at 55 °C and can survive for several months in a desiccated environment. Most strains can use citrate and glucose as sole sources of carbon, but cannot use L-sorbose as a sole carbon source. They produce gas from sugars with gas production from starch being an important diagnostic characteristic. The gastrointestinal tracts of warm-blooded animals and humans can be colonized with *Klebsiella* with bacterial cell numbers reaching up to 10^8 CFU per gram in the feces. Carriage rates of 50% have been reported in premature infants in NICUs and these organisms can persist for one month or longer in infant feces.

Clinical Manifestation of Infections Linked to *Klebsiella*

Klebsiella can be disseminated following consumption of a contaminated food (Table 1), contact with a contaminated water supply during bathing and by person-to-person contact in the hospital environment. This genus is a common pathogen in the community but more importantly it is a major cause of morbidity and mortality in NICUs. It is responsible for a significant proportion of hospital-acquired infections such as necrotizing enterocolitis (NEC), septicemia, UTIs, soft tissue infections, and pneumonia. *Klebsiella* is a cause of community-acquired pneumoniae (CAP). CAP manifests as a sudden onset of breathing problems, hemoptysis, toxemia, and high fever. Early treatment with antibiotics can improve the prognosis; nevertheless CAP remains a leading cause of death especially in patients at the extremes of age. Clinically, the most important member of the genus is *K. pneumoniae*. In many developing countries, *Klebsiella* are one of the leading causes of meningitis in newborns. Between 1997 and 2002 *Klebsiella* was responsible for 7–10% of all hospital-acquired bloodstream infections across Europe, Latin American, and North America.

Klebsiella can cause serious infections in animals that can lead to a significant economic loss. They can cause mastitis in cows, leading to poor milk yields and result in the death of the animal and contagious equine metritis in horses, which can be costly to eradicate.

Antimicrobial Resistance Among *Klebsiella* Species

In recent years *Klebsiella* species have become resistant to a wide range of antibiotics. *Klebsiella* are reported to have exhibited resistance to penicillins, especially ampicillin and carbenicillin via the penicillinase enzyme SHV-1. Historically these organisms were susceptible to cephalosporins and gentamicin; more recently however *K. pneumoniae* has developed a novel mechanism of resistance to carbapenems, known as *K. pneumoniae* carbapenemases (KPCs). KPC represent a new class of bacterial enzyme capable of inactivating carbapenems. High-level antibiotic resistance is conferred by a plasmid-encoded KPC that can hydrolyze all cephalosporins, monobactams, and carbapenems. Spread of these resistance plasmids into other Gram-negative bacteria such as *Enterobacter*, *K. oxytoca*, *E. coli*, *Serratia marcescens*, and among *Pseudomonas* species has been reported. The first isolate of KPC-producing bacteria was described in a *K. pneumoniae* isolate recovered from a hospital in North Carolina in 2001. However, KPC-mediated resistance is now a worldwide problem and has been reported in China, Colombia, Brazil, France, Israel, and the US.

Epidemiology of *Klebsiella*

Should *Klebsiella* species be implicated in one or more cases of food-borne illness an investigation would be necessary to describe the associated epidemiology. This approach would include the genotyping of isolates recovered from patients and the implicated food source. Various foodstuffs especially those high in sugar content can harbor *Klebsiella* and are capable of colonizing the bowel. Its presence at low levels in PIF makes it a bacterial pathogen of concern due to the susceptibility of vulnerable neonates. *Klebsiella* species may be detected in food or water as a coliform along with other Enterobacteriaceae; however, as *Klebsiella* is also found throughout the

environment it may not necessarily indicate fecal contamination. Enteral feeding contamination by *K. oxytoca* has been recognized as a source of colonization of the throat and stool in premature infants. *Klebsiella* in food may give rise to the subsequent colonization of the gastrointestinal tracts of patients in hospitals, an important first step in the development of systemic infection.

In one study over a four-week period discriminatory typing was used to trace *Klebsiella* isolates through a hospital environment. A correlation was reported between food and fecal serotypes, and patient clinical isolates. This study explored more fully the role of contaminated food as a source for *Klebsiella* colonization and subsequent infection. An investigation, including environmental sampling, was undertaken after four leukemia patients in the same hospital ward developed serious infections with *Klebsiella aerogenes*, the source of this organism, was found to be a food blender used for preparing milk-based drinks on the ward, common to all four patients.

Culture Methods Used for Isolation of *Klebsiella* Species

The majority of *Klebsiella* strains grow well on Simmons' Citrate Agar and Møller's KCN medium. Some respiring strains grow very poorly on MacConkey agar. Chromogenic agar can be used for the isolation and enumeration of *Klebsiella* species, such as HiCrome *Klebsiella* Selective Agar. A chromogenic substrate in the media is cleaved specifically by *Klebsiella* to produce purple-magenta colored colonies.

Control and Preventative Measures to Limit Dissemination of *Klebsiella*

Klebsiella are well adapted to survival in the environment, even more so than other enteric bacteria. The main route of transmission of this pathogen within the hospital setting is through poor hand hygiene. Of concern to public health is the increase in antibiotic resistance reported among isolates of this genus. The high mortality rates associated with infectious pathogens will continue to increase due to the expanding antibiotic resistance profiles reported and the increased risks of therapeutic failures. Therefore, the ability of *Klebsiella* to adapt to new antimicrobial compounds requires new approaches to better manage hospital infections with *Klebsiella* species.

The restriction on the use of third generation cephalosporins and effective and consistent hand hygiene will help to reduce the prevalence of healthcare-associated infections.

Pantoea Species

Background to the Genus *Pantoea*

The genus *Pantoea* was proposed in 1989 to include several species that had formally resided in other genera of the *Erwinia herbicola–Enterobacter agglomerans* complex. The creation of the genus *Pantoea* allowed strains previously known as *E. agglomerans* and *E. herbicola* to be assigned to this new genus. Until recently the species of the genus included *Pantoea agglomerans, Pantoea ananatis*, *Pantoea citrea*, *Pantoea dispersa*, *Pantoea punctata*, *Pantoea stewartii*, and *Pantoea terrea*. In 2009, four new *Pantoea* species were described following their isolation from eucalyptus leaves and shoots showing symptoms of blight and die-back. These species were named: *Pantoea vagans*, *Pantoea eucalypti*, *Pantoea deleyi*, and *Pantoea anthophila*.

More recently, three new *Pantoea* species were discovered: *Pantoea gaviniae, and Pantoea calida* were isolated from infant formula and an infant formula production environment. *Pantoea allii* was recovered from onion and onion seed. There remains considerable taxonomic diversity within the *Pantoea* genus with some isolates of the *Erwinia herbicola–Enterobacter agglomerans* remaining ungrouped. Much work remains to be done to clarify the phylogenic classification of this genus.

Characteristics of the Genus *Pantoea*

Pantoea species share most of the general phenotypic characteristics associated with other genera within the Enterobacteriaceae. Most strains grow well at 4 °C and *P. dispersa* grows well at 41 °C where most other strains do not. *Pantoea* species are lysine, ODC, and arginine dihydrolase negative. They usually ferment glucose, lactose, mannitol, and sucrose, and are indole- and urease-negative. Most clinical isolates produce a yellow pigment. The genus *Pantoea* meaning all sorts and sources is intended to designate bacteria from diverse geographical and ecological sources. These bacteria are widely distributed in nature and have been found in soil, water, food (Table 1), humans, and animals. *Pantoea agglomerans* is associated with plants as an epiphyte or an endophyte and some isolates have been reported to be tumorogenic pathogens. They can easily be recovered from cotton and have been linked to cotton fever among intravenous drug abusers. Cotton pledgets are continuously used by nurses and physicians in a hospital environment, which can become contaminated in several ways.

Pantoea ananatis is considered an emerging plant pathogen as it has shown an increase in incidence, host and geographical range. Its occurrence in plants and trees can lead to sporadic plant disease outbreaks resulting in severe economic losses in eucalyptus, corn melons, Sudan grass rice, pineapple, and total losses have been reported in the case of onions. This bacterium may not be pathogenic to all plants, as some may possess both antibacterial and antifungal activities. *Pantoea ananatis* have been observed to increase root and shoot growth in papaya plants and promote growth and induce systemic resistance against *Xanthomonas axonopodis* pv. *vesicatoria* in pepper.

Clinical Manifestation of Infections Linked to *Pantoea*

Because of its confusing taxonomy and the difficulty in designating *Pantoea* species, little is known about their pathogenicity. *Pantoea agglomerans* is both a commensal and pathogen of animals and humans and is commonly isolated in hospitals. The latter is also the most commonly isolated *Pantoea* species in humans. They are considered an opportunistic pathogen that rarely causes disease in healthy individuals. The role of other *Pantoea* species is relatively unknown. The most common infection caused by *P. agglomerans* is septic arthritis or synovitis,

which has been traditionally reported after thorn injury. *Pantoea agglomerans* is also associated with pondylodiscitis, tibial osteitis, colelithiasis, occupational respiratory infections, skin allergy and has been linked with other organisms in polymicrobial peritonitis. It has been associated with contaminated intravenous products, a parental nutrition outbreak, the anesthetic agent propofol, blood products and contamination of transference tubes. In the 1970s *P. agglomerans* (then referred to as *E. agglomerans*) was implicated in a nationwide epidemic of septicemia caused by contaminated bottles of infusion fluids: 25 hospitals were affected with 378 cases reported. In 2002 it was reported that a *P. dispersa*-like strain was the only detectable microorganism cultured from an immunocompromised female with a rare combination of acute myeloid leukemia (AML) and multiple myeloma (MM).

Antimicrobial Resistance Among *Pantoea* Species

Limited information is available on the prevalence of antimicrobial resistance among members of this genus. Some *Pantoea* species have been reported to be resistant to most antibiotics used in an NICU. In 2000, a clinical isolate of *P. agglomerans* recovered from an individual with septic arthritis was reported to be highly resistant to fosfomycin and mecillinam.

Epidemiology of *Pantoea*

The epidemiology of plant diseases caused by *P. ananatis* on different hosts is relatively unknown. Environmental factors affect the severity of the disease caused by *P. ananatis*. Strain subtyping of *Pantoea* is important for epidemiological purposes and to identify those strains that are pathogenic to plants or cause infection in humans. Molecular subtyping methods including fluorescent- AFLP (F-AFLP) and PFGE have been successful for typing *Pantoea* strains.

Culture Methods Used for Isolation of *Pantoea* Species

Pantoea species generally grow well on most laboratory media, such as blood agar, nutrient agar, tryptic soy agar, and some media specifically designed for the isolation of the Enterobacteriaceae. However, *P. agglomerans* grows poorly on MacConkey agar and is not hemolytic on blood agar. A selective medium PA 20 was developed for the isolation and enumeration of *P. ananatis* from plant material, specifically from onion seed. It was reported to inhibit the growth of most of the common saprophytes associated with the seed. Phenotypic identification of *Pantoea* species may be difficult as they share many similar characteristics. The most promising approach for the speciation of *Pantoea* is the use of multilocus sequence analysis (MLSA). By using four housekeeping genes, MLSA could clearly differentiate between *Pantoea* species.

Other Information Relevant to the Genus *Pantoea*

Pantoea agglomerans are among the most promising biocontrol agents for a variety of bacterial and fungal plant diseases, particularly fire blight in apples and pears. However, this species is designated as a biosafety level 2 organism because it is an opportunistic human pathogen which hiders its commercial registration as a biocontrol product. These bacterial strains are also effective against other bacterioses, such as basal kernel blight of barley and postharvest fungal diseases of pome fruits.

Serratia Species

Background to the Genus *Serratia*

The first member of the Enterobacteriaceae family was discovered by a Venetian pharmacist Bartolomeo Bizio in 1823, who identified *S. marcescens*, growing on an Italian barley dish. The red-pigmented organism was described as a case of bleeding polenta. *Serratia marcescens* was the first species recognized within this genus and it is the primary pathogenic species. Rare reports of infection have been documented with *Serratia plymuthica*, *Serratia liquefaciens*, *Serratia rubidaea*, *Serratia odorifera*, and *Serratia fonticola*. All species with the exception of *Serratia entomophila* have been isolated from humans.

Characteristics of the Genus *Serratia*

Serratia are considered to be ubiquitous in the environment and the organism is found in water, soil, plants, insects, animals, mammals including humans and food (**Table 1**), particularly those rich in starch. Most *Serratia* isolates are motile with petrichous flagella. Optimum growth of *Serratia* has been observed at pH 9 and at temperatures ranging from 20 to 37 °C. Only *S. marcescens* and *S. rubidaea* fail to grow at 4 °C. The former is smaller in size when compared to the average coliform bacteria. *Serratia* can produce alternate forms of flagella that can provide the cell with different types of motility; depending on what medium the organism is grown on.

Serratia species can be distinguished from other genera by its production of three enzymes DNAase, lipase, and gelatinase. Members of this genus give positive reactions for citrate, Vogues–Proskuer, ortho-nitrophenyl-β-D-galactoside (ONPG) and can ferment mannitol and trehalose. If present, lactose fermentation occurs slowly. *Serratia marcescens* is unique among enteric bacteria in many respects as it is one of the most effective bacteria capable of degrading chitin, through the production of chintenases and a wetting agent or surfactant called serrawettin, which assists in the colonization of surfaces. Swim and swarming motility along with extracellular enzymes including nucleases, proteases, and haemolysin may contribute to pathogenesis. *Serratia* produce two types of bacteriocins. Group A bacteriocin producing strains are resistant to chloroform, heat, proteolytic enzymes, and active against other *Serratia* strains. Group B bacteriocin producing strains are susceptible to these latter agents but are active against other enterobacteria but not against other *Serratia* strains.

Serratia produce a cell-associated red color pigment called prodigiosin or 2-methyl-3-amyl-6-methoxy-prodigiosene. Pigmented strains have caused alarm by simulating the appearance of drops of blood on starchy foodstuffs. Production

of prodigiosin is highly variable between strains and it is only formed under aerobic conditions at specific temperatures. There is no correlation between optimum growth conditions and pigment production. Some isolates may grow at optimum temperature producing no pigment whereas at a lower temperature pigment production may be in abundance. Although some *S. marcescens* strains produce a red pigment, a majority of clinical strains are nonpigmented (>90%) and are usually found in various ecological niches.

The elaboration of fimbriae, production of potent sidreophores, presence of cell wall antigens, production of proteases, and the ability to resist the bactericidal action of serum have all been identified as potential virulence factors in *Serratia*.

Clinical Manifestation of Infections Linked to *Serratia*

Until the 1950s, *S. marcescens* was believed to be a nonpathogenic saprophyte and rarely isolated from human patients. In the past 30 years there has been an increase in nosocomial infections linked to *S. marcescens*. The first report of *S. marcescens* infection occurred in 1951 in Stanford University Hospital where 11 cases were reported over a 6-month period. This bacterium is now recognized as an opportunistic pathogen in humans and may spread in epidemic proportions causing nosocomial infections in hospitalized patients. Clinically *S. marcescens* causes a range of infections including cystitis, arthritis, eye infections, respiratory tract infections, UTIs, septicemia, meningitis, and wound infections.

Serratia marcescens has been reported to cause endocarditis in hospitals and in the community and affects the left hand side of the heart, which is in contrast to other Gram-negative bacteria. The bacterium is relatively uncommon accounting for only 1% of bloodstream infections reported to the National Healthcare Safety Network (NNIS) between 1986 and 1989. However, the mortality rate of *Serratia* is high and has been reported to be between 33% and 52%. *Serratia* infection linked to meningitis is also very rare but its high mortality rate (45%) exceeds that of other Gram-negative bacteria with surviving patients usually having neurological deficits. *Serratia marcescens* nasopharyngeal colonization in hospitalized patients commonly leads to respiratory infections. According to the NNIS, *Serratia* are responsible for 4% of nosocomially-acquired pneumonia.

Antimicrobial Resistance Among *Serratia* Species

Serratia marcescens can utilize a wide range of nutrients, which aids its ability to survive and grow under extreme conditions, including in the presence of disinfectants and antiseptics such as those used in contact lens washing solutions containing chlorhexidine, cotton balls and antiseptic solutions containing benzalkonium chloride and antiseptic soaps containing triclosan. Antibiotic resistance has been reported against several antimicrobial classes including penicillins (first- and second-generation cephalosporins, β-lactamase-mediated carbapenem resistance), aminoglycosides, and polymyxins. Infections with *S. marcescens* may be difficult to treat due to its resistance to multiple antibiotics. Nonpigmented strains are generally more resistant as they usually harbor resistance plasmids.

Epidemiology of *Serratia*

Several methods have been described for successfully subtyping *S. marcescens*, including PFGE, serotyping, phage typing, biotyping, one-dimensional sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis (PAGE), bacteriocin typing, antimicrobial susceptibility testing, and plasmid analysis (which is limited by the fact that only 25% of strains contain plasmids). Multilocus enzyme electrophoresis (MLEE) and electrophoresis of periplasmic proteins are alternative typing methods.

Culture Methods for the Isolation of *Serratia*

Differential and selective media have been developed for the isolation and presumptive identification of *Serratia*. These bacteria can readily grow on routine microbiological culture media (such as brain heart infusion broth, trypticase soy, nutrient agars). Isolation of *Serratia* from nonsterile sources such as food, environmental habitats, and hospital samples may require more specialized culture media. In this case deoxyribonuclease-toluidine blue-cephalothin agar (DTC) and caprylate-thallous (CT) agar may be required.

Control and Preventive Measures to Limit the Dissemination of *Serratia*

Major recalls involving *Serratia* include a recall in early 2008 by the US Food and Drug Administration (FDA) who issued a nationwide recall of one lot of Pre-Filled Heparin and Normal Saline Flushes that were contaminated by *S. marcescens*. The primary reservoir for *S. marcescens* is hospitalized patients, usually those with UTIs, or those individuals colonized or presenting with infected respiratory tracts. Infections transmitted from person-to-person are the most common dissemination route and are generally very difficult to control. Hospital infection control recommendations should stress the importance of strict adherence to hand-hygiene guidelines. More research is required to understand the relationship between *Serratia* species cultured from foods.

Other Information Relevant to the Genus *Serratia*

The production of the red pigment prodigiosin is a valuable marker and has been used in experiments such as the transmission of aerosols. In the US *S. marcescens* has been used in biological warfare population-vulnerability experiments. Prodigiosins display immunosuppressive, proapoptotic and anticancer properties and can therefore be potentially interesting candidates for drug development. *Serratia*-elaborated chintenase can be used in the treatment of chitin containing wastes, biocontrol agents against pathogenic fungi, production of adhesives and wound dressings, heavy metal recovery from waters, and dialysis membranes.

Other Genera Less Often Encountered

This section briefly describes the less frequently encountered pathogenic genera in the Enterobacteriaceae family including *Cedecea*, *Edwardsiella*, *Hafnia*, *Kluyvera*, and *Leclercia*.

Cedecea Species

The name of this genus was derived from the letters CDC, the abbreviation used for the Center for Disease Control and Prevention in Atlanta, where the organism was originally discovered. *Cedecea* species, based on their biochemical characteristics most closely resemble *Serratia*. Three species have been named (*Cedecea davisae*, *Cedecea lapagei*, and *Cedecea neteri*) and a further two species remain to be named (and are referred to as *Cedecea* species 3 and 5). These bacteria are usually motile and produce gas from glucose. *Cedecea* grow well on nonselective laboratory media producing convex colonies at 37 °C. *Cedecea* are predominately isolated from the respiratory tract but their importance in causing disease has not yet been demonstrated, although *C. davisae* and *C. neteri* have been reported to cause bacteremia and pneumonia.

Edwardsiella Species

The genus *Edwardsiella* originally consisted of only a single member (*Edwardsiella tarda*), however currently, at least three species are now known to exist (and are denoted as *Edwardsiella hoshinae*, *Edwardsiella ictaluri*, *Edwardsiella tarda*). *Edwardsiella* have a limited environmental habitat and host range. These bacteria are frequently isolated from freshwater and marine environments. They may be pathogenic for animals associated with these ecological niches especially cold-blooded vertebrates including, catfish, eels, lizards, and birds sometimes causing an economic loss.

Edwardsiella tarda is the only species in this genus, known to be pathogenic for humans where it is primarily associated with sporadic cases of gastroenteritis. *Edwardsiella* isolated from the gastrointestinal tract accounts for more than 80% of all clinical strains. Other infections associated with this bacterium include wound infections and systemic diseases such as cholecytitis, meningitis, osteomyelitis, and septicemia. *Edwardsiella tarda* is rarely found in the feces of healthy individuals. Risk factors associated with *E. tarda* are exposure to aquatic environments, exotic animals, people with underlying illness, including liver disease and the ingestion of raw fish. Studies have shown the invasive properties of *E. tarda* where most strains invaded HEp-2 cell monolyers, produced hemolysin and siderophores, and bound Congo red dye. Furthermore, *Edwardsiella* species are susceptible to commonly used antibiotics, including penicillin a feature that is unusual for genera such as Enterobacteriaceae. A serotyping scheme has been developed to further identify these bacteria. This serotyping system comprises of 61 O-groups and 45 H-antigens. Some *E. tarda* isolates produce bacteriocins that are effective against other *E. tarda* strains and some *Yersinia* species.

Hafnia Species

The current *Hafnia* genus consists of only one species, *Hafnia alvei*. This bacterium was previously known as *Enterobacter hafnia* or *Enterobacter alvei*. *Hafnia alvei* have been isolated from different environmental and clinical sources. Food products that commonly yield *Hafnia* include ground (minced) beef and vacuum-packed beef, pork products, dairy products, freshwater fish, and honey (Table 1). These organisms do not ferment lactose and can resemble *Salmonella* on specific *Salmonella* isolation agar. *Hafnia* species demonstrate biochemical reactions that are similar to those of *Enterobacter* and *Serratia*. No specific media has been developed for their isolation and detection. *Hafnia* is often isolated from clinical material yet its role in infection is poorly understood. They have been associated with extraintestinal disease usually in immunocompromised individuals. However, the majority of *Hafnia* strains are not considered pathogenic. They are usually isolated from feces and rarely recovered from other body sites.

Kluyvera Species

Kluyvera was first proposed as a genus in 1956 by Asai and coworkers, with two species *Kluyvera citrophila* and *Kluyvera noncitrophila*. However, the genus *Kluyvera* was not defined completely until 1981 when Farmer *et al*. used DNA–DNA hybridization technique to fully characterize the genus. The original species names proposed by Aasi and coworkers were not used, as they did not fit the CDC's species descriptions. Previously known as enteric group 8 and API group 1, the current genus consists of four species, *Kluyvera ascorbata*, *Kluyvera cryocrescens*, *Kluyvera georgiana* (formerly species group 3), and *Kluyvera cochleae*. *Kluyvera* have been isolated from milk, sewage, soil, water, kitchen food (Table 1), an oriental fruit fly, and a hospital sink. Human isolations are more common with the organism being cultured from sputum, urine, stool, blood cultures, peritoneal fluid, wound specimen, and a Broviac catheter. Clinical signs related to *Kluyvera* infection can vary and affect a wide range of age groups. Each of these species has been isolated from human clinical specimens except *K. cochleae*. *Kluyvera* species can cause bacteremia, soft tissue infections, intra-abdominal abscesses, and UTIs. Reports suggest that this organism can also cause disease in healthy immunocompetent children. *Kluyvera* isolates can demonstrate resistance to penicillin and some first- and second-generation cephalosporins (cefalotin and cefuroxime) due to the production of β-lactamase enzymes.

Leclercia Species

The genus *Leclercia* was proposed for those species previously known as *Escherichia adecarboxylata* or CDC Enteric group 41. Even though *Leclercia adecarboxylata* has been isolated from a variety of sources its natural habitat is unknown. Most of the early isolates studied were recovered from food, water, and environmental sources. However, *Leclercia* has also been found in human clinical specimens including blood, feces, sputum, urine, and wounds with cases reported from around the world. Some strains are known to produce a yellow pigment and grow well on general laboratory media. Although *Leclercia* are generally susceptible to most antibiotics in clinical use today, there are reports of resistant strains.

See also: Bacteria: *Cronobacter (Enterobacter) sakazakii* and other *Cronobacter* spp.; Shiga Toxin-Producing *Escherichia coli* and Other Pathogenic *Escherichia coli*; *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*. Characteristics of Foodborne Hazard and Diseases: Drug Resistant Pathogens; Pathogenesis and Virulence.

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Francisella tularensis

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Glossary

Biological warfare agent An agent (bacteria, viruses, or fungi) that could be used to kill or incapacitate humans, animals, or plants.

Coagulation necrosis A type of necrosis that develops when the protein elements coagulate and thus the affected cells or tissue form a dry and homogeneous eosinophilic mass.

Colony-forming unit The viable number of a bacterium or a fungus.

Enzyme-linked immunosorbent assay An immunoassay that uses an enzyme linked to an antigen or antibody as a marker for the demonstration of a specific protein, generally an antibody or antigen.

Fluorescent antibody test A test for the demonstration of agents expressing a specific protein by binding antibodies specific for the protein and visualizing these complexes by fluorescent labeling of the antibody.

***Francisella*-like endosymbiont** These are *Francisella* variants with unknown pathogenicity which are harbored by ticks.

Granuloma A structured nodular aggregation of mononuclear inflammatory cells and connective tissue.

Immunohistochemistry This is a method for the detection of specific antigens in tissue sections by staining with antibodies marked with pigmented or fluorescent material.

Lethal dose 50 (LD_{50}) The dose of a substance that would be expected to kill half of a population of exposed organisms.

Polymerase chain reaction A molecular technique for amplifying a single or a few copies of deoxyribonucleic acid (DNA) by separating the DNA into two strands and incubating it with oligonucleotide primers and DNA polymerase to generate millions of copies of that particular DNA sequence.

Background

Francisella tularensis is the etiological agent of tularemia, a lethal zoonotic disease and potential biological warfare agent. It was first isolated and characterized by McCoy and Chapin in 1912 during an outbreak of a 'plague-like' disease in ground squirrels in Tulare County, CA, USA. The genus designation honors the American pathologist, Edward Francis, who examined tularemia during the 1920s and 1930s; the name of the disease and species name derived from Tulare County.

Characteristics of the Organism

Four subspecies of *F. tularensis* are recognized: the highly virulent *F. tularensis* ssp. *tularensis* (type A), moderately virulent *F. tularensis* ssp. *holarctica* (type B), and *F. tularensis* ssp. *mediasiatica*, and low virulent *F. tularensis* ssp. *novicida*. The *F. tularensis* ssp. *tularensis* is found in North America; *F. tularensis* ssp. *holarctica* is found throughout Europe, Asia, and North America, and was recently discovered in Tasmania; *F. tularensis* ssp. *mediasiatica* has been isolated only in Kazakhstan and Turkmenistan, whereas *F. tularensis* ssp. *novicida* has been linked to waterborne transmission in Australia and the US.

Francisella tularensis is an intracellular bacterium. It is an obligate aerobe, small (0.2–0.7 μm × 0.2–1.7 μm), Gram-negative, nonmotile, and pleomorphic coccobacillus covered by a carbohydrate-rich capsule. *Francisella tularensis* is oxidase negative, weakly catalase positive, and cysteine is required for its growth. Utilization of glycerol is an effective tool to differentiate *F. tularensis* ssp. *tularensis* (glycerol positive) and *F. tularensis* ssp. *holarctica* (glycerol negative).

Survival of *F. tularensis* in nature is dependent on a variety of factors such as temperature (1 h at 60 °C) or direct exposure to sunlight (3 h at 29 °C). General survival in carcass tissues is 3–4 weeks and 4 months in water at 4–6 °C. *Francisella tularensis* does not form resistant structures and is relatively sensitive to all standard inactivation procedures. Therefore, autoclaving is suitable for the inactivation of *F. tularensis*. The bacterium is sensitive to hypochlorite and other commonly used chemical decontaminants and readily inactivated on exposure to ultraviolet irradiation.

Francisella tularensis has ~1.9 mega base pair size genome (*F. tularensis* ssp. *holarctica* LVS NC_007880 and *F. tularensis* ssp. *tularensis* SCHU S4 NC_006570). It is a highly clonal bacterium, which means that it inherits deoxyribonucleic acid (DNA) in a vertical manner and does not transfer DNA laterally between cells.

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Epidemiology

Natural infections with *F. tularensis* have been reported in a range of vertebrates including mammals, birds, amphibians, and fish. Despite its broad host range, tularemia is primarily a disease of the orders Lagomorpha and Rodentia. In the New World, the cottontail rabbit (*Sylvilagus* spp.), black-tailed jackrabbit (*Lepus californicus*), and snowshoe hare (*Lepus americanus*) are important in the ecology of tularemia, whereas the European brown hare (*Lepus europaeus*), mountain hare (*Lepus timidus*), and Japanese hare (*Lepus brachyurus*) are associated with tularemia in the Old World. Voles (*Microtus* spp., *Arvicola amphibius*, and *Myodes glareolus*) are most frequently involved in tularemia epizootics but other rodent species are also infected. Hematophagous arthropods have substantial role both in maintaining *F. tularensis* in the nature and disease transmission. Ticks are believed to be the most important arthropods (biological vectors) in the ecology of tularemia throughout the Northern Hemisphere, but other bloodsucking arthropods (e.g., mosquitoes – Scandinavia and Russia, and horseflies – Russia and the US) transmit *F. tularensis* mechanically. There are two known cycles of tularemia: the terrestrial and aquatic. In the terrestrial cycle, hares and rodents are the most important mammalian hosts, whereas hematophagous arthropods play a role as vectors. In the aquatic cycle, voles and maybe muskrats and beavers serve as the main host species, shedding live bacteria into the environment. It was found that a protozoon species (*Acanthamoeba castellanii*) can be an important aquatic-environment reservoir of *F. tularensis.*

Humans are highly susceptible to *F. tularensis*. People can get infected in several ways such as direct contact, through wounds or small cuts, ingestion, inhalation, or via the conjunctiva. *Francisella tularensis* can be transmitted by the infected animals' tissues or fluids, contaminated water or food, aerosols, or by the bites of infected arthropods.

Through ingestion of contaminated water or food, the oropharyngeal form (see section 'Clinical Manifestation and Pathology') of tularemia can be acquired. The water of streams and wells may become contaminated with *F. tularensis* and remain contaminated for more than a month when carcasses of infected mammals (e.g., rodents or lagomorphs) remain in the water. The meat of several animals (e.g., hares and rabbits) may be infected but other food items could also be contaminated with the feces of *F. tularensis* infected animals (e.g., rodent). In Turkish tularemia outbreaks between 1936 and 2004, 387 out of 507 cases (77%) were referred to as the oropharyngeal form, while 32 out of 1100 recorded human tularemia cases in Finland between 1967 and 1983 were diagnosed as oropharyngeal form. In Italy in 1982, consumption of water from an unchlorinated water system caused a waterborne tularemia outbreak with 49 human cases. In early 2000, 327 food- and water-related human tularemia cases were recorded in Kosovo , at the end of a 10-year-long political crisis and war. The rodent population increased during wartime, which allowed epizootic spread of *F. tularensis* infection among rodents and resulted in the contamination of food and water sources.

Pathogenesis

Francisella tularensis is a highly infectious agent but its virulence factors have not been well characterized. As low as 10 colony-forming unit (CFU) of *F. tularensis* ssp. *tularensis* is enough to cause fatal infection in mice, guinea pigs, or rabbits and a similarly small dose is enough to induce a severe or sometimes fatal infection in humans. *Francisella tularensis* ssp. *holarctica* causes lethal infection in mice and guinea pigs at a similarly small inoculation dose, however, a higher dose is needed to induce the disease in rabbits (lethal dose 50 (LD_{50}): $>10^6$ CFU) or humans (LD_{50}: $>10^3$ CFU).

After entering the body (via inoculation by hematophagous arthropods or wounds, through the conjunctiva, by inhalation of infected aerosols, by ingestion of contaminated meat, or by water), bacteria multiply locally causing ulceration and necrosis and then invade the blood and lymph vessels and spread to the lymph nodes and organs such as liver, spleen, lung, kidney, serosal membranes, and bone marrow, causing multiple foci of coagulation necrosis. *Francisella tularensis* is a typical intracellular pathogen with a high predilection not only to growin macrophages but it can also infect many other cell types, such as epithelial cells, hepatocytes, muscle cells, and neutrophils. *Francisella tularensis* septicemia occurs at the end stage of the disease when bacteria invade the blood vessels without causing lesions indicative of tissue response. Only this septicemic form is seen in highly sensitive animals, which die within 2–10 days. Relatively resistant animals and humans can survive the infection and develop protective immunity. Synergy between phagocytes, antibodies, and T cell-derived cytokines appears to be critical for achieving immunity against *F. tularensis* and clearing infection. In humans, the antibody response is measurable by the second week postinfection. Antibody levels are highest during the second month after infection and decline gradually thereafter.

Clinical Manifestation and Pathology

Clinical cases of tularemia are infrequently observed in free-ranging wildlife as infected animals are usually found moribund or dead. Nonspecific signs include depression and pyrexia. Local inflammation or ulceration at the portal of entry and enlargement of the regional lymph nodes may be observed. Highly sensitive animals develop fatal septicemia and may be nonresponsive before death. In hares, depression, stupor, loss of body weight and lack of fear, and facilitating capture are observed in the late stages of the disease. The pathology of tularemia differs considerably between different animal species. In highly sensitive species with acute disease (e.g., mouse), the usual macroscopic finding is the enlarged spleen and, less frequently, liver. Pinpoint white foci could be seen in these organs showing multifocal coagulation necrosis by histology. In moderately sensitive species (e.g., European brown hare) a more chronic form of disease can be observed and characterized by granulomatous lesions with central necrosis, particularly in the lungs and kidneys and occasionally in the liver, spleen, bone marrow, and lymph nodes. The granulomas contain heterophils, macrophages, and giant cells.

Seven clinicopathological forms of tularemia have been described in human medicine: ulceroglandular, glandular, oculoglandular, oropharyngeal (after food- or waterborne infection), pneumonic, typhoidal, and septicemic. The most frequent clinical signs in humans are inflammation and later ulceration at the primary site of infection, with swelling of regional lymph nodes, which may become abscessed. Generally, the course of the clinical disease includes sudden onset of fever, generalized aches, inflammation of the upper respiratory tract with nasal discharge, vomiting, malaise, and anorexia. In oropharyngeal tularemia, people show an ulcerative–exudative stomatitis and pharyngitis, with or without tonsillar involvement, and excessive regional neck lymphadenitis.

Analytical Methods

Field examination of pathological samples is not recommended when there is a suspicion of tularemia because of the potential for human exposure and risk of contaminating the environment. Special precautions, including wearing gloves, masks, and eyeshields are advised when handling infective materials. Procedures should be performed within appropriate biosafety containment facilities (biosafety level 2 or 3).

Francisella tularensis appears as numerous Gram-negative small-sized bacteria in impression smears or in histological sections of spleen, liver, lung, kidney, bone marrow, lymph node as well as in blood smears. *Francisella tularensis* can be detected specifically by direct or indirect fluorescent antibody tests. Immunohistochemical assay is a very useful and sensitive method for the detection of *F. tularensis* in domestic and wild animals.

Francisella tularensis can be identified by culture. The most adequate samples for culture are, in acute cases, heart blood, spleen, liver, or bone marrow, whereas in chronic cases, granulomatous lesions should be examined. Owing to the highly fastidious culture requirements of *F. tularensis*, isolation can be difficult as it grows poorly in conventional culture media. Francis medium, McCoy and Chapin medium, or modified Thayer-Martin agar are recommended. Isolation of *F. tularensis* from carcasses may be difficult due to the overgrowth of contaminant bacteria. Selective culture media can be prepared by adding penicillin, polymyxin B, and cycloheximide. When the primary isolation of *F. tularensis* is difficult, it may be isolated following inoculation of tissue suspension from suspect cases into laboratory animals such as mice. The colonies of *F. tularensis* are small, round, and they do not appear within the first 48 h of incubation at 37 °C and 5% CO_2. Because *F. tularensis* ssp. *tularensis* and *holarctica* differ in citrulline ureidase activity and glycerol fermentation, biochemical assays can be used for differentiation based on glycerol fermentation or citrulline ureidase activity.

Several polymerase chain reaction (PCR) methods have been described for the detection of *F. tularensis* DNA in both environmental and clinical specimens. Conventional PCR assays target the 16S ribosomal ribonucleic acid gene and genes encoding the outer membrane proteins, *fopA* or *tul4*, they show good specificity and allow rapid detection of *F. tularensis* in specimens. However, *Francisella*-like endosymbionts of ticks can produce nonspecific positive results in these assays. Real-time PCR methods show no evidence of cross reactivity with non-*F. tularensis* agents (other vectorborne and environmental organisms) and the high sensitivity increases the chance of detecting very low numbers of *F. tularensis* organisms in environmental samples. Molecular methods are also applied to *F. tularensis* isolates to provide resolution among the *Francisella* species, subspecies, and within-subspecies strains.

Serology can be carried out for investigating exposure to *F. tularensis*. A slide agglutination test, using one drop of stained bacteria and one drop of whole blood, is a widely used field method. The standard serological tests are the tube agglutination and microagglutination tests. Cross reaction with *Brucella abortus*, *Brucella melitensis*, *Brucella suis*, *Legionella* spp., and *Yersinia* spp. may occur. The enzyme-linked immunosorbent assay allows an early diagnosis of tularemia. Western blotting, indirect immunofluorescence assay, and flow cytometry have also been assessed for the serological diagnosis of tularemia.

Control/Preventive Measures

Francisella tularensis has an extremely broad host range and very complex ecological transmission cycles, therefore it is difficult to control. Monitoring of wildlife, arthropod vectors, and surface water for tularemia activity in enzootic areas provide useful information for public health authorities, veterinarians, and wildlife managers.

Infection in humans with *F. tularensis* can be treated with antibiotics such as quinolones (ciprofloxacin and levofloxacin), aminoglycosides (streptomycin and gentamicin), and tetracyclines (tetracycline and doxycycline). Publication of epizootic events and providing information on treatment and protection are important. Rubber gloves should be worn by trappers or hunters when skinning carcasses of species commonly associated with tularemia to prevent contact transmission. The use of insect repellent, protective clothing, and frequent body searches for prompt removal of ticks can greatly reduce the risk of infection. Meat from potentially infected animals (e.g., hares and rabbits) should be well cooked. The food which may be contaminated with animal feces should be avoided. Untreated water from lakes and streams should not be consumed, used for washing, or for brushing teeth. Food stores and water sources should be protected from contact with possibly infected animals (e.g., rodents and lagomorphs). Diagnostic laboratory procedures should be performed within secure biosafety containment facilities (biosafety level 2 or 3) observing appropriate biosafety procedures.

Generally vaccination has not been widely applied but it has been used under high-risk situations, typically for laboratory researchers. Many vaccine candidates including acellular subunit, killed whole cell, and live attenuated vaccines have been developed in the recent years but none of them have been licensed yet.

See also: Analytical Methods: Transmissible Spongiform Encephalopathy Diagnosis. Bacteria: *Brucella*; *Yersinia enterocolitica*

and *Yersinia pseudotuberculosis*. Public Health Measures: Food Defense: Prevention of Sabotage and Bioterrorism. Veterinary Drugs Residues: Antibacterials

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Helicobacter pylori

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Glossary

Achlorhydric Absence of chlorhydric acid (gastric fluid).
Cag PAI *Cag* pathogenicity island.
Ferrets Used as wild hunters for rats and mice, they are today domesticated mammals.
Lymphoma General term for malignant neoplasms from lymph and reticuloendothelial tissues.
MALT lymphoma Gastric mucosa-associated lymphoma: Extensive lymphoma formed of large B cells with a high malignant degree of pathogenicity.
Microaerophilic A bacteria that does not require a high rate of oxygen supply.
Omeprazole A proton pump inhibitor.

History, Background, and Taxonomy

For a long time before the discovery of the potential role of an unknown bacteria, gastric ulcers were considered to be the result of hyper acidic gastric mechanism. Patients were treated with drugs neutralizing acidic content, such as bismuth subsalicylate that was efficient and has been used for long. However, bismuth being a toxic metal, this treatment has been limited. The hypotheses of bacterial potential role in peptic ulcer were suggested by several authors at the end of the nineteenth century. In early 1950s, several microbiologists described a bacterium in gastric acid secretions and in mucosal biopsies, until Robin Warren and Barry Marshall identified in 1982 a Gram-negative, microaerophilic organism, named initially *Campylobacter pyloridis* and later *Helicobacter pylori*. It is helix-shaped with 3 mM lengh and it possesses 4–6 flagella that confer motility to pathogenic strains. It produces oxidase, catalase, and urease. *H. pylori* strains can form biofilms, capable of converting to viable coccoid form, which is a potential epidemiological factor and contributes to resistance of the organism in acidic conditions. Its taxonomy has been defined as that of a completely new Genus, belonging to Family '*Helicobacteriaceae*' Genus *Helicobacter*, Species *H. pylori*. In the late twentieth century with the development of molecular biology, sequencing of the *H. pylori* genome contributed to establishing taxonomy bases on the deoxyribonucleic acid (DNA) level and to understanding virulence factors. Moreover, genomic analyses have unraveled a large diversity of strains: Genetic differences should further permit a better understanding of pathogenesis of *H. pylori*. Some Helicobacter-like organisms (HLOs) have been detected by PCR (specific tests). The first HLOs have been described in ferrets (*Helicobacter mustelae*) and in Syrian hamsters (*Helicobacter hepaticus*). Thus, some taxonomic changes should be expected in the near future.

Bacteriology of *H. pylori*

In Microbiology laboratories classical techniques can be applied for isolation and identification of Helicobacter, as have been done early in the 1980s, by Robin Warren and Barry Marshall. Bacteriological cultures have been achieved by using bacterial growth from biopsies taken from gastric mucosa (of both the body and the antrum). Culture methods have been described by specialists but not standardized: The biopsy specimens must be homogenized and then sowed in selective media (Agar Pylori, BioMérieux) or nonselective media (such as Columbia III Agar with 5% sheep blood). Incubation requires microaerophilic conditions for 10–14 days (37 °C) and then further techniques require experience to identify colonies and carry out subcultures for identification tests. However, bacteriological and molecular methods remain limited to specialized expert centers: Positive or negative results based on invasive methods must be confirmed by noninvasive methods, such as detection of *H. pylori* antigens. The latter methods have been applied to stools of patients, confirming the potential intestinal carriage or occasional excretion of the pathogen in stools.

Epidemiology, Contamination, and Transmission

Investigations carried out after the discovery by Marshall and Warren have shown that *H. pylori* is a common organism found in more than 50% of the world's population, and 80% of individuals infected with the organism are asymptomatic. This bacterium lives probably exclusively in human stomach and is the only bacterial species capable of surviving in such an acidic environment. The habitat of *H. pylori* is mainly the upper digestive tract. By using appropriate techniques, *H. pylori* can be isolated in stools, saliva, and dental plaques, which suggests transmission by several routes, such as

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contaminated water or foods. Despite that it is not classified among foodborne organisms, its transmission may occur *via* stools contaminated from foods and water, as well as by oral–oral route or fecal–oral route.

Recent research has shown the presence of other helicobacter species in mammal animals (*Helicobacter felis, Helicobacter muridarum)* and in some species of birds (*Helicobacter pullorum*). Another specific helicobacter species is *H. mustelae* widespread in ferrets (*Mustela putorius*): These wild animals, known since Ancient Egypt, used for hunting rats and mice, have become popular as domestic pets: They can develop ulcerative gastritis due to *H. mustelae (*isolated from feces of ferrets, which support fecal–oral transmission of gastric *Helicobacter*). An interesting discovery resulting from an archeology research group has shown the presence of *H. pylori* in the stomach of *Homo sapiens* 58 000 years ago, before the large migrations of Africans to Asia and Europe; they observed that the genetic diversity of strains reduces as much as the people move far from East Africa.

In the western area, the level of carriage of *H. pylori* varies significantly with the countries. In developing countries higher levels of incidence have been determined, probably due to poor hygiene conditions, contaminated water and food, as well as promiscuous conditions of living. Contaminated children have been found in those countries, as mentioned in the Maastricht 2-2000 Consensus Report. In the US, the predominant contaminated population is that of elderly people **(** >60 years old), whereas the incidence is $<20\%$ in people below 40 years. This apparently contradictory statement can be explained by an early contamination of elderly people at the time when Helicobacter was unknown, or related to intestinal disorders or mucosal diminished immune defense to bacterial adhesion described in older population, even in developed countries.

Clinical Profile, Signs and Symptoms, and Associated Diseases

Helicobacter infection can be either clinically silent or symptomatic. Several symptoms associated with the presence of *H. pylori* can be nonspecific, varying with time. When inflammation of the gastric mucosa develops, gastric or abdominal pain, acidic reflux, regurgitation, vomiting, and nausea constitute the majority of visible symptoms. However, 70 or 80% of infections remain asymptomatic.

Besides the classical peptic ulcer or gastro-duodenal ulcer, several syndromes have been related to the infection with *H. pylori*, such as chronic gastritis and atrophic gastritis associated with achlorhydric syndrome. Several extragastric diseases have been attributed to *H. pylori*: mucosa-associated lymphoma (MALT lymphoma), coronaritis, gastro-oesophageal reflux disease, iron deficiency anemia, skin disease, and rheumatological manifestations. The association of chronic helicobacter infection with alterations in gastric mucosal cell proliferation is recognized worldwide as well as association between *H. pylori* infection with gastric adenocarcinoma and gastric lymphoma. The role of helicobacter in those associations remains uncertain and needs confirmation of its potential responsibility in gastric and extragastric diseases.

Inflammatory Factors

H. pylori infections are a source of inflammatory processes mediated by disulfide-bridged proteins: helicobacter cysteine-rich proteins (*Hcp*), particularly Hcpa (*hp0211*), triggers an immune response through the differentiation of monocytes into macrophages. They interfere with host cell functions, change the monocyte morphology, the expression of surface marker protein (CD11b), phagocytic activity, and cell adherence. Colonization of the stomach by *H. pylori* results in chronic inflammatory process of the stomach lining and the severity of the inflammation forms the basis for *H. pylori*-related diseases. Resulting from severe inflammation, acid and pepsine in the stomach lumen overwhelm the mechanisms of protection of stomach and duodenal mucosa, which results in duodenal and stomach ulcers. In people producing large amounts of acid, *H. pylori* colonizes the antrum of the stomach where *H. pylori* avoids the acid secreting parietal cells located in the main body of the stomach. The inflammatory response to the bacteria induces G cells in the antrum to secrete *gastrin*, and the latter product stimulates the parietal cells to secrete even more acid in the stomach lumen. The increased acid load can damage the duodenum with production of an ulcer. Chronic inflammation induced by bacteria is associated with further diminished acid production, atrophy of the stomach lining resulting in gastric ulcer, and the risk of gastric cancer.

Structure, Genome, and Pathogenesis

Some structural characteristics of helicobacter contribute to the pathogenicity of this organism. Like other Gram-negative bacteria, *H. pylori* possesses an outer membrane (OM) comprising phospholipids, a lipopolysaccharide (LPS), and the O antigen of LPS (toxin) may be fucosylated, mimicing Lewis blood group antigens found on the gastric epithelium. The OM also contains cholesterol, glucosides as found in few other bacteria. Genetic studies have shown genetic differences between strains. Approximately 29% of the loci in the pathogenetic strains have a 40 kb-long *Cag* pathogenicity island (*cagA* gene) that contains 40 genes: This pathogenicity island is not found in strains from asymptomatic carriers of *H. pylori*. *CagA* gene codes for one of the major virulence proteins and those strains having *CagA* gene are associated with the ability to cause ulcers.

To colonize the stomach, *H. pylori* avoids the acidic pH of the lumen by means of its flagella, burrows in the mucus to reach the stomach epithelial cell layer, and swims to the neutral environment of the epithelial cell surface. *H. pylori* can also be found on the inner surface of the gastric epithelial cells and produces adhesins, which permit attachment of the bacteria to epithelial cells. One important characteristic of *H. pylori* is its abundant production of the enzyme *urease,* which is present inside and outside the bacteria. The *urease* destroys *urea* normally secreted in the stomach: This results in carbon dioxide and ammonia, converted to ammonium neutralizing gastric acid: Therefore, the survival of *H. pylori* is dependent on its urease production. In addition, the ammonia produced is toxic to the epithelial cells, as well as other products of *H. pylori*, such as proteases, cytotoxin A (Vac A), and certain phospholipases.

The addition of the roles of urease production, ammonium, and toxic products are convergent factors in favor of persistence and growth of *H. pylori* in the gastric area.

H. pylori and Cancers

The *Cag pathogenicity island* (*cag* PAI) predominates in approximately 50–70% *H. pylori* strains in Western countries. Patients infected with those strains have a strong inflammatory response in the stomach with a higher risk of developing ulcers and gastric cancers. Type IV secretion system expressed by the *cag* PAI strains, 'injects' peptidoglycan, the inflammation-inducing agent, from their cell wall into the epithelial cells, followed by expression of cytokines (promoting inflammation). The same system disrupts cytoskeleton, adherence to adjacent cells, cell polarity, and once inside the cell, the *CagA* protein allosterically activates *phosphatase/protooncogene Shp2*. Pathogenic *H. pylori* can activate the Epidermal Growth Factor (EGFR), a membrane protein with a tyrosine kinase domain. Activation of EGFR by *H. pylori* is associated with altered functions of epithelial cells and their contribution to pathogenesis.

Among those complex mechanisms of pathogenesis, with a great diversity of *H. pylori* strains, two related mechanisms by which the organism may promote cancer are being studied: one mechanism involves increased production of free radicals near *H. pylori* with an increased rate of host cell mutation; the other mechanism called 'a perigenetic pathway' involves enhancement of transformed host cell phenotype by alterations in cell proteins (adhesins). *H. pylori* induces inflammation, local high levels of tumor necrosis factor-α, alteration of gastric epithelial cells, dispersion and mutation of cells (without additional mutations in tumor suppressor genes). Further studies will permit a better understanding of cancer development related to *H. pylori*.

Detection Methods

The detection and evaluation of the incidence of a disease such as *H. pylori* infection is necessary to develop measures for the control of a potential spread of the infection. In different populations the variability of the incidence is a function of hygiene and contamination conditions. Thus the control of the incidence requires development of means for detection of the organism and this needs to proceed to diagnostic tests, such as detection of antibodies, reliable to diagnose the infection in a series of patients. Serological tests can also control the efficacy of the treatment with antibodies persisting for months after a potential eradication of *H. pylori*. However, none of the methods are totally reliable. They must be chosen as a function of clinical profiles of patients and of availability of diagnostic tests.

The test with radioactive 13C- or 14C-labeled urea metabolized by the bacteria (resulting in labeled carbon dioxide detected in the patient's breath) requires hospitalized patients and cannot be used in large groups of populations for evaluation of the incidence and developing methods for control of transmission. Similarly, detecting the presence of *H. pylori* in a biopsy check during endoscopy, histology, and microbial culture should permit the control of treatment efficacy, but is only applicable in patients examined while in hospital. Few other tests encounter the same limits and do not permit a significant control of the patients' conditions and incidence in a given population.

Prevention and Control Measures

Prevention of contamination by *H. pylori* requires detection in potentially contaminated populations. As modes of transmission have been established and carriage of the bacteria in the upper gastrointestinal tract being silent in many cases, the contamination may occur by means of water or food in close contact with such contaminated population. The lack of hygiene contributes to such transfer (oral–oral or fecal–oral routes). Therefore detection is important for prevention, but suitable methods may not be available in developing countries with highly contaminated population. Rising antibiotic resistance does not permit empirical antibiotic treatments in those populations. Vaccine studies have been approached in mouse models with promising results. Different routes and adjuvants have been assayed to find the most appropriate mode of immunization. However, although attempts are promising in animals, trials in humans were only recently conducted. Prophylactic vaccines seem to be more appropriate than vaccines to cure the infection and to eradicate *H. pylori* or to reduce the bacterial density from gastric mucosa. New directions for immunization are studied with growing interest in the use of DNA or living vectors, but they are still at an early stage. An intramuscular vaccine against *H. pylori* infection is being investigated in Phase I clinical trials. Presently the most effective method of prevention remains education of public in food and water hygiene.

Omeprazole (or derivatives) combined with active antibiotics have proven therapeutic efficacy and decreasing incidence in developed countries.

Conclusion

Analyzing the knowledge acquired during recent years, the late discovery (1980) of *H. pylori* has resulted in a relatively limited knowledge of the organism: in terms of characteristics of the bacteria, growth, metabolism, chemical, and genetics, requirements for *in vitro* culture and *in vivo* behavior. The literature on *H. pylori* is limited and mainly devoted to pathogenicity and mode of clinical pathogenesis. There is still more research to be done in the years to come.

Omeprazole (or derivatives combined with active antibiotics have proven therapeutic efficacy and decreasing incidence in developed countries.

See also: Characteristics of Foodborne Hazard and Diseases: Pathogenesis and Virulence. Risk Analysis: Estimating the Burden of Foodborne Disease

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BACTERIA

Listeria monocytogenes

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Glossary

Biofilm An aggregation of microorganisms attached to a surface and embedded in an extra-cellular polymeric matrix.
Epidemic clone Group of individuals with a common ancestor that are evolved clonally.
Invasion Mechanism by which pathogenic microorganisms infect human host cells.
Outbreak A group of at least two cases of a single illness that are demonstrated by epidemiological investigation to have a single common exposure or source.
Water activity or a_w The amount of water that is available to microorganisms.

Background

Listeria monocytogenes is a foodborne pathogen that causes the disease known as listeriosis in humans and in several animal species. The official discovery of *Listeria* dates back to 1924, when Murray, Webb, and Swann isolated *L. monocytogenes* as the etiological agent of a septicemic disease affecting rabbits and guinea pigs in their laboratory at Cambridge in England. This strain was named *Bacterium monocytogenes*, as it was observed to infect monocytes of the blood. The following year, Pirie isolated an identical bacterium from the liver of several gerbils, also known as the African jumping mouse. He named it *Listerella hepatolytica*. Until 1940, there was considerable confusion in the nomenclature of *L. monocytogenes*. Pirie chose *Listerella* as the generic name in honor of Lord Lister, the well-known pioneer in the field of bacteriology. However, this name had already been applied to a group of slime molds (Mycetozoaire). The generic name *Bacterium* as applied by Murray and collaborators was undesirable because the organism does not possess the characteristics of this genus. The resolution of the Committee on Nomenclature, Third International Congress for Microbiology, New York, 1939, was that in all duplications of generic names, only the one first applied should be considered valid, invalidating the generic name proposed by Pirie, and thus he suggested the name *Listeria* in 1940. *Listeria* was adopted in the sixth edition of *Bergey's Manual of Determinative Bacteriology* and approved by the Judicial Commission on Bacteriological Nomenclature and Taxonomy, and it is now the official genus name.

Although the first cases of human listeriosis were reported in 1929 in Denmark, the oldest preserved laboratory culture of *L. monocytogenes* dates from 1921, isolated from a patient with meningitis by Dumont and Cotoni in France. For many years, clinical *Listeria* isolates were a laboratory rarity, and the epidemiology of the disease was an unresolved mystery. However, at the end of the 1970s and the start of the 1980s, the number of reports on *Listeria* isolations began to increase and, in 1983, the first outbreak in human directly linked to the consumption of *L. monocytogenes* contaminated foodstuffs (coleslaw salad) in Canada was reported. Subsequent investigations of a series of epidemic outbreaks in humans in North America and Europe clearly established *L. monocytogenes* as a foodborne pathogen.

Characteristics of *Listeria monocytogenes*

The Organism

Listeria monocytogenes is a member of the genus *Listeria*, a group of Gram-positive bacteria with low G + C content closely related to *Bacillus* and *Staphylococcus*. The genus *Listeria* includes seven other species: *Listeria ivanovii*, *Listeria welshimeri*, *Listeria innocua*, *Listeria seeligeri*, *Listeria grayi*, *Listeria marthii*, and *Listeria rocourtii*, the latter two having been described in 2009. Although *L. monocytogenes* is recognized as the causative agent of listeriosis in humans, rare cases of infection by *L. innocua*, *L. ivanovii*, and *L. seeligeri* have also been reported.

Listeria monocytogenes is catalase-positive, oxidase-negative, regular short rod with a diameter of approximately 0.5 μm and a length of 0.5–2.0 μm (Figure 1). They are facultative anaerobes that do not form a capsule, do not form spores, and are motile by peritrichous flagella when cultured at 20–25 °C and nonmotile at 37 °C. They have the capacity to hydrolyze aesculin and sodium hippurate but not urea, gelatin, or casein. Production of acid is observed when *L. monocytogenes* is cultured in media with L-rhamnose, but not D-mannitol or D-xylose.

Through examination of group-specific surface proteins, such as somatic (O) and flagellar (H) antigens, at least 13

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serotypes (i.e., 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e, and 7) have been recognized in *L. monocytogenes*.

Factors Affecting Survival and Growth

The organism's ability to grow and reproduce in harsh conditions makes it a foodborne pathogen of great concern. In common with other organisms, the environmental conditions like temperature, pH, water activity, and the atmospheric environment are the main factors affecting the growth of *L. monocytogenes* in foods (Table 1).

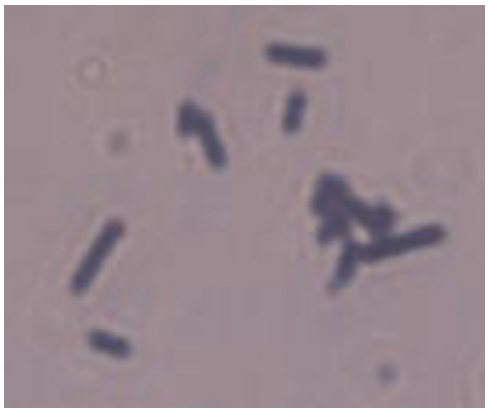

Figure 1 Gram staining of *Listeria monocytogenes*.

Temperature

Listeria monocytogenes can grow over the temperature range of −1.5 to 45 °C, with an optimum between 30 and 37 °C.

Listeria monocytogenes is not considered as a heat-resistant organism, being eliminated by conventional milk pasteurization. As with other organisms, heat resistance increases with decreasing water activity and after exposure to some sublethal treatments. A list of *D*-values reported for *L. monocytogenes* in the scientific literature is presented in Table 2. It is important to note that these values may vary according to the experimental methodology and the strains tested. Significant differences in the resistance of different strains of *L. monocytogenes* to different stress conditions have been observed.

Listeria monocytogenes has been isolated from several frozen foods, including dairy products, meats, vegetables, and fish products. Cell numbers of *L. monocytogenes* Scott A inoculated into ice cream did not alter during storage at −18 °C for 5 months.

Table 1 Reported generation times for growth of *Listeria monocytogenes* under different conditions

Temperature (°C)	*pH*	*Media*	*Generation time (h)*
−1.5	NR	Vacuum-packaged sliced roast beef	100
0.0	6.6	UHT milk	77
2.5	6.6	UHT milk	24
5	NR	Vacuum packed ham	33.1
5	6.41	Anthotyros cheese	20.2
10	NR	Shredded lettuce (chlorine treated and modified atmosphere storage)	10.8
10	NR	Melon pulp	7.12
10	4.9	Papaya	15.1
10	NR	Vacuum-packed ham	13.4
20	NR	Melon pulp	1.74
20	4.9	Papaya	6.4
30	NR	Melon pulp	0.84
30	4.9	Papaya	1.2

NR, not reported.

Table 2 Reported *D*-values for *Listeria monocytogenes* under different conditions

Temperature (°C)	*pH*	*Media*	*Water activity*	D-*value (min)*
60	5.5	Beef	NR	3.8
61	NR	Surface-pasteurized turkey bologna	NR	2.1
63.3	NR	Liquid egg yolk	0.989	0.20
63.3	NR	Liquid egg yolk plus 10% w/v sucrose	0.978	0.72
63.3	NR	Liquid egg yolk plus 10% w/v NaCl	0.865	11.5
66.1	NR	Raw milk	NR	0.25
70	NR	Beef	NR	0.13
71.1	NR	Raw milk	NR	2.7×10^{-2} (1.62 s)
71.7	NR	Sterile whole bovine milk	NR	5.0×10^{-2} (3.0 s)
71.7 (prior heat shock at 48 °C 15 min)	NR	Sterile whole bovine milk	NR	7.7×10^{-2} (4.62 s)

NR, not reported.

pH

Listeria monocytogenes grows over a wide range of pH values (4.3 to 9.6) with an optimum between 6 and 8. These values, however, may vary with the acidulant used and temperature.

When inoculated into orange serum or cut strawberries (pH 3.6–3.8), log reductions of 0.5–1 cfu g^{-1} were observed during storage for 7 days at 4 °C. However, 4 to 5 log reductions were observed from 2 to 5 days when orange serum was incubated at 30 °C. Similarly, when inoculated into apple juice at pH 3.42 (initial cell concentration 10^6 cfu ml^{-1}), no bacteria were recovered after 48 h of incubation at 26 °C.

Prior adaptation of *L. monocytogenes* to mildly acidic conditions has been demonstrated to enhance the survival of the pathogen in low-pH foods. As an example, when inoculated into Crescenza cheese (pH 5.0–5.6), cells previously exposed to pH 3.5 increased by 3 log cycles, whereas nonadapted cells decreased 0.8 log cycles after 14 days storage at 4 °C.

Water Activity

In optimum conditions of pH and temperature, the minimum water activity (a_w) value that allows growth is 0.90. However, it has been recovered from the sludge in cheese brines (at salt saturation, ca. 26% w/w, a_w 0.75, and cool ambient temperature of 14–16 °C) over a long period of time, showing considerable resistance to very low water activities. In a saturated salt solution in (TSB) at 8 °C, survival was 50% after 1 year, but at 22 °C survival was ca. 45% after only 4 months. In dry-cured fermented salami with water activities from 0.79 to 0.86, *L. monocytogenes* survived for 12 weeks at 5 °C.

Atmosphere

Growth of *L. monocytogenes* occurs in aerobic conditions, but is improved by anaerobic conditions and the bacteria can tolerate up to ca. 20% CO_2; however, CO_2 concentrations approximately above 50% are considered inhibitory.

Irradiation

Although there is limited information available, it is generally assumed that *L. monocytogenes* exhibits a resistance to gamma and UV irradiation similar to and lower than, respectively, other Gram-positive bacteria, and is more resistant to irradiation than Gram-negative bacteria. As with other organisms, irradiation in air is more lethal than in vacuum or modified atmospheres (low O_2 tension). A 6 to 7 log reduction can be achieved by 2–4 kGy.

Listeria monocytogenes in Food-Processing Environments

Listeria monocytogenes is generally found widely distributed in natural environments and has been isolated from soil, decaying vegetation, stream water, sewage, urban environments, and human and animal feces. This common environmental distribution and its unusual ability to adapt and survive under extreme conditions, when compared to other pathogens, make *L. monocytogenes* a considerable challenge in terms of eradication and control in the food-processing environment, and a great concern for the food industry. Although methods of basic quality assurance have been developed and implemented to control *L. monocytogenes* contamination throughout the food processing stages, several studies indicate that the most important source of food product contamination is cross-contamination from equipment and the general environment after the foods have been processed. Moreover, it has also been shown that *L. monocytogenes* can persist – a specific subtype of a strain is repeatedly isolated over an extended period of time – in food processing plants. A number of studies support the observation that persistent strains can often be isolated from the environment of food-processing plants (e.g., drains, equipment, etc.), and particularly from the sites close to food-contact surfaces (e.g., slicing machines), rather than raw materials. The finding that persistent strains are often recovered from the environment and equipment after cleaning and sanitizing, emphasizes the risk of growth and establishment of *L. monocytogenes*, particularly in sites difficult to access leading to ongoing food product contamination. The reasons why certain *L. monocytogenes* strains are able to persist is not well understood. Some authors consider that these strains are better adapted to the processing environment and possess characteristics that influence survival against stresses in the processing environment, such as resistance to sanitizers and improved ability to adhere to food-contact surfaces to form biofilms. Although it has been demonstrated that *L. monocytogenes* is capable of rapid attachment to different food-processing surfaces like buna-N rubber, cast iron, stainless steel, nylon, Teflon®, polyester floor sealant, and glass, few studies have provided strong evidence of the ability of this pathogen to actually form biofilms. Therefore, there is still some controversy as to whether or not *L. monocytogenes* forms a classic biofilm, or if the more common methods used to quantify biofilm formation (e.g., enumeration or crystal violet staining of adhered cells) merely reflects the cell adsorption to a surface (a reversible state, as the bacteria can be removed from the surface by moderate washing), rather than a true biofilm (adherence of planktonic cells followed by production of extracellular polysaccharides and resistance to cleaning and sanitizing).

Listeria monocytogenes Control in the Food-Processing Environment

Sanitizers

No unusual resistance to biocides has been reported for *L. monocytogenes* and, in general, it is considered less resistant than *Pseudomonas* spp. The efficacy of biocides, however, decreases when they are tested against attached cells. Previous work on the evaluation of eight commercial disinfectants demonstrated that aldehyde and acid/peroxide-based products were ineffective at the recommended concentrations. Only three products, those containing an amine, quaternary ammonium compound, and peroxide and chlorine-based products caused 5 log reductions in *L. monocytogenes* cell numbers. Acquired resistance to biocides in *L. monocytogenes* has been considered a very rare event, but during the last few years, resistance to different agents has been increasingly documented for this species.

Antilisterial Bacteriophages

In October 2006, the FDA proclaimed the 'generally regarded as safe' (GRAS) status for LISTEX™ P100 (MICREOS Food Safety, the Netherlands), a commercial preparation of antilisterial phages, against *L. monocytogenes* in cheese. The extension to all products susceptible to *Listeria* opens the door for the meat and fish industry to apply LISTEX™. Recently, the Dutch designated inspection office SKAL confirmed the 'organic' status of LISTEX™ under the EU law, as a result of which it can be used in the EU in regular and organic products. Natural bacteriophages may prove to be a unique solution, where increased safety does not come at the expense of product characteristics. US food processors can now benefit from LISTEX™, like their European counterparts.

Natural Antimicrobials, Bacteriocins

Bacteriocins are simply defined as biologically active protein moieties with an antibacterial mode of action. The family includes a diversity of proteins in terms of size, microbial targets, modes of action, and immunity mechanisms. They differ from traditional antibiotics in one critical way: they are ribosomally synthesized rather than from secondary metabolites, they have a relatively narrow spectrum of activity, are only toxic to bacteria closely related to the producing strain, and the genes responsible for production and immunity are generally found clustered in operons. They have been found in all major lineages of bacteria, but are most generally found and studied in lactic acid bacteria (LAB). These bacteriocins can serve as natural food preservatives and processing aids that are GRAS.

To date, the only commercially produced bacteriocins are nisin, produced by *Lactococcus lactis*, (marketed as Nisaplin™), pediocin PA-1, produced by *Pediococcus acidilactici*, (Danisco, Copenhagen, Denmark), and ALTA™ 2431 (Kerry Bioscience, Ireland). Nisin is used in more than 48 countries and has the Food and Drug Administration approval. Nisaplin™ is sold as a natural food protectant and has been shown to be effective in a number of food systems, inhibiting the growth of a wide range of Gram-positive bacteria, including *L. monocytogenes*. The possibility of including live bacteriocin-producing LAB in foods has been a promising area: protective culture concept. Bioprotective cultures may act as starter cultures in the food fermentation process, such as dry sausage manufacturing process, or they may protect foods without any detrimental organoleptic changes. This system of incorporating a bacteriocin-producing culture into a food provides it with its own built-in biopreservation, thereby returning to a more natural method of shelf-life extension and improving the safety of food. This concept has been studied in cold-smoked fish and in some artisanal meat sausages.

Listeriosis: Epidemiology and Clinical Features

Listeriosis had generally been thought of as a veterinary disease until the 1980s when the rise in the numbers of human cases of listeriosis in several countries, together with evidence for foodborne transmission, renewed interest in this disease. Listeriosis is an atypical foodborne illness of major public health concern because of its severity, the high case-fatality rate, the long incubation period, and the predilection for individuals who have an underlying condition which leads to impairment of T-cell-mediated immunity. Most cases of human listeriosis are foodborne; however, the disease process is complex with multiple routes of infection.

Clinical Manifestations

Listeriosis in humans caused by the consumption of contaminated food can occur in two forms: the more severe, invasive systemic disease, or a febrile gastroenteritis (Table 3). The establishment of the disease depends on three major variables: the number of bacteria ingested with food, the pathogenic potential of the strain, and the immunological status of the host. For example, only 4 of the 13 serotypes (i.e., 1/2a, 1/2b, 1/2c, and 4b) have been involved in the majority of human listeriosis cases reported.

Listeria monocytogenes has the ability to cross the intestinal, blood–brain, and fetoplacental barriers; septicemia, central nervous system infections, miscarriages, and stillbirths are the most common forms of the invasive infection.

Several outbreaks of listeriosis associated with consumption of foods with high levels of *L. monocytogenes*, in which the symptoms are confused with those of other foodborne gastroenteritis, have been reported.

Listerial dermatitis, appearing as papular or pustular lesions on the arms or hands of veterinarians or other people, can occasionally occur as an occupational hazard from infected animals.

Mechanism of Infection

Host cell infection begins with the internalization of the bacteria either by phagocytosis in the case of macrophages or induced phagocytosis (invasion) in the case of normal nonphagocytic cells. *Listeria monocytogenes* displays a large variety of surface proteins, known as internalins, that mediate bacterial invasion into human cells. The surface protein internalin A (InlA) was identified as the main bacterial factor involved in the invasion of polarized cells. The cellular receptor for InlA in epithelial cells is E-cadherin, present in several human barriers. It has been demonstrated that the E-cadherin/InlA interaction is critical for the bacteria to cross barriers such as the intestinal, the feto-placental, or the blood–brain barrier. The protein InlB is also critical for *L. monocytogenes* invasion, exhibiting, however, a broader range of target cells than InlA. The main signaling receptor for InlB is the hepatocyte growth factor (HGF) receptor Met, a ubiquitous tyrosine kinase receptor involved in the development of organs such as the liver or placenta. Escape of the pathogen from the vacuole within infected cells requires the expression of listeriolysin O (LLO), a pore-forming toxin which induces the lysis of this compartment, which in some cells can function synergistically with, or be replaced by a phosphatidylinositol-specic phospholipase C (PI-PLC). Once the phagosome is broken, the bacteria are freed into the cytoplasm. Intracellular movement requires expression of the bacterial surface protein ActA, required for the polymerization of actin-enriched structures that

Table 3 Clinical manifestations, diagnosis, and treatment of foodborne listeriosis

Types of listeriosis	*Sources*	*Incubation period for the disease*	*Clinical manifestations*	*Diagnosis*	*Treatment*
Severe gastroenteritis (generally occurs in healthy individuals)	Consumption of foods contaminated with high levels of *L. monocytogenes*	A few hours to 2 days	Fever, vomiting, and diarrhea, sometimes progressing to septicemia	Isolation of *L. monocytogenes* from blood (severe cases) Isolation of *L. monocytogenes* from suspected foods Isolation of *L. monocytogenes* from feces is of little relevance	Ampicillin or ampicillin in combination with aminoglyco-side or cotrimoxazole
Adult infections (more common in elderly and immunocompromised)	Consumption of foods contaminated with *L. monocytogenes*	A few days to several months	Asymptomatic or flu-like symptoms, may progress to meningitis, encephalitis, or septicemia	Isolation of *L. monocytogenes* from blood, CSF, or affected organs; observation of Gram-positive bacilli in the CSF	
Infection during pregnancy (more frequent in the third trimester of pregnancy)	Consumption of foods contaminated with *L. monocytogenes*	A few days to several months	Asymptomatic or flu-like symptoms associated or not, with gastrointestinal disorders, uterine infection, miscarriage, stillbirth, or neonate infection	Isolation of *L. monocytogenes* from blood, amniotic fluid, or vaginal fluids	
Neonatal infection	Transplacental dissemination	A few hours after birth to 48 h (early-onset syndrome)	Early neonatal septicemia (similar to infection caused by group B *streptococci*)	Isolation of *L. monocytogenes* from blood, CSF, umbilical cord, gastric exudates, or affected organs; observation of Gram-positive bacilli in the CSF	
	Contamination during delivery	1–2 weeks (late-onset syndrome)	Meningitis, encephalitis, and septicemia (closely associated with prematurity)	Isolation of *L. monocytogenes* from blood, CSF, or affected organs; observation of Gram-positive bacilli in the CSF	
	Hospital-acquired infection (by contact with infected newborns or with contaminated postnatal environment)				

CSF, cerebrospinal fluid.

enable *L. monocytogenes* to move from one infected cell to adjacent cells, without being exposed to the extracellular environment. Lysis of the second vacuole is performed by a lecithinase (PC-PLC) and LLO.

Six of the crucial virulence genes, involved in the different steps of *L. monocytogenes* infectious cycle, are clustered on a pathogenicity island known as 'positive regulatory factor A (PrfA)'-dependent virulence gene cluster. These genes are: *hly* (encoding LLO); *plcA* (encoding PI-PLC); *actA* (encoding ActA); *plcB* (encoding PLCB); *mpl* (encoding a metalloprotease involved in the maturation of pro-PlcB); and *prfA* (encoding, PrfA) that activates the transcription of all genes located in this pathogenicity island, including its own. The internalins InlA and InlB are encoded by the *inlAB* operon located in another gene cluster. PrfA is also involved in the regulation of the expression of other genes involved in *L. monocytogenes* virulence that are dispersed on the chromosome, including the internalin locus *inlAB*. Mutants lacking a functional PrfA protein are avirulent and present significantly reduced transcript levels of the virulence genes.

Listeria monocytogenes is well adapted to survive both as a saprophyte, in soil and decaying organic matter, and as a pathogen inside eukaryotic host cells. A variety of physicochemical signals such as temperature, pH, salt, carbon sources, and several stress conditions seem to play a crucial role in the expression of the *L. monocytogenes* virulence genes and in the efficient transition between extracellular and intracellular lifestyles. Outside host cells, *L. monocytogenes* represses the synthesis of PrfA, considered the master regulator of the virulence genes, resulting in the reduction of virulence gene expression. Following ingestion by a mammalian host, the increased temperature and reduced pH of the stomach results in increased production of stress-response proteins, internalins, and PrfA, initiating the transition to virulence. The alternative sigma factor σ^B, encoded by *sigB*, also contributes to the regulation of PrfA expression as a response to several types of stresses such as low pH (~pH 2) of the stomach, high osmolarity and activity of bile in the upper intestine, and thereby *L. monocytogenes* virulence gene transcription. After surviving the acidity of stomach, the bacteria are able to cross the intestinal barrier and are thought to disseminate from the mesenteric lymph nodes to the spleen and the liver.

Epidemiology

Statistical Data on Prevalence and Incidence of the Disease

Listeriosis is reported mainly in the industrialized countries, and data from Africa, Asia, and South America are scarce. The absence of diagnostic and surveillance systems or testing facilities, the high incidence of other pathogens and pathologies, different consumption patterns and dietary habits, or different host susceptibilities are the possible reasons for the lack of data in these continents.

In Europe, the incidence of listeriosis in 2008 was three cases per million inhabitants. A decrease was observed in 2007 and 2008. However, in recent years, an increase in the rate of listeriosis has been reported in countries such as Estonia, Germany, Ireland, Italy, Latvia, Lithuania, Netherlands, Poland, Spain, Sweden, and the UK.

In Canada and in the USA the reported incidence is approximately four and three cases per million inhabitants per year, respectively.

Although listeriosis can cause disease in all populations, the majority of cases (more than 50%) occur in those aged 65 years and more, but most elderly patients often have an underlying immunocompromising condition.

Reservoir of Pathogens, Vehicle of Transmission, Examples of Food Incriminated and Data on Prevalence of Contamination or Distribution in Nature

Listeriae are ubiquitous microorganisms that have been isolated from a diversity of sources, including soil, water, effluents, a large variety of foods, and human or animal feces. They live as saprophytes in decomposing plant matter, which appears to be their natural habitat. However, the prevalence of *L. monocytogenes* in urban sites is higher than in natural sites. It has been isolated in low numbers from a variety of environmental samples associated with the primary production of food.

A wide range of food types has been associated with the transmission of listeriosis, namely milk and dairy products, meat products (cooked chicken, sausages, paté and rilletes, pork tongue, hot dogs, ham, salami, chicken wraps, and deli turkey breast), fish products (shrimp, smoked fish, smoked trout mousse, shellfish, and smoked mussels), vegetables (coleslaw salad, alfalfa, rice salads, and celery), and other products like sandwiches. Generally, these foods have an extended shelf life, do not require further cooking at home, and can support the growth of high levels of *L. monocytogenes*.

The prevalence of *L. monocytogenes* in foods has been evaluated by many researchers and food authorities or food inspection agencies. Each year, The European Food Safety Authority publishes a report with the data collected in each country.

Causes of the Outbreaks/Incidences

Food products were not considered as a vehicle of listeriosis until the outbreak which occurred in Canada in 1981 was investigated, and provided evidence to link ingestion of food contaminated with *L. monocytogenes* with listeriosis. Since then, severe outbreaks have been reported worldwide (Table 4), although the investigation of invasive listeriosis outbreaks is complex due to the long incubation period (5–70 days). This makes it very difficult to obtain accurate food histories from case-patients and also because listeriosis primarily affects persons with an immunocompromised status.

Specific Factors of Risk

Listeriosis is a rare disease among the general population with a reported incidence much lower than many other foodborne diseases. Certain groups within the general population which have an underlying condition which leads to impairment of T-cell-mediated immunity are particularly susceptible to infection, namely immunocompromised persons by medication

Table 4 Food products implicated in invasive listeriosis outbreaks occurring worldwide between 1981 and 2009; number of cases and deaths reported and serotype involved

Year	*Location*	*Total number of cases (deaths)*	*Maternal cases*	*Food implicated (suspect)*	*Serotype/EC*
1981	Nova Scotia, Canada	41 (18)	34	Coleslaw	4b, ECI
1983	Massachusetts, USA	49 (14)	7	Pasteurized milk	4b, ECIV
1985	California, USA	142 (48)	94	Mexican-style cheese	4b, ECI
1983–1987	Switzerland	122 (34)	65	Vacherin Mont d'Or cheese	4b, ECI
1987–1989	UK	>366 (>90)	NR	Pâté	4b, ECIV
1989–1990	Denmark	26	3	Blue mold cheese	4b, ECI
1992	New Zealand	4 (2)	NR	Smoked mussels	1/2a & 4
1992	France	279 (85)	0	Pork tongue in jelly	4b, ECI
1993	France	38	31	Rillettes	4b
1998–1999	Multiple states, USA	101 (21)	NR	Hot dogs	4b, ECII
1999	Finland	25 (6)	0	Butter	3a
1999–2000	France	10	3	Rillettes	4b
1999–2000	France	32 (10)	9	Pork tongue in aspic	4b
2000	Multiple states, USA	30 (7)	8	Delicatessen turkey RTE meats	1/2a, ECIII
2000	North Carolina, USA	13 (5)	11	Home-made Mexican-style Cheese	4b
2002	Multiple states, USA	54 (8)	12	Delicatessen turkey RTE meats	4b, ECII
2002	Quebec, Canada	17 (0)	3	Cheese made from raw milk	
2003	UK	14 (NR)		Butter	4b
2003	Texas, USA	12 (NR)	NR	Mexican-style cheese	4b
2005	Switzerland	10 (3)	2	Tomme cheese	1/2a
2006	Czech Republic	78 (13)		Soft cheese	1/2b
2008	Chile	119 (5)		Cheese	
2008	Canada	23 (1)		Soft cheese	
2008	Multiple Provinces, Canada	57 (23)		Meat products: Maple Leaf Foods	1/2a
2009	Chile	3 (3)	2	Meat products?	
2009–2010	Germany, Austria	12/(7)		Acid curd cheese	
2009–2010	Portugal	19(6)	2	Unknown	4b
2010	USA	7 (4)		Celery	

NR, not reported.

or disease (e.g., organ transplant or cancer patients), HIV-infected individuals, pregnant women, fetuses/newborn babies, and the elderly. In these higher risk groups, associated mortality rate can reach 40%. Within these susceptible populations, bone marrow transplant patients, those with blood-borne cancers, and individuals with full-blown AIDS are at the highest risk of severe listeriosis resulting in a fatal outcome. However, cases in individuals not showing any apparent predisposition have been reported.

Demographic changes and medical advances have resulted in an increase in the size of certain at-risk groups, namely the elderly and immunocompromised patients; therefore, an increase in listeriosis in older patients is likely to occur. In fact, the majority of the cases are already being reported worldwide in those aged 65 years and more.

Analytical Methods for Organism and Toxins

Detection, Isolation, and Enumeration of *Listeria monocytogenes*

The analysis of *L. monocytogenes* is performed to determine compliance of the food industry with standards and guidelines, to assess the safety of foods, or to support foodborne disease investigation.

There are several established culture-based methods for the detection of *L. monocytogenes*, all of them requiring an initial step to increase the viable cell count to detectable numbers. This is generally achieved using a selective enrichment in liquid medium, which usually takes from 24 to 72 h. Enrichment media include University of Vermont I broth (UVM I), buffered Listeria enrichment broth (BLEB), Fraser broth, and 1/2 Fraser broth, in which the inhibitory components acriflavine and nalidixic acid are used in similar concentrations; Fraser broth also has lithium chloride as a selective agent. Enrichment steps are followed by plating onto agars containing selective/differential agents. Oxford agar (OXA) was the medium of choice of the International Organization for Standardization (ISO) until 1998, when it was replaced by PALCAM agar (**Figure 2**). In 2004, it was replaced by a chromogenic agar, ALOA, (agar Listeria acc. to Ottaviani & Agosti), which allows the differentiation of *L. monocytogenes* and *L. innocua* (**Figure 3**). The detection in the first two plating media is based on the hydrolysis of aesculin which does not differentiate between species. In ALOA, the detection is based on the phosphatidylinositol phospholipase C enzyme (PI-PLC) activity, present in *L. monocytogenes* and in *L. ivanovii*, and β-glucosidase activity detected by a chromogenic substrate. The confirmation of suspected colonies can be performed by testing a limited number of biochemical markers such as hemolytic activity (CAMP test, **Figure 4**) and sugar fermentation patterns.

Figure 2 Growth of *Listeria monocytogenes* in PALCAM agar.

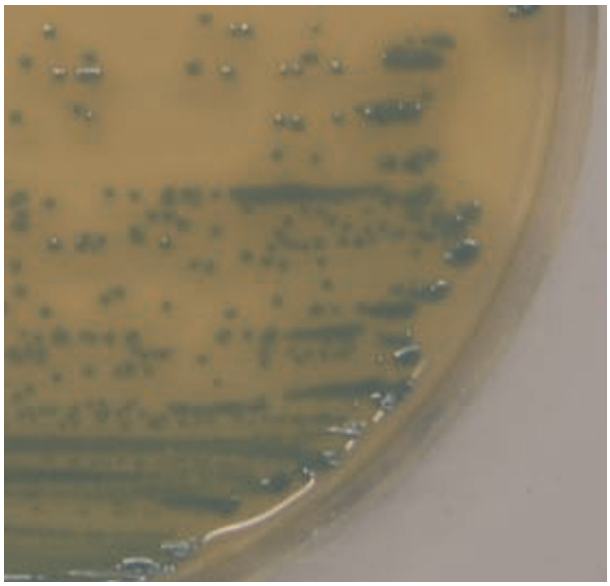

Figure 3 Growth of *Listeria monocytogenes* in ALOA plates.

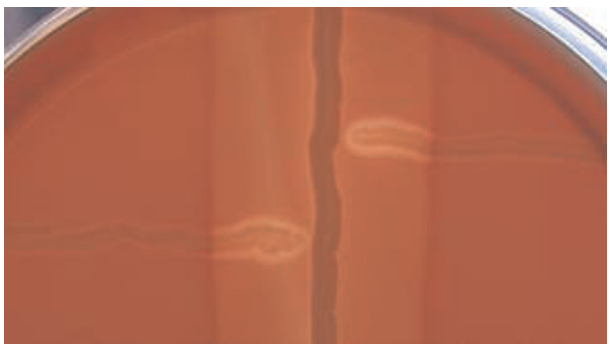

Figure 4 CAMP test for *Listeria monocytogenes*.

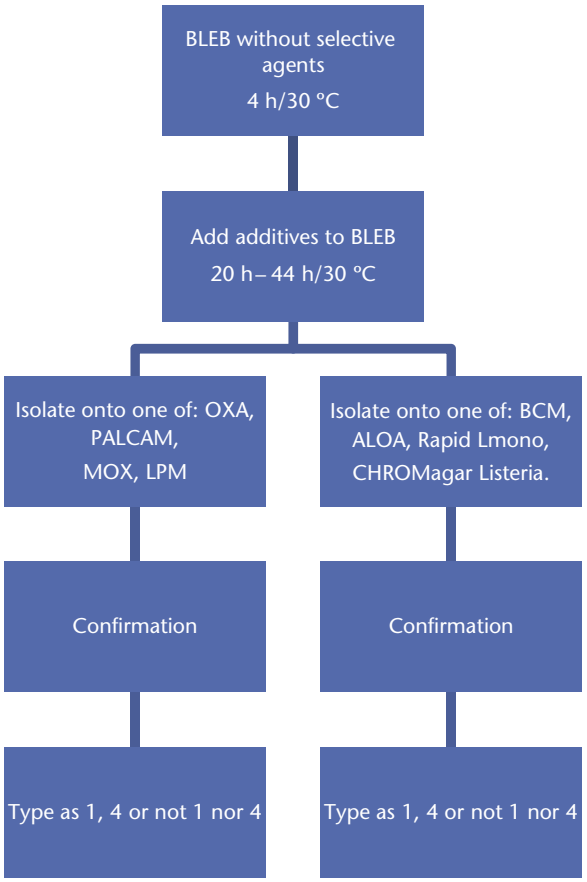

Figure 5 Schematic representation of FDA BAM method for the detection of *Listeria monocytogenes* in foods.

The FDA Bateriological Analytical Manual (BAM) method, as shown in Figure 5, uses one enrichment medium (BLEB) which has a first step of incubation of 4 h without selective agents, followed by an incubation of 20–44 h in the complete medium at 30 °C. After this step, subculturing by streaking is performed on selective solid media. There is a recommendation to use a second medium to differentiate species. After biochemical confirmation, the method requires serological subtyping (using commercial antisera) in order to characterize isolates as type 1, 4, or neither type 1 nor 4 (types 3, 5, 6, etc.).

The ISO method is summarized in Figure 6. The methodology includes two enrichment steps in 1/2 Fraser and in Fraser broth, and the use of two solid media for isolation of *L. monocytogenes*, ALOA and the second solid medium are chosen by the laboratory. Some alternative methodologies based on the ISO method are available in the market, such as the VIDAS LMO II (bioMérieux, France) or 'short protocols' AFNOR validated (Figure 7).

Enzyme-linked fluorescent assay (ELFA) is the basis of the method used by the automated VIDAS for the detection of antigens of *L. monocytogenes*. Results are obtained in 48 h if negative. Otherwise, further confirmation has to be performed following the reference method or through another alternative method.

More recently, a set of kits has been launched based on real-time PCR methods, such as the iQ-Check *L. monocytogenes*

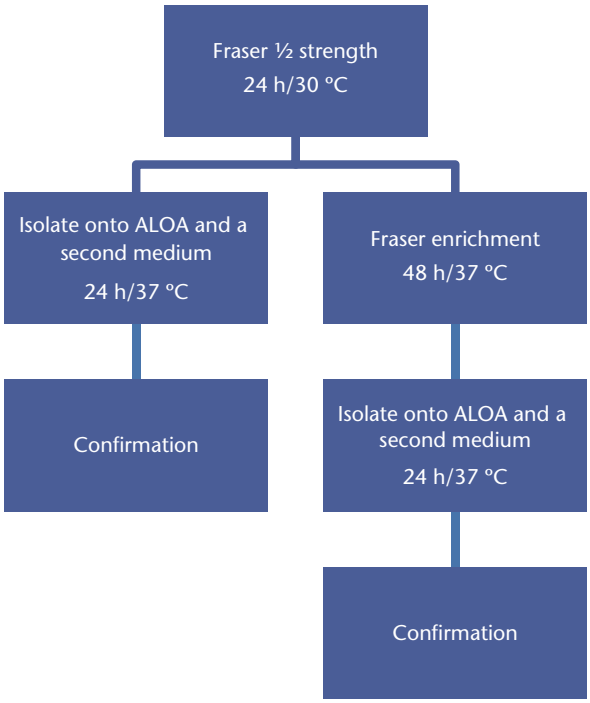

Figure 6 Schematic representation of ISO 11290-1:1996, amended. 1:2004 method for the detection of *Listeria monocytogenes* in foods.

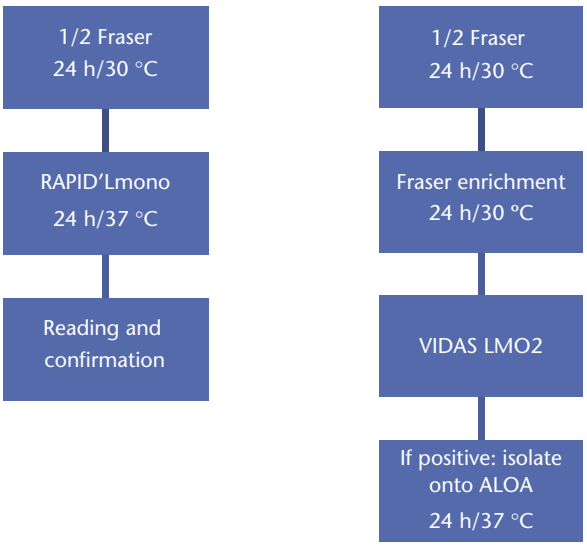

Figure 7 Schematic representation of protocols AFNOR validated using Rapid'Lmono (left) and VIDAS bioMérieux (right) for the detection of *Listeria monocytogenes*.

from Bio-Rad which has AFNOR validation according to ISO 16140 (Figure 8). After the enrichment steps in 1/2 Fraser or in Listeria special broth (LSB) for 24 h at 30 °C, lysis of bacteria and DNA extraction are performed using reagents included in the kit and finally, a PCR is run. Results are obtained in 24 h. According to AFNOR validation, positive results

Figure 8 Schematic representation for the detection method of *Listeria monocytogenes* by real-time PCR.

should be confirmed by the reference method or using another alternative method.

The most probable number (MPN) is a procedure to estimate the microbial concentration in a test sample; it is particularly useful when low numbers of organisms are present. Generally, three 10-fold serial dilutions are used in either a three- or five-tube MPN series. Based on positive results achieved, an MPN table is used to calculate the concentration of bacteria in the original sample.

Direct counts of *L. monocytogenes* can be performed following the reference method described in ISO 11290-2, Amd. 2004. It should be noted that due to the low pH and high salt concentration in cheeses, some cells may be in an injured state and therefore unable to grow on selective media such as PALCAM, which was the major plating media used until 2004. This direct method is also characterized by a detection limit of 10–100 cfu g^{-1} and, therefore, is not well adapted to the enumeration of low levels of *L. monocytogenes* in foods.

Subtyping of *Listeria monocytogenes*

Several methods of subtyping of isolates of *L. monocytogenes* have been used over the past decades. These include serotyping, antimicrobial resistance testing (e.g., arsenic, cadmium, and tetracycline resistance), bacteriocin typing, phage-typing, multilocus enzyme electrophoresis (MEE), and more recently, several molecular, genetic methods such as amplified fragment length polymorphisms (AFLPs), random amplification of polymorphic DNA (RAPD), and ribotyping, but the current and internationally accepted method is pulsed-field gel electrophoresis (PFGE), although gene sequencing techniques (multilocus sequence typing (MLST) and multiple-locus variable-number tandem repeat analysis (MLVA)) are being developed as a supplement to PFGE.

Through characterization by phylogenetic and population genetic studies, *L. monocytogenes* isolates can be grouped into major evolutionary lineages, namely, lineage I (that includes isolates belonging to serotypes 1/2b, 3b, 4b, 4d, and 4e), lineage II (that includes isolates belonging to serotypes 1/2a, 1/2c, 3a, and 3c), and lineage III that subdivides into lineages IIIA, IIIB, and IIIC (all of which contain serotype 4a, 4c, and atypical serotype 4b isolates). Phylogenetic studies have

demonstrated that isolates classified as subgroup IIIB represent a distinct fourth lineage of *L. monocytogenes.* Although isolates from all four lineages have been associated with human listeriosis, lineage I isolates are significantly overrepresented among human listeriosis cases, in particular, those of serotype 4b. Lineage II strains have a higher prevalence among isolates from environmental samples and foods, and lineage III strains are rare and mainly associated with listeriosis in animals.

Subtype characterization of *L. monocytogenes* isolates from listeriosis outbreaks that have occurred over the past three decades has suggested that many outbreaks have been caused by a small number of *L. monocytogenes* epidemic clones (ECs), i.e., a group of closely related individuals with a common ancestor. Four *L. monocytogenes* ECs have been reported to date, designated as ECI, ECII, ECIII, and ECIa (ECIa has also been referred to as ECIV) (Table 4).

Level of Characterization Needed to Link Human Cases to the Implicated Food or to Each Other

Typing of bacterial isolates, that is the generation of patterns of parameters which can contribute to the differentiation between closely related taxa, reveals the relationship between them. Typing can be used for a number of purposes, including the determination of the primary sources of contamination and to link people who are ill following the consumption of contaminated foods, to the sources of bacterial contamination. Also, the differentiation beyond the species level provides a tool to better understand the ecology of *L. monocytogenes* strains and subtypes, including differences in their ability to cause human foodborne disease. The characterization of bacterial isolates can be based on their phenotypic traits, such as metabolic reaction profiles, antibiotic susceptibility profiles, and serotypes, or by the nature of the DNA of the genome, by using molecular typing techniques.

Serotyping

Serotyping is most often an early step in a typing scheme and, although not being highly discriminatory, it can be useful in preliminary investigations. Under the sponsorship of the World Health Organization, serotyping was one of the first typing methods to be studied, evaluated, and standardized. Although the methodology is easy and simple to perform, problems with serovars that are neither 1/2a nor 4b were found and related to the difficulty in preparing good-quality antisera.

A commercial serotyping kit: *Listeria* antiserum Seiken kit (Denka Seiken Co., Tokyo, Japan) is available in the market and widely used instead of antisera produced by individual laboratories, which are used in only a small number of reference laboratories. Palumbo *et al.* (2003) developed an enzyme-linked immunosorbent assay (ELISA) to be used in conjunction with the Denka Seiken kit that made the process more efficient.

More recently, a multiplex PCR was developed allowing the molecular segregation of isolates into four groups: IIa, which includes serotypes 1/2a and 3a; IIb, which includes serotypes 1/2b, 3b, and 7; IIc, which includes serotypes 1/2c and 3c; and IVb which includes serotypes 4b, 4d, and 4e.

Pulsed Field Gel Electrophoresis Typing

PFGE is a method that has been successfully used in typing *L. monocytogenes.* Restriction enzymes cut genomic DNA infrequently producing simple profiles that could be easily compared. PFGE is considered the standard subtyping method to detect listeriosis outbreaks.

PFGE, which was used for the first time in 1991, uses a standardized DNA macrorestriction analysis by PFGE for *L. monocytogenes* using restriction enzymes *Asc*I and *Apa*I, and the establishment of a universal size standard strain for use with the standardized PFGE protocols. With the aid of computerized gel scanning and analysis, software data banks of PFGE patterns have been created enabling the comparison of strains and identifying its phylogenetic relationship with other strains.

In the USA, PulseNet is the national molecular surveillance network for foodborne infections (*Escherichia coli* O157:H7, nontyphoidal *Salmonella* serotypes, *L. monocytogenes,* and *Shigella*) and was established in 1996. The establishment of international surveillance networks, like PulseNet, in Canada, Europe, the Asia Pacific region, and Latin America, would be crucial for early warnings on foodborne disease outbreaks.

One of the disadvantages of PFGE is the time needed to complete the analysis (3–4 days). To analyze large numbers of samples, the use of a more rapid typing method, although less discriminatory, is of practical importance.

Ribotyping

This technique also uses infrequently cutting restriction enzymes, but targets ribosomal DNA. Ribosomal genes are relatively conserved across the bacteria. This allows tracing of lineages through the appearance of mutations over time. For ribosomal typing of *L. monocytogenes*, the restriction enzyme *Eco*RI is used and the DNA fragments are separated by electrophoresis. The bands are then probed with DNA probes that specifically detect those DNA fragments that code for ribosomal RNA (rRNA). A fully automated ribotyping system (Riboprinter®, Dupont Qualicon) has enabled large-scale typing of isolates with less effort and expertise than PFGE. The technique can also be used with other restriction enzymes, improving the discrimination of strains.

DNA Sequencing-Based Molecular Subtyping Methods

In recent years, DNA sequencing-based molecular subtyping methods for *L. monocytogenes* have been developed as alternatives for band-based molecular subtyping techniques (e.g., PFGE). These approaches have been used not only to assess genetic diversity among isolates, but also to study the evolution and phylogeny of *L. monocytogenes*.

Multilocus Sequence Typing

MLST is based on the DNA sequencing of multiple genes (usually seven) or gene fragments to uncover allelic variants in conserved genes of bacterial species. Sequences of each gene obtained for the isolates to be characterized are aligned and the differences observed, even at a single nucleotide, are

assigned as distinct alleles. An MLST type is then assigned to each isolate according to the unique combination of polymorphisms observed for each of the loci. MLST schemes involving sequencing of multiple housekeeping genes, of multiple virulence genes, or of a combination of housekeeping and virulence genes have been used to characterize *L. monocytogenes* isolates.

One of the advantages of MLST over other molecular typing methods is that sequence data are unambiguous and are portable between different laboratories, being easily compared with web-based databases.

Multiple-Locus Variable-Number Tandem Repeat Analysis

MLVA takes advantage of the polymorphism of tandem-repeat DNA sequences (TRs). Bacterial genomes are rich in TRs. In each repeat sequence locus, the repeat copy number can vary between different strains, therefore these sequences are often referred to as 'variable-number tandem repeats' or VNTRs. In MLVA, the VNTR array is amplified using PCR. Forward primers are fluorescently labeled so that the PCR product can be detected and sized using high-resolution capillary electrophoresis (automatic sequencer). Use of multiple different fluorescent tags enables multiplexing targets with overlapping fragment sizes. At each locus, the number of copies of each repeat is assigned based on the observed fragment sizes (allele type). Owing to the rapid accumulation of changes in VNTR regions, MLVA is particularly useful in short-term evolution studies of bacterial species.

Whole genome sequences of several *L. monocytogenes* isolates have been crucial for the identification VNTRs and the development of MVLA typing schemes for *L. monocytogenes* isolates.

PFGE is still considered the more discriminatory typing technique for *L. monocytogenes* and the selected routine typing technique for surveillance and listeriosis outbreak investigations. However, its cost, labor-intensive, and difficult interlaboratory comparisons of results lead to the constant search for other subtyping methods. MLVA has already become the preferred typing technique for other foodborne pathogens, for example, *E. coli* O157:H7, and has proven to be more discriminatory than PFGE in some cases. Therefore, in the future, this technique may replace or complement PFGE typing of *L. monocytogenes*.

Control Measures

Control of *L. monocytogenes* in the food-processing environment has been the subject of a number of research projects. Owing to its ubiquitous nature and growth capability at refrigeration temperatures, it is impossible to assure its absence in all ready-to-eat (RTE) foods at the time of consumption. Therefore, it is unanimously accepted that an integrated multipartner approach – the government, the trade, and the consumers – is required to control this organism.

Regulatory Control

The microbiological criteria for *L. monocytogenes* are a matter of discussion between several international food safety authorities. Commercialization of foods in a globalized world makes it absolutely necessary that regulations relating to *Listeria* in foods be harmonized.

A 'zero-tolerance' policy is applied in the US. The EU, Australia, and Canada permit less than 100 cfu g^{-1} in specific foodstuffs depending on the risk associated or the capability of the bacterium to grow during the shelf life of the product in question.

Concerning *L. monocytogenes*, three relevant categories of foodstuffs with different limits were established by the EU Regulation 2073/2005, amended by EU Regulation 1441/2007: RTE foods intended for infants or for special medical purposes (absence in 25 g for products placed in the market during their shelf-life); RTE foods able to support the growth of *L. monocytogenes*, other than those intended for infants and for special medical purposes (absence in 25 g before the food has left the immediate control of the food business operator, who has produced it, and the product will not exceed the limit of 100 cfu g^{-1} throughout the shelf-life); and RTE foods unable to support the growth of *L. monocytogenes*, other than those intended for infants and for special medical purposes (100 cfu g^{-1} for products placed in the market during their shelf-life).

Control at Industry Level

The regulator's efforts to control *L. monocytogenes* in foods will be not effective, if the indusrty is not committed to ensuring the production and distribution of safe food. Control strategies are needed at all stages in the food chain (farm, processing, retail, foodservice, and domestic environment) with the following objectives: (1) to prevent contamination and growth in raw materials; (2) to destroy/reduce the pathogen if present in raw materials; (3) to prevent recontamination after a listericidal process; and (4) and to prevent/reduce the growth when present in final products to avoid levels considered unsafe at the time of consumption. The most effective strategies to reach these objectives include: implementation of environmental controls and using effective disinfection systems; implementation of Hazard Analysis and Critical Control Points (HACCP) programs at all stages of the food industry, giving particular attention to time/temperature controls and cross-contamination during processing and at retail; frequent revision of the HACCP programs so that they can more accurately identify the critical control points along the production line; combination of factors that are more effective in controlling listeria growth in RTE foods, for example, lactic acid, bacteriocins, and starter cultures; and application of post-packaging treatments (e.g., high-pressure processing) to inactivate *L. monocytogenes*.

Food Safety Education

In addition to regulatory and industry changes, educational initiatives on listeriosis prevention are needed.

The education of at-risk individuals, of their food preparers, and of the health professionals providing care for them is proposed as a key strategy in the reduction of the incidence of listeriosis – information on high-risk foods to be avoided,

and education on the four simple practices (clean, separate, cook, and chill) that can help reduce the risk of foodborne illness in general and listeriosis in particular.

Costs

A report published by the Centers for Disease Control and Prevention (CDC, USA) estimated that 2518 cases of listeriosis occurred every year in the USA, which result in 2322 hospitalizations and 504 deaths in the US each year. However, these results are based on a 1999 report and, therefore, are outdated because the food industry has invested heavily in improving its food safety practices. In the EU, the reported cases in 2008 (data from 25 countries) were 1389 and 134 deaths.

Illness caused by the consumption of contaminated foods has a considerable economic and public health impact worldwide, which are difficult to quantify. Generated costs include health-related expenses (e.g., medical costs and quality-of-life losses), and expenses related to food-processing companies, for example, food product recalls and bad publicity. A recent and illustrative example was the listeriosis outbreak that occurred in Canada in 2008 caused by hams and charcuterie products, which had a direct cost of $19 million to the company (Maple Leaf Foods) due to the recall of contaminated meat.

See also: Characteristics of Foodborne Hazard and Diseases: Cost of Foodborne Diseases; Pathogenesis and Virulence. Disciplines Associated with Food Safety: Food Microbiology. Food Safety Assurance Systems: Hazard Analysis and Critical Control Point System (HACCP): Principles and Practice; Microbiological Testing, Sampling Plans, and Microbiological Criteria. Foodborne Diseases: Foodborne Diseases and Vulnerable Groups. Public Health Measures: Foodborne Disease Outbreak Investigation; Modern Approach to Food Safety Management: An Overview; Surveillance of Foodborne Diseases

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Mycobacterium avium ssp. *paratuberculosis*

ECD Todd, Ewen Todd Consulting LLC, Okemos, MI, USA

Glossary

High temperature short time pasteurization 71.7–72°C for at least 15 s for high temperature short time (HTST) pasteurized milk products, which is a continuous pasteurization process as compared with batch pasteurization at lower temperatures for a longer time. This results in five log (99.999%) or greater reduction in harmful bacteria. HTST is also used for beverages, such as fruit juices. Currently, many milk processing plants exceed these minimum requirements for fluid milk products. Compared to other pasteurization processes, HTST pasteurization maintains color and flavor better, but some cheeses are found to have varying responses to the process. For fluids, HTST must be used in conjunction with sterile fill technology to prevent postpasteurization contamination.

Lymph nodes Small bean-shaped scattered masses of tissue in the lymphatic system that act as filters and immune monitors, removing fluids, bacteria, or cancer cells along the route of the system.

Macrophage Any large, mononuclear, highly phagocytic cells derived from monocytes that occur in the walls of blood vessels and in loose connective tissue. They are components of the reticuloendothelial system. Macrophages are usually immobile but become actively mobile when stimulated by inflammation; they also interact with lymphocytes to facilitate antibody production.

Nucleotide-binding oligomerization domain-containing protein 2 (NOD2) The official name of the *NOD2* gene is nucleotide-binding oligomerization domain containing 2 on chromosome 16 in humans. The *NOD2* gene provides instructions for making a NOD2 also known as caspase recruitment domain-containing protein 15 (CARD15) or inflammatory bowel disease protein 1 (IBD1). NOD2 plays an important role in the immune system. It recognizes bacterial molecules (peptidoglycans) and stimulates an immune reaction.

Phagocytosis The process includes five steps by phagocytes (white blood cells): (1) invagination, (2) engulfment, (3) internalization and formation of the phagocyte vacuole, (4) fusing of lysosomes to digest the phagocytosed material, and (5) release of digested microbial products.

Polymerase chain reaction In polymerase chain reaction testing, numerous copies of a gene are made by a rapid technique for *in vitro* amplification of specific DNA or RNA sequences, allowing small quantities of short sequences to be analyzed. The two strands of DNA containing the gene segment are separated, using DNA polymerase to make a copy, and then continuously replicating the copies.

Pulsed-field gel electrophoresis (PFGE) PFGE is used especially to separate large DNA fragments through agarose gels by periodic changing the direction of the electric current in order to minimize overlap of the bands.

Restriction fragment length polymorphism (RFLP) Fragments of DNA, cut by restriction enzymes, are different in length and the restriction fragments are separated according to their lengths by gel electrophoresis. RFLP analysis is used to trace relationships among organisms.

Subclinical infection Infection associated with no detectable symptoms although caused by microorganisms capable of producing easily recognizable diseases. This may occur in an early stage, with signs and symptoms appearing during the later stage of the infection, or the symptoms and signs may never appear (asymptomatic). Such infections can only be detected by laboratory testing.

Subpasteurization Slightly lower temperatures and/or shorter times than for the standard milk pasteurization temperatures, typically used to retain flavor in cheese making but with a risk of pathogen survival.

Background

Mycobacterium avium subspecies *paratuberculosis* (MAP) was first identified in 1895, Germany, by Johne and Frothingham at the Veterinary Pathology Institute in Dresden. The organism was causing chronic inflammation of the intestine in a cow, and this condition affecting the ruminants (cows, sheep, and goats) subsequently bore the title of Johne's disease (JD). This disease was later diagnosed in nonruminants: dogs, pigs, horses, chickens, primates, and, occasionally, carnivores like weasels. Clinical JD in dairy cattle typically causes loss of condition, a reduction in milk yield, weight loss, and periodic episodes of diarrhea. In a cattle herd with one or two clinically diseased animals, up to 50% of the other apparently healthy

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animals will be subclinically infected. Infections are most likely to occur in calves and young cattle, because MAP is transmitted from cow to calf through the colostrum and milk, and directly between animals in crowded and contaminated farm environments. MAP can persist in the intestinal tract of subclinically infected animals for years without causing clinical disease, only to manifest itself when the animal undergoes physical or psychological stress such as calving or overcrowding, respectively. There is no treatment and the disease is invariably fatal, domestic animals are usually euthanized once the disease has progressed to the point of rendering them unproductive. There is increasing evidence today that Crohn's disease (CD) in humans, being somewhat similar to JD in animals, is also probably because of MAP.

Characteristics

As the name indicates, MAP is a member of the *M. avium* complex (MAC) (Nichols *et al.*, 2004). MAP is a small mycobacterium of approximately 0.5 μm by 1–2 μm in size and is an obligate intracellular pathogen that can cause chronic inflammation of the intestine in many animals. It is Gram- and acid-fast positive, and MAP cells also have intracellular vacuoles or inclusions common to mycobacteria. The environmental distribution of MAP is markedly different from that of most other members of the MAC. Although the DNA of MAP is >99% identical with that of *M. avium*, MAP only occurs in environments contaminated by feces of infected animals. MAP compensates for its inability to assimilate iron and replicate in the environment (due to lack of mycobactin) by producing an extra tough waxy rough cell wall (made of lipids and polysaccharides) thus enabling its prolonged survival until ingestion by a susceptible host. Like other mycobacteria, MAP has the capacity to replicate inside host macrophages (white blood cells). As part of the immune system, macrophages are capable of destroying a wide variety of bacterial pathogens. Mycobacteria, including MAP, however, are one of the few types of bacteria that not only can survive the antibacterial effects of macrophages, these facultative intracellular bacterial pathogens are able to replicate inside macrophages and cause disease. MAPs accomplish this by secreting factors that avoid or neutralize the production of antibacterial chemicals inside macrophages, and they are also able to suppress or modulate the host's immune response.

MAP grows very slowly under culture and is difficult to identify (see under Analytical Methods for MAP). Like many mycobacteria, MAPs demonstrate both environmental and heat resistance. Pasteurization of milk (typically 72 °C for 15 s) was designed to destroy *Mycobacterium bovis* (and *tuberculosis*) excreted from cattle into milk. However, MAP is more thermotolerant than *M. bovis*, and experimental work has shown that pasteurization at 72 °C for 15 or 25 s would reduce the number of viable organisms, but may allow some survivors. This conclusion may be supported by surveys of pasteurized milk where MAP has been found at low prevalence (see under Epidemiology of JD in Animals). Some MAP species can colonize swimming pools, aquaria, and cold water distribution systems, whilst others are more commonly associated with hot water systems. If liquids of injections contain mycobacteria, these can cause abscesses, whereas other iatrogenic infections are linked to contaminated endoscope washers and renal dialysis fluid.

Epidemiology of JD in Animals

JD, or paratuberculosis, is a disease concerning domestic livestock of cattle, sheep, and goats, that was first noticed a little over 100 years ago in Europe and North America, but is likely to be much more widespread today. The risk of infection is not only an economic one for farmers but also a human health concern because there is evidence that MAP is also pathogenic for people. Most work with MAP has been focused on cattle, and less is known about its presence in other species (Eltholth *et al.*, 2009). In beef cattle, herd prevalence from several studies is higher, as to be expected, than for individual cows (2–54% vs. 0·4–19%). For dairy cattle, herd prevalence is 3–70%, and for individual animals, 1–18%. Although these data reveal much variability between studies, the overall results indicate that MAP is frequent in both beef and dairy cattle. MAP has been isolated from the intestinal mucosa, mesenteric, head and lymph nodes, liver and spleen from naturally infected animals, and thus the contamination of the milk could be from direct shedding of the MAP in the udder or fecal contamination from an infected animal during or after milking. MAP has been detected in raw and pasteurized milk from cows, sheep, and goats, and in cheeses made thereof. In raw cow milk, the prevalence of polymerase chain reaction (PCR) and culture positive samples in several studies has ranged between 2–49% and 0.3–1.6%, respectively. In a Swiss survey of bulk milk, 23% sheep samples and 24% of goat samples were MAP positive. Meat may also be contaminated though transmission from systemically infected animals as surfaces of beef carcasses have shown the presence of MAP DNA. All this indicates that humans may be potentially exposed to MAP through consumption of dairy products (less likely from meat) or working with infected animals. Different studies have been applied to investigate the effect of different time temperature combinations on MAP inactivation. It has been estimated that MAP inactivation by high temperature short time pasteurization at 72 °C for 15 s ranges from 4 to 7 log. The rate of inactivation may depend on the primary concentration of MAP in raw milk. Subpasteurization temperature of milk for cheese production may not be sufficient for complete inactivation of MAP (65.58 °C for 16 s; Stabel and Lambertz, 2004) and MAP is not completely destroyed in milk that is artificially contaminated with a high inoculum even after a combination of high pressure (600 MPa) and pasteurization. Surveys of pasteurized milk indicate survival does occur (up to 3% culture and 15% positive by PCR). Survival may depend on the level of MAP present at pasteurization but this was not measured in the surveys. A more limited set of surveys of cheeses have shown that these may be contaminated (up to 4% culture positive and 32% by PCR).

There are also concerns for MAP in infant formula. Fifty one dried milk baby food products from 10 producers operating in seven European Union countries were tested by competitive real time quantitative PCR (Hruska *et al.*, 2011). From 48 to 32 500 MAP cells g^{-1} of powdered infant milk were found in 18 out of the 51 samples (35%). More than

10 000 cells g^{-1} were present in four samples (7.8%). Such concentrations mean that one package of milk could contain 5 million MAP cells, which is ingested by a bottle-fed infant over the course of several days. Even though they do not suffer from an immediate infection, premature babies and bottle-fed newborns can be affected by pro-inflammatory triggers from the muramyldipeptide and other components of the mycobacterial cell wall arising from the MAP present that later develop into chronic inflammatory diseases, such as CD.

In many countries, clinically diseased JD ruminants may be slaughtered and the meat and offal used for human consumption. During the slaughter and processing of the animals, large numbers of MAP, particularly in the lymph nodes and liver, would be released into the slaughterhouse environment to contaminate other carcasses, and possibly infect the workers. Any manure or slurry from the holding pens or slaughterhouse area may be sold as fertilizer and, thus, produce grown in soils containing this manure would expose the crops and their consumers to viable MAP, because pathogen survives well in the environment and may resist mild cooking.

Epidemiology of CD in Humans

CD is named after Burrill B. Crohn, an American gastroenterologist, for identifying the disease in 1932, although the disease has been recognized as early as 1913 by a Scottish surgeon, who has documented at least nine cases of inflamed intestinal tract. Typically affecting $<1\%$ of the population, CD is a chronic intestinal inflammation of humans, of which there is some evidence that MAP is a possible cause, or at least a contributor. Metaanalysis studies indicate that there is an association between MAP and CD, even though the etiology of CD is not yet clear. CD was first recognized in the past century, initially appearing in developed societies in temperate regions with intensive farming, more commonly in northern regions of Europe and North America, and less so in Asia and Africa. However, CD seems to be increasing in countries such as Iran, India Brazil, China, and Japan, which have substantially increased production and consumption of dairy products. This rising trend may also be linked to both the westernization of lifestyles such as changes in dietary habits and environmental changes such as improved sanitation plus industrialization (Thia *et al.*, 2008). Distinct regions of Canada (Prairie provinces and Nova Scotia), UK (Scotland, particularly west and north), France (Amiens), the Netherlands (Maastricht), Scandinavian region (Stockholm), and New Zealand (Canterbury) represent the highest incidence areas (Economou and Pappas, 2008). The highest levels of CD worldwide seem to be in Canada with an incidence rate of 20.2/100 000 in Nova Scotia (Bernstein *et al.*, 2006). Reasons for this specific distribution is not known. A convincing link between MAP and CD has been found from long-term studies up to the 1990s in Iceland (Hermon-Taylor and El-Zaatari, 2004). Before 1930, MAP infection and JD in Iceland were virtually unknown. Then in 1933, a few subclinically infected sheep were imported from Germany and distributed to 14 farms. By the late 1950s, the disease became an epidemic in Iceland with almost 30% of sheep farms affected. The mean incidence of CD (number of cases 100 000^{-1} $year^{-1}$) rose each decade, from 0.4 in1950–59 to 5.6 in the early 1990s, with the highest annual figure of 8.2 in 1992. Young people were particularly affected. This upward trend would probably continue (**Figure 1**).

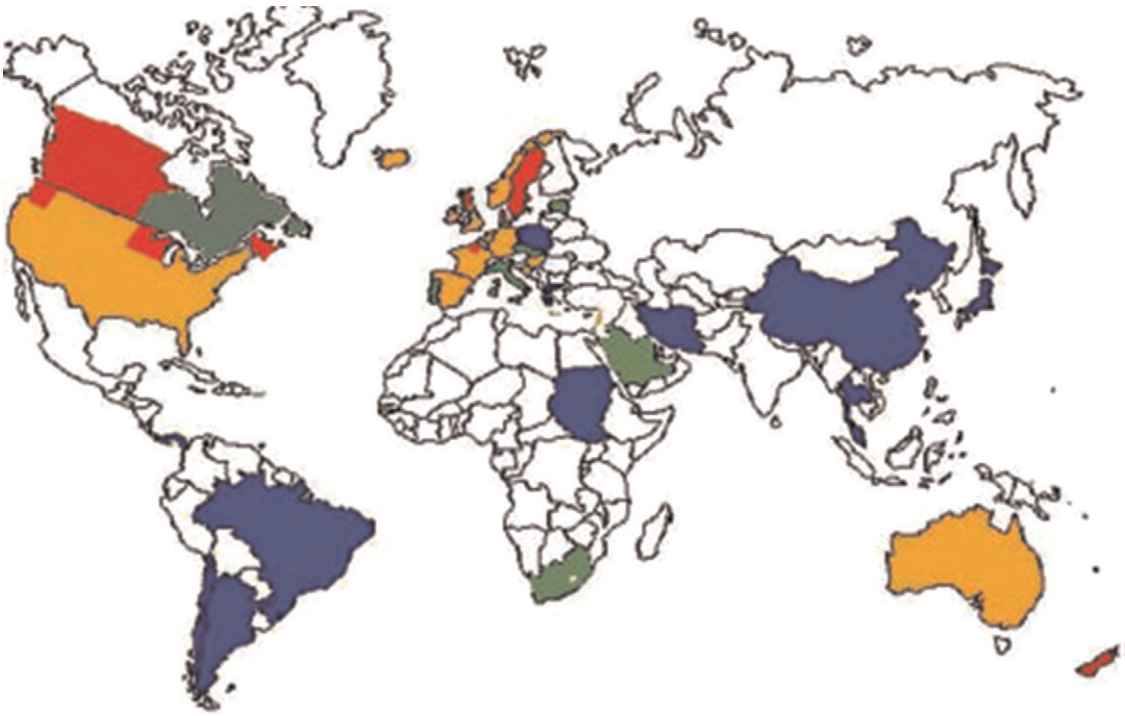

Figure 1 The global map of CD: Red refers to annual incidence above 7/100 000, orange to incidence 4–7/100 000, green to incidence 1–4/100 000, blue to incidence <1/100 000. Absence of color indicates absence of data. Reproduced from Economou M and Pappas G (2008) New global map of Crohn's disease: Genetic, environmental, and socioeconomic correlations. *Inflammatory Bowel Diseases* 14(5), 709–720.

CD affects any part of the gastrointestinal tract (GI) from mouth (nonhealing sores) to anus (itchiness or abscesses), but particularly the ileum and the colon. Symptoms vary in relation to abdominal pain, diarrhea, vomiting, and weight loss, which is classified on the whole as a type of inflammatory bowel disease. Flatulence, bloating, and blood in the stool may also occur. Associated problems are skin rashes, arthritis, inflammation of the eye, tiredness, and lack of concentration. CD also increases the risk of cancer, particularly colon cancer, where there is inflammation in the GI. The usual onset is between 15 and 30 years of age, but it is not easy to diagnose and may begin at earlier ages. There can be periods of remission followed by painful symptoms. Most people seem to be asymptomatic because of frequent exposure during a lifetime; some of these may be colonized with a risk for later acute symptoms resulting from a stress event if they are susceptible. The disease is probably underdiagnosed, and is present worldwide; it is now recognized as a major healthcare problem. CD is now known to be caused by interactions between environmental, immunological, and bacterial factors in genetically susceptible individuals. The chronic inflammation of CD is caused when the adaptive immune system tries to compensate for a deficient innate immune system. Over 30 gene mutations have been associated with CD that runs in families, and a biological function is known for most of them. An impaired cytokine secretion by macrophages contributes to impaired innate immunity, and leads to a sustained microbial-induced inflammatory response in the colon where there are billions of bacteria. NOD2, a gene toward CD genetic susceptibility, is associated with less effective macrophage destruction by MAP. Those with CD have polymorphisms that change the amino acids in the protein NOD2, each impairing the responses to peptidoglycan, which is a component of bacterial cell walls (Abraham and Cho, 2009). These polymorphisms occur with increased frequency in persons of European ancestry but are not present in Asian patients, and are significantly less frequent in African Americans with CD. Also, MAP produces mannins, which protect both itself and various bacteria from phagocytosis, and may allow it to cause a variety of secondary infections thereof. However, other bacteria could be implicated in CD (independently or synergistically with MAP) such as adherent invasive *Escherichia coli*, because they are found more frequently in CD patients than in controls. An interesting argument for the recent rise in CD is the possible exposure to large numbers of psychrotrophic bacteria such as *Yersinia* and *Listeria*, which are more likely to be in foods because of the widespread use of refrigerators. The protozoan *Blastocystis* may also be an important facilitator of infection. Environmental components that could play a role in risks for CD are increased consumption of animal protein, milk protein, and an increased ratio of omega-6 to omega-3 polyunsaturated fatty acids, but not vegetable protein and fish. Smoking and the use of contraception hormone pills also seem to increase the risk of CD or exacerbate the symptoms. There is no cure for CD and remission would neither be possible nor prolonged if achieved. In cases where remission is possible, relapses can be prevented and symptoms can be controlled with medication, lifestyle and dietary changes, changes to eating habits (e.g., eating smaller amounts more often), reduction of stress, moderate activity, and exercise.

Analytical Methods for MAP

The principal diagnostic tests for MAP infection in animals are individual or pooled fecal culture, ELISA, and IFNγ release from activated white cells in response to MAP antigens. Even though PCR diagnostics have also been introduced, ELISA remains as the cheapest and most convenient screening test hitherto. However, commercially available ELISA kits lack the sensitivity and specificity to diagnose subclinical MAP infection at an early stage. Although fecal cultures are among the preferred confirmation methods, fecal MAP is historically difficult to isolate, and strains from sheep or humans may require months or years of incubation before their gradual emergence becomes visible – and bovine strains can be even slower to demonstrate colonial growth. Some strains of MAP cannot be grown at all. If they can be cultivated, MAP may take an initial 16 weeks or longer to produce visible colonies on primary cultures, and requires exogenous mycobactin an iron-transport protein for *in vitro* growth. On solid media such as Middlebrook 7H11, MAP colonies appear rough and translucent; on Herrold's media containing egg yolk, they are smooth and opaque. As the cultures become older and the medium dries, the colonies take on a crumbly appearance. In liquid media, MAP grows in characteristic tight clumps. Culturing has been improved by the commercial availability of BD BACTEC™ and the Mycobacteria Growth Indicator Tube System (BD MGIT™) media (Becton Dickinson). More than 30 different MAP strains have been identified using methods such as restriction endonuclease analysis, IS900 restriction fragment length polymorphism (RFLP), and pulsed-field gel electrophoresis (PFGE). Typing of MAP isolates obtained from all over the world indicating that there are differences between ovine and bovine strains, signify a host adaption. Because most strains grow very slowly or not at all, PCR-based typing procedures for MAP are highly desirable, and success has been found with random amplified polymorphic DNA patterns and a multiplex PCR with a common IS900 primer and a locus-specific primer.

Routes of Transmission

Apart from consumption of milk and other foods being contaminated with MAP, these mycobacteria can infect persons from environmental sources too. As MAP survives well in the environment, it may be transmitted to people through water; this can be directly through consumption of potable water and also possibly from exposure to aerosols because of flooding, irrigation of fields, misting of plants indoors, besides that due to greenhouses, and shower heads. When lakes and rivers are contaminated by agriculture runoff from heavily grazed pastures, MAP will be present as cells within protozoa. Bacterial pathogens such as *Legionella pneumophila* and members of the MAC can be taken up by protozoa like amoebae and ciliates without undergoing digestion.

Bacterial proliferation in laboratory cultures was noticeably enhanced in the presence of amoebae, and biofilms rapidly formed in mixed amoebae and bacteria cultures (Marciano-Cabral *et al.*, 2010). This indicates that synergistic effects may occur in the environment to enhance the survival and growth of MAP in water systems. Filtration may remove some of these

if they are trapped in the larger particles but those passing through any filtration systems may not be destroyed by chlorination. These pathogens can form biofilms in distribution pipes arriving at both industrial and domestic outlets, and MAP can accumulate over time in cold and hot water storage and delivery systems. There have been anecdotal reports to link clusters of CD cases with water supplies. In a rural English community, 12 persons developed CD between 1960 and 1983, which is higher than normal prevalence. With its own water supply from local springs, the village was in a hollow surrounded by upland pastures that were grazed by cattle in which clinical JD was evident. The second CD cluster occurred in a Minnesota town, where there were 7 cases of CD amongst 285 graduates of the high school class of 1980. All seven had been swimming in local ponds and lakes where there was rich agricultural grazing land, and high fecal coliform counts indicated extensive contamination with cattle fecal runoff; unfortunately, no testing for MAP was done.

Prevention and Control

Because MAP may survive in milk at subpasteurization and even in pasteurization temperatures due to its heat resistance, the best way to prevent exposure of consumers to MAP, is the eradication of the JD and by sourcing milk and meat from JD free herds. In particular, since powdered or liquid milk substitutes the breast feeding of premature babies and newborns, it should be produced from MAP-free milk.

On the medical side, research on linking MAP with CD should continue, including possible synergistic effects with other pathogens. Less is known regarding the source and extent of MAP in the food supply. New methods are required for early detection of infected animals in order to decrease infection between animals as well as contamination of the environment, particularly in milking parlors, dairy plants, slaughterhouses, and meat processors. Although estimates of the prevalence of JD are scarce, particularly for nondairy herds, the available data suggest that the likelihood of contamination of raw milk with MAP in most studied regions is substantial. These locations are, therefore, critical points in reducing the risk of MAP contamination. Along the food production chain from farm to processor, certain steps are likely to increase or decrease the level of MAP contamination in the final product. There are many data gaps that need to be filled before more specific prevention and control procedures can be implemented. These include estimating: (1) the extent of dissemination of MAP in tissues of infected animals; (2) the amount of MAP shedding in milk and feces; (3) the level of fecal contamination of milk and carcasses; (4) to what extent farm and slaughterhouse workers could be infected; and (5) effect of different processing steps of milk and other dairy products and meat on the inactivation of MAP. There should be a careful comparison in prevalence and concentration of MAP at different steps in the processing of milk and meat products from known JD infected farms and those apparently unaffected by the disease.

Those who are immunocompromised (young, elderly, and ill) are more likely to be infected, if exposed. Although mass immunization of these persons cannot yet be considered feasible or practical (an improved and enhanced TB vaccine could be effective against MAP as well as *M. bovis* and *M. tuberculosis*), risks can be reduced by limiting their exposures to some potential sources, such as unpasteurized dairy products, undercooked meat, farms with ruminants and slaughterhouses, whirlpool baths, and showers. However, it would seem that MAP is so widespread in the environment that everybody is exposed to low levels of these organisms on a regular basis and most people seem to be able to cope without developing acute symptoms.

See also: Analytical Methods: Transmissible Spongiform Encephalopathy Diagnosis. Bacteria: *Mycobacterium bovis*; Food Safety Assurance Systems: Management of Biofilm Risk. Food Technologies: Pasteurization. Foodborne Diseases: Foodborne Diseases and Vulnerable Groups; Overview of Biological Hazards and Foodborne Diseases. Public Health Measures: Safe Use of Wastewater for Agricultural Production. Safety of Food and Beverages: Milk and Dairy Products

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Johne's Information Center, School of Veterinary Medicine, University of Wisconsin, USA.

BACTERIA

Mycobacterium bovis

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Glossary

Anthroponosis An infectious disease in which the etiological agent is carried by humans and is transferred to other humans and animals.
Foodborne disease Any disease resulting from the consumption of contaminated food, pathogenic bacteria, fungus, viruses, or parasites that contaminate food, as well as chemical or natural toxins such as poisonous mushrooms.
Organism life cycle A period involving all different generations of a species succeeding each other through means of reproduction, whether through asexual reproduction or sexual reproduction (a period from one generation of organisms to the same point in the next).
Surveillance In public health and epidemiology, the discipline of continuously gathering, analyzing, and interpreting data about diseases, and disseminating conclusions of the analyses to relevant organizations, to intervene in their patterns in order to control and prevent them.
Taxonomy The science of classification, in microbiology the arrangement of microorganisms into a classification.
Zoonosis Any infectious disease that can be transmitted between species (by different ways, by a vector or by their products, food) from animals to humans or from humans to animals (less common).

Introduction

Mycobacterium bovis is a slow-growing (16–20 h generation time), aerobic bacterium, Gram positive and acid-fast, and the causative agent of tuberculosis (TB) in cattle (known as bovine TB) (ICD-10 A16), although it can produce infection in other animals (in addition to cattle, important maintenance hosts of the pathogen include goats, bison, deer, and badgers in Ireland and the UK; buffalo in Africa; moose in Canada; and the common brushtail possum in New Zealand).

Characterisitics of the Pathogen

Mycobacterium bovis is a member of the *Mycobacterium tuberculosis* complex (Table 1), which includes *M. tuberculosis*. Related to *M. tuberculosis* – the bacterium which causes TB in humans – *M. bovis* can also jump the species barrier and cause TB in humans. Bovine TB is not only a relevant zoonosis, with an important economic burden (up to hundreds of millions of euro annually in Europe), but also a relevant human health threat that can spread to humans through inhalation of infectious droplet nuclei and by ingestion of raw milk, than being relevant for public health. This human health threat, however, has been largely neglected for many reasons, which will be discussed later. Both species share genetic identity of over 99% at the whole genome level, although according to recent data *Mycobacterium microti* is more closer phylogenetically than *M. bovis* to *M. tuberculosis*.

Additionally, *M. bovis* and *M. tuberculosis* also share key aspects such as developing similar lesions and immune responses, which often result in colonization and spread to the same organs, namely lungs and lymphatic tissues.

Besides its occurrence in humans and cattle, in the past two decades domestic outbreaks of *M. bovis* infection have also been reported in pigs and other animals. *Mycobacterium bovis* often does not produce acute disease, persists in the carrier stage, has multiple nonhuman reservoirs, and easily crosses species.

In general, animal TB is a disease of high economic relevance within the context of livestock farming as it directly affects animal productivity and also influences international trade of animal products. Bovine TB was completely eradicated in many USA herds at a cost of USD $450 million over a period of 50 years using a 'test and slaughter' program combined with meat inspection. However, data suggest that in states bordering Mexico, over 70% of TB cases would still be due to *M. bovis*. *Mycobacterium bovis* infections have also been detected in wildlife and can have severe consequences for the ecosystem. For example in Canada, TB was endemic in plains bison and occurred in elk, moose, and mule deer in Buffalo National Park (BNP), Alberta during the 1920s and 1930s. Bison were moved from BNP to Wood BNP (WBNP), where TB became, and remains, endemic in bison, posing a challenge to efforts to restore bison in northern Canada. TB was found in a white-tailed deer

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Table 1 Main species included in the *M. tuberculosis* complex, showing the strains characterized and included in the NCBI Taxonomy Browser (2012)

Mycobacterium africanum
Mycobacterium bovis
Mycobacterium bovis AF2122/97
Mycobacterium bovis BCG
Mycobacterium bovis BCG str. ATCC 35733
Mycobacterium bovis BCG str. ATCC 35740
Mycobacterium bovis BCG str. ATCC 35743
Mycobacterium bovis BCG str. China
Mycobacterium bovis BCG str. Mexico
Mycobacterium bovis BCG str. Moreau RDJ
Mycobacterium bovis BCG str. Pasteur 1173P2
Mycobacterium bovis BCG str. Tokyo 172
Mycobacterium bovis str. Rabéenle
Mycobacterium canettii
Mycobacterium microti
Mycobacterium pinnipedii
Mycobacterium tuberculosis
Mycobacterium sp. 'sarcoidosis patient #1a'
Mycobacterium sp. 'sarcoidosis patient #1b'
Mycobacterium sp. 'sarcoidosis patient #2'
Mycobacterium sp. 'sarcoidosis patient #3'
Mycobacterium sp. 'sarcoidosis patient #4'
Mycobacterium sp. 'sarcoidosis patient #5'
Mycobacterium sp. 17263
Mycobacterium sp. 68/7171
Mycobacterium sp. 9502227
Mycobacterium sp. ANT 07
Mycobacterium sp. ANT 08
Mycobacterium sp. ANT 09
Mycobacterium sp. CHTN-E
Mycobacterium sp. CIPT 140060001
Mycobacterium sp. CIPT 140070001
Mycobacterium sp. CIPT 140070002
Mycobacterium sp. CIPT 140070003
Mycobacterium sp. CIPT 140070005
Mycobacterium sp. CIPT 140070007
Mycobacterium sp. CIPT 19980863
Mycobacterium sp. N256
Mycobacterium sp. N405
Mycobacterium sp. N406

Source: NCBI Taxonomy Browser (2012) (http://www.ncbi.nlm.nih.gov/taxonomy).

in Ontario in 1959, and in an infected elk near Riding Mountain National Park (RMNP), Manitoba in 1992. Intense surveillance has resulted in detection of 40 elk, 8 white-tailed deer, and 7 cattle herds infected between 1997 and 2008 in the RMNP area. Moreover, animal TB bears a zoonotic potential and is therefore of public health concern currently.

Like in humans, aerosolized bacteria are the most common source of infectious organisms in cattle, and the primary site of natural infection is the respiratory tract (Figure 1). In bovine TB, airborne infection is considered the most common route of transmission, and more than 20% of cattle with bovine TB shed the mycobacteria, mainly during early infection (Figure 1). The intermittent nature of bacilli shedding from infected animals, after a short initial postinfection period has been documented. Several studies indicate that between 5% and 30% of naturally infected animals release mycobacteria in clinical samples, but the precise duration of the secretion period is unknown. In humans, transmission can occur due to the consumption of contaminated milk from infected cattle, which has not been pasteurized or boiled (Figure 1).

Different studies in the world have supported the fact that although slight differences are found in the genome sequence of *M. tuberculosis* and *M. bovis*, the physiology and host range spectrum are quite different. In recent years, using multiple approaches, progress has been made regarding the molecular basis that might, at least partially, explain how mycobacteria are able to remain as a persistent or latent infection in its human host, and it has also been suggested that this particular stage of disease exists in cattle, by analogy with human TB; nevertheless, until now little attention has been paid to this form of infection in bovine hosts, either from the academy or from government sectors.

TB produced by *M. bovis* is an important human zoonosis associated with the consumption of dairy products contaminated with the bacilli, and to labor risk (such as direct contact or droplet transmission) in farms or slaughterhouses (Figure 1). In developed countries eradication programs have significantly reduced the prevalence of this disease, but reservoirs in wildlife make complete eradication difficult. *Mycobacterium bovis* is classified as a Risk 3 pathogen for public health. Close to 40% of the Latin American and Caribbean countries have reported the occurrence of TB due to *M. bovis*, and a conservative estimate would be that it causes 5% of the total pulmonary TB cases (considering that in many developing countries only radiological and clinical diagnosis of human TB is made in many cases). Of the approximately 375 million cattle in Latin America and the Caribbean, 70% are held in areas where rates of *Mycobacterium bovis* infection in cattle are higher than 1%. The remaining 30% are in countries where infection affects less than 1% of the cattle, including 65 million in countries where bovine TB infection is virtually nil. For example, it is estimated that in certain endemic areas of Mexico more than 15% of cattle would be infected.

Historically, *M. bovis* has been associated with extrapulmonary TB in infants and children, usually occurring due to the consumption of milk from infected cattle, which had not been pasteurized or boiled. It could be estimated that *M. bovis* can cause up to 10% of the extrapulmonary TB cases, particularly in these epidemiological settings. Despite the low reported cases of *M. bovis* infections in humans, it is accepted that zoonotic transmission is negligible in most of the developed world, but not in developing countries, and this deserves and should be investigated in many places over the world.

The World Health Organization (WHO) in conjunction with the Food and Agriculture Organization and the World Animal Health Organization (*Office International des Epizooties* (OIE)) recently classified bovine TB as a neglected zoonosis, with special reference to developing countries. In the world's most vulnerable communities, animal diseases, which are transmissible between livestock and humans, not only have the potential to impact human health directly but also threaten human livelihoods by compromising sustainable food supply, income, and social status.

According to the OIE data available at the World Animal Health Information System which is coupled with the World

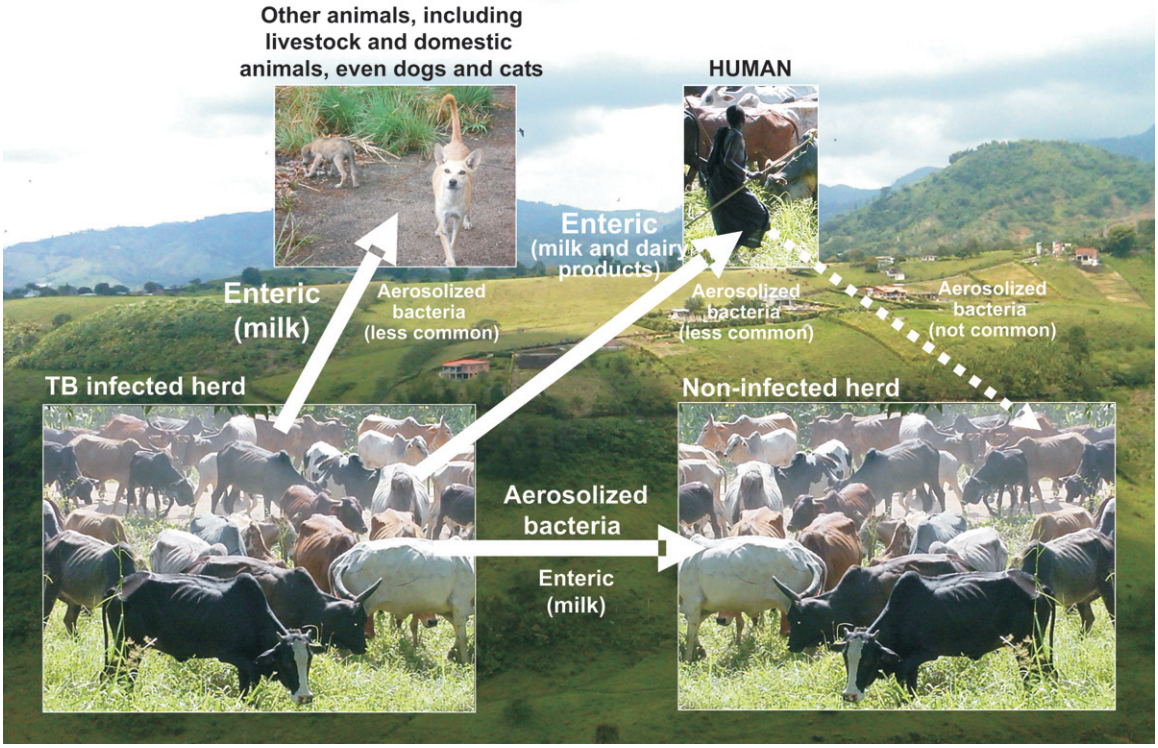

Figure 1 Main ways of transmission of *M. bovis* between animals and human.

Animal Health Information Database interface (Handistatus), during 2004, 13 countries in the America reported bovine TB (Argentina, Bolivia, Brazil, Canada, Chile, Colombia, Costa Rica, Cuba, Dominican Republic, El Salvador, Guatemala, Guyana, Honduras, Mexico, Nicaragua, Panama, Paraguay, Peru, USA, Uruguay, and Venezuela) (Table 2). This represents a significant remaining burden of disease in this region, although in such databases there is no precise information on the number of cases of bovine TB reported each year from these countries (Table 2).

In other regions of the world, such as Africa and Asia, a considerable number of countries have reported bovine TB (Table 3). Totalizing, there are in these three regions of the world more than 125 countries that have reported bovine TB prevalence as enzootic or of high prevalence in the last two decades. Adding those countries who have sporadically reported it (17), indicates that in total 142 countries have described cases of bovine TB. However, there are 29 with no data and finally just 26 (less than 20% of the countries) with a test and slaughter policy (Table 3).

At the epidemiological surveillance web system ProMEDMail, during 2011 (1 January–31 December 2011), 18 reports of bovine TB, from Canada, France, New Zealand, UK, and USA were published. In 2010, there were 16 reports, from the same countries. Until 1 May 2012, seven reports have been published, from Poland, UK, and USA.

Moreover, it is also estimated that 70% of the human population live in countries where cattle undergo no control or only limited control for bovine TB, constituting a potential infectious source. Ideally, all cattle that give positive readings to diagnostic tests should be killed or culled, as they may well represent a source of ongoing infection. However, under financial constraints, for example in developing countries, programs to combat bovine TB require a more accurate targeting of cattle that represent the greatest threat of spreading infection. Even more there are countries where no control program exist, are not even part of a zoonosis or foodborne programs and the human TB control programs usually gets attention only if it is *M. tuberculosis* but not infections due to other mycobacteria including *M. bovis*. This situation would be even more important in rural areas where cattle-human contact is high and where consumption of raw milk and dairy products would be considerable. In the context of animal health, we must consider the inability of current assays to discriminate between active and a likely latent infection in cattle, which could make the difference between sacrificing truly contagious animals or only suspected ones. Research and development on latent infection diagnostic techniques in cattle, as has been fully implemented in human TB due to *M. tuberculosis*, should be investigated.

Mycobacterium bovis has a wide range of hosts, but it primarily infects cattle (Figure 1). Cattle (as well other animals with less frequency) can transmit the agent to humans through the consumption of unpasteurized, contaminated dairy products, making bovine TB a zoonotic foodborne disease and a risk for food safety in many countries (Figure 1).

Table 2 Report of bovine tuberculosis by countries of the Americas, according the World Animal Health Organization, 1996–2004

Country/Territory	*Year*								
	1996	*1997*	*1998*	*1999*	*2000*	*2001*	*2002*	*2003*	*2004*
Antigua and Barbuda	...	–	...	+	...	...	–	...	...
Argentina	+	+	+	+	+	+	+	+	+
Bahamas	0	0	...	...	...	0	0	...	...
Barbados	(1976)	(1976)	...	(1976)	(1976)	(1976)	(1976)	...	(1976)
Belize	(1991)	(1991)	(1991)	(1991)	...	(1991)	(1991)	(1991)	(1991)
Bermuda	...	...	–	–	...	...	...	...	–
Bolivia	+	...	+	+	...	+	+	+()	+()
Brazil	+()	+()	+	+	+	+	+	+	+
British Virgin Islands	0000	0000	0000	0000	0000	...	...	...	0000
Canada	+	+	+	+	(1999)	+	+()	+()	+()
Cayman Islands	...	...	–	–	–	–	–	–	–
Chile	+	+	+	+	+	+	+	+	+
Colombia	(1992)	+	+	+	+	+	+	+	+
Costa Rica	+	+	+	+	+	+	+	+	+
Cuba	+	(1996)	+	(1998)	(1998)	(1998)	(1998)	+	+
Curaçao (Netherlands Antilles)	0000	...	...	...	...	...	–	...	–
Dominica	...	0000	...	...	...	...	...	...	...
Dominican Republic	+	+	+	+	+	+	+	+	+
Ecuador	+	...	...	...	+	+	+	...	...
El Salvador	+	+	...	+	+	+	+	+	+
Falkland Islands/Malvinas	0000	0000	0000	0000	0000	0000	0000	0000	0000
French Guiana	0000	0000	0000	0000	0000	0000	0000	0000	0000
Grenada	...	(1996)	...	...	...	...	...	(1996)	...
Guadeloupe (France)	0000	0000	0000	0000	0000	0000	0000	0000	0000
Guatemala	...	...	+	...	+	+	+	+	+
Guyana	...	...	+	+	...	...	...	...	+
Haiti	+	...	?	+	+	...	...	?	?
Honduras	...	+	...	...	...	...	+()	+()	+()
Jamaica	...	(1989)	...	...	...	(1989)	(1989)	(1989)	(1989)
Martinique (France)	+	+	+	+	...	(1999)	(1999)	(1999)	(1999)
Mexico	+()	+()	+()	+()	+()	+()	+()	+()	+()
Nicaragua	+	...	...	...	+	+()	+()	+()	+()
Panama	(1982)	+()	+()	+()	+()	+()	+()	+()	+()
Paraguay	+	+	+	+	+	+	+	+	+
Peru	+()	+()	+()	...	+()	+()	+()	+()	+()
Puerto Rico	...	...	...	...	...	...	...	0000	...
Saint Kitts and Nevis	–	–	–	...	...	0000	0000	0000	0000
Saint Vincent and the Grenadines	...	...	...	...	...	...	...	0000	0000
Suriname	...	+	...	...	...	...	–	...	...
Trinidad and Tobago	(1985)	(1985)	+	...	+	+	(2001)	(2001)	(2001)
USA	+	+	+	+	+()	+()	+()	+()	+()
Uruguay	+	+	+	+	+	+	+	+	+
Venezuela	+()	+()	+	+	+	+	+	+	+

Notes: 0000, disease never reported; –, disease not reported (date of last outbreak not known); (month/year), date of the last reported occurrence of the disease in previous years; ?, disease suspected but presence not confirmed; +, reported present or known to be present; +?, serological evidence and/or isolation of the causal agent, but no clinical signs of disease; (), disease limited to specific zones; ..., no information available.

Foodborne *M. bovis* Infection

In developed countries pasteurization of milk as well testing and culling of infected cattle have resulted in steep decreases in the incidence of *M. bovis* TB. *Mycobacterium bovis* caused as much as 25% of the cases of human TB in developed countries in the late nineteenth and early twentieth centuries. Human TB cases in developed countries caused by *M. bovis* usually affected persons who acquired the infection locally before the implementation of control measures, especially from the habit of drinking raw milk. In developing countries where control measures have not been fully implemented it can occur more often and in fact probably occurs, although it is not regularly reported. Despite that reports of *M. bovis* TB in developed countries still are found in the literature and in some epidemiological reports as has been shown. One study during the period 2001–04 described that this zoonotic foodborne disease occurred in New York, USA. Investigators concluded that recent foodborne transmission within the USA had occurred, because there was a lack of evidence of airborne, person-to-person transmission, and because none of the patients <5 years of age (one of whom died because of peritoneal TB) had

Table 3 Reports of the prevalence of bovine tuberculosis and use of the test and slaughter policy in countries within the WHO regions

Region	*Number of countries reporting*		*Not reported*	*No data*	*Number of countries with test and slaughter policy*
	Enzootic or high prevalence	*Sporadic or low prevalence*			
Africa	55	8	25	18	7
Asia	36	1	16	9	7
Latin America and Caribbean	34	8	12	2	12
Developing regions	125	17	53	29	26

Source: Data from Cosivi O, Grange JM, Daborn CJ, *et al.* (1998) Zoonotic tuberculosis due to *Mycobacterium bovis* in developing countries. *Emerging Infectious Diseases* 4: 59–70.

a history of international travel for exposure in known bovine TB endemic areas or countries. A significant risk of *M. bovis* infection remains in certain segments of population at endemic countries in the form of: (1) continuing on-farm consumption of unpasteurized cows' milk, (2) retail sales by approved establishments of unpasteurized milk and dairy products, and (3) occupational exposure to infectious aerosols from tuberculous animals and their carcases.

In Europe, Great Britain, particularly in southwest England and Wales, has recently reported that bovine TB has increased over the past two decades. Despite increased controls during a breakdown, many breakdowns recur within a short period of time; nationally approximately 23% recur within 12 months and 38% within 24 months. Recurrent breakdowns can have a damaging effect on the well being of individual farmers and are demanding on government resources for repeated testing and compensation (paid to farmers for slaughtered animals). Even more, a recent report of zoonosis in Great Britain, concluded that 18 of 35 strains isolated from humans had genotypes associated with cattle in the UK and that this was suggestive of domestic acquisition of disease.

Bovine TB can affect humans but today the risks are considered to be very low due to routine testing and slaughtering of cattle and the pasteurization of milk. Although the risk is small, it should not be ignored, particularly in developing countries. However, in developed countries exposure to unpasteurized dairy products imported from countries where *M. bovis* is prevalent is currently recognized as prevalent problem, especially when the world is becamingmore globalized and food importation and trade is routine.

In this context, another issue is the identification of the problem raised. The reporting of human TB in many countries generally does not distinguish between TB cases caused by different *Mycobacterium* species, particularly in developing countries, where more specific diagnostic techniques are not available and in most cases diagnosis only reaches *M. tuberculosis* complex infection. It has also been suggested that owing to a lack of laboratory facilities in many regions, prevalence of human TB due to *M. bovis* in most tropical countries is likewise unknown. *Mycobacterium bovis* infection surveillance in many countries is not regularly done and in other cases is not accurate. Food quality control is not assessed and done in most places, not necessarily for acute foodborne diseases such as zoonotic TB. Even more, the possible presence of *M. tuberculosis* infection in cattle is an issue to be considered while designing monitoring and surveillance systems. The inespecific diagnosis of *M. bovis* in developing countries together with the fact that a considerable proportion of extrapulmonary cases can be caused by *M. bovis*, given its access to the body through foodborne transmission, can lead to the thinking that cases of apparent human extrapulmonary TB, particularly in rural areas where cattle is largely present, can in fact be caused by *M. bovis*. More epidemiological studies are needed to address this question.

Cumulative data from the period 1997 to 2003 show that 31% of the reported human cases caused by *M. bovis* in the EU were found in the UK; that is, one of the EU member states with a higher reported prevalence of bovine TB.

Inspite of this, information on human disease due to *M. bovis* shown in developed and developing countries is scarce. From a review of a number of zoonotic TB studies, published between 1954 and 1970 and carried out in various countries around the world, it was estimated that the proportion of human cases due to *M. bovis* accounted for 3.1% of all forms of TB: 2.1% of pulmonary forms and 9.4% of extrapulmonary forms.

Human disease caused by *M. bovis* has been confirmed in African countries. In an investigation by two Egyptian health centers, the proportions of sputum-positive TB patients infected with *M. bovis*, recorded during three observations, were 0.4%, 6.4%, and 5.4%. In another study in Egypt, 9 of 20 randomly selected patients with TB peritonitis were infected with *M. bovis*, and the remaining with *M. tuberculosis*.

Isolation of *M. bovis* from sputum samples of patients with pulmonary TB has also been reported from Nigeria. Of 102 *M. tuberculosis* complex isolates, 4 (3.9%) were *M. bovis*. Another study in Nigeria reported that 1 of 10 mycobacteria isolated from sputum-positive cultures was *M. bovis*.

In a Zaire study, *M. bovis* was isolated from gastric secretions in 2 of 5 patients with pulmonary TB. In the same study, the prevalence of the disease in local cattle was approximately 8% by tuberculin testing and isolation of *M. bovis*.

In a recent investigation in Tanzania, 7 of 19 lymph node biopsies from suspected extrapulmonary TB patients were infected with *M. tuberculosis* and 4 with *M. bovis*. No mycobacteria were cultured from the remaining 8. Although the number of samples was low, the high proportion (21%) of *M. bovis* isolates is of serious concern.

In Latin America, a conservative estimate would be that 2% of the total pulmonary TB cases and 8% of extrapulmonary TB

cases are caused by *M. bovis*. These cases would therefore account for 7000 new TB cases per year, a rate of nearly 2 per 100 000 inhabitants. From a nationwide study in Argentina during 1982–84, 36 (0.47%) of 7672 mycobacteria cultured from sputum samples were *M. bovis*. However, in another study in Santa Fe province (where most of the dairy cattle industry is concentrated) during 1984–89, *M. bovis* caused 0.7–6.2% of TB cases.

Very limited data on the zoonotic aspects of *M. bovis* are available from Asian countries. However, cases of TB caused by *M. bovis* were not reported in early investigations in India.

Mycobacterium bovis has been classically assumed, dogmatically, that it is less virulent than *M. tuberculosis* in humans and is rarely transmitted from person to person. However, recent literature indicates that *M. bovis* can produce severe disease.

Clinical Presentation of Foodborne TB

The clinical presentation of tuberculosis due to *M. bovis* depends on the route of transmission. In general, zoonotic TB due to *M. bovis* is indistinguishable clinically or pathologically from TB caused by *M. tuberculosis*. Foodborne disease, acquired through contaminated milk or related diary products from infected cattle usually leads to cervical or mesenteric lymphadenopathy and others forms of extrapulmonary disease. Aerogenous infection from cattle or humans leads to pulmonary TB. Pulmonary bovine TB is clinically, radiologically, and pathologically indistinguishable from TB caused by *M. tuberculosis*.

A high index of clinical suspicion is needed in symptomatic patients with a history of possible exposure. At risk groups include animal workers, farmers, meat packers, vets, and zoo keepers.

In immunocompromised subjects, particularly those infected with the human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome, risk of disease is increased. HIV infection can exacerbate the risk following infection from cattle causing more disseminated disease than in those not immunocompromised. The mycobacteria isolated in these cases can turn out to be resistant to rifampin and pyrazinamide.

In general, human TB due to *M. bovis* can certainly be as severe as that due to *M. tuberculosis.* Recent data from San Diego, CA, USA, revealed that persons with *M. bovis* were 2.55 times as likely to die during treatment than those with *M. tuberculosis*.

Diagnosis of *M. bovis*

Diagnosis of *M. bovis* will depend on the availability of diagnostic techniques. Conventional methods for diagnosis and epidemiological studies of bovine TB and other mycobacterial diseases are far from ideal. In the last two decades, molecular biology has provided new approaches which have enabled detailed studies to be made of the molecular characteristics of *M. bovis*. In most developed countries final confirmation of this agent would be made using molecular techniques as PCR and restriction fragment analysis of genomic deoxyribonucleic acid (DNA) from isolates of *M. bovis*.

Human contact tracing includes clinical examination, chest X-ray and Mantoux test or purified protein derivative (PPD). A gamma interferon release assays tests (such as QuantiFERON-TB gold® or T-Spot®) can be utilized in some cases. In developing countries, the intradermal delayed type hypersensitivity (DTH) skin test, using PPD from culture of *M. bovis* (PPD-B) or *M. avium* (PPD-A), is the most frequently used test for diagnosis of TB or detection of *M. bovis* infection in cattle. Many improvements have been made to the original tuberculin test, and molecular approaches to identify and clone antigens may lead to improved specificity and sensitivity of DTH skin tests.

Advances in technology in the past decade have allowed the development of new *in vitro* techniques, such as antibody-based, cell-mediated immunity-based and nucleic acid-based diagnostics, which allow more rapid diagnosis than bacteriological culture. The choice of diagnostic technique should consider both the population being investigated (e.g., apparently healthy animals or a herd with a high prevalence of clinical infection) and aim of the testing (e.g., the screening of healthy animals or confirmation of infection in animals strongly suspected to be infected). Moreover, any evaluation of a diagnostic test must use a carefully selected control population which is representative of the population to be tested in terms of relative proportions of infected and noninfected animals.

Until recently, none of the *M. bovis* typing techniques permitted a satisfactory differentiation of isolates. During the past decade, the genome of pathogenic mycobacteria has been extensively studied, and phylogenetic analyses have shown that all (except *M. avium*) belong to a single genetic species: the *M. tuberculosis* complex.

Finally, as an approach to diagnosis in any case all contacts should be screened, including cattle and humans.

Treatment of *M. bovis*

The treatment of human bovine TB is the same as for the disease due to *M. tuberculosis*, although as *M. bovis* is naturally resistant to pyrazinamide, this agent is usually not recommended. However, susceptibility to other anti-TB drugs is usually similar to that of *M. tuberculosis*. Reported effective treatments include isoniazid, rifampicin, and ethambutol (for 2 months), followed by isoniazid and rifampicin for a further 7 months. Quinolones such as levofloxacin and ofloxacin have been also reported as helpful together with other anti-TB drugs.

The World Health Organization 2003 guidelines on the therapy of bovine TB have no specific recommendations. In practice, patients are treated with the standard course of anti-TB regimens, but pyrazinamide is omitted from the regimen. The American Thoracic Society recommends an initial 2-month regimen of isoniazid, rifampicin, and ethambutol followed by a 7-month continuation phase of isoniazid and rifampicin. For chemoprophylaxis this includes isoniazid and rifampicin for latent TB infection.

Vaccination and Prevention

Vaccination could potentially be used as a practical means of controlling bovine TB in countries in which a wildlife reservoir of the disease is present, and also in those countries which cannot afford conventional control strategies. The development of cattle vaccines would be the best option for long-term

control of TB. Protection of cattle against bovine TB by vaccination could be an important control strategy in countries where there is persistence of *M. bovis* infection in wildlife and in developing countries where it is not economical to implement a 'test and slaughter' control program. Early field trials with Bacille Calmette Guerin (BCG) *M. bovis* vaccine in cattle produced disappointing results, with induction of tuberculin skin-test reactivity following vaccination and low levels of protection. However, other studies using a low dose of BCG vaccine in cattle have produced more encouraging results and field trials.

In general, vaccination of cattle currently is not accepted in many countries because the vaccine could interfere with the skin reaction to the tuberculin test in the field (as happens in humans). Efficacy of *M. bovis* BCG in protecting bovine and other animal species against tuberculous infection has received considerable study. Vaccination of cattle prevents the spread of the disease in populations by reducing the number and size of the lesions, and the load of bacteria (rather than by preventing infection; very similar to what happens in humans, vaccine does not prevent infection but can reduce severe extrapulmonary forms).

A number of new TB vaccines including attenuated *M. bovis* strains, killed mycobacteria, protein, and DNA vaccines are under development and many are being assessed in cattle. Recent results have revealed several promising vaccine candidates and vaccination strategies. In cattle, neonatal vaccination with BCG appeared to be more effective than vaccination of 6-month-old calves and in most situations no other vaccine has been shown to be better than BCG. However, prime-boost strategies involving combinations of BCG with a protein or DNA vaccine, to improve on BCG vaccination alone, have produced very encouraging results.

Effective vaccination strategies would have a major impact in countries that cannot afford expensive test and slaughter-based control strategies. For these reasons there is a concerted international effort to evaluate vaccines for use in cattle populations and to define vaccination strategies which will eliminate the disease from infected herds. DNA, protein and genetically modified vaccines inoculated in a single dose, given as prime-boost or injected concurrently, will elicit significant protection against challenge with *M. bovis* under controlled conditions. However, vaccines and vaccination strategies require evaluation under field conditions in all epidemiological settings. Furthermore, complementary strategies are under development to differentiate immune responses that follow vaccination from those following disease.

In conclusion, recent studies in cattle and wildlife have demonstrated the practicality and effectiveness of vaccinating animals against TB and provide much impetus for future use of vaccines.

Conclusions

Bovine TB is still one of the largely neglected foodborne zoonotic diseases in the world, particularly in developing countries. However its occurrence would be still important in many places. Thus, this zoonosis deserves further research and efforts to establish the real burden of disease in animals as well in humans. Developing strategies of interaction between academia and health care public sectors, including medical and veterinary disciplines, would generate more accurate data. Even more, as human TB due to *M. tuberculosis* is still a public health concern internationally, in developing countries where diagnosis is usually not fully based on molecular and more specific diagnostic techniques, other than microscopy examination for acid-fast bacilli in sputum and other biological samples as well as culture, *M. bovis* would be causing many of *M. tuberculosis*-attributed human disease, particularly in the case of extrapulmonary forms of disease, even more in rural areas where both diseases can overlap and where human–cattle contact as consumption of raw milk and dairy products contaminated with *M. bovis* would be considerable. Finally, zoonosis control programs and human TB control programs, specially in those settings, should consider the importance of *M. bovis* and begin to introduce operational research into their activities as well surveillance to control and prevent disease from this zoonotic agent.

See also: Food Safety Assurance Systems: Microbiological Testing, Sampling Plans, and Microbiological Criteria. Institutions Involved in Food Safety: Trust in Animals and Food Safety (TAFS) Forum; World Health Organization (WHO). Public Health Measures: Surveillance of Foodborne Diseases. Safety of Food and Beverages: Dairy Products: Cheese

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BACTERIA

Pasteurella multocida

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Glossary

Adjuvant An adjuvant is an agent that may stimulate the immune system and increase the response to a vaccine without having any specific antigenic effect on itself.
Enzyme-linked immunosorbent assay An immunoassay that uses an enzyme linked to an antigen or antibody as a marker for the demonstration of a specific protein, generally an antibody or antigen.
Gel diffusion precipitin test Precipitin test made in a layer of agar that permits radial diffusion, in both the horizontal dimensions, of one or both reactants. Double (gel) diffusion in two dimensions (Ouchterlony test) incorporates antigen and antibody solutions placed in separate wells in a sheet of plain agar, permitting radial diffusion of both reactants; this method is widely used to determine antigenic relationships; the bands of precipitate that form where the reactants meet in optimal concentration are of three patterns, referred as reaction of identity, reaction of partial identity (cross-reaction), and reaction of nonidentity.
Mitogen A mitogen is a chemical substance that encourages a cell to commence cell division, triggering mitosis. A mitogen is usually some form of a protein.
Opportunistic pathogen An organism that exists harmlessly as part of the normal body environment and does not become a health threat until the body's immune system fails. The host's resistance may be lowered, for example, by other diseases or drugs.
Polymerase chain reaction A molecular technique for amplifying a single or a few copies of a piece of deoxyribonucleic acid (DNA) by separating the DNA into two strands and incubating it with oligonucleotide primers and DNA polymerase to generate millions of copies of that particular DNA sequence.
Septicemia Pathogenic bacteria in the bloodstream.
Zoonosis Zoonosis, also called zoonotic disease refers to diseases that can be passed from animals, whether wild or domesticated, to humans.

Background

Pasteurella multocida is the type species of the genus *Pasteurella* that was named in honor of Louis Pasteur who first recognized the significance of *P. multocida* in the etiology of fowl cholera (FC) and performed successful vaccination studies with the agent during the 1880s. *Pasteurella multocida* is known to be a widespread veterinary pathogen and also has the potential to cause zoonotic infections in humans. As a primary infectious agent, it plays the principal role in inducing FC in poultry, hemorrhagic septicemia (HS) in cattle and buffaloes, atrophic rhinitis (AR) in swine, and snuffles in rabbits. As a secondary invader, it is associated with pneumonia in swine and ruminants, with respiratory tract diseases in rodents, and with sporadic human infections resulting generally from injuries caused by household pets.

Characteristics of the Organism

Pasteurella multocida is a Gram-negative, nonmotile, nonspore forming, coccoid or rod-shaped facultative anaerobic bacterium. Cells usually measure $0.2–0.4 \times 0.6–2.5\ \mu m$ but after repeated subculturing they tend to be converted into pleomorphic forms. The bacterium is surrounded by a polysaccharide capsule that may be lost during *in vitro* maintenance.

Pasteurella multocida displays considerable phenotypic and genotypic variation. Three subspecies (subsp.) are distinguished by DNA–DNA hybridization and by their carbohydrate fermentation pattern, respectively: *P. multocida* subsp., subsp. *septica*, and subsp. *gallicida* although in recent times subsp. *P. multocida* and *gallicida* have been shown to belong to only one distinct phylogenetic lineage and thus their distinction has become questionable.

Based on the polysaccharide antigens of the capsule, *P. multocida* strains can be classified into five capsular serogroups (A, B, D, E and F) with the application of an indirect hemagglutination assay. This traditional technique is increasingly being replaced with a highly specific multiplex capsular polymerase chain reaction (PCR) assay. Comparing the somatic (cell wall) antigens, the commonly used Heddleston typing system distinguishes 16 *P. multocida* serotypes by recognizing heat stable lipopolysaccharide (LPS) antigens in a gel diffusion precipitin test.

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Pasteurella multocida strains show considerable diversity in fermentation of different carbohydrates and on this basis 14 biovars can be differentiated at this time.

Promising efforts are currently in progress to develop advanced molecular typing methods for more specific characterization of *P. multocida* isolates. This will be a prerequisite for a better understanding of the epidemiology of *P. multocida* caused diseases.

Epidemiology

Pasteurella multocida is a natural inhabitant of the oropharyngeal and gastrointestinal floras of a wide range of vertebrates, including terrestrial and aquatic mammals and birds. It can survive in the upper part of the respiratory tract of apparently healthy animals, and these carriers are believed to serve as the main spreading sources of the bacteria. Transmission is most common via contaminated saliva, various excretions, or aerosol droplets. Contaminated animal housing equipment may also serve as potential sources of infection. It has been demonstrated that *P. multocida* can survive for a long time in organic materials and up to 1 year in water. Thus the inhalation of aerosolized *P. multocida*-contaminated water droplets may be another possible route of transmission. Moreover, it has been established that *P. multocida* may survive in free-living amoeba representing additional probable *P. multocida* reservoirs in the soil and water ecosystems.

Pasteurella multocida strains are usually restricted to one host species; however, cross infections between different species have also been described.

The importance of zoonotic human infections following companion animal attacks has increased recently. Animal bites create a significant public health problem, the vast majority of them being caused by dogs (85–90%) and less frequently by cats (5–10%). Nevertheless, infections occur more often after cat bites (50% incidence) than dog bites (10–15% incidence) that may be explained by the fact that, among host species, cats have the highest rate of oropharyngeal colonization by *P. multocida*. Although infections associated with cat and dog bite wounds are usually polymicrobial in nature, *P. multocida* is responsible for the highest number of such infections. Severe *P. multocida* infections may also occur in the absence of animal bites or scratches. In the majority of those cases, licking of intact or injured skin by pet animals are the most probable source of entry for the bacterium. Respiratory tract and intra-abdominal infections associated with possible inhalation of the microorganisms have also been reported.

As obvious from the above, human *P. multocida* infections are rare and generally appear to be linked to exposure to animals. There is no evidence that these bacteria pose a threat to the human food chain. Nevertheless, *P. multocida* has the ability to cause serious human infections. Coupled to that, the mitogenic effect of *P. multocida* toxin (PMT), whose gene is found in some human isolates of *P. multocida*, is a cause of concern; such strains are potentially carcinogenic. Therefore, it appears likely that *P. multocida* is a low risk to food safety, but this is a topic that should be kept under surveillance.

Pathogenesis

Pasteurella multocida can produce a broad spectrum of various pathological conditions in a great number of host species. This observation allows us to hypothesize that the pathogenesis of these diseases, beside certain mutual mechanisms, might show specific characteristics. Interestingly, with the exception of AR, our knowledge about the details of the pathogenesis of various diseases caused by *P. multocida* is very limited. There may be a number of reasons for the great variety in disease manifestations such as host adaptation of strains; differences in the production of special virulence factors; and a number of host factors such as ecology, anatomic characteristics, and inborn immunity.

A clear association has been recognized between capsule type and certain hosts and diseases. FC is generally caused by type A and F strains, whereas HS is produced only by types B and E. AR strains belong to type D and A. However, the molecular and cellular basis of these host and disease associations is still waiting clarification.

Pasteurella multocida produces several potential virulence determinants; nonetheless, their contribution to pathogenesis is generally poorly understood. In many cases the virulence of *P. multocida* will possibly be defined by cell-surface expressed components. The most important of these are the polysaccharide capsule and certain typical constituents of the outer membrane of the cell wall such as LPS, a limited number of major proteins, and several minor proteins. During infection, these polysaccharides might help the bacterium to avoid the innate immune mechanisms of the host, such as phagocytosis or complement-mediated killing. By contrast, as antigenic determinants, they stimulate the antibody production of the adaptive immune system. The exact role of the outer membrane proteins during the course of infection is unclear, but they may be the mediators of bacterial interaction with the host environment. They could modulate the cytokine production of innate immune cells of the host. Consequently, alteration in these surface structures may influence the immunogenicity of the strains by increasing antigenic variability and thus helping the bacterium to avoid the defense mechanisms of the host.

AR is a specific manifestation of pasteurellosis and is a typical example of diseases of polymicrobial etiology: *P. multocida* infection needs assistance by *Bordetella bronchiseptica* to establish itself on the intact nasal epithelial surface. Toxin production by *P. multocida* is the key factor in the complex etiology of AR in swine. Toxigenic strains carry the *tox*A gene that encodes a potent exotoxin called PMT. This toxin has the unusual properties of being a very potent mitogen with the capacity to drive proliferation in a number of cell types and in animal models. The *tox*A sequence has a low G–C content (35%) in contrast with the rest of the *P. multocida* genome, suggesting that the gene has a relatively recent origin, and indeed *tox*A is found on an inducible bacteriophage. This observation makes horizontal gene transfer probable and so the spread of the ability of PMT production among various *P. multocida* strains may be predicted.

The toxigenic status of human isolates of *P. multocida* is rarely examined. However, the limited data available would suggest that isolates found in infections linked to companion animals are rarely toxigenic, whereas those linked to exposure

to swine are more likely to be toxigenic. These latter infections mainly affect the respiratory tract, but some cases of wound infections have also been found. However, very little work has been carried out either on the role of PMT in the pathogenesis of human disease, or indeed on the human pathogenesis of nontoxigenic *P. multocida*.

Pasteurella multocida appears to be an opportunistic pathogen in humans; therefore the predisposing conditions decisively determine the prognosis and final outcome of the disease. *P. multocida* infected human wounds typically manifest as local skin or soft tissue infections at the site of the injury. Furthermore, in patients with serious underlying disorder or at advanced age systemic infections can develop that can be fatal.

Pasteurella multocida can cause meningitis via direct inoculation with an animal bite or scratch, by contamination from contiguous infected wounds after trauma or neurosurgery, or by extension from an adjacent infected site by retrograde spread through lymphatic or blood circulation.

Clinical Manifestation and Pathology

FC is an economically important widespread avian disease affecting all types of birds throughout the world. It may occur as peracute to acute septicemia, with high morbidity and mortality, or in chronic forms with localized infections of wattles, nasal sinuses, joints, ovaries, or other tissues. Torticollis may be seen when the meninges, middle ear, or the cranial bones are infected.

HS is a fatal systematic disease of ungulates causing great losses mostly in Asia and Africa, and is characterized by a rapid course; edematous swelling in the throat, pharyngeal, and brisket region; swollen and hemorrhagic lymph nodes; and the presence of subserous petechial hemorrhages on the visceral surfaces throughout the body.

In pigs, beside pneumonia, AR is an important disease resulting considerable economic losses in the affected herds. The major signs are twisting or shortening of the nose due to turbinate atrophy and nasal septum deviation.

As a secondary invader, *P. multocida* frequently plays a major role in the progression to severe form of pleuropneumonia in swine and ruminants. The lesions caused by *P. multocida*, added to those induced by the primary infection, are usually an acute or subacute fibrinous to fibrinopurulent bronchopneumonia, fibrinous pleuritis with adhesion, pericarditis and lobular consolidation.

Wound infections in humans caused by *P. multocida* due to animal bites, scratches, or licks most commonly lead to local cellulitis or abscess formation but may also induce bone and joint infections, respiratory tract infections, intra-abdominal infections, endocarditis, meningitis, and even septicemia. In a typical infection, onset of pain, erythema, and swelling frequently occurs at the site of the lesion within hours of a bite. Pneumonia is the most common manifestation of respiratory tract infections by *P. multocida* accompanied by cough, fever, and pleuritic chest pain. Symptoms of *P. multocida* induced meningitis include headache, pyrexia, neck stiffness, vomiting, and altered level of consciousness.

Diagnosis and Analytical Methods

Early diagnosis is crucial, particularly in immunocompromised patients. Clinical manifestation is diverse and usually nonspecific. Conclusive diagnosis requires isolation and identification of *P. multocida*. Primary isolation from clinical samples is usually performed on enriched agar media supplemented with 5% bovine, horse, or sheep blood among aerobic conditions with the preferable addition of 5% CO_2. In the case of greatly contaminated specimens, the use of one of the several available selective media is highly recommended. Suspected colonies can further be classified with the use of commercially available automated identification systems, like API 20NE system (bioMerieux, Marcy l'Etolie, France), Vitek 2 system (bioMerieux, Marcy l'Etolie, France), and Biolog Microstation ID System (Biolog, Hayward, CA, USA), although it should be emphasized that these devices are not always able to distinguish *P. multocida* from some other closely related *Pasteurella* spp. This problem can be overcome by sequencing of 16S ribosomal ribonucleic acid genes that is increasingly applied as a rapid, accurate, and alternative microbiological technique to phenotypic methods of bacterial identification.

Production of PMT can be detected by its biological activity and immunological characteristics using an appropriate enzyme-linked immunosorbent assay, or the identification of the *tox*A gene by PCR.

Pasteurella multocida strains show high heterogeneity when biochemical characteristics, serotype, pathogenicity, and antibiotic sensitivity are examined. Nevertheless, these parameters do not provide enough details to understand the relationships of the strains. PCR based assays, especially the repetitive extragenic palindromic and enterobacterial repetitive intergenic consensus techniques specific to conserved repetitive sequences have high discriminatory power and provide reproducible results. These methods have proved to be useful tools in epidemiological studies on *P. multocida* isolates originating from various host species.

Control and Preventive Measures

Human infections often occur in the elderly and are difficult to avoid. If animal bites occur, rapid diagnosis is required to enable the immediate and adequate medical care of the injuries.

Accurate information on the susceptibility to antibiotics is essential for successful treatment of bacterial infections. *Pasteurella multocida* is almost always sensitive to penicillin and ampicillin, thus these antibiotics appear to be the most appropriate choices. However, penicillin resistant isolates have also been reported and therefore susceptibility testing must constantly be performed. Third generation cephalosporins have also been used successfully, often in combination with benzylpenicillin and cefotaxime. If there is an obvious history of allergy to β-lactam antibiotics, chloramphenicol may be proposed as an alternative treatment. The optimal duration of antibiotic treatment is not well established and each case has to be evaluated individually. Usually, 2 weeks of treatment is

appropriate for most patients but may need to be extended depending on the clinical response.

In the case of food producing animals, vaccination is the preferable option for preventing *P. multocida* caused diseases as the intensive use of antibiotics significantly increases the hazard of the selection of resistant bacterium variants that may lead to severe public health concerns.

HS: Vaccination is the principal means of prevention. Oil-adjuvanted inactivated whole-cell vaccines have appeared to be the most effective. Success of live vaccine has also been reported.

AR: Combination of *B. bronchiseptica* and *P. multocida* killed vaccine with a satisfactory amount of inactivated PMT content provides good results against the clinical manifestation of the disease and may help the eradication of the toxigenic *P. multocida* from the herd, at least over time.

FC: Adjuvanted polyvalent killed vaccines are widely used and generally provide satisfactory protection. Autogenous bacterins are recommended when polyvalent bacterins are ineffective.

No *P. multocida* vaccine is available for human use.

See also: Analytical Methods: Transmissible Spongiform Encephalopathy Diagnosis. Veterinary Drugs Residues: Antibacterials

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Plesiomonas shigelloides

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Glossary

Aeromonas A genus consisting of Gram-negative, polarly flagellated bacterial rods, facultatively anaerobic, bacterial species, members of which produce acid and gas from the fermentation of glucose.

Bacteremia Bacteria in the blood.

Choloangitis An infection of the common bile duct, the tube that carries bile from the liver to the gallbladder and intestines.

Cryptosporidium parvum A protozoan species that causes cryptosporidiosis, a parasitic disease of the mammalian intestinal tract resulting in acute, watery, and nonbloody diarrhea.

Diabetes mellitus A group of metabolic diseases in which a person has high blood sugar, because either the body does not produce enough insulin, or cells do not respond to the insulin that is produced.

Enterotoxin A protein toxin released by a microorganism in the intestine causing diarrhea associated with food poisoning.

Extraintestinal Situated or occurring outside the intestines.

Gastroenteritis Inflammation of the gastrointestinal tract, involving both the stomach and small intestine and resulting in acute diarrhea. Can be due to an infectious virus or bacteria.

Gram-negative Gram-negative bacteria do not retain the dye crystal violet in the Gram stain procedure when alcohol is applied. A red counterstain (safranin) is added after the decolorizing, alcohol step, staining all Gram-negative bacteria red. In contrast, Gram-positive bacteria retain the crystal violet and appear violet under the microscope.

gyrB Deoxyribonucleic acid (DNA) gyrase gene that encodes DNA gyrase which unwinds bacterial double helix DNA allowing DNA replication.

***β*-Hemolytic** The ability of the colonies of certain bacteria to induce complete lysis (hemolysis) of red blood cells when grown on blood agar resulting in clear zones surrounding the colonies.

Holecystitis Inflammation of the gallbladder that occurs most commonly because of an obstruction of the cystic duct by gall stones.

Immunocompromising A state in which the immune system's ability to fight infectious disease is compromised or entirely absent.

Liver cirrhosis A consequence of chronic liver disease characterized by replacement of liver tissue by fibrotic scar tissue leading to loss of liver function. Cirrhosis is most commonly caused by alcoholism, hepatitis B and C, and fatty liver disease.

Meningitis Life threatening inflammation owing to infection of the protective membranes covering the brain and spinal cord, known collectively as the meninges.

Osteomyelitis Bacterial infection of the bone marrow.

Plesiomonas shigelloides A Gram-negative, polarly flagellated rod, facultatively anaerobic, enteropathogenic bacterium, sensitive to the vibriostatic agent O/129, which produce acid but no gas from the fermentation of glucose.

Primary hemochromatosis Hereditary iron overload of the body.

Proteus A genus consisting of Gram-negative, facultatively anaerobic, peritrichously flagellated, bacterial rods.

Pseudomonas A genus consisting of Gram-negative, polarly flagellated rods, obligately aerobic, bacterial species.

Renal insufficiency Kidney failure.

Sepsis A pathological state, resulting from the presence of microorganisms or their toxins in the blood or in other tissue of the body.

Serotyping Distinguishing microbial isolates of the same species on the basis of antibodies produced against antigens such as the cell wall and/or flagella.

Shigella A genus consisting of Gram-negative, nonmotile rods, facultatively anaerobic, and enteropathogenic bacterial species.

Sickle-cell anemia A congenital form of anemia occurring mostly in African-Americans; characterized by abnormal red blood cells having a crescent or sickle shape, resulting in enhanced cell fragility, afflicting approximately 1 in every 500 African-American births and 1 in every 1000–1400 Hispanic-American births.

Splenectopy Surgical excision of the spleen.

Vibrio A genus consisting of Gram-negative, polarly flagellated curved bacterial rods, facultatively anaerobic, sensitive to the vibriostatic agent O/129, and whose species produce acid but no gas from the fermentation of glucose.

Vibrionaceae A bacterial family consisting of Gram-negative bacterial genera, polarly flagellated, and facultatively anaerobic.

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Introduction

Plesiomonas shigelloides is a Gram-negative polarly flagellated rod native to aquatic animals and environments. The organism has been ranked third as a cause of travelers diarrhea in Asia. Mild to severe self-limiting diarrhea is the most frequent symptom derived primarily from uncooked shellfish although extra-intestinal infections of high mortality are known to occur, particularly among children and immunocompromised individuals. The organism produces a cholera-like (CL) enterotoxin, a thermostable (TS) and a thermolabile (TL) enterotoxin. A large plasmid (>120 mDa) has also been found to facilitate invasion. Methodology has been developed for the direct polymerase chain reaction (PCR) detection of as few as 60 CFU of *P. shigelloides* per gram of shellfish tissue.

Characteristics of the Organism

Plesiomonas shigelloides is a unique Gram-negative polarly flagellated pathogenic bacterium native to aquatic animals and environments. The genus *Plesiomonas* consists of a single homogeneous species. Its metabolism is similar to that of the genus *Vibrio* in that sugars are fermented with acid production but no gas. 5S ribosomal deoxyribonucleic acid (rDNA) sequencing has indicated the organism to be closely related to the genus *Proteus*. Mild to severe diarrhea is the major symptom although extraintestinal infections of high mortality including septicemia and meningitis are known to occur, particularly in immunocompromised individuals. The utilization of inositol with acid production is a unique characteristic of the organism that is exploited with several agar media developed for its selective and differential isolation. The organism is β-hemolytic and produces a CL enterotoxin in addition to a thermostable (TS) and a thermolabile labile (LT) enterotoxin. A large plasmid (>120 mDa) has also been found to facilitate invasion.

Nomenclature and Taxonomy

Plesiomonas shigelloides was first described in 1947 and was indicated to have certain properties in common with *Shigella*. The initial strain possessed the major somatic antigen of *Shigella sonnei* but differed biochemically. This strain, designated C27 and similar isolates were subsequently placed chronologically into the genera *Pseudomonas*, *Aeromonas*, and *Vibrio*). The genus *Plesiomonas* was initially established in 1962 in the family Vibrionaceae based on a number of its unique features and its similarity to the genus *Aeromonas* ('plesio,' neighbor, 'monas,' *Aeromonas*). In common with other members of the family Vibrionaceae, it is polarly flagellated, facultatively anaerobic, and cytochrome oxidase positive. In addition, isolates have in common with members of the genus *Vibrio* sensitivity to the vibriostatic agent O/129. 5S rDNA sequencing has indicated the organism to be closely related to the genus *Proteus*.

Physiological and Biochemical Characteristics

The minimum temperature range for growth is 8–10 °C and maximum is 42–45 °C depending on the specific strain. Most strains do not grow below 8 °C, however, one strain has been reported to grow at 0 °C. Most strains have an optimum growth temperature between 35 and 38 °C. A temperature of 42–44 °C is recommended for isolation to eliminate aeromonads. Although most isolates are able to grow from a pH of 4.0–9.0, strains have been reported to be killed rapidly at a pH of 4.0 and below. Most isolates exhibit growth from 2.0% to 3.0% NaCl. Some strains have been found to grow in 5.0% NaCl. NaCl however is not an absolute requirement for growth.

Identification of *P. shigelloides* is biochemically based on the organism being cytochrome oxidase positive, lysine and ornithine decaboxylase positive, and arginine dihydrolase positive, with production of acid from inositol (Table 1) and the fermentation of sugars without gas production.

Table 1 Metabolic characteristics of *Plesiomonas shigelloides*

Metabolic property	*% of strains positive*
Phenylalanine deaminase	0–4
Arginine dihydrolase	100
Lysine decarboxylase	100
Ornithine decarboxylase	100
Cytochrome oxidase	100
NO_3 reduction to NO_2	100
Indole production from tryptophan	100
Methyl red test	90–100
Motility (36 °C)	92–95
DNase	0–100
β-Galactosidase	90–99
Gelatinase	0
β-Glucosidase (aesculine hydrolysis)	0
Hydrogen sulfide	0
Urease	0
Voges-Proskauer test	0
Citrate utilization	0
Fermentation of:	
Adonitol	0
L-Arabinose	0
Cellobiose	0
Dulcitol	0
Erythritol	0
Glucose	100
Glycerol	35–66
Lactose	76–81
Maltose	100
Mannitol	0
D-Mannose	70–95
Melibiose	70–95
α-Methyl-D-glucoside	0
Mucate	0
Inositol	95–100
Raffinose	0
Rhamnose	0
Sorbitol	0
Sucrose	0–5
Trehalose	95–100
D-Xylose	0

Ecological Distribution

The primary habitats of *P. shigelloides* are freshwater ecosystems (rivers, lakes, and surface waters) and marine

Table 2 Agar media for selective isolation of *Plesiomonas shigelloides*

Inositol brilliant green bile salt agar[a]		Plesiomonas *agar*[b]		Plesiomonas *differential agar*[c]	
	$g\ l^{-1}$		$g\ l^{-1}$		$g\ l^{-1}$
Peptone	10.0	Peptone	1.0	Peptone	7.5
Beef extract	5.0	NaCl	5.0	Beef extract	7.5
NaCl	5.0	Yeast extract	2.0	NaCl	5.0
Bile salt mixture	8.5	Mannitol	7.5	Meso-inositol	10.0
Brilliant green	0.00033	Arabinose	5.0	Bile salt mixture	8.5
Neutral red	0.025	Inositol	1.0	Brilliant green	0.00033
Meso-inositol	10.0	Lysine	2.0	Neutral red	0.025
Agar	13.5	Bike salts No. 3	1.0	Agar	13.5
pH 7.2, 42 and 44 °C for 48 h		Phenol red	0.08	pH 7.4, 44 °C for 24 h	
		Agar	15.0		
		pH 7.4, 42 °C for 24 h			

[a]Schubert RHW (1977) Ueber den Nachweis von *Plesiomonas shigelloides* Habs und Schubert, 1962, und ein Lectivemedium, den Inositol-Brillantgrün-Gallesalz-Agar. *Ernst Rodenwaldt Archiv* 4: 97–103.
[b]Miller ML and Koburger JA (1985) *Plesiomonas shigelloides:* An opportunistic food and water borne pathogen. *Journal of Food Protection* 48: 449–457.
[c]Huq A, Akhtar A, Chowdbury M and Sack D (1991) Optimum growth temperature for the isolation of *Plesiomonas shigelloides* using various selective and differential agars. *Canadian Journal of Microbiology* 37: 800–802.

estuaries in tropical climates. It is rarely isolated in temperate and cold climates. *Plesiomonas shigelloides* occurs as free-living cells in aquatic systems, including fish, crabs, shrimp, mussels, and oysters. The organism has been isolated from soil and a number of terrestrial animals (amphibians, monkeys, birds, polecats, and reptiles) in addition to domestic animals (sheep, swine, cattle, cats, dogs, and goats). *Plesiomonas shigelloides* has also been reported to occur at a low incidence ($\sim$0.008%) in healthy humans. Uncooked oysters are the major food incriminated in outbreaks in the US.

Toxins and Invasive Factors

Plesiomonas shigelloides produces a CL enterotoxin, a TS and a TL enterotoxin. A large plasmid ($>$120 mDa) has also been found to facilitate invasion.

β-Hemolysis

Although several reports have indicated the absence of β-hemolysin production, an agar overlay system has been found to yield β-hemolysis. More than 90% of isolates have been reported to produce a cell associated β-hemolysin detected with agar overlay and contact hemolysis assays. The observation that surface colonies fail to yield hemolysis is of particular significance, and suggests that either oxygen tension or viscosity may influence β-hemolysin production or its release from cells. Hemolysis has been found to depend on the medium and method used for cultivation of the organism, which may explain the negative results obtained by several authors.

Isolation

Enteric agars were first used for the isolation and identification of *P. shigelliodes* but are not ideal. Growth of most isolates of *P. shigelloides* is obtained on MacConkey, *Salmonella–Shigella*, desoxycholate, Hektoen enteric, and xylose lysine desoxycholate agars, however, some strains may be inhibited. The first selective medium for the specific isolation of *P. shigelloides* was developed in 1977 and designated inositol brilliant green bile salts (IBB) agar (Table 2). Its differential property is based on the fermentation of inositol and selective ability on bile salts. IBB agar has been found to be highly effective for selective isolation of *P. shigelloides* from human feces following enrichment in alkaline peptone water. *Plesiomonas* (PL) agar (Table 1) was developed in 1985 and contains the nonfermented carbohydrates mannitol and arabinose at levels of 0.75% and 0.5% respectively. The fermentable carbohydrate inositol is present at a critically low level of 0.1% along with 0.2% lysine and notably low level of 0.1% bile salts No. 3. The initial pH is adjusted to 7.4 before autoclaving. Contaminating colonies fermenting mannitol and arabinose will be red. Fermentation of the low level of inositol and decarboxylation of lysine will result in a near neutral pH so that typical colonies of *P. shigelloides* are pink. With the plating of aquatic samples, both IBB and PL agars should be used simultaneously at 35 °C for maximum recovery. IBB was found to have a greater recovery rate and PL agar is less inhibitory to injured cells. *Plesiomonas* differential agar (PDA) was developed in 1991 (Table 2) and is a modified *Salmonella–Shigella* agar in which lactose is replaced with inositol. PDA at 44 °C and with a 24 h incubation period has been found to be a suitable medium for isolating *P. shigelloides* from water samples and possibly also clinical samples where the presence of aeromonads poses a problem.

Serology

Serotyping of *P. shigelloides* isolates is based on the detection of O (somatic) and H (flagellar) antigens. The first antigenic typing scheme for *P.* shigelloides established in 1978, was based on 30 O antigenic groups and 11 H antigens. Subsequent studies indicated more than 100 serovars. One of the

most frequently encountered serovars of *P. shigelloides* is 017, which is involved with protection against shigellosis due to *S. sonnei* via a common lipopolysaccharide in the cell walls of both species.

Epidemiology and Outbreaks

A total of 20–50% of persons traveling abroad suffer from diarrhea usually within their first week of travel. Many of these infected individuals report to have consumed seafood or uncooked food in the 2 weeks before the onset of diarrhea. One of the major agents causing travelers diarrhea appears to be *P. shigelloides* which has been ranked third as a cause of travelers diarrhea in various areas of Asia including China and Japan.

A total of 23% of patients suffer from underlying immunocompromising conditions such as diabetes mellitus, renal insufficiency, liver cirrhosis, bowel disease, pregnancy, cancer, primary hemochromatosis (excess deposition of iron throughout the body), splenectopy, or sickle-cell anemia. Most of these predispositional factors suggest that the availability of iron may be a major factor preventing or limiting infections in healthy individuals due to *P. shigelloides*.

The first outbreak of gastroenteritis due to *P. shigelloides* occurred in Japan in 1963 and was due to contaminated cuttlefish salad involving 275 cases of diarrheal infection out of 870 individuals who consumed the salad. Salted mackerel resulted in an outbreak in 1966 in Japan involving 53 cases. Subsequent outbreaks in Japan have involved water-borne diarrhea affecting 978 out of 2141 persons.. Uncooked shellfish is most frequently implicated in cases involving seafood.

Plesiomonas shigelloides is also responsible for a variety of extraintestinal infections, particularly among children and immunocompromised individuals with underlying maladies such as holecystitis and choloangitis, bacteremia and sepsis, arthritis, and polyarthritis, osteomyelitis, neonatal meningitis, and pneumonia. The severity of a generalized *P. shigelloides* infection is reflected by the fact that 1978 to 2010, 8 out of 12 neonatal patients developing bacteremia globally succumbed, even after several of the 8 received appropriate antibiotic treatment.

A suspected factor of predisposition to infection by *P. shigelloides* is coinfection by other intestinal pathogens. Results from monoinfection of BALB/c mice with *P. shigelloides* revealed long-term colonization of the neonatal mouse intestine, along with associated pathological lesions. The effects of coinfection of *P. shigelloides* with *Cryptosporidium parvum* were characterized by bacteremia and heavy colonization of the intestine by *P. shigelloides*. In addition, necrotizing inflammatory changes in the ileum and colon were accompanied by diarrhea and deaths of infected mice. In contrast, the results from monoinfections of neonatal mice with the *Aeromonas* spp. exhibited only short-term colonization of the intestine by these pathogens. However, when mice were coinfected with *Aeromonas hydrophila* and *C. parvum*, growth of the bacterial species was prolonged with no clinical or histopathological changes observed in the mice. A detailed study of *P. shigelloides* associated with gastroeneritis in Ecuador from 2004 to 2008 indicated little evidence that single infection with *P. shigelloides* causes diarrhea but stronger evidence indicated that coinfection with rotavirus causes diarrhea. One study from Germany indicated that from the years 2000 to 2001 *P. shigelloides* was found to cause gastroenteritis 6 times in Ludwigshafen, Germany. Not all of these patients reported a trip to foreign countries or consumption of seafood.

Most isolates of *P. shigelloides* are resistant to ampicillin and mezlocillin. Successful antibiotic therapy of neonates has usually involved the administration of cefotaxime or meropenum.

Application of the PCR

Conventional PCR

PCR assays for confirming the identity of *P. shigelloides* isolates and for the detection and quantitation of the organism in environmental samples have utilized primers that amplify sequences of the 23S rRNA gene, DNA gyrase gene (*gyrB*), and *hugA* gene that encode an outer membrane receptor HugA, required for heme iron utilization (Table 3).

Methodology has been developed for the direct PCR detection of as few as 60 CFU of *P. shigelloides* per gram of clam tissue. Various factors have been found to affect quantitative PCR assays of *P. shigelloides*. Different *Taq* polymerase preparations, varying sets of primers, different DNA stains, and different cell lysing agents have been found to significantly influence the linear relationship between the fluorescent intensities of DNA bands and log of CFU per PCR. Primer dimers formed in the PCR can be eliminated by using different *Taq* polymerase preparations and different sets of primers to run the PCR. In addition, the use of ethidium bromide monoazide has been successful in allowing the PCR to distinguish viable from nonviable cells of *P. shigelloides*. The use of two random primers (Table 2) with random amplified polymorphic DNA (RAPD) has been found effective in distinguishing a number of *P. shigelloides* isolates from diverse sources.

Real-Time PCR

The application of real-time PCR (Rti-PCR) to *P. shigelloides* has primarily employed SYBR green for the reporter molecule. Rti-PCR methodology has allowed the detection of 1×10^3 CFU of *P. shigelloides* per gram of oyster tissue, equivalent to the DNA from 25 CFU per Rti-PCR.

Control and Prevention of *P. shigelloides* Infections

As *P. shigelloides* gastrointestinal infections are frequently the result of the consumption of raw shellfish, and other forms of raw seafood, all seafoods should be adequately cooked to destroy the organism. As much as the organism is associated with fresh and marine aquatic habitats, water consumed directly and also used for food preparation, or rinsing of foods should always be of a potable nature, usually achieved by adequate chlorination. Control of *P. shigelloides* in food

Table 3 Polymerase chain reaction (PCR) primers and deoxyribonucleic acid (DNA) probes

Primer or probe	*Sequence (5′→3′)*	*Size of amplified sequence (bp's)*	*Gene or DNA target sequence*	*References*
PS23FW3	CTC-CGA-ATA-CCG-TAG-AGT-GCT-ATC-C	284	23S ribosomal DNA (rDNA)	González-Rey *et al.* (2000)
PS23RV3	CTC-CCC-TAG-CCC-AAT-AAC-ACC-TAA-A			
PSG237-F	TTC-CAG-TAC-GAG-ATC-CTG-GCT-AA	68	*gyrB*	Fuchushima and Tsunomori (2005)
PAG110R	ACC-GTC-ACG-GCG-GAT-TAC-T			
Forward	AGC-GCC-TCG-GAC-GAA-CAC-CTA	112	23S rDNA	Loh and Yap (2002)
Reverse	GTG-TCT-CCC-GGA-TAG-CAG			
Probe	LCRed640-GGT-AGA-GCA-CTG-TTA-AGG-CTA-GGG-GGT-CAT-C-P			
PS-F	GCA-GGT-TGA-AGG-TTG-GGT-AA	628	23S rDNA	Gu and Levin (2006a)
PS-R	TTG-AAC-AGG-AAC-CCT-TGG-TC			
RAPD: LMPB1	GGA-ACT-GCT-A			Gu *et al.* (2006) from Boerlin *et al.* (1995)
RapD: LMPB4 AAG-GAT-CAG-C				
Forward	GCC-AGC-GGG-AAG-GGA-AGA-ACC	435	*hugA*	Herrera *et al.* (2006)
Reverse	GTC-GCC-CCA-AAC-GCT-AAC-TCA			

products consists of adequate refrigeration in that most strains are unable to grow at refrigerated temperatures. Adequate refrigeration applies to slaughter, processing, shipping, and storage. As *P. shigelloides* is an intestinal organism, during evisceration of both warm blooded animals and fish species, the intestine should be removed intact so that no intestinal contents are placed in contact with the tissue to be consumed. After handling raw meat, shellfish, or other marine foods, one should thoroughly wash hands with soap or detergent to prevent cross-contamination of other foods, particularly those to be eaten raw such as fruits, vegetables, and salads. Raw meat or marine foods should never be stored in contact or on the same refrigerator shelf as raw fruits and vegetables. Food handlers with active gastrointestinal infections should not be allowed in contact with foods during preparation.

See also: Bacteria: *Shigella*. Characteristics of Foodborne Hazard and Diseases: Pathogenesis and Virulence. Disciplines Associated with Food Safety: Food Microbiology. Food Safety Assurance Systems: Food Safety and Quality Management Systems; Good Animal Husbandry Practice; Good Practices in Fisheries and Aquaculture. Food Technologies: Food Irradiation. Foodborne Diseases: Foodborne Diseases and Vulnerable Groups; Foodborne Diseases in Travelers; Overview of Biological Hazards and Foodborne Diseases; Overview of Emerging Food Technologies; Prevalence of Foodborne Diseases in Europe; Prevalence of Foodborne Diseases in North America; Prevalence of Foodborne Diseases in South East and Central Asia; Prevalence of Foodborne Diseases in Western Pacific Region; Prevalence of Foodborne Diseases in Africa. Public Health Measures: Challenges of Developing Countries in Management of Food Safety; Challenges of Industrialized Countries in Food Safety Management. Risk Analysis: Risk Assessment: Microbiological Hazards. Safety of Food and Beverages: Seafood

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Proteus

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Glossary

Dienes typing A method to distinguish *Proteus* strains based on Dienes' phenomenon that *Proteus* strains of the different Dienes types do not merge into each other but leave inhibition lines between strains on the solid medium.
Immunodeterminant A specific structure of an antigen recognized by antibodies or membrane receptors of B cells or T cells; also called antigenic determinant.
Swarming A process whereby bacteria leave colonies and migrate together.
Urease This enzyme hydrolyzes urea into carbon dioxide and ammonia. The urease serving as a pathogenic factor has been observed in many bacteria and fungi. The test of urease activity could diagnose the presence of pathogens.

Background

Proteus spp. are Gram-negative aerobic bacteria. Their size is 0.4–0.8 μm in diameter and 1.0–3.0 μm in length. They are named based on their ability to undergo morphological changes of colonies. With peritrichouse flagella, *Proteus* spp. are motile. Some characteristics of a *Proteus* culture are swarming and an ammonia smell. The *Proteus* habitat is widely distributed in the environment. As an opportunistic human pathogen, *Proteus* is found in the human and animal gastrointestinal tract, skin, and oral mucosa, as well as in feces, soil, water, and plant. *Proteus* causes food spoilage of raw meat, seafood, vegetables, and canned food. The detection of *Proteus* spp. indicates that affected food is not prepared in hygienic surroundings. In autumn, the rate of *Proteus* detection is higher. *Proteus* spp. do not form spores, and they can grow on most culture media and liquefied gelatin. When *Proteus* grows in milk, the milk curds, and then liquefies. *Proteus* strains are able to grow in temperatures ranging from 10 to 43 °C. The optimal temperature for *Proteus* is 25 °C. The swarming occurs between 20 and 37 °C. *Proteus* spp. decompose organic substances. They also oxidatively deaminate amino acids, hydrolyze urea, exhibit proteolytic activity, and produce hemagglutinins and hemolysins.

The genus *Proteus* belongs to the tribe Proteeae of family Enterobacteriaceae and includes five species: *Proteus vulgaris*, *Proteus mirabilis*, *Proteus penneri*, *Proteus myxofaciens*, and *Proteus hauseri*. Besides these, there are three unnamed *Proteus* genomospecies. *Proteus hauseri* and the three unnamed species were characterized as four new genomospecies from a biogroup that was considered as *P. vulgaris*. The three genomospecies left unnamed are due to the lack of phenotypical discrimination standards. *Proteus mirabilis* is the dominant *Proteus* spp. causing human infections. Approximately 25% people carry *P. mirabilis* in the intestine. There is also *P. vulgaris* or *P. penneri* infection in humans, which is mostly nosocomial infection. *Proteus myxofaciens*, isolated from the larvae of gypsy moths, is not considered to be an important species causing human infections.

The tribe Proteeae comprises three genera, *Proteus*, *Providencia*, and *Morganella*. The genus *Morganella* includes only one species, *Morganella morganii*, which was formerly *Proteus morganii*. Based on trehalose fermentation, *M. morganii* is divided into two subspecies, *M. morganii* subsp. *morganii* (trehalose-negative) and *M. morganii* subsp. *sibonii* (trehalose-positive). Another genus in the tribe Proteeae is the genus *Providencia*. All five *Providencia* spp. (*Providencia alcalifaciens*, *Providencia heimbachae*, *Providencia stuartii*, *Providencia rettgeri*, and *Providencia rustigianii*) have the phenylalanine-deaminating activity that *Proteus* spp. possess. Not all *Providencia* strains have urease activity.

Characteristics of the Organisms

The movement across a solid medium surface is not a unique characteristic of *Proteus* spp. The Gram-negative bacteria of *Serratia* spp. and *Vibrio* spp. and the Gram-positive bacteria of *Bacillus* spp. and *Clostridium* spp. also have this swarming phenomenon. During the process of swarming, the *Proteus* colonies display periodic cycles on a solid medium, and the appearance of bacteria cells becomes elongated and flagellated. *Proteus* appears as short rods in broth cultures with the size of approximately 0.6 μm width and 1–2 μm length. After being inoculated to suitable solid media, the cells enlarge to approximately 0.8 μm wide and 2–4 μm long, and then some peripheral cells of colonies gradually elongate to approximately 0.7 μm wide and 20–80 μm long. The morphological change is largely due to the change of flagellates. The shorter flagella become longer, whereas the number of flagella

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increases. The swarm cell has 50–500 times more flagella than that of original cells. With the facilitation of the abundant longer flagella, *Proteus* spp. more efficiently move outward from colonies. Besides flagella, some extracellular slimes produced by *Proteus* spp., such as cell surface polysaccharides, may also help the movement process by reducing friction. The swarming process includes three stages, which are differentiation, migration of bacterial mass, and consolidation. After the multicellular differentiation, the swarmers migrate away from the colonies. Concentric rings of the cell population form in the peripheral region of the colonies. The swarming continues until the cells spread out on the surface of the medium. Once the swarming stops, the long cells divide into several shorter form normal cells, which is called consolidation. The swarming occurs only in the solid media. When the swarmers are transferred to liquid media, they divide to the septa of shorter swimmers. The flagellar number reduces as well. Furthermore, the swarming is a population behavior. A single *Proteus* cell loses the swarming ability. The swarming of *Proteus* not only requires the mobility and differentiation ability but also coordinating multicellular behaviors. Mutations affecting multicellular coordination prevent the swarming of *Proteus*.

The morphological change of *Proteus* is correlated with the biochemical change. The number of nucleoids increases as the swarmers turn longer. Rather than producing lipopolysaccharides (LPS) with both long and short O-antigenic side chains, the long O-antigenic side chains are dominant type in the swarmers. There are also changes in the protein expression level, such as urease, metalloprotease, hemolysin, and flagellin. Approximately 40–60 genes are involved in the swarming process. There are genes controlling flagellar synthesis, rotation of the flagellar filament, synthesis of cell surface structures (LPS and peptidoglycan), cell division, etc.

The swarming is a disadvantage factor for strain isolation. The inhibition of swarming facilitates *Proteus* strain isolation. Because the swarming relies on the moisture of solid medium surfaces, a dryer medium surface inhibits swarming. The differentiation and swarming of *Proteus* display a glutamine-dependent pattern and can be inhibited by the glutamine analog, γ-glutamyl hydroxamate. Furthermore, the flagella of *Proteus* function as a sensor for the outside environment. Thus, antibodies against flagella also inhibit swarming.

The same *Proteus* strain swarms and coalesces. Different strains repel each other. Therefore, narrow buffer regions are left between swarming *Proteus* strains when two different types of *Proteus* strains are inoculated to a solid medium. This method of *Proteus* strain discrimination is called Dienes typing. The clear line between *Proteus* strains is named the Dienes demarcation line.

Clinical Manifestation, Pathogenesis, and Treatment

Proteus can cause gastroenteritis, urinary tract infections, and wound infections. The ingestion of food contaminated by *Proteus* may contribute to the sporadic and epidemic cases of gastroenteritis, which may cause symptoms such as vomiting, fever, abdominal pain, severe nausea, diarrhea, and dehydration. The incubation period is short, usually 1–3 days. The illness duration is approximately 40 h. Sometimes, blood can be found in patients' vomitus. *Proteus mirabilis* and *P. penneri* are often isolated from diarrheal fecal samples of gastroenteritis patients. The incidence rate of acute intestinal infection of *Proteus* is higher in young children as well as older and immunosuppressed persons, due to their low immunity. *Proteus* is thought to increase the pathogenicity of other microbes. When *Proteus* infection occurs together with other microbes, infant diarrhea is more severe. As a secondary pathogen, *P. vulgaris* has been frequently observed in coinfection with streptococci, staphylococci, *Bacillus coli*, *Bacillus lactis aerogenes*, *Bacillus welchii*, *Bacillus diphtheriae*, etc.

Infection by the genus *Providencia*, another member of the tribe of Proteeae, is rare. *Proteus alcalifaciens*, *P. heimbachae*, *P. rettgeri*, and *P. rustigianii* are usually related to gastroenteritis whereas *P. stuartii* is usually related to urinary infections. The *Providencia*-associated gastroenteritis leads to abdominal pain, vomiting and diarrhea. Some patients may have fever. Most case reports of the *Providencia*-associated gastroenteritis are related to fecal contamination. The common incubation period from the ingestion of contaminated food is 80–90 h. *Proteus alcalifaciens* has been identified as an enteric pathogen. Both *in vitro* cell invasion tests and animal models have proved the pathogenicity of *P. alcalifaciens*.

The third member of the tribe Proteeae is *Morganella* sp. The presence of common food spoiled by *M. morganii* is fish, including mackerel, marlin, mahi-mahi, tuna, and bluefish. Both *Proteus* spp. and *M. morganii* have the histidine dehydrogenase activity to produce histamine. The temperature of 15 °C is a critical point for histamine production of *M. morganii*. When the temperature is lower than 15 °C, the histamine production by *M. morganii* is significantly reduced. In general, *M. morganii* does not produce toxic concentration of histamine below 7 °C. The elevated level of histamine and the factors influencing histamine absorption synergistically lead to symptoms after the ingestion of spoiled food. The symptoms include headache, diarrhea, redness of the face and neck, a feeling of heat, itching, etc. The time elapsed between food intake and symptom onset ranges from minutes to 3 h. Usually, 100 mg dL^{-1} of histamine is the minimum level to cause symptoms, although 20 mg dL^{-1} of histamine may cause symptoms in some individuals. The histamine level in fresh fish is normally 1 mg dL^{-1}, and 50 mg dL^{-1} is the hazardous level. The cases of *M. morganii* outbreaks have been found associated with either raw fish or processed fish consumption. Therefore, cooking is not an effective way to eliminate the toxicity.

In terms of treatment, *Proteus* spp. have varied sensitivity and resistance to antibiotics. Most *Proteus* spp. are sensitive to penicillin, gentamicin, furagin, ciprofloxacin, levofloxacin, and nevigramone, but they are resistant to nitrofurantoin, tetracycline, bacitracin, cecropin, polymyxin, and colistin. The antibiotic resistance of *Proteus* spp. is transferred through plasmids encoding antibiotic-resistant genes. *Proteus* spp. have a high content of phosphate-linked 4-aminoarabinose in their LPS. The less acidic bacterial surface makes them inherently resistant to polycationic antibiotics, such as cecropin and polymyxin. Polymyxin B binds to the negatively charged lipid A portion of LPS. In *P. mirabilis*, L-arabinoso-4-amine substituting the ester-linked phosphate group of lipid A can lead to the resistance to polymyxin B. The first-choice antibiotic

against *P. mirabilis* is ampicillin and the alternative antibiotics are aminoglycoside and cephalosporin. Resistance to fluoroquinolones has been seen in *P. mirabilis* isolates. Most *P. mirabilis* strains are sensitive to ampicillin and cephalosporin, but *P. vulgaris* and *P. hauseri* are not sensitive to them. The first-choice antibiotics to treat *P. vulgaris* and *P. hauseri* are cefotaxime and ceftizoxime, and the alternative antibiotics are cefoxitin and trimethoprim (TMP)-sulfamethoxazole (SMX). In addition, *P. vulgaris* and *P. hauseri* are sensitive to ceftazidime, ceftriaxone, imipenem, ciprofloxacin, netilmicin, sulbactam, meropenem, and levofloxacin. In contrast to other *Proteus* spp., *P. penneri* is resistant to chloramphenicol. Therefore, combinations of antibiotics are more effective treatment against *Proteus*, such as gentamicin with carbenicillin, gentamicin with ampicillin, monomycin with ampicillin, Zosyn (piperacillin and tazobactam), and Unasyn (ampicillin and sulbactam).

Most *M. morganii* strains are resistant to penicillin and cephalosporin and are susceptible to aztreonam, aminoglycoside, and quinolone. *Providencia* is highly resistant to penicillin G, ampicillin, chloramphenicol, colistin, polymyxin B, nitrofurantoin, and nalidixic acid, but it is sensitive to aminoglycoside, quinolone, carbapenem, aztreonam, and modern cephalosporin.

Virulence Factors

Several virulence factors of *Proteus* have been identified. The presence of fimbriae is one of the virulence factors. The *Proteus* spp. adhere to surfaces using fimbriae. The adhesive ability of *Proteus* to intestinal epithelium has been proved. Based on the diameter, *Proteus* fimbriae are classified as the thicker type ($\sim$7 nm) and the thinner ($\sim$4 nm) type. The thicker fimbriae are resistant to mannose and named *Proteus*-like fimbriae. Their acronym is MR/P. The thinner fimbriae are also resistant to mannose and named *Klebsiella*-like fimbriae with the acronym of MR/K. The MR/K type is positive in tannic acid-treated erythrocyte agglutination test whereas the MR/P strain is positive in untreated erythrocyte agglutination test. Owing to the difference in abilities of adherence to epithelial cells, the MR/P and MR/K types exhibit different pathogenicities. For nosocomial infections, the MR/P type of *Proteus* enhances the risk of acute pyelonephritis because the MR/P fimbriae facilitate the strains to colonize in the upper urinary tract. In contrast, the MR/K type has a different tissue-binding pattern, which binds to the tubular basement membranes and glomerular capsules by MR/K hemadhesins. Besides MR/P and MR/K, there are other types of fimbriae, such as *P. mirabilis* fimbriae (PMF), ambient-temperature fimbriae (ATF), *P. mirabilis* P-like fimbriae, etc.

Other surface structures, such as outer membrane proteins and polysaccharides, also play a significant role in the *Proteus* virulence. Outer membrane proteins serve as mitogens for B cells. OmpA protein, one identified outer membrane protein, elevates the level of IgG O-specific antibodies. Immunization of mice with outer membrane proteins shows protective role in pyelonephritis. Another antigenically important surface structure is LPS. The diversity of the O-specific polysaccharides of LPS results in the antigenical heterogeneity of *Proteus*. One feature of the *Proteus* O-specific polysaccharides is the substitution of uronic acids by amino acids. The O-specific polysaccharides of *Proteus* are typical, including hexoses, hexosoamines, and uronic acids, and 6-deoxyamino sugars. The unusual components are lactic acid ethers, (R)-hydroxybutyryl, pyruvic, and phosphate groups. Most of the time, the unusual components are not immunodominant. N-acetyl-D-glucosamine linked to the β-D-GlcA-L-Lys and phosphoethanolamine linked to the β-D-Glc-NAc are immunodominant in the O-specific polysaccharides of *P. mirabilis*. An immunodeterminant of *P. mirabilis* O-specific polysaccharides is the substitution of D-galacturonyl-1,4-D-galactosamine disaccharide by lysine. Furthermore, the flagella and other cell surface components produced during the differentiation and swarming are antigenic and contribute to the *Proteus* virulence.

The *Proteus* enzymes and secretory components are another class of virulence factors. Firstly, the urease of *Proteus* is a virulence factor and contributes to its pathogenicity. The infection ability of the urease-defective mutant is approximately 1000-fold less than that of normal strains. Over 200 Gram-negative bacteria species have urease activity. The urease metabolizes urea into carbon dioxide and ammonia. The alkaline environment is more suitable for *Proteus* to survive in gastric acids. The ammonia produced by *Proteus* may result in gastric and peptic ulcers. Secondly, the proteolytic enzymes expressed by *Proteus* are associated with virulence. The production of proteolytic enzymes to degrade host immunoglobulins is one strategy of protecting *Proteus* from the attack of the host immune system. *Proteus* is capable of IgA protease synthesis. One characteristic of *P. mirabilis* IgA proteases and some *P. vulgaris* IgA proteases is that the protease cleaves IgA heavy chain at a site different from the cleavage site of other microbial IgA proteases. There is also an extracellular proteolytic enzyme produced by *P. mirabilis* cleaving IgA, IgG, and some non-immunoglobulin proteins. Thirdly, the production of extracellular hemolysin is correlated with a higher cytotoxic activity. *Proteus* not only adhere to epithelial cells, but also invade the cells. The penetration ability is correlated with the cell-associated hemolytic activity. The hemolytic activity of *Proteus* is due to the production of cytotoxic hemolysins. Most *P. mirabilis* and *P. vulgaris* strains can degrade erythrocytes. Typically, the hemolytic phenomenon relies on the living cell of *Proteus*. Therefore, the intracellular or cell-associated hemolysis has been found in all strains of *P. vulgaris* and *P. mirabilis* whereas the extracellular or cell-free hemolysis has been observed only in some strains. In contrast, the extracellular hemolysin has been found in all *P. penneri* strains tested, and intracellular hemolysin has been found in some *P. penneri* strains. Lastly, *Proteus* secrets siderophores, an iron chelator, which also contributes the *Proteus* virulence.

Analytical Methods

Proteus strains can be isolated from vomitus and fecal specimens of patients. An isolation method is to grow the sample in dextrose broth fermentation tubes, and then the bacterial cells are streaked on agar or Endo plates. Stool samples can be streaked directly on Endo plates. A pure strain is obtained by successive passage of colonies. Characteristic colonies are

examined by Gram staining, a colorless and round appearance, and motility. Except Gram staining, most common staining methods stain *Proteus*. One staining procedure is summarized briefly as follows. After heat fixing, the bacterial cells are stained by methyl violet for 1 min and Gram's iodine for 1 min. Then, the slide is rinsed with alcohol and water and is stained with 0.1% basic fuchsin for 10 s. The suspected colonies can be further tested by biochemical confirmatory tests. *Proteus* spp. show positive results of hemolytic activity, hydrogen sulfide production, acid production, gas production, gelatin hydrolysis, decomposition of glucose, decomposition of sucrose, and growth in potassium cyanide. Furthermore, *Proteus* is positive in methyl red test and has phenylalanine deaminase activity. None of *Proteus* spp. can ferment lactose, D-adonitol, L-arabinose, D-arabitol, D-mannitol, D-mannose, melibiose, sorbitol, or side marigold alcohol. The species level identification requires both phenotypical characters and genotyping. There are special phenotypical characteristics for some *Proteus* spp. For example, *P. vulgaris* and *P. hauseri* are different from other *Proteus* spp. in indole production. *Proteus vulgaris* uses maltose, salicin, esculin, and amygdalin, but not lactose or mannitol. *Proteus penneri* is salicin-negative, esculin-negative, and chloramphenicol-resistant. The unique character of *P. mirabilis* is that it does not use maltose and has ornithine decarboxylase activity. *Proteus myxofaciens* is negative in the tyrosine clearing test. *Proteus mirabilis* ferments sucrose much slower than others do. Other than classical biochemical tests, the serological test, electrophoresis pattern of multilocus enzymes, and DNA similarity are also used to identify and discriminate *Proteus* strains. The DNA-based genotyping techniques include pulsed-field gel electrophoresis (PFGE), sequencing of 16S rRNA genes, restriction fragment length polymorphisms (RFLP), etc.

Genera *Providencia* and *Morganella* do not have the swarming phenomenon. Furthermore, Genera *Providencia* and *Morganella* can be distinguished from Genera *Proteus* by the negative results in gelatin hydrolysis and acid production from D-Xylose. *Providencia* is different from *Morganella* in acid production from D-Mannose. *Providencia* is negative in acid production from D-Mannose whereas *Morganella* is positive. *Morganella morganii* has urease activity whereas only *P. rettgeri* strains from *Providencia* constantly show urease activity.

Prevention

Proteus may exist on vegetables, meats, poultry, etc. Between approximately 17 and 22 °C, *Proteus* is the dominant spoiler of crustacean meats. *Proteus vulgaris* produces custard-like rot in eggs and sourness in ham. However, *Proteus* still has beneficial effects on food, such as its contribution to cheese flavor. *Proteus* spp. are one of the most common species on the surface of food processing equipment. The biofilm formed by *Proteus* with other bacteria has been found in the aging tank of dairy plants. Effective cleaning and disinfecting processes are required to eliminate accumulated *Proteus* on the equipment surface and prevent biofilm formation. Establishing closed systems can reduce the chance of contamination. During the food storage and distribution process, the temperature and hygiene conditions are critical factors to prevent the growth of *Proteus* spp. For example, fish and crustacean meats could be spoiled by handlers and storage containers. At temperatures between approximately 17 and 22 °C, *Proteus* becomes the dominate spoiler. The carrier rate of *Proteus* is high in raw meat. During a cooking process, sharing chopping boards or containers for raw and cooked food may cause cross-contamination. At the postprocess stage, it is also necessary to reduce the time of placing cooked food above 20 °C. The *P.* vulgaris infections caused by the spoiled overnight food have been reported.

Morganella morganii has been isolated from various seafoods, among which canned mackerel and tuna are the major source. *Morganella morganii* is commonly isolated in the outbreak of histamine fish poisoning. The fish handling and processing procedure is critical to prevent *Morganella* spoilage. To reduce the exposure time to ambient temperature, immediate freezing or chilling in ice upon fish harvest is recommended. To prevent crosscontamination, the fish contacting surfaces should be sanitized regularly. The modified atmosphere packaging, such as 40% carbon dioxide and 60% oxygen, effectively prevents the histamine formation by psychrotolerant *M. morganii*-like bacteria.

Further Reading

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BACTERIA

Pseudomonas

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Glossary

Abscess A localized collection of pus resulting from an infection.
Disinfection The reduction, by means of chemical agents and/or physical methods, of the number of microorganisms in the environment, to a level that does not compromise food safety or suitability.
Endemic A disease that is constantly present to a greater or lesser degree in a human population in a specific geographic location. Enzootic is the comparable term referring to diseases associated with animal populations.
Food safety An assurance that food will not cause harm to the consumer when it is prepared and/or eaten according to its intended use.
Foodborne disease Any disease of an infectious or toxic nature caused by or thought to be caused by consumption of food or water.
Incidence The rate or range of occurrence or influence of something, especially of something unwanted.
Infection The invasion and multiplication of microorganisms such as bacteria, viruses, and parasites that are not normally present within the body.
Infectious dose The amount of pathogen (measured in numbers of organisms) required to cause infection in the host.
Inoculation The introduction of a substance into a body in order to induce an immune response.
Intoxication An abnormal state that is essentially poisoning.
Morbidity The incidence of disease; the rate of sickness.
Mortality The number of deaths in a given time or place; the proportion of deaths to population.
Pathogen An organism capable of causing disease.
Percutaneous Through the skin.
Quarantine A strict isolation imposed to prevent the spread of disease.
Surveillance A type of observational study that involves continuous monitoring of disease occurrence within a population.
Virulence The relative capacity of a pathogen to overcome body defenses.

Background

Pseudomonas, first discovered by Gessard in 1882, is a genus of bacteria belonging to the family Pseudomonadaceae. *Pseudomonas* spp. are free-living bacteria commonly found in soil, ground water, plants, and animals, including humans. Although members of the genus *Pseudomonas* are known to be true pathogens of plants, *Pseudomonas aeruginosa* has become increasingly recognized as an emerging opportunistic pathogen of clinical relevance worldwide. *Pseudomonas aeruginosa* is the most common cause of pseudomonal infection, which generally accounts for a necrotizing inflammation (e.g., necrotizing enterocolitis). *Pseudomonas* spp. are generally not considered as foodborne pathogens responsible for typical gastrointestinal (GI) illnesses, and are mainly associated with spoilage of a variety of food, including fruits and vegetables. However, *P. aeruginosa* may spread among particularly immunocompromised individuals by direct contact with or by the ingestion of contaminated foods and water (for further discussion on transmission, see Section Epidemiology), and this organism has been implicated in perirectal infection, pediatric diarrhea, typical gastroenteritis, and necrotizing enterocolitis. Reports suggest that *Pseudomonas* is associated with typhlitis (also termed as neutropenic enterocolitis) in neutropenic patients, who suffer a sudden onset of fever, abdominal distension and worsening abdominal pain as a result of this disease. Pseudomonal infection can trigger a diarrheal disease known as Shanghai fever, which involves high fever, prostration, headache, and diarrhea. Also, this bacterium can potentially cause bacteremia or septicemia by the invasion of GI tissues leading to its lethal entry into the bloodstream. In addition, *Pseudomonas* plays a common role in eliciting the magnitude of food safety as an indicator organism. Food spoilage by this bacterium warns that other harmful bacteria, including those capable of food poisoning could also grow under the same conditions of the food matrices.

Characteristics of the Organism

Nomenclature/Taxonomy and Classification

The word *Pseudomonas* has derived from Greek *Pseudo* (false) and Latin *monas* (single unit). The genus *Pseudomonas* is included in the class Gammaproteobacteria, which constitutes

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one of the largest groups of bacteria, and have conserved macromolecules, such as 16S ribosomal ribonucleic acid (rRNA) gene sequences. The phylum Pseudomonad once constituted a large number of *Pseudomonas* species, but recent reclassification has identified a few groups that are of great medical interest. Of these, the true or fluorescent *Pseudomonas* comprises *P. aeruginosa*, *Pseudomonas fluorescens*, and *Pseudomonas putida*. Another medically significant group includes *Pseudomonas cepacia*, *Pseudomonas pseudomallei*, and *Pseudomonas mallei*, which are currently categorized as *Burkholderia* spp. implicated in many human and animal infections.

Morphology

Pseudomonas spp. are nonspore forming, Gram-negative rod (bacilli) bacteria measuring 0.5–0.8 μm by 1.5–3.0 μm, and are motile due to possession of single or multiple polar flagella (Figures 1 and 2). Unlike other *Pseudomonas*, *P. mallei* is a nonmotile coccobacillus. Three colony types of *P. aeruginosa* are observed: small, rough colony isolated from nature; large, smooth, colony with flat edges and an elevated appearance from clinical samples; and mucoid colony frequently obtained from respiratory and urinary tract secretions. The smooth and mucoid colonies are thought to be highly associated with colonization and virulence.

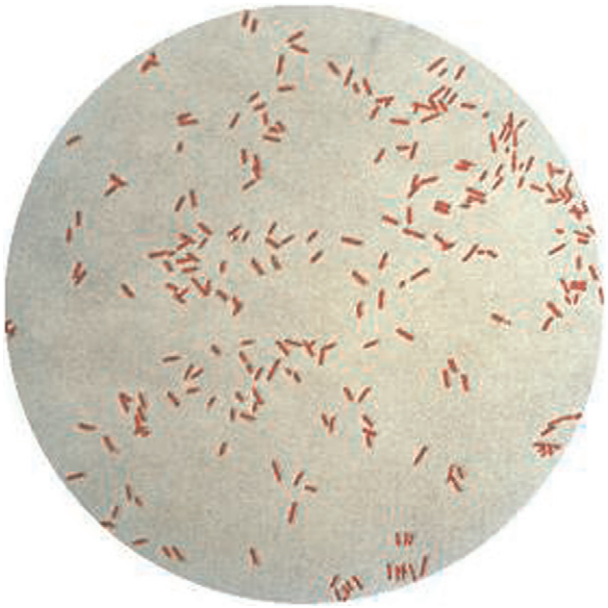

Figure 1 Gram stain of *Pseudomonas aeruginosa* cells. Reproduced from US Centers for Disease Control and Prevention (CDC).

Metabolism

Most *Pseudomonas* spp. are obligate aerobes, and do not depend on fermentation for their metabolism. However, these organisms are able to grow in the absence of oxygen provided that NO_3 is available as a respiratory electron acceptor in the environment. *Pseudomonas* spp. produce acid from glucose or other carbohydrates only in the presence of oxygen. They are usually catalase and oxidase positive, but lactose negative on MacConkey agar. This bacterium is known to have minimal nutritional requirements, and can even grow in distilled water. A simple medium consisting of acetate as a source of carbon and ammonium sulfate as a source of nitrogen is sufficient for pseudomonal growth in the laboratory.

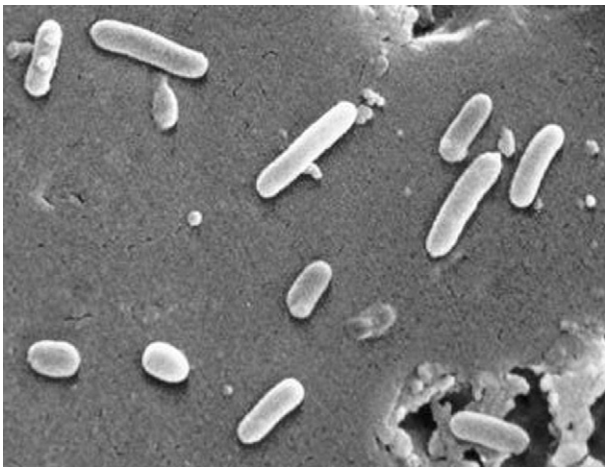

Figure 2 *Pseudomonas aeruginosa* scanning electron micrograph. Reproduced from US Centers for Disease Control and Prevention (CDC).

Survival and Growth Characteristics in Food and Environment

Most *Pseudomonas* spp. can easily thrive in a variety of food materials (e.g., fruits, vegetables, grains, and animal feeds), and may either promote growth of certain foods or subject some food to deterioration. Because of the predilection for growth in moist environments, these bacteria are ubiquitous in soil and water, and found on surfaces in contact with soil or water. *Pseudomonas* is distinguished by the characteristic fruity (grape like) odor of its colonies. *Pseudomonas* species are tolerant to a wide array of physicochemical conditions. *Pseudomonas aeruginosa* not only prefers to grow at 37 °C but is also able to tolerate higher temperatures up to 42 °C.

Biofilm Architecture

Pseudomonas spp. have widely been described in many studies on biofilms. Particularly, *P. aeruginosa* has been used as a paradigm for the study of bacterial biofilm formation. These bacteria have remarkable ability to secrete exopolysaccharides, such as alginate, Psl, and Pel associated with the biofilm formation. It has also been demonstrated that these pathogens perform cell to cell communication by a subtle strategy called quorum sensing via the production of small molecules called acyl homoserine lactones. This mechanism is believed to play a vital role in the biofilm development, and is being investigated further as a therapeutic target for control of chronic infections with *Pseudomonas*. Researchers have already performed a number of studies into the development of an effective quorum sensing blocker in order to limit biofilm-associated infections. Biofilm facilitates the bacterium with

survival through adverse environmental changes. The glycocalyx biofilm formation by *B. pseudomallei* (also *P. pseudomallei*) and transformation into several phenotypic variants protect the bacterium from the hostile environment and aid in its survival. Biofilm in drinking water systems is an important concern worldwide, and can be a reservoir for *P. aeruginosa*. Specific tap assembly units from neonatal wards in Northern Ireland have revealed the presence of slimy *Pseudomonas* aggregates during an investigation following four fatal cases of *P. aeruginosa*-associated bacteremia. Biofilms can be a particular problem in home water treatment devices that utilize carbon filters or membranes. Moreover, the ability of *Pseudomonas* spp. to slow their metabolism allows them to survive within biofilm matrices in the bottled or packaged drinking water for months. The capacity to metabolize a wide variety of nutrients scattered in nature has enhanced the potential of *Pseudomonas* for building such sticky communities in almost every place of the environment (Figures 3 and 4).

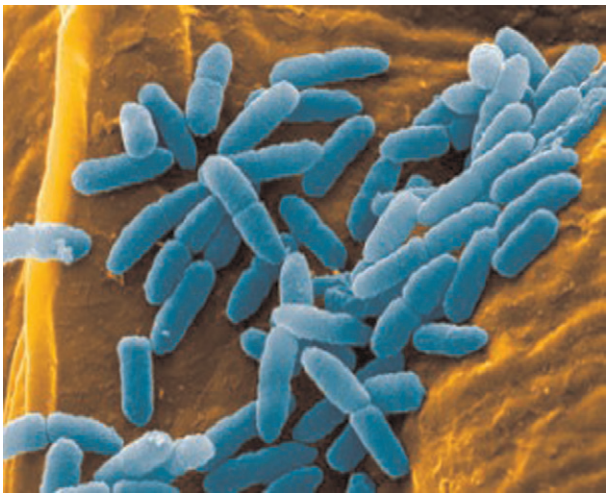

Figure 3 Biofilm matrix of *P. aeruginosa*.

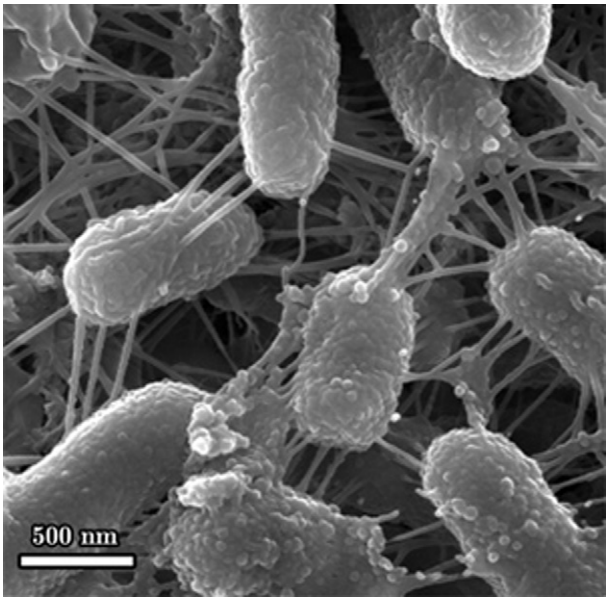

Figure 4 *Pseudomonas putida* biofilm architecture.

Production of Pigments

Another important characteristic of this bacterium, which enables isolation and differentiation of majority of the strains, is the production of certain diffusible pigments. Many *Pseudomonas* species secrete a fluorescent yellow-green siderophore called pyoverdine (fluorescein) under conditions of iron limitation. *Pseudomonas aeruginosa* is capable of producing a blue phenazine pigment pyocyanin, a reddish brown pigment pyorubin, and a brown to black pigment pyomelanin which help differentiate this species from others of the genus. Pyocyanin is produced in large quantities in low iron-containing media and helps with iron metabolism in the bacterium. Infections caused by *P. aeruginosa* are suppurative as a result of secretion of this pigment (derived from 'pyocyaneus' or 'blue pus') at the site of infection. Pyocyanin is also involved in inflammatory response and tissue damage during pathogenesis. *Pseudomonas fluorescens* synthesizes a secondary siderophore quinolobactin, which results from the hydrolysis of the unstable molecule 8-hydroxy-4-methoxy-2-quinoline thiocarboxylic acid (thioquinolobactin) and is yellow, dark green in the presence of iron.

Description of Pseudomonal Toxins

Majority of *Pseudomonas* infections are both invasive and toxinogenic. *Pseudomonas aeruginosa* invades tissues by the production and subsequent release of extracellular enzymes and toxins that break down physical barriers and damage host cells. Two extracellular proteases associated with virulence are elastase and alkaline protease. The bacterium produces three other soluble proteins that have implications in invasion: a pore-forming cytotoxin and two hemolysins. *Pseudomonas* endotoxin lipopolysaccharide (LPS) may contribute to resistance to phagocytosis and the serum bactericidal response. The lipid A moiety of LPS mediates typical pathogenesis of Gram-negative septicemia. *Pseudomonas aeruginosa* also produces two extracellular protein toxins, exoenzyme S and exotoxin A, which are associated with virulence and disease severity. Capsular polysaccharide and type III secretion systems of *P. pseudomallei* have association with intracellular survival. The extracellular polysachharide capsule of *P. mallei* is postulated to be a critical determinant of virulence. Certain *Pseudomonas* spp. are capable of producing a lethal toxin called tetrodotoxin (TTX) harbored by a variety of marine animals (for further discussion on this topic please see Section Tetrodotoxin-Producing *Pseudomonas*).

Genetic Factors of Virulence

Pseudomonas spp. have the ability to exchange genes by conjugation or horizontal gene transfer that may lead to the spread of virulence among strains and species. Expression of certain genes during infections plays an influential role in the pathogenesis and toxicity. *Pseudomonas* is able to regulate the genes to activate and control its virulence. Molecular interactions of homologs of the transcriptional regulators PtxR and PtxS play a key role in the regulation of exotoxin A expression,

which is a primary virulence factor of *P. aeruginosa*. The presence of multiple drug resistance in most *Pseudomonas* sp. is inherent. *R*-factor inheritance and transfer of *R*-plasmids contribute to resistance in mucoid *Pseudomonas*.

Resistance to Different Factors and Survival in the Host

One of the primary clinical concerns relating to *Pseudomonas* is its natural resistance to multiple antimicrobial agents, including penicillin and many related β-lactam antibiotics, as a consequence of the permeability barrier conferred by its characteristic Gram-negative outer membrane. Its widespread resistance to many conventionally used antibiotics is well known and a significant problem in health and agriculture. *Pseudomonas* has developed irrevocable resistance to naturally occurring antibiotics by living in association with bacilli, actinomycetes, and molds in the soil. Horizontal spread of resistance genes among species and strains of *Pseudomonas* poses further challenges in tackling infections with this pathogen. It is also resistant to rich salt and dye concentrations and weak antiseptics. The ability to form biofilms on surfaces also makes this pathogen impermeable to standard therapeutic concentrations of various antibiotics. Alginate production develops the characteristic slimy (mucoid) shield of the bacterium upon colonization on surfaces or substrates, and in the host environment, including the GI system protecting pseudomonads from typical phagocytic killing by mammalian white blood cells (WBCs), such as macrophages. This unique property increases the ability of the pathogen to stick to host cells. The exopolysaccharides are also thought to contribute to the biofilm architecture of *P. aeruginosa*, and to play a critical role in viability of cells in biofilms.

Pseudomonas characteristics are diverse by nature, and have largely contributed to its ecological success as an opportunistic pathogen. Many of these characteristics also provide this bacterium with the scope for its survival and growth under extreme environmental conditions as well as adaptive evolution through atmospheric changes.

Clinical Manifestation in Animals

Glanders and Melioidosis

Pseudomonas mallei (now known as *B. mallei*) is the predominant cause of glanders, which is a contagious and acute or chronic disease, mainly found in horses. This pathogen has also been isolated in other equids like donkeys and mules, and goats, sheep, cats, and dogs. Glanders is difficult to diagnose, and often fatal. Another pseudomonal infection caused by *B. pseudomallei* (formerly *P. pseudomallei*) is known as melioidosis (also called Whitmore disease), which clinically resembles glanders, but the bacterium exists mainly in the contaminated environment unlike *B. mallei*. The occurrence of this disease has been reported in horses, swine, goats, sheep, dogs, and cats. Owing to its wide spectrum of manifestations, i.e., the involvement of most body organs, melioidosis is considered to be one of the 'great imitators.' Infection could be acute, subacute, or chronic (asymptomatic). Both *B. mallei* and *B. pseudomallei* also have ability to afflict humans seriously, and are now considered as potential bioterrorism agents. Owing to their highly pathogenic characteristics, these organisms can be exploited in deliberate-release events.

Incubation

Incubation period for both glanders and melioidosis is unknown, but deemed variable. The average time taken for the onset of the diseases has been reported to be 1–2 weeks after inoculation. Both diseases have been described with potential for reactivation from latent state after decades of incubation.

Signs and Symptoms

The disease is characterized by serial development of ulcerating nodules in the upper respiratory tract, lungs, and skin. The acute form of glanders occurs in affected animals within 2 weeks of incubation period. They usually show symptoms of respiratory discomfort following the onset of septicemia and high fever (up to 106 °F). A thick, mucopurulent nasal discharge is observed in these animals. The severity of the clinical signs accounts for death of the infected animal within a few days, if not treated with appropriate antibiotics. Glanders can potentially affect both nasal mucosa and the lungs of animals. The disease can be recognized by the development of nodules in the nasal septum or the lungs. These nodules further degenerate into ulcers in deep tissues leading to enlarged and edematous lymph nodes. Broken contents of the nodules in the lungs may be released into the bronchioles, and may give rise to upper respiratory tract syndrome.

Melioidosis is clinically presented with local suppurative infections, ulcerative skin, cutaneous abscesses, fulminant bacteremia, or septic shock with multiple abscesses in virtually all organs of the body. Pneumonia has been documented to be the most common syndrome of *B. pseudomallei* infection, particularly in endemic regions. Other syndromes include prostatic abscesses, paralytic encephalomyelitis, or intraabdominal abscess formation, which may require surgery. Mortality rates among those with bacteremia or septic shock are significantly high.

Sequelae/Chronic Effects and Complications

The chronic form of glanders called 'farcy' causes ulceration in the skin and subcutaneous tissues followed by swelling of the affected area. This condition persists and affects surrounding lymphatic vessels, which thicken and consolidate to form 'farcy pipes' or 'farcy buds.' The nodules produce a highly infectious and sticky mucus, which may spread onto other sites of the body. The chronic stage of glanders may also affect liver and spleen, and develop intramuscular abscesses in the arms or legs. The disease sustains for months or years in the infected animals, which may not develop immunity even after recovery.

Chronic symptoms of melioidosis mimic those of tuberculosis. Cutaneous and intramuscular (in the limbs) abscesses are common. Liver and spleen may also be affected by chronic melioidosis. This form of the disease may relapse and recur over the course of years.

Dose of Infections

A significantly low infectious dose (ID_{50}) of *B. pseudomallei* for animals may be sufficient for a successful onset of

characteristic illnesses. The lethal dose (LD_{50}) by inoculation in some susceptible animals has been found to be less than 10 viable organisms. ID_{50} for humans is still unclear, but may vary considerably according to host resistance. Most infections with *B. pseudomallei* have occurred through gross contamination, such as contaminated war wounds and laboratory accidents. Very little is known about the infective dose of *B. mallei* for both humans and animals, but it seems that the parasite is potentially more dangerous than *B. pseudomallei*. Its ID_{50} has not yet been determined in terms of colony-forming units, but has been merely estimated as an inoculum concentration of 0.2 mg by subcutaneous inoculation.

Pseudomonas aeruginosa-Associated Diseases

Infections with *P. aeruginosa* are significant among various animals. It is a clinical problem in mainly neutropenic animals, including mice that are irradiated or treated with chemotherapeutic compounds. This pathogen transits the nasopharyngeal or gut mucosal barrier of these seriously immunodeficient animals, and becomes the cause of systemic bacteremia and sepsis leading to death.

Signs and Symptoms

In mice, clinical presentations of pseudomonal infection include conjunctivitis, nasal discharge, or typical signs of rodent illness, such as stooped posture, anorexia, and ruffle fur. The bacterium is also associated with otitis media, pneumonia, septicemic enteritis, and fulminant death in chinchillas (*Chinchilla lanigera*). *Pseudomonas aeruginosa* is one of the leading causative agents of otitis externa in many animals, including dogs. The disease presents with head shaking or ear scratching, purulent exudates, malodor, swelling of ears followed by inflammation and pain, ulceration, and otitis media. Reports suggest that otitis media is highly associated with preceding otitis externa in dogs. Clinical signs of otitis media include persistent and chronic otitis externa, neurological abnormalities, and ruptured or bulging, hemorrhagic tympanic membranes with exudates in the middle ear. *Pseudomonas* is also known as a secondary pathogen in canine upper respiratory tract infection, and can cause severe bronchopneumonia in dogs that requires immediate clinical attention. *Pseudomonas aeruginosa* is now considered as one of a few unusual pathogens implicated in outbreaks of clinical mastitis in cattle, sheep, and goat. The infection is generally characterized by acute or subacute exacerbations. Some cows may have high mortality following an onset of severe peracute mastitis with toxemia, whereas others may present signs of subclinical infections.

Tetrodotoxin-Producing *Pseudomonas*

Certain species of *Pseudomonas*, such as *P. putida* are capable of producing a potent neurotoxin called tetrodotoxin (TTX) primarily found in marine puffer fish belonging to the family Tetraodontidae. Other than *Pseudomonas*, bacterial species including *Vibrio*, *Bacillus*, *Actinomyces*, *Serratia*, *Aeromonas*, and *Microbacterium* have been reported as TTX producers in marine animals. The first identified bacterial strain producing the toxin was *Vibrio*. TTX was discovered in 1909 by a Japanese scientist Yoshizumi Tahara. This toxin has also been isolated from other marine animals largely different to puffer fish, such as several species of blue-ringed octopuses of the genus *Hapalochlaena*, rough-skinned newt, ribbon worms, parrotfish, frogs of the genus *Atelopus*, xanthid crabs, horseshoe crabs, starfish, and certain angelfish. Puffer fish are now considered as the second most poisonous vertebrates in the world, after the golden poison frog. The toxin is believed to potentially exist in the gonads, liver, intestine, and skin of these fish at levels sufficient to cause rapid and violent death. Bioaccumulation of the toxin is thought to contribute to its high concentrations in the food-chain. Despite potential life-threatening toxicity of the fish, its flesh is widely recognized as a delicacy in Japan, where it is handled and prepared with caution by specially trained and certified puffer chefs. However, cases of TTX poisoning as well as deaths among individuals from puffer fish consumption continue to be reported in this country.

Signs and Symptoms

The onset of clinical sings of intoxication may be visible in minutes to hours of ingestion. Major symptoms develop usually within 24–48 h of incubation. Early common signs of the poisoning are paresthesias (lip, tongue, and face), numbness, and abdominal pain associated with salivation, nausea, vomiting, and diarrhea. Major symptoms involve paralysis of voluntary muscles, including the diaphragm, cardiac dysfunction, loss of sensation, central nervous system dysfunction (e.g., coma) and respiratory failure leading to death. Death usually occurs within 6–24 h, but if the patient survives after 24 h, prognosis improves. There are sporadic cases of TTX poisoning in animals, especially in wild populations. TTX-containing organs or puffer fish can be extremely toxic to most animals when eaten. Common clinical signs of toxicity in dogs include vomiting, wobbliness, drop in the heart rate, and lethargy. Some animals could variably have excess salivation, diarrhea, involuntary contractions or twitching of the muscles, seizures, unusual heart beat, respiratory failure, and death. Consumption of organisms containing TTX is also known to kill dogs, cats, and humans. A dose of approximately 10 mcg kg^{-1} has been estimated to be sufficient for serious toxicity of TTX in humans.

Possible Association with Crohn's Disease

Crohn's disease, also called regional enteritis, is a human inflammatory bowel disease (IBD) or colitis that mainly affects the intestines, but may occur virtually in any part of the GI system from the mouth to the end of the rectum (anus) triggering a variety of symptoms. It is widely hypothesized as an autoimmune disorder characterized by the faulty destruction of healthy body tissues by one's own body immune system. Nevertheless, current understanding of more realistic scenario of the disease pathogenesis proposes its underlying relation to innate immune dysfunction in affected humans.

Clinical Presentations

Crohn's disease is often misdiagnosed as irritable bowel syndrome. Crohn's disease patients suffer chronic inflammation of the GI tract, which results in thickening of the intestinal wall. The disease can be primarily presented with severe abdominal cramps, bloody or mucoid stools, watery diarrhea, nausea and vomiting, constipation, loss of appetite, GI or rectal bleeding, fistulas around the rectal area, and ulceration in the mouth. Crohn's colitis may also cause extra-GI complications, such as fever, fatigue, inflammation and lesion in the eye, skin sores, joint pain and swelling, defective or failed growth and sexual impairment in children, vitamin B_{12} deficiency, and weight loss. Major consequences of the disease, which could pose life-threatening conditions, may include hemorrhage, dilation of the large intestine (toxic megacolon), and even colon rupture. There is no complete cure for the disease, and if it worsens due to inappropriate or lack of medications, patients might be at serious risk of small bowel or colon cancer. Although prolonged medications are crucial in Crohn's disease, its severity in some cases may not fully respond to conventional medical therapy. In such instances, patients may need to undergo surgery for removal of a damaged portion of the intestine or the entire colon, with or without the rectum.

Etiology

Although the true cause of Crohn's disease still remains unclear, various factors are assumed to play an underlying role in the development of this disease. Among these, genetic, immunologic, microbial, and dietary factors may have implications. Infectious agents like *Mycobacterium avium* subspecies *paratuberculosis* (MAP), *Pseudomonas*, adherent-invasive *Escherichia coli*, *Listeria monocytogenes*, *Yersinia pseudotuberculosis*, and measles virus have been proposed as triggers of Crohn's disease, predominantly on the basis of seroreactivity. The association of MAP with the pathogenesis of Crohn's disease has been hypothesized most extensively, but the presence of mycobacterial deoxyribonucleic acid (DNA) in lesions remains controversial. Reduced tolerance to normal GI microbiota has also been argued to be somewhat responsible for Crohn's disease. It is now suggested that *P. fluorescens* has a strong correlation with the disease in humans. Crohn's and similar illnesses have also been reported in animals, such as livestock, cats, and dogs, but their association with *Pseudomonas* spp. has not yet been described. Despite being long known as a psychrotrophic organism, *P. fluorescens* is found as a low-level commensal in the ileal mucosa of humans. Nevertheless, colonization of these bacteria appears to be extensive in the susceptible colonic mucosa in Crohn's disease. The pathogen may become opportunistic in the disordered mucosal environment in genetically vulnerable individuals, and contribute to Crohn's disease mucosal damage through the expression of T cell superantigen, I2 encoded by *PfiT* gene. Studies have revealed that the bacterium is able to adhere to intestinal epithelial cells (IECs), exert cytotoxic effects, and induce proinflammatory response. The presence of specific antibodies against some strains of this pathogen in the serum of patients further indicates its potential role in Crohn's disease pathogenesis. Also, *P. fluorescens*-specific I2 antigen sequence is systematically detected in the ileal mucosa of patients. A positive correlation is found between the level of anti-I2 antibodies and severity of the disease. Certain strains of this species induce secretion of proinflammatory cytokine, interleukin (IL)-8 in human intestinal and colorectal tumor cell lines through a pathway different to that observed in *P. aeruginosa*-activated IL-8 secretion. *Pseudomonas fluorescens* can also alter intestinal epithelial permeability by the rearrangement of *F*-actin microfilaments, and translocate across Caco-2/TC7 cell monolayers via the transcellular pathway. Thus, *P. fluorescens* demonstrates its potential for significant interaction with IECs. Crohn's disease patients with elevated antibody titers against *P. fluorescens* I2 antigen are more susceptible to advancement to perforating or fibrostenotic disease, thereby requiring more surgery.

According to recent theories, Crohn's disease is linked to malfunctioning of innate immune system of the body that recognizes microbe-associated molecular patterns by pattern recognition receptors (PRRs), such as toll-like receptors (TLRs), and subsequent release of cytokines and chemokines. Prolonged exposure to bacterial antigens results in reduced expression of surface TLRs in colonic epithelial cells. Furthermore, polymorphisms in PRRs, including TLRs and nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) NOD2 (also known as caspase recruitment domain (CARD)-containing protein, CARD15) play a role in Crohn's disease susceptibility. Mutations of TLRs and NLRs may also contribute to vulnerability to the disease and progression to fibrostenosis. Therefore, defective sensing of bacterial pathogens or their derivatives is possibly involved in the etiopathogenesis of Crohn's disease. It is, however, ambiguous whether *P. fluorescens* or other bacterial pathogens can mimic or exploit any host cell signaling complex within the GI tract, and induce polymorphisms or mutations in these cytoplasmic receptors. Other fluorescent *Pseudomonas* species may also have implications in the pathology of Crohn's disease. There is evidence of a high prevalence of *Pseudomonas brenneri*, *Pseudomonas migulae*, *Pseudomonas panacis*, and *Pseudomonas proteolytica* in children with Crohn's disease. A former member of pseudomonads, *Stenotrophomonas* (*Pseudomonas*) *maltophilia* broadens the idea of pseudomonal contribution to the occurrence of Crohn's disease. DNA sequences with homology to *P. maltophilia* DNA have been found in inflamed tissues of Crohn's disease patients. In addition, historical review of Crohn's disease suggests that cell wall variants of this bacterium and other *Pseudomonas*-like bacteria may have a relationship with the disease. In fact, there is a coexistence of *Pseudomonas* and other closely related bacteria with the pathophysiology of Crohn's disease. Characteristic inflammation resulting from the disease is considered as a sign of the dysfunctional immune response to the corresponding pathogen. In recent years, *P. fluorescens* has received increased attention as a candidate human pathogen, which plays a putative role in specific magnitudes of Crohn's disease and corresponding immune disorder. Further systematic investigations are needed to elicit a definitive picture confirming its exact implication in the etiology of the disease.

Table 1 Clinical presentations and outcomes of melioidosis in northern Australia

Parameter	*Number of patients*	*Number of deaths*	*Mortality in percentage*
Bacteremic	117	43	37
Pneumonia	66	31	47
Genitourinary infection	23	4	17
Osteomyelitis, septic arthritis	4	1	25
Other diagnoses	24	7	29
Nonbacteremic	135	6	4
Pneumonia	61	4	7
Genitourinary infection	14	0	0
Skin abscess	32	0	0
Soft tissue abscess	10	0	0
Neurological	10	2	20
Osteomyelitis, septic arthritis	5	0	0
Other diagnoses	3	0	0
Total	252	49	19

Source: Reproduced from Currie BJ, Fisher DA, Howard DM, *et al.* (2000) Endemic melioidosis in tropical northern Australia: A 10-year prospective study and review of the literature. *Clinical Infectious Diseases* 31: 981–986.

Epidemiology

Occurrence of Glanders and Melioidosis

Geographic Distribution, Prevalence, and Incidence

Glanders is a common problem among domestic animals in Central America, South America, Asia, Africa, and the Middle East, but has not occurred in the US since the 1940s. Melioidosis caused by *B. pseudomallei* is endemic in Southeast Asia, and most cases are reported in Malaysia, Singapore, Thailand, Vietnam, Cambodia, Laos, and Myanmar. It is estimated that 5–20% agricultural workers in endemic areas have antibody titers to this pathogen. This bacterium is a major cause of morbidity (an estimated 2000–3000 cases per year) and mortality in northeastern Thailand. Major incidences of this disease are also recorded in northern Australia. Table 1 represents the overall scenario of human melioidosis in this region in accordance with the clinical presentations. During the Vietnam War, melioidosis was referred to as the Vietnamese Time Bomb when American Troops returned from Vietnam, and were diagnosed as carriers of latent infection with likelihood of recurrence. Other regions, where melioidosis is recognized in humans and animals, include Africa, India, China, Taiwan, Brunei, the Middle East, and South Pacific. Incidences have also been reported from Sri Lanka, Bangladesh, and Pakistan. Confirmed periodic cases of the disease have been reported in Iran. Israel has by far had no reports of melioidosis. During the twentieth century, sporadic cases in western countries were documented mainly among returning travelers or military veterans. As cases are being increasingly documented from outside the classic endemic regions for melioidosis, global warming is assumed to play a critical role in further expansion of the endemic boundaries of the disease.

Transmission

Animals, particularly equids are the predominant reservoirs of *B. mallei*. Transmission of glanders in animals commonly takes place via inhalation or direct inoculation with broken or ulcerated skin of carrier animals. Transmission of *B. mallei* between humans is rare, but feasible. It is likely to occur in humans through percutaneous or direct inoculation of nasal mucosa of animals suffering glanders. Therefore, people who are highly vulnerable to the infection are chiefly occupational personnel, such as laboratory workers, butchers, horse handlers, and veterinarians. Infection due to ingestion of food and water contaminated with nasal discharges from these animals is also possible. Chronic glanders or 'farcy' is assumed to occur through direct inoculation of *B. mallei* from affected equines. The organism is considered as a greatly adapted parasite of horses, which cannot continue to survive in nature outside of its host. Modes of transmission of melioidosis in animals are very similar to that of glanders, except ingestion that is paradoxically much less common for the former despite the natural persistence of *B. pseudomallei* in the environment. Transmission is by far believed to occur in humans through percutaneous inoculation being the predominant portal of entry, inhalation, and more rarely sexual transmission. Inhalation of dust from contaminated soil is a likely route of transmission in case of pulmonary infection. Aspiration or drinking of unchlorinated contaminated water may also lead to infection. Two outbreaks of melioidosis in Australia were linked to potable water supplies. Human-to-human as well as zoonotic transmission is rare.

Infections by *P. aeruginosa*

Certain bacterial infections in both humans and animals are being considered as serious problems that question the efficacy of conventional therapeutics and control measures. Some of these problems can be directly attributed to *P. aeruginosa*, which is the causative agent of many opportunistic infections worldwide.

Prevalence and Incidence

This pathogen reportedly occurs in more than 50% of humans, and is routinely detected among many hospital patients. A number of different epidemiological studies have

found the occurrence of *P. aeruginosa* in nosocomial infections that are difficult to treat with most conventional antibiotics due to its extensive antimicrobial resistance. The bacterium is claimed to be the fourth most commonly isolated nosocomial pathogen associated with nearly 10% hospital-acquired infections. These infections primarily include pneumonia, urinary tract infections, surgical wound infections, and septicemia. *Pseudomonas aeruginosa* is the second most common cause of nosocomial pneumonia and the most common cause of intensive care unit pneumonia. It has also been isolated from throat (5%) and stool (3%) of nonhospitalized patients. This ubiquitous pathogen is disseminated in moist hospital environments (e.g., food, disinfectant, sinks, toilets, taps, showers, mops, sewage, respiratory and dialysis equipments, etc.) as well as in nature. In a study conducted on the prevalence of *Pseudomonas* in Indonesian soil samples, 78% of the isolates were *P. aeruginosa*. It is known to be a notorious chronic pathogen associated with long-term infections in individuals with the heritable disease cystic fibrosis (CF). *Pseudomonas aeruginosa* becomes highly resistant to host response and antimicrobials after prolonged colonization of the CF lung. The bacterium is likely to colonize in the lung of approximately 80% of the CF patients. Increasing longevity of young CF patients, particularly children has largely contributed to a dramatic proliferation in the proportion of adult CF patients (from 8% in 1969 to 33% in 1990).

Factors Contributing to Infections

Pseudomonas aeruginosa can rapidly spread in the respiratory and GI tracts of hospitalized patients, particularly those under antibiotic treatment and bedridden for extended periods. Interestingly, young black men have been reported to be more susceptible to pseudomonal endocarditis. *Pseudomonas aeruginosa* reenters the hospital via fruits, plants, and vegetables. Contact with or ingestion of food materials contaminated with this organism may lead to not only general extraintestinal infections but systemic bacteremia or sepsis as well because of its ability to enter the bloodstream through the GI barrier. Visitors as well as patients transferred between facilities can reintroduce the bacterium into a germ-free hospital environment. Spread occurs through hands of healthcare personnel, direct contact with contaminated reservoirs, or ingestion of contaminated foods and water. Certain medical procedures, such as surgery and insertion of catheter or respiratory tube, also predispose the patients to serious infection with this pathogen.

Mortality

Infections with this pathogen have a high mortality rate among the immunocompromised individuals, mostly with syndromes of bacteremia or lower respiratory tract infections. Rates of mortality vary from 15% to 20% of patients (with severe ear infections) to 89% of patients (with left ventricular dysfunction).

Animal Morbidity and Mortality

Pseudomonas aeruginosa is commonly detected in watering systems of animals and humans that include both the drinking water matrix and wash water. It is likely that many animals can transiently harbor this organism. Long-term treatment with antibiotics plays a contributing role in aggressive disturbance of animal gut flora and leads to prolonged colonization of this pathogen. Frequent incidences of mastitis caused by *P. aeruginosa* among cattle, goat, and sheep were reported in past decades. Herd infections have been found to be associated with generous exposure to contaminated wash water, teat cup liners, or intramammary treatments provided by milkers. Infections within the mammary glands may be established due to the use of unhygienic techniques or tools in udder therapy or milking. In the early 1970s, high incidence (80.1%) of *P. aeruginosa* pyocine type 1 in bovine mastitis was recorded in Israel with a high rate of fecal carriage of this organism both in adult cattle and in calves. Pseudomonal mastitis in sheep and goat has also occurred in Israel. More than 15 Israeli dairy sheep and goat herds have been reported for outbreaks with clinical presentations of gangrenous infections. *Pseudomonas aeruginosa*-associated high early mortality rates in chicks have been experienced in chick hatcheries in AL, USA.

Transmission of *P. aeruginosa* between animals is not well documented. Most common mode of entry is exposure to contaminated water. These bacteria can also be transmitted from animals to humans or vice versa via the ground water used for washing by means of ingestion or direct inoculation. Although shedding of mastitic *P. aeruginosa* into raw milk has been poorly documented, it is still likely through infected udder or contaminated milking devices. Animal neutrophils provide the first line of host defense against transmission of this pathogen through wounds or mucosal barrier. Recent emergence of resistance to many therapeutic antibiotics in this bacterium has gained much interest among clinicians and researchers. High prevalence of extended-spectrum β-lactamase-producing *P. aeruginosa* has become an alarming issue in hospitals and laboratories.

Marine Poisoning and Tetrodotoxin

Geographic Distribution, Prevalence, and Incidence

Puffer fish poisoning has been reported to a great extent in many parts of the world, particularly in coastal areas. This poisoning has mostly been associated with the consumption of puffer fish from waters of the Indo-Pacific ocean regions. According to recent evidences, *Pseudomonas* spp. have been considered as putative TTX producers in puffer fish and other TTX-harboring marine animals. The bacteria were isolated from the skin of a puffer fish *Takifugu poecilonotus* in 1987, and production of TTX and its derivatives was subsequently confirmed in the laboratory. During 1974–83, the incidence of puffer fish intoxication was estimated to be approximately 200 cases annually, with almost 50% mortality. Approximately 50 people die of puffer fish poisoning in Japan every year. Cases of death are also found in Hong Kong, Singapore, and Australia. TTX intoxication is also frequently reported from some cities in China. Episodic cases have been documented in the US. Recent cases of morbidity and mortality in Bangladesh due to consumption of puffer fish have also been described. The fishes have been found to be most dangerous if eaten just before or during their reproductive season due to a positive relationship between gonadal activity and toxicity. Other than

Table 2 Levels of TTX poisoning in patients of some Asian countries during 1989–2008

Incident time	*Location*	*Patients involved*	*LOD (ng ml^{-1})*	*TTX concentration in urine (ng ml^{-1})*	*TTX concentration in plasma/serum (ng ml^{-1})*
2008	Bangladesh	141	Not mentioned	0.4–75.4	<1.6–13.7
2007	Hong Kong	1	0.13	88.2	Not available
2006	Hong Kong	4	0.13	30.7–460.5	<0.13
2006	Taiwan	3	0.1	Not available	3.3
2005	Hong Kong	3	0.13	59.3–109.6	<0.13
2005	Taiwan	6	1.0	169–325	<1–8
2004	Japan	7	0.1	15–150	0.9–1.8
2001	Taiwan	6	4.9	15–109.7	<4.9–13
1989–96	Japan	6	2.0	6–102	Not available
Not mentioned	Japan	11	0.5	15–650	2.5–320

Abbreviations: LOD, limit of detection; TTX, tetrodotoxin.

Source: Reproduced from Leung KS-Y, Fong BM-W and Tsoi Y-K (2011) Analytical challenges: Determination of tetrodotoxin in human urine and plasma by LC–MS/MS. *Marine Drugs* 9: 2291–2303.

puffer fish, edible marine gastropod-associated poisoning is also a concern in China and Taiwan. TTX poisoning by horseshoe crab is evident in Thailand. Table 2 summarizes detection of various TTX levels in Asian patients between 1989 and 2008.

Transmission

The main portals of entry of the toxin are ingestion, inhalation, and injection. It can also enter the body through abraded skin.

Crohn's Disease Scenario

Distribution, Prevalence, and Incidence

Pseudomonas fluorescens is widespread in refrigerated food products, soil, and water. Parallel emergence of the pathogen and rise of Crohn's disease has been suggested to be linked to the vast use of refrigerated food in western countries. Surprisingly, it has been isolated from clinical environment, and the I2 antigen specific for this bacterium has been detected in the serum of 54% patients with ileal Crohn's disease. Studies have demonstrated positive test results for *Pseudomonas* spp. in 58% children with Crohn's disease, whereas the percentage has been much less (33%) in non-IBD children. The incidence of Crohn's disease has mounted over the past few decades. Incidences have been estimated to be between 400 000 and 600 000 in North America. Recorded prevalence of Crohn's disease in the US is approximately 7 cases per 100 000 populations, whereas 27–48 cases per 100 000 have been reported in Northern Europe. Crohn's disease incidence in Asia, Africa, and Latin America is postulated to be lower than in Europe. Considerably high incidence and prevalence of Crohn's disease have been reported in Canterbury, New Zealand.

Concerning Factors

Prevalence of Crohn's disease is possible at any age, but the most susceptible age group for the disease is usually 15–35 years. Among all patients diagnosed to be Crohn's disease positive in the USA, nearly 20–30% patients are aged below 20 years. White children seem to be more vulnerable than black children in this country. Similarly, the proportion of whites with IBD has been found to be 97.5% in New Zealand. Family history of incidence and smoking affect the causation of the disease. Although males and females are affected by Crohn's disease almost equally, prevalence is more likely among females in some countries. It is also suggested that Jewish people are more prone to this disease.

Analytical Methods

Typical signs (e.g., nodules, ulcers, scar formation, and debilitated condition) may provide sufficient evidence for a clinical diagnosis of glanders. However, as the symptoms do not develop in the early stage of the disease, the mallein test is a common method of choice for detection of the disease. Complement fixation is another widely used testing technique, but may give rise to a false-positive result. Enzyme-linked immunosorbent assay (ELISA) has been found to be more accurate than complement fixation. Immunofluorescent microscopy on pus or secretions can be a rapid diagnostic tool in endemic areas. Traditional culturing of exudates from lesions could help identify the causative agent. Polymerase chain reaction (PCR) for amplification of 16S and 23S rRNA gene sequences can be used for specific detection and phylogenetic analysis.

Clinical diagnosis of melioidosis is quite similar to that of glanders. A gentamicin-supplemented liquid transport broth called Ashdown's medium is also used for selective growth of *B. pseudomallei*. Repeated indirect hemagglutination test or ELISA renders successful positive results, and is an important technique for testing the seroprevalence among tourists returning from a melioidosis-endemic region. Both *Burkholderia* species can be identified by combining API 20NE or 20E biochemical kit with a simple screening system using Gram stain, typical growth characteristics, and oxidase reaction along with antibiotic susceptibility testing (resistant to polymyxin B and colistin).

Infections with classic *Pseudomonas* spp. in both humans and animals are identified by conventional culture and biochemical methods. Use of cetrimide agar or other commercially available *Pseudomonas* agar media is quite effective in rendering selective confluent growth of colonies with typical

pigment production. On MacConkey agar, *P. aeruginosa* colonies are colorless (nonlactose fermenting) without pigment production, but the colonies appear blue green on ordinary nutrient agar. Culture results are available in 2–3 days. The ability of *P. aeruginosa* to grow at 42 °C may aid in laboratory identification. Hemolytic activity (β-hemolysis) is confirmed by using blood agar (e.g., 5% sheep blood agar). The characteristic grapelike odor of the colonies is also helpful during phenotypic analysis. Biochemical identification includes positive catalase and oxidase reactions and citrate utilization. Blood sample screening and site-specific investigations, such as urine, stool, sputum, pus, cerebrospinal fluid cultures, or drainage from an infected ear or eye are very useful methods. Also, fluorescence is used to detect the bacteria in wound specimens. X-rays and other imaging approaches, including computed tomography (CT) and magnetic resonance imaging (MRI) can be performed to evaluate infections in deep organ tissues. Otitis can be assessed by swab cytology of the otic exudates, where *P. aeruginosa* is found in the presence of neutrophils under high-dry ($\times$400 to $\times$800) or oil-immersion ($\times$ 1000) magnification. Genotypic analysis includes PCR and DNA hybridization. Serotyping and pulse-field gel electrophoresis can be carried out to obtain type-specific information and molecular patterns. Sequence analysis provides further accuracy in species identification as well as antibiotic resistance determination.

TTX poisoning can be assessed in food matrices by mouse bioassay. Production of the toxin and its derivatives can be determined by high-performance liquid chromatography (HPLC), matrix-assisted laser desorption/ionization–time of flight (MALDI-TOF) mass spectrometric detection, and ELISA. Quantification of the toxin in the serum, whole blood, or urine may confirm diagnosis of poisoning. Currently, most analytical methods recruit separation by gas chromatography (GC) or HPLC followed by mass spectrometry (GC–MS or LC–MS). Other proposed methods comprise immunoaffinity chromatography, HPLC with postcolumn derivatization and fluorescence detection (HPLC-FLD), and HPLC with ultraviolet (HPLC-UV) detection. Recently, an optical technique making use of surface plasmon resonance immunosensors to detect TTX has been reported to be capable of rapid screening with low reagent consumption. Also, biosensors offer themselves as promising biotools for detection of marine toxins. A novel method called liquid chromatography–electrospray ionization–mass spectrometry (LC-ESI-MS) coupled with a column switching technique is claimed to be helpful in sensitive determination of TTX.

Usual diagnosis methods to detect Crohn's disease encompass barium enema or upper GI series, colonoscopy or sigmoidoscopy, CT scan of the abdomen, endoscopy, MRI, and enteroscopy. Associated microbial infections may be identified by stool and blood culture, serology, WBC count, ileal or colonic mucosal biopsy (pinch biopsy), and 16S PCR.

Control/Preventive Measures

The widespread nature of *Pseudomonas*-associated diseases makes it literally difficult to eradicate many infections caused by the bacteria. Many efforts could further be undermined by the fact that transmission of endemic strains and genetic variants is likely to reoccur. However, appropriate control measures can provide some success in minimizing the number of potential incidences relating to *Pseudomonas* infections.

Possible spread of glanders and melioidosis can be controlled by early detection and elimination of affected animals. Immediate disinfection and quarantine of vulnerable areas may offer great benefits. Airborne and contact precautions must be taken to avoid potential inoculation. CF patients are advised to avoid traveling to high-risk areas. Only melioidosis patients suffering from severe suppurative pneumonia with productive sputum should be isolated. On the contrary, isolation of all infected persons is recommended for glanders. Laboratory personnel should perform handling of specimens and isolation procedure in a biosafety level-3 facility. Respiratory protection is also recommended for manipulating the organisms outside of a biosafety containment capacity. Standard laboratory guidelines must be followed to ensure further safety. Certain antibiotics, including carbapenems, doxycycline, streptomycin, gentamicin, ceftazidime, cefepime, and combinations of sulfazine or sulfamonomethoxine with trimethoprim have been found to be quite effective in the prevention of experimental glanders in endemic areas. Potential vaccine candidates are being investigated. Education in mainly endemic regions to minimize exposure to wet season soils and surface water is also expected to be of significance. Public health surveillance and action procedures may have great impact. Concerns of potential bioterrorism (deliberate release event) using *B. mallei* or *B. pseudomallei* have driven further international alert in recent times.

Infections with *P. aeruginosa* are far more difficult to control as the bacterium is ubiquitous. However, individuals at high risk, particularly immunocompromised patients must be more precautious about any possible incidence caused by this pathogen. This organism can be found on the surface of many raw fruits and vegetables. Hence, patients particularly with neutropenia should not consume these foods because of the risk of subsequent GI colonization and bacteremia. As *P. aeruginosa* is often found in raw milk as a spoilage organism, appropriate heat sterilization of the milk should be applied before consumption considering the potential for pathogenicity of this bacterium. Healthy lifestyle can help prevent many associated diseases. Much of colonization of animals with the bacterium can be prevented by raising them in strict bioexclusion housing. Regular monitoring of water quality is necessary for detection of potential prevalence. Sterilized or chlorinated water must be used for immunodeficient animals, especially those that are neutropenic. Routine application of disinfectants, such as chlorine and chloramine, where appropriate, prevents possible spread of the bacteria from the environment, and may interfere with the biofilm formation. Installation of silver–copper ionization equipment and the use of UV and ozone can help control much of the biofilm growth in various ground and drinking watering systems. Rederivation of animals by embryo transfer or hysterectomy into *P. aeruginosa*-free dams helps achieve healthy (sterilized) progeny. Health professionals should be highly cautious about washing hands with disinfectants before dealing with patients. They should also ensure adequate sterilization of all vulnerable medical devices, and regular monitoring of this action. Aseptic procedures must be followed during surgery and other insertion practices

(e.g., catheterization). Burn patients should be strictly isolated. Potential vaccines to immunize individuals as well as CF patients are under development. Control of extensive or inappropriate prescription and treatment with antibiotics may discourage emergence of new antimicrobial resistance.

Perhaps, harboring of specific bacterial pathogens in marine animals cannot be disturbed, but legislative restrictions on trade and consumption of puffer fish and other TTX-containing gastropods could be enforced. Adequate removal of toxic organs, such as gonad and liver by qualified cooks may provide some protection against possible poisoning. Currently, there is no known antidote available for TTX.

Appropriate therapies support great improvement of the prognosis of Crohn's disease. Cessation of cigarette smoking may reduce the risk of occurrence of the disease and its serious complications. Direct association of *P. fluorescens* and other phylogenetically close bacteria with this disease still needs confirmation for the development of effective measures to control colonization of the microflora in the colon or other parts of the GI system.

Research Needs

Latest advancement in technologies provides global researchers with enormous support in studying comprehensive molecular profiles of infectious bacteria and the etiopathophysiology of various diseases. Th'erefore, it is important to conduct further research into *Pseudomonas* virulence and its spectrum of pathogenesis. Detailed investigations need to be carried out to establish a true relationship between the expression of *P. fluorescens* proteins and the occurrence of Crohn's disease. Another area of great interest would be the underlying functions potentially harbored by this bacterium to predispose the innate immune system to genetic polymorphisms. Understanding the molecular mechanism recruited by this bacterium in the modulation of intestinal epithelial-barrier architecture may provide further insight into the disease. Also, development of an effective compound to inhibit biofilm formation on indwelling medical devices and in wounds remains a challenge.

See also: Characteristics of Foodborne Hazard and Diseases: Drug Resistant Pathogens. Food Safety Assurance Systems: Management of Biofilm Risk. Institutions Involved in Food Safety: Trust in Animals and Food Safety (TAFS) Forum. Natural Toxicants: Tetrodotoxin. Organisms of Concern but not Foodborne or Confirmed Foodborne: Spoilage Microorganisms. Public Health Measures: Food Safety in Hospitals and Other Healthcare Settings. Veterinary Drugs Residues: Antibacterials

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Salmonella Non-Typhi

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Glossary

All-in/all-out A production system whereby animals are moved into and out of facilities in distinct groups. By preventing the commingling of groups, spread of disease is limited. Production facilities should be cleaned and disinfected thoroughly between groups of animals.
Flagella A tail- or hair-like projection that protrudes from the cell body of certain bacteria and functions in locomotion.
Organoleptic The sensory properties of a particular food: the taste, color, odor, and feel.
Outbreak The occurrence, in a community, of an illness with a frequency that is clearly in excess of normal expectancy. For foodborne illness, it is usually defined as the occurrence of two or more cases of a similar foodborne illness resulting from the ingestion of the same food.
Phage A bacteriophage is a virus that infects bacteria. Phage typing is a typing method that is based on the reaction of bacterial isolates toward experimental infection with a series of specific bacteriophages.
Preharvest Measures to improve food safety applied before slaughter or harvest, that is, usually at the farm level.
Postharvest Measures to improve food safety applied at or after slaughter or harvest, that is, during the processing and preparation of the food.
Type III secretion system Type III is a protein appendage found in several Gram-negative bacteria. It consists of a needle-like structure used by the bacteria as a probe to detect the presence of eukaryotic organisms and secrete proteins that help the bacteria infect them. The proteins are secreted directly from the bacterial cell into the eukaryotic cell, also known as 'the host' cell.

Background

Bacteria of the genus *Salmonella* are widespread and important causes of foodborne infections. Serotypes Typhi (*S.* Typhi), *S.* Paratyphi, and *S.* Sendai are highly adapted to humans. The nontyphoid *Salmonella* serotypes are widely distributed in nature, including the gastrointestinal tract of mammals, reptiles, birds, and insects. Most clinical infections of humans are transmitted from healthy carrier animals to humans through food. Nontyphoid *Salmonella* was among the earliest of the so-called emerging pathogens with an important impact on the public health and economy in industrialized countries.

Historical Aspects and Current Perspectives

In 1885, the bacteriologist Theobald Smith, who worked in the US Department of Agriculture, isolated *S.* Choleraesuis from porcine intestine, and the genus *Salmonella* was named after Salmon, his laboratory chief. The first report of a laboratory confirmed outbreak of foodborne salmonellosis was described by Gärtner: 58 persons in 25 different families who had eaten beef developed acute gastroenteritis, one died. Gärtner isolated the 'Gärtner-bacillus' from the infected cow from which the meat came, and from organs of the fatal case. In the following years, several outbreaks of salmonellosis affecting man or animals were reported, and the old concept of 'meat poisoning' was linked with the etiological agent *Salmonella*.

Although *S.* Typhi became an enormous problem in the US in the early industrial era, the disease burden associated with nontyphoid *Salmonella* was low before World War II. Improvements in sanitation nearly eliminated *S.* Typhi as a cause of indigenous infections in the US. However, decades later, the incidence of nontyphoid salmonella infections started to increase, as seen from Figure 1. A similar picture was seen in many other developed countries. In the 1980s and 1990s, two *Salmonella* serotypes in particular, *S.* Enteritidis and *S.* Typhimurium, became major causes of foodborne illness in Western countries as well as in countries that currently are adopting industrialized food production. By contrast, in most developing countries a more diverse range of *Salmonella* serotypes are found in humans.

In many countries, food safety programs have recently resulted in declines in the number of reported salmonella infections. However, salmonella continues to be the most frequent cause of bacterial foodborne disease outbreaks. In addition, new vehicles are recognized as causes of infections in man and the problems are aggravated by an increase in the number of salmonella infections caused by multidrug-resistant strains. Finally, outbreak investigations, trace back investigations, and efforts to improve food safety are becoming increasingly challenging due to the globalization of the food supply; a trend that will continue in the coming years.

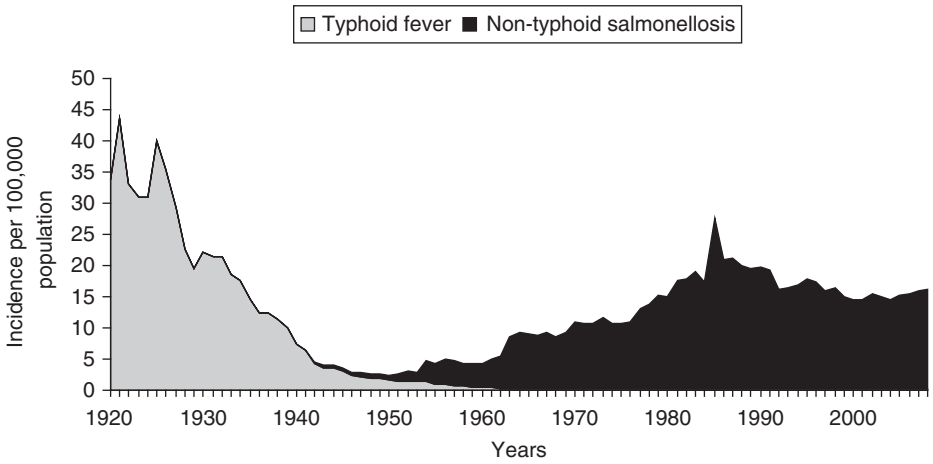

Figure 1 The number of registered infections per 100 000 population caused by *S.* Typhi (gray shading) and nontyphoid salmonella (dark shading) in the US from 1920 to 2008. Data from the CDC kindly provided by Rob Tauxe.

Characteristics

Nomenclature/Taxonomy and Classification

The zoonotic salmonella differs from most other pathogens by the existence of a very large number of different named salmonella types of which *S.* Enteritidis and *S.* Typhimurium are the two most frequently occurring. The classification into these types is based on serological reactions by different surface proteins. An extensive serotyping scheme was developed by Fritz Kauffmann and David White in the 1930s and 40s and is currently maintained by the World Health Organization (WHO) collaborating center for salmonella serotyping at the Institute Pasteur. According to this so-called White–Kauffmann–Le Minor scheme, serotypes are distinguished and named according to reactions to sera against two different groups of antigens: the cell surface lipopolysaccharide (LPS) proteins (for historical reasons called 'O' antigens) and flagellar proteins ('H' antigens). The 'H' antigen (the flagella) derives from German '*hauch*' (breath), first used to describe the swarming of highly motile organisms. 'O' derives from German '*ohne*' (without), first applied to nonswarming (i.e., nonflagellated) bacteria, but now used as a generic term for the LPS somatic antigens of enteric bacteria including *Salmonella*. The Vi antigen was thought to be responsible for virulence. Salmonella strains will typically express either of two sets of genes encoding the flagellar antigens, and two distinct H-antigen 'phases' are therefore said to exist and both need to be expressed in order for the serotyping to be performed. For a small number of serotypes, biochemical reactions or reactions against capsule proteins (Vi antigens) are also included. According to the established notation, the antigenic formula is written with numbers and letters, with antigenic groups separated by colons. Thus, for instance, *S.* Typhimurium has the formula 1,4,5,12:i:1,2 because it carries an LPS protein reacting against the *1,4,5*, and *12* antisera and the *i* phase I H antigen and the *1,2*, phase II H antigen. Today, more than 2570 different serotypes are recognized. Although serotype names were originally inspired by symptoms or by the names of their discoverers, serotypes are now typically named after the geographic location in which they were first characterized (for instance, *S.* Heidelberg, *S.* London, and *S.* Napoli).

According to current bacteriological taxonomy, *Salmonella* constitutes a genus within the Enterobactericeae family. The genus has two species, *Salmonella enterica* and *Salmonella bongori*. More than 99% of serotypes belong to *S. enterica* which is further subdivided into six subspecies: *enterica*, *salamae*, *arizonae*, *diarizonae*, *houtenae*, and *indica*. The salmonella serotypes associated with disease in humans are typically found within *S. enterica* subsp. *enterica*, which contain most of the known serotypes. Formally, the taxonomically correct name of, for instance, the salmonella type that is commonly referred to as *S.* Typhimurium would thus be: *S. enterica* subsp. *enterica* serovar Typhimurium. In general, *S. enterica* subsp. *enterica* are associated with warm-blooded animals, whereas the five remaining subspecies of *S. enterica* are predominantly found in cold-blooded animals (in particular reptiles) and the environment.

Morphology, Metabolism, and Growth

Salmonella enterica is a Gram-negative rod-shaped enterobacterium. The size of the rods ranges from 0.7–1.5 μm to 2.2–5.0 μm; *Salmonella* produces colonies of approximately 2–4 mm in diameter. They have peritrichous flagella, although they are sometimes nonmotile. They are facultative anaerobic chemoorganotrophs. They are oxidase negative and catalase positive. They are generally: able to reduce nitrate to nitrite, able to grow on citrate as sole carbon source, capable of producing acid and gas from glucose, able to produce hydrogen sulfide on triple sugar iron and decarboxylate lysine and ornithine, and able to hydrolyze indole and urea.

Salmonellae are adapted to life in the animal gut and their optimal growth temperature is 37 °C. Some serotypes may be found in a number of different hosts whereas others have adapted to specific hosts. Serotypes may be said to be 'host adapted' if they are prevalent in one particular host, and also able to colonize and perhaps cause disease in other hosts. An example of this would be *S.* Dublin which infects cattle, but rarely humans; however, when doing so causes systemic

infections at a very high rate (resulting in case fatality rates of 20–30%). *S*. Kentucky, common in poultry but less common in humans, is another example of a host-adapted serotype. Some serotypes are host specific, i.e., are able to grow in one host only. A special example hereof is *S*. Typhi and *S*. Paratyphi that are found in humans only, where they cause enteric fever (and are thus not zoonotic salmonella types). In general, the mechanisms underlying host adaptability are poorly understood.

Salmonella may survive for long periods outside of the body and may persist for long periods in, for instance, food production environments. *Salmonella* has rarely given rise to outbreaks via drinking water, but may survive and multiply in foods, and storage of foods at room temperature for prolonged periods before consumption has been found to be a risk factor for outbreaks to occur on multiple occasions. *Salmonella* will not normally grow at refrigerator temperatures; the temperature range of growth is 7–45 °C. *Salmonella* normally survives freezing. Optimum pH for growth is 6.5–7.5, but salmonellae can proliferate in the range of pH 4.5–9.5. Growth of *Salmonella* has not been reported in foods with an a_w of less than 0.93. Nonetheless, bacteria may survive very well in dry foods.

Genetic Factors of Virulence

Salmonella generally infects via the fecal–oral route, survives passage through the stomach acid, and causes lesions in the lower end of the small and upper end of the large intestine. These lesions and the following disease manifestations follow colonization of host cells associated with the gut epithelium. Nontyphoid salmonella strains generally cause self-limiting, transient enterocolitis with watery diarrhea. On rare occasions, they may also cause system infections. In contrast, other strains primarily cause systemic infection and bacteremia, including several of the host-adapted strains, such as *S*. Typhi in humans. Much knowledge has been gained about bacterial virulence factors and pathogenicity through *in vitro* and cell culture studies and through studies of *S*. Typhimurium in mice. The latter model resembles that of systemic infections more than that of general diarrhea, whereas a cattle intestinal loop model has been used for the study of the diarrheal pathogenesis. It is fair to say, however, that the mechanisms behind the induction of enterocolitis are still poorly understood.

Salmonella Typhimurium is a well-adapted pathogen which contains a large number of different virulence genes, many of which are organized in chromosomal pathogenicity islands. Salmonella can enter cells via several routes. Through phagocytosis they are taken up by macrophages where they persist in a special vacuole, called the salmonella-containing vacuole. Factors that are part of a type III secretion system encoded by the salmonella pathogenicity island 2 are required for this. Another mechanism which is probably of more relevance for the induction of diarrheal disease is bacterial-mediated endocytosis. Through fimbriae and other surface molecules, the bacteria attach to epithelial cells. Here they induce endocytosis by use of a separate type III secretion system. This is encoded by the so-called salmonella pathogenicity island 1 and consists of a needle-complex that introduces effector proteins into the cell membrane and cytosol of the host cell. This, in turn, induces cytoskeleton rearrangements and membrane ruffling and eventually uptake of the bacterium. Following invasion, the bacterium induces diarrhea through interplay of a number of virulence factors. Disruption of the normal epithelial control of the passage of ions and immune cells in addition to the generation of localized inflammatory responses following complex signaling with different types of host cells have been suggested to be important elements in this process.

Clinical Manifestations

Exposure of humans to nontyphoid salmonella from contaminated foods or from environmental sources occurs frequently and may lead to clinical or subclinical infection. The serotype, number of organisms ingested, vehicle of infection, microbial environment of the gut, and several other host-associated factors are important in determining the outcome of exposure.

Incubation Time

Within 6–48 h after ingestion of contaminated food, diarrhea occurs. Most patients develop symptoms 24–48 h after exposure, but with ingestion of a high dose the incubation period may be as short as a few hours. In other situations, patients may initially be subclinically colonized, and symptoms develop more than a week after exposure.

Symptoms

Nontyphoid salmonella infection often presents as an acute gastrointestinal illness, indistinguishable from that due to many other gastrointestinal pathogens. In addition to diarrhea, other common symptoms include abdominal pain or cramps, fever, chills, nausea, vomiting, pain in the joints, headache, myalgia, general malaise, and weight loss. Diarrhea varies in volume and frequency, and may be blood-containing. Between 3 and 7% of immunocompetent persons infected with *S*. Typhimurium or Enteritidis have positive blood cultures. The risk of blood stream infection is higher for less common serotypes like Dublin, Choleraesuis, Oranienburg, and Virchow. The proportion of blood isolates is higher in patients of extreme ages, and particularly in the elderly.

Immunosuppression and chronic underlying illness, including inflammatory bowel disease, organ transplantation, malignancy, and malnutrition, are risk factors for severe gastroenteritis, as well as blood stream infection. Subsets of patients develop a primary septicemia without prominent gastrointestinal symptoms.

Duration

In uncomplicated cases, fever usually resolves within 48–72 h, and the average time to recovery from gastroenteritis is 1–2

weeks. However, many patients (up to 25%) report changes in bowel habits up to 6 months after the acute phase.

Carrier Duration

The mean duration of carriage of nontyphoid salmonella is 4–5 weeks after resolution of the gastroenteritis. This duration varies with age of the patient and the serotype. For some less common serotypes, such as *S.* Panama, *S.* Muenchen, and *S.* Newport, more than 20% are still positive after 20 weeks, whereas 90% of *S.* Typhimurium patients are negative at 9 weeks postinfection. A higher proportion of infants than adults have prolonged shedding, but the delayed clearance in neonates and infants does not result in permanent carriage. Treatment with antimicrobials may lead to longer excretion. A chronic carrier state is defined as the persistence of salmonella in stool or urine for periods greater than a year. It is estimated that between 0.2% and 0.6% of patients with nontyphoid salmonella infections develop chronic carriage, which is less than for *S.* Typhi.

Dose

Salmonella infections are acquired by the fecal–oral route, although the swallowing of contaminated aerosols may cause infections in rare situations. Data on the number of salmonella organisms required to cause disease come from volunteer studies and investigation of outbreaks in which the numbers of bacteria in the food vehicles have been determined. Volunteer studies are not generalizable because of the limited number of subjects included, because the physical and chemical properties of the vehicle may not represent relevant food vehicles, and because laboratory passage strains may be less virulent than those found in food animals. Furthermore, higher doses of salmonella may be required to cause disease in healthy volunteers than in populations of high risk. There is evidence of a correlation between dose and disease severity for several common serotypes, including *S.* Typhimurium, *S.* Enteritidis, *S.* Infantis, *S.* Newport, and *S.* Thompson.

Under certain circumstances, very small inoculae have been sufficient to cause the disease. In an outbreak of *S.* Typhimurium infection caused by contaminated chocolate, less than or equal to 10 *S.* Typhimurium bacteria per 100 g of chocolate was found. The authors concluded that the infectious dose was fewer than 10 organisms. In an outbreak caused by paprika and paprika-powdered potato chips contaminated by a variety of serotypes (*S.* Saintpaul, *S.* Rubislaw, and *S.* Javiana), the infective dose was estimated at 4–45 organisms, with an attack rate of only 1 in 10 000 exposed persons. Another example was a massive outbreak of *S.* Enteritidis linked to commercially distributed ice cream made from a liquid premix that had been transported in tanker trucks used previously to haul liquid raw eggs. The highest level of product contamination documented in this outbreak was only six organisms per half-cup (65 g) serving of ice cream. In these three outbreaks, the estimated concentration of *Salmonella* is likely to have been similar to that of the product at the time of consumption. These studies confirm that low-level contamination of food by salmonella, and thus extremely small infectious doses, can cause disease in humans. Ingested salmonella must pass the acid barrier of the stomach, which is a first line of defense against enteric infections. Most strains of *S. enterica* survive poorly at normal gastric pH (less than 1.5), but survive well at pH more than or equal to 4.0, and may have an adaptive acid-tolerance response that may promote survival at low pH. If the salmonellae are suspended in a lipid vehicle, the kill from the acid barrier is reduced, and consequently the infectious dose is reduced. Examples of such vehicles include ice cream, several other types of desserts, some sauces, chocolate, cheese, and peanut butter.

Therapy with antimicrobial drugs has been shown to be a risk factor for salmonella infections. Recent administration of antimicrobials may provide a relative advantage for the Gram-negative flora, a so-called competitive effect. In addition, if the salmonella is resistant to the drug, it has a selective advantage compared with other bacteria in the gut. Chronic illness, including immunosuppressive disease, malignant disease, and diabetes may also decrease the number of bacteria needed to cause infection. A lower dose of bacteria may cause illness in neonates, infants, and seniors than children and young adults.

Sequelae, Chronic Effects, and Complications

Nontyphoidal salmonella gastroenteritis is often described as a mild disease without any complications; without spread to the bloodstream, most infections are self-limiting. However, carefully conducted epidemiological studies suggest that complications are experienced by a significant proportion of patients. Persistence of bowel symptoms is common and is responsible for considerable morbidity and health-care costs. More severe complications are less common. Among 3328 Danish patients, extraintestinal disease was present in 4% and complications in 7%, including 27 unnecessary appendectomies. Complications include endocarditis or arterial infections, intestinal perforation and abdominal, soft tissue, and urogenital infections. Between 2% and 15% of episodes of *Salmonella* gastroenteritis are followed by reactive arthritis, Reiter's syndrome, or erythema nodosa (all correlated with HLA-B27 antigen). Arthritis occurs at an average of 10 days after the onset of diarrhea. Acute phase mortality (case-fatality) has been estimated to be 1.3% in the US, 1.4% in Spain, and 1.2% in Denmark. Case-fatality is higher after septicemia than gastroenteritis only, and is higher in the very old than in individuals below 60 years of age. In addition, infants may have a higher case-fatality. In a registry-based study, 1-year mortality risk was 3.1%, 2.9 times higher than a matched sample of the Danish population, suggesting that the mortality after salmonella infections may be underestimated.

Epidemiology

Surveillance Systems, Sources of Knowledge

Based on symptoms, salmonella infections cannot be distinguished from gastroenteritis caused by several other infectious agents (e.g., *Campylobacter* spp.) and surveillance, therefore, has to rely on laboratory confirmed infections.

Many countries have surveillance systems by which information on diagnosed patients is reported to the national surveillance center from all or a subset of primary diagnostic laboratories. Many countries also have systems by which strains are referred to central or reference laboratories for confirmation and serotyping, and in some cases also additional phenotypic or molecular typing. A number of countries also examine veterinary or food specimens for salmonella in a structured manner, and a substantial added value may be achieved when data from the human surveillance systems are analyzed together with such data from the food chain.

International data on salmonellosis are collected and collated by a number of different institutions or networks, of which some prominent examples are given below. In Europe, the European Centre for Disease Prevention and Control (ECDC) has collected national surveillance data from the EU member states beginning from 2007. The predecessor for salmonella, the EU-supported network 'Enternet,' collected data from European and other countries from 1994 to 2006. In the US, Centers for Disease Control and Prevention (CDC) collects surveillance data from the individual states. In addition, surveillance data are actively collected from a large number of state diagnostic laboratories through the national surveillance network FoodNet. Another important surveillance network is PulseNet US, a typing network coordinated by the CDC in which a subset of salmonella patient isolates from the entire country is subjected to molecular typing using the PFGE or, more recently, the MLVA method, and compared centrally. The aim of this network is to find outbreaks in a timely manner and the network has been extended beyond the US to other countries and regions. By comparing and linking 'fingerprints' of *Salmonella*, subtyping networks are able to discover general outbreaks even when they include single or only few cases in each geographic location. National and international outbreaks have been frequently detected by this approach. International surveillance networks also exist, one example being the Global Foodborne Infections Network (formerly known as the Global-Salm-Surv), a WHO-supported network that collects surveillance data globally and works to improve laboratory detection and typing methods in a number of countries.

The above mentioned systems have improved surveillance of salmonella infections and a paralleled development has been seen in several countries at both national and local levels in recent years. Public health departments are increasingly cooperating and using data from surveillance systems and also alerted by food control authorities, clinicians, and the public professionals. Sharing of information has the potential to detect foodborne outbreaks that would have gone unnoticed in the past and to identify the food source and remove it from the market as soon as possible and, thereby, prevent outbreaks from becoming larger than if no action were taken.

Trends

Table 1 shows the 10 most frequent serotypes in recent years in selected countries. In all countries, either *S*. Enteritidis or *S*. Typhimurium or both are the most prevalent. In the US, the same five serotypes have been found at the top of the list for several years, whereas in many European countries, the serotypes following *S*. Enteritidis and *S*. Typhimurium will vary from year to year. Typically, however, 10 or 20 different serotypes will account for 9 out of 10 infections each year with the remaining 10% of the infections being due to a large number of rarely seen serotypes. Also notable from the table are the relative differences in the number of registered infections, this point is covered in the next section below.

In most of the industrialized world, the number of infections with nontyphoid *Salmonella* types clearly went up in the 1980s and 1990s. From being a relatively unknown organism in the general population, owing to the fairly low level of infections in the 1960s and 1970s, the rising number of infections and the frequently publicized outbreaks helped salmonella to gain notoriety, and consumers began to be aware of the risks of handling food (particularly poultry and eggs) in their homes. During the recent decade, the number of infections has been stable or has followed a downward trend in some countries. Several reasons may be identified behind the overall emergence of *Salmonella* infections, however, most are associated with modern-society, industrialized food production. A number of factors characteristic for the changes in food production systems that have taken place in the past 3–4 decades have facilitated the transmission of salmonella (Table 2). Large-scale production of animals and crops and breeding pyramids of specially bred, genetically similar food animals are vulnerable to salmonella infections. Intensified farming may make animals more prone to infections and trade with live animals can, if they become infected, efficiently distribute the infections from one country to another. New types of foods, for instance the increased use of ready-to-eat foods, and new production systems may sometimes also be liable to contamination. Also, it may take some time before producers or consumers become aware that certain foods are risk products.

In most industrialized countries, *S*. Enteritidis and *S*. Typhimurium are the two most frequently occurring serotypes. In Europe, they together constitute more than 80% of all serotypes. *S*. Enteritidis is the most frequent serotype generally responsible for approximately two-thirds of the infections in Europe (Figure 2). In the US, *S*. Typhimurium is generally more frequent than *S*. Enteritidis and their collective share is approximately 35–40% of all infections.

Because of the high number of infections with these two serotypes, they need to be categorized into smaller groups, necessitating the use of identification methods that go beyond serotype. Phage typing, a phenotypic typing method based on the reaction of *Salmonella* strains toward experimental infection with a series of specific bacteriophages, is a method that has been used widely, and phage-typing systems have been developed for *S*. Enteritidis, *S*. Typhimurium, and few other common serotypes. The antibiogram of strains, though not *per se* a typing system, has also been used widely to categorize serotypes into finer subgroups. Using such methods, it has been possible to obtain a better understanding of the spread of drug-resistant subtypes, and in some instances trends in the number of infections have been found to be constituted by the rise and fall of specific subtypes. Such subtypes, sometimes termed 'success-clones,' have had the genetic make-up that allowed them to establish themselves in important food-production animal reservoirs from where they have, for various reasons, spread from one to several countries.

Table 1 Top 10 nontyphoid *Salmonella* serotypes of laboratory confirmed infections in selected countries/regions and the percentage each serotype constitute of all isolated nontyphoid serotypes

No.	*England and Wales*[a]		*Germany*[b]		*Spain*[a]	
	Serotype	*Number (%)*	*Serotype*	*Number (%)*	*Serotype*	*Number (%)*
1	Enteritidis	66 201 (59)	Enteritidis	239 263 (64)	Enteritidis	23 213 (53)
2	Typhimurium	15 001 (13)	Typhimurium	74 785 (20)	Typhimurium	10 597 (24)
3	Virchow	2736 (2)	Infantis	3417 (1)	Hadar	2275 (1)
4	Newport	1961 (2)	Bovismorbificans	1553 (0)	Infantis	606 (1)
5	Hadar	1213 (1)	Virchow	1460 (0)	Virchow	571 (1)
6	Infantis	1127 (1)	Derby	1192 (0)	Rissen	532 (1)
7	Stanley	1093 (1)	Hadar	1144 (0)	Newport	343 (1)
8	Agona	1077 (1)	Goldcoast	915 (0)	Ohio	335 (1)
9	Braenderup	1006 (1)	Brandenburg	767 (0)	Bredeney	282 (1)
10	Java	933 (1)	Newport	673 (0)	Derby	275 (1)

No.	*Scandinavia*[a,c]		*USA*[d]		*Australia*[e]	
	Serotype	*Number (%)*	*Serotype*	*Number (%)*	*Serotype*	*Number (%)*
1	Enteritidis	27 653 (41)	Typhimurium	41 388 (20)	Typhimurium	17 773 (41)
2	Typhimurium	11 757 (17)	Enteritidis	34 075 (16)	Virchow	2819 (6)
3	Stanley	2161 (3)	Newport	21 202 (10)	Saintpaul	2370 (5)
4	Virchow	1951 (3)	Heidelberg	10 806 (5)	Enteritidis	1904 (4)
5	Newport	1387 (2)	Javiana	8443 (4)	Birkenhead	1444 (3)
6	Hadar	1315 (2)	Montevideo	4932 (2)	Chester	1083 (2)
7	Agona	1212 (2)	Muenchen	4180 (2)	Infantis	920 (2)
8	Infantis	858 (1)	Saintpaul	3790 (2)	Hvittingfoss	809 (2)
9	Saintpaul	749 (1)	Oranienburg	3538 (2)	Bovismorbificans	804 (2)
10	Braenderup	715 (1)	Braenderup	3150 (2)	Muenchen	804 (2)

[a]Data from 2001–2008 from the Enternet and Tessy databases.
[b]Data from 2001–2006 from the German national surveillance system. *Source*: the SurvStat@RKI server at the Robert Koch Institute.
[c]Covering Denmark, Norway, Sweden, and Finland. Swedish data are from 2001 to 2005 only.
[d]Data from 2001 to 2006 from the National *Salmonella* Surveillance System reports, at the CDC.
[e]Data from 2001 to 2006 from the Enternet database.

Table 2 Examples of factors that have had a major contribution for the emergence of nontyphoid salmonellosis in industrialized countries in the past 3–4 decades

Level of food chain	*Factor*	*Effect*
Consumer level	Less familiarity with preparation of new risk foods	Increased incidence of salmonellosis, in particular following emergence of new risk foods
	Increasing number of elderly or immunosuppressed consumers	
Retailers and restaurants	Occasional break down in safety barriers, cross-contamination in large kitchens, use of exotic fruits and vegetables	Outbreaks, sometimes large
Food production/ processing	Large-scale production of minimally processed and ready-to-eat products	Amplification of contamination, widespread dissemination of contaminated products
	Globalized trade	
Animal production systems	Trading of live infected animals for food production or pets (e.g., reptiles)	Spread of infection (and new 'success-clones') from one country or continent to another
	Increased use of genetically similar animals in breeding pyramids	Infection of large number of animals if infection occurs at top of pyramid
	Large-scale production systems	
	Long-distance transport of animals	Transport-induced stress enhances shedding and spread of salmonella
Feed and antimicrobial drugs for food animals	Compound feed, international trade with feed	Changes in intestinal ecology, dissemination of serotypes
	Antimicrobial growth promoters and antimicrobial treatment	Changes in intestinal ecology, selection of resistant bacteria that are passed on to humans

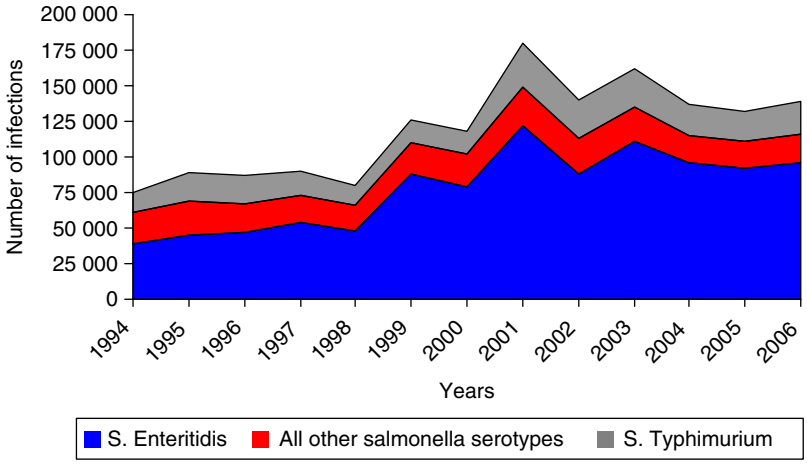

Figure 2 Number of human infections with *S.* Enteritidis, *S.* Typhimurium and remaining serotypes 1994–2006 as registered in the Enternet network database from all European countries supplying data to the network (n = 1 555 464).

One example is the *S.* Typhimurium phage type DT104 which in its original form carried a genetic cassette conferring resistance to five different antimicrobials ('penta-resistance'): ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline. This was among the genetic factors that not only helped made it 'successful,' but has also served as a means of identifying the strain where phage typing has not been used. *S.* Typhimurium DT104 was largely nonexistent before 1980, but then appeared in the UK where it gradually spread in the 1980s from cattle to other food production animals and from thereon in the 1990s, spread to continental Europe and North America and other parts of the world. DT104 is common in a broad range of food animals, such as poultry, pigs, and sheep, and in many countries is, or has been, the most common subtype of *S.* Typhimurium isolated from humans. In later years, however, this type has been declining again in several countries.

During primarily the 1980s and 1990s, *S.* Enteritidis successfully infected chickens used for egg production and have since given rise to millions of human infections, transmitted via the consumption of eggs. A number of necessary or contributory factors have been found to lie behind this very severe food safety problem. Genetically, *S.* Enteritidis has the unique capacity of being able to infect the ovaries of chickens without causing symptoms. Thereby the bacterium may enter the egg internally and thus both spread to the offspring and transmit to consumers. It has been argued that *S.* Enteritidis has filled an ecological niche that became vacant following the control of the host-specific serotypes *S.* Gallinarum and *S.* Pullorum, both of which gave symptomatic infections. In the kitchens, before this problem arose, eggs were safe to eat raw and a number of common dishes and desserts contained raw eggs. Consumers were, therefore, caught unguarded and needed to adapt to the new situation. Salmonella may multiply within the egg or in the prepared dish before consumption, thus sometimes growing to high numbers, and increasing the potential for causing disease. Also at the level of production systems, major changes have been introduced. Small-scale egg production has been substituted for large often battery chicken production systems. These rely on genetically clonal animals that are all offspring of the same few ancestor birds, which have been bred for high performance and are supplied by commercial companies. When, as it happened, infected chickens are delivered into the top of such breeding pyramids, a very large number of birds will quickly become infected. This way the infection was able to spread very efficiently.

Many phage types of *S.* Enteritidis have been recognized as forming part of the problem, some being more prominent in certain geographical regions. *S.* Enteritidis phage type 4 was particularly predominant in Europe and North America where it spread in the 1980s and 1990s. Figure 3 shows the situation in the UK, where this type was particularly important and caused what was in reality a 20-year long outbreak. The decline that started in the late 1990s was concomitant with the introduction of vaccination of egg-laying hens against serovar Enteritidis. Other factors such as improved biosecurity in egg-laying flocks, a build-up of immunity in other animals, and the rise in the number of livestock infections with host-adapted serovars of *Salmonella* have also played a part in this decline.

The Surveillance Pyramid and Differences Between Countries

National and international salmonella surveillance data will never truly reflect reality and, therefore, should be interpreted with great care. This relates to the fact that salmonellosis is a transient and comparably mild disease and so will always be under-diagnosed. However, only diagnosed cases show up in the statistics. Salmonellosis is normally a self-limiting disease and most patients will choose not to see a doctor. For those who do, the doctor may often choose not to request a stool sample examination, and even when examination is performed, only a subset of the true patients will be correctly diagnosed and reported to the national registration systems. Salmonella surveillance systems will therefore only capture a fraction of the true cases and will tend to capture the more severe manifestations. Population studies performed in the Netherlands, the UK, and US indicate that the true number of symptomatic cases in the community is from 3.8 to 38 times as high as the surveillance data would indicate, and other studies have shown even higher conversion factors.

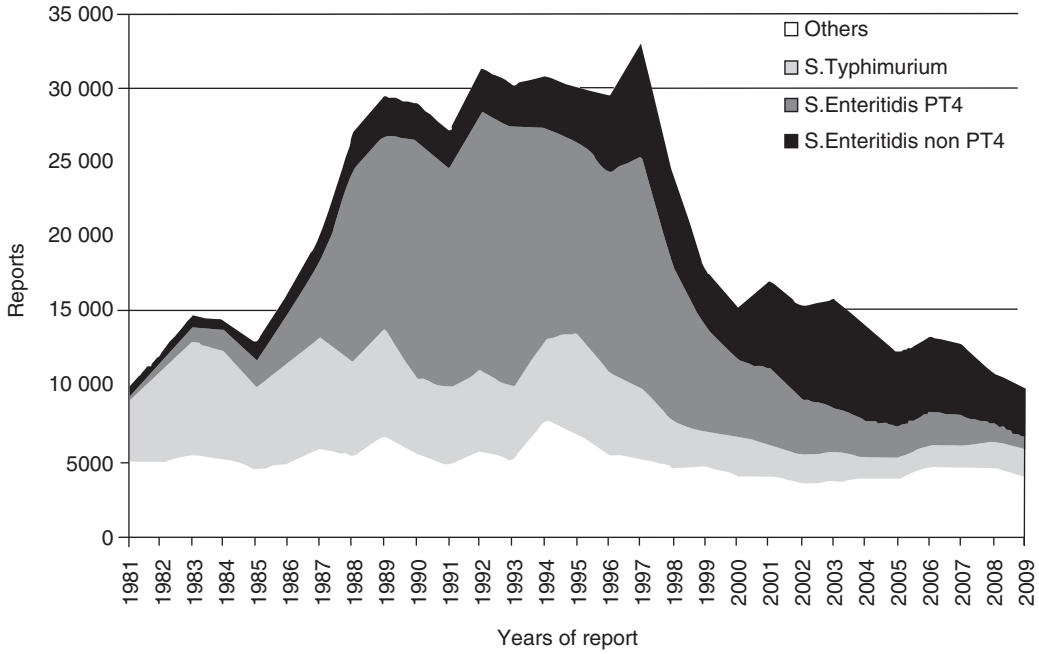

Figure 3 Incidence of reported infections by most frequent types of Salmonella, England, and Wales, 1991–2009. Data from the national surveillance systems in England and Wales. Courtesy of Chris Lane, HPA, London.

For the same reasons it is generally also not possible to directly compare rates of salmonellosis between countries. This is evident from Table 1. Local practice, the priority given to foodborne illnesses, and financial factors will influence heavily on how many samples are analyzed for salmonella. Also, laboratory methods may vary and serotyping may not be complete. Furthermore, strains and information may not always be routinely collected centrally, or may be collected from a subset of laboratories only. Therefore, a low number of cases in a given country or region may sometimes be little more than a reflection of a suboptimal diagnostic or surveillance system. This is true not only for human cases, but also for surveillance data covering the food chain.

To make comparisons between countries, surveys or special studies have to be performed. An example from the veterinary perspective is the so-called baseline studies that have been conducted jointly in the EU member states recently. As part of these, the levels of salmonellosis in pigs, egg layers, and broilers have each been assessed through a uniform scheme of sampling and testing. Recent attempts to compare the level of human infections have involved comparison of burden-of-illness studies, sero-epidemiological studies, and evidence from infected travelers. For instance, studies of returning infected travelers from Sweden, which have a very low level of endemic salmonellosis, have been used to address the degree of underreporting in national statistics and the risk of infection in different countries, which in Europe largely was found to follow a north–south gradient.

Developing Countries

Infectious diarrhea is a massive problem in many developing countries and carries a high mortality, particularly among young children. Although salmonellosis in developing countries is often well characterized and subject to various forms
of control programs, the situation is quite different in the developing world. Owing to poor sanitation, *S*. Typhi and *S*. Paratyphi continues to constitute significant health problems in many countries. However, although not well documented, nontyphoid salmonella infections also appear as a considerable health problem in developing countries in so far as they are frequently the etiological agent in infectious diarrhea. In addition, they will often cause blood stream infections in immunocompromised persons, and may therefore seriously complicate the clinical picture of malaria or HIV-infected individuals. One estimate holds that 10% of HIV-positive adults in Sub-Saharan Africa will develop nontyphoid salmonella bacteremias each year, resulting in considerable mortality. The problem in developing countries is often complicated by high levels of antimicrobial drug resistance in a variety of salmonella serotypes that causes invasive infections in children.

Age, Gender, and Seasonality

Figure 4 shows the incidence year by age and gender of the total number of notified laboratory confirmed nontyphoid salmonella infections in Denmark. The incidence of *Salmonella* is substantially higher in infants and young children, a result of both increased susceptibility and a diagnostic bias, because relatively more samples from children will be examined for salmonella. The incidence rate also increases slightly in young adults in their twenties, but then remains fairly constant. A similar picture is seen in many other industrialized countries, sometimes with a slight additional increase among elderly persons. In the US, the overall yearly reported incidence ranged from 19 to 13 per 100 000 population from

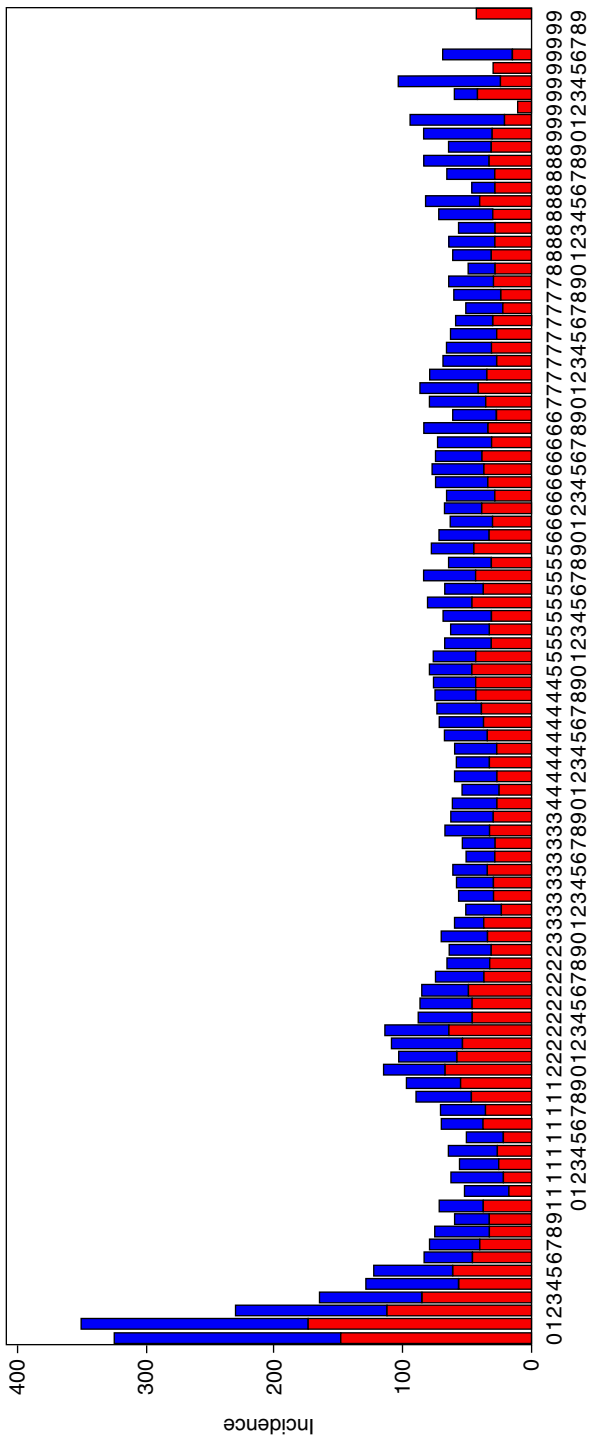

Figure 4 Age- and gender-specific incidence of human laboratory confirmed salmonella episodes in Denmark 2005–2009 (n = 11 063). Cases are shown per 100 000 Danish residents by gender and at each year of age. Reproduced from Statens Serum Institut, Denmark.

1987 to 1997, whereas the incidence in infants was as high as 122 per 100 000. Slight differences between genders are also frequently seen, typically with a higher incidence in boys than in girls, but among adults a higher incidence in women than men. The reason behind this may involve health-seeking behavior, physician's behavior, age- and gender-specific exposures, transmission patterns as well as hormonal and immunological factors.

In temperate climates, salmonella infections exhibit a marked seasonal pattern, typically with a several times higher incidence of domestically acquired infections in the warmer months compared with the colder. Depending on their reservoirs, some serotypes may however deviate from this pattern.

Geographic Distribution of Certain Serotypes

A few serotypes are geographically restricted. A good example is *S.* Weltewreden, which is widespread throughout South Asia and South East Asia, but rarely occurs in Europe except as imported infections. Other examples are *S.* Eastbourne in West Africa or *S.* Concord in Ethiopia. Some serotypes are typical for geographical location they take their name from; *S.* Napoli is frequently found in Italy and *S.* Mississippi in limited areas of the Eastern and South Eastern USA. *S.* Javiana is common in young children in Eastern US. Infections with such serotypes may be caused by direct or indirect contact with a particular animal reservoir or be associated with a local and limited production of food items. Knowledge about geographical restriction may, similar to knowledge about predominance of certain serotypes in certain animal reservoirs, be very helpful when trying to find sources of human infections.

Isolation, Identification, and Enumeration

Conventional Detection of *Salmonella enterica*

Culture-based detection and isolation procedures for salmonella principally consist of four steps: nonselective preenrichment, selective enrichment, isolation, and confirmation. However, minor differences in the use of media, incubation time, and temperature can be found in various standard methods.

Food, animal feed, and environmental samples usually contain low numbers of potentially stressed salmonella bacteria and therefore preenrichment in nonselective media is needed to aid the recovery of sublethally injured salmonella. Even though salmonellae are viable and able to cause disease under the right conditions, the organisms can easily be killed if they are grown under selective pressure, such as high temperature or in the presence of chemical additives. Buffered peptone water and lactose broth are two of the most widely used media for preenrichment.

The second step is enrichment in selective media. This increases the number of salmonella to a level where detection on selective agar plates is possible, whereas at the same time inhibiting growth of accompanying microflora by selective agents in the media. The most commonly used media for selective enrichment are Rappaport-Vassiliades soy broth, selenite cysteine broth, and tetrathionate broth. Modified semisolid Rappaport-Vassiliades is another selective media particularly useful for detecting salmonella in feces and environmental samples.

Isolated colonies are obtained by streaking out the selective enrichment broths on the surface of selective solid media. Growth of accompanying bacteria is repressed by the addition of inhibitory compounds and salmonellae are differentiated from other bacteria by, for instance, the production of H_2S or acid from sugars. Most standard methods recommend employing two media exerting different selective pressures. Commonly employed media include brilliant green, xylose lysine Tergitol-4, bismuth sulfite, Hektoen enteric, and xylose lysine deoxycholate agars. Recently, novel chromogenic agars have been compared favorably with these common agars, being more sensitive and specific.

Finally, presumptive *Salmonella* colonies are subcultured on nonselective plates in order to get well-isolated colonies that can be used for further characterization by biochemical and serological analysis. Several biochemical reactions are tested, i.e., triple sugar iron, mannitol, urea, ornithin decarboxylase, and lysine decarboxylase. A serological verification by determining the antigenic composition is performed. The O and H antigens are determined by agglutination testing using polyvalent antisera. Miniaturized tests such as API 20 E (bioMérieux) and BBL™ Enterotube™ II (BD Diagnostics) have been developed for confirmation and they present an efficient and labor-saving alternative. Identification of isolated colonies by polymerase chain reaction (PCR) is a reliable, cost-efficient, and labor-saving option, becoming widely applied in microbiological laboratories. Several well-validated PCR assays are described for this purpose, some of them providing additional discrimination between strains or information on antibiotic resistance depending on the target gene(s) of choice.

Human gastroenteritis caused by salmonella is diagnosed by culturing of fecal samples. Normally, isolation is accomplished by direct plating on selective agar plates from fecal samples containing high numbers of target bacteria. An enrichment step in selenite broth has been reported to increase the sensitivity of the direct culture, but this has to be weighed against an additional time for diagnosis of 24 h. Confirmation of presumptive salmonella is performed as described earlier.

Conventional methods for detection of *Salmonella* are time consuming and labor intensive. Furthermore, these methods may suffer from poor specificity due to, for instance, difficulties in recovering sublethally injured cells, problems in the identification of atypical colonies, a high degree of false-positive results, and lack of detection of viable but nonculturable bacteria. However, among the advantages of conventional culture methods are that they are universally accepted, have a history of good reliable performance, and produce an isolate that can be characterized further.

Detection of *Salmonella enterica* by Alternative Methods

Polymerase Chain Reaction

PCR offers many advantages compared to conventional culture-based detection methods regarding sensitivity, specificity,

speed, and possibility of automatization. Numerous PCR assays for the detection of salmonella in a variety of sample matrixes have been described in the literature. The majority of them deal with detection in food, reflecting the epidemiology of this human pathogen. The PCR-based detection methods in food commonly employ a preenrichment step combined with subsequent PCR detection. The preenrichment times reported vary from 6 to 24 h, depending on the artificial inoculation level of *Salmonella* in the experimental design of the studies. The studies taking into consideration the limit of detection of 1 colony-forming unit (CFU) per 25 g in food samples report a minimum of preenrichment of 8 – 10 h. The majority of these PCR assays amplify part of the *invA* gene, encoding a protein involved in the invasion of epithelial cells, however, it has been shown that *invA* is lacking in some strains.

The methodology, i.e., enrichment media, time and temperature, sample preparation, PCR primers, probes, and thermal profile, varies according to the food matrix in question, and with the abundance of methods and lack of standardization it can seem overwhelming to identify a suitable method. A description of some of the more recent validated methods for detection in relevant food matrices and a review of molecular methods for detection and discrimination of foodborne salmonella may be found in the literature list at the end of the article.

The major advantage of PCR-based detection of a foodborne pathogen like *Salmonella* is the reduction in time of analysis. A negative sample is usually diagnosed within 24 h with PCR, compared to 3–4 days using culture-based detection. Furthermore, a higher degree of automation of PCR-based detection is achievable, making high-throughput analysis possible.

Multiplex PCR assays simultaneously detecting a range of relevant pathogenic bacteria are likewise described in the literature. Such methods would constitute highly effective screening tools for determining the microbiological safety of certain food commodities; however, implementation of the majority of these assays is hampered by a lack of validation.

PCR detection methods have been described for human clinical samples as well. A number of them are designed to target specific serotypes and have little use as diagnostic screening tools, but more broad methods for *S. enterica* have compared favorably with conventional culture-based methods in several studies.

Other Methods

Methods combining immunomagnetic separation (IMS) with detection steps like enzyme immunoassays (EIAs) or PCR assays have been developed. The salmonella cells are separated from their matrix by IMS of magnetic beads coated with salmonella-specific antibodies, and subsequently detected by PCR or EIA. This primary concentration of target cells should theoretically result in an improved limit of detection, however, the findings are not convincing.

Loop-mediated isothermal amplification (LAMP) methods for detection of *Salmonella* in food matrices and clinical samples are being increasingly developed, and are reported to be more sensitive than equivalent PCR methods. Because there are no instrument needs with this type of assay, the application possibilities are far greater, and LAMP has the potential to be applied as a simple screening assay in the field or at the point of care by clinicians.

DNA microarrays for detection and characterization of pathogens such as salmonella are being developed more frequently. The microarray oligonucleotides are designed to recognize a variety of *Salmonella* genes involved in virulence, antibiotic resistance, or serovar-specificity, and information on presence and expression can be obtained. DNA microarrays targeting and partly characterizing multiple pathogens including salmonella have been described in the literature as a diagnostic tool for rapid and simultaneous identification of pathogens involved in clinical diarrhea.

Enumeration

Assessment of the illness a pathogen can cause in a given population is a key factor in food safety. Because the severity of salmonellosis is associated with the degree of contamination (dose–response relationship), these risk assessments are based on information on the number of salmonella.

Culture-based enumeration of salmonella relies either on the most-probable-number (MPN) technique if low numbers are expected, or on direct isolation on selective solid media if high numbers (more than 100 CFU g^{-1} or ml) are believed to be present. Although the MPN technique is quite laborious, results obtained from direct isolation can be influenced by the amount of background flora and the physical state of the salmonella cells.

PCR-based enumeration of salmonella would, on several levels, be a welcome alternative to culture-based enumeration. However, the sensitivity of PCR still makes detection, without prior enrichment or an improved sample preparation step to concentrate target cells, impossible in the case of low-level contamination.

Food Sources of Infection and Preventive measures

Routes of Transmission of *Salmonella enterica* to Humans

Nontyphoid salmonella infections are generally acquired via food, though in the developing world contaminated drinking water can also be a route of infection. Person-to-person transmission is normally rare because of the relatively high infectious dose of the organism, but infections may be transmitted through direct animal contact. Transmission from reptiles held as pets is well documented, particularly in the US. However, foodborne infections are by far the most common, and salmonellosis has traditionally first and foremost been transmitted as zoonotic infection via consumption of products from infected animals, in particular eggs, poultry meat, pork, and beef. Increasingly however, other types of foods are also seen as sources of infection, including fresh produce.

Principles of Control

Animal food production systems can be viewed as a chain of successive steps, each of which holds the potential for introduction of salmonella infection or contamination. Efficient control of salmonella is, therefore, only reached when all steps in this chain are monitored and targeted by quality programs.

Animal feed may be infected and, therefore, needs to be controlled. Animals may become infected at the farm via contact with wild animals, insects, the environment, farmers or veterinarians, or the introduction of infected animals in the flocks or herds. At slaughter, meat from noninfected animals may become cross-contaminated via infected animals that introduce salmonella into the slaughterhouse environment. Contaminated meat products may give rise to a large number of infections if processed in a way that allow for growth of salmonella or if processed into ready-to-eat products without sufficient reduction of counts or inhibition of growth. At retail, breach of the cold-chain or improper handling of products may pose a hazard.

Finally, contaminated products in kitchens in the homes or in commercial establishments may give rise to illness if not handled properly, as for instance through lack of separation of raw meat and salad ingredients, improper storage, or through improper cooking procedures. On this basis, education of consumers and food handlers in respecting safe food-preparation measures forms a part of the overall attempts to control salmonella. Important elements include cooking poultry, meat, and eggs thoroughly, keeping food at safe temperature, separating raw and cooked food, using safe water and safe raw materials, and careful personal and kitchen hygiene.

Countries to varying degree have legislation and food control and inspection systems that cover the later steps in the food chain, ensuring that hygienic standards are in place and that quality assurance systems, hazard analysis and critical control points (HACCP) programs, and own-check programs are followed. However, at farms and slaughterhouses, *Salmonella* is more difficult to control and much research has been focused on controlling or limiting the introduction of *Salmonella* at these points in the chain. As a general principle, it is better to control the problem early in the food chain, thus arguing for prioritizing preharvest rather than postharvest control programs. Obviously, sampling and testing at different steps in the chain are also important in order to monitor the effect of the control programs. An integrated process, where data are shared and steps agreed among different stakeholders in the food chain is very much advisable, though this may be challenging to establish because of the required cooperation between different types of sectors, both commercial (farmers, slaughterhouses, and retailers) and public (different ministries and levels of administration). There are historic examples of successful salmonella control. In Sweden, a large *S.* Typhimurium outbreak occurred in 1953, killing 90 people. This led to a national action plan against salmonella, decades before this infection reached the agenda of other countries. As a result, Sweden has been officially free of *S.* Enteritidis and *S.* Typhimurium for more than 20 years. Sweden and Finland were able to maintain their 'salmonella-free' status when they entered the EU. To what extent experiences from the Nordic countries can be adapted by other countries with different and larger production systems remains a debatable issue.

Major Transmission Reservoirs and Their Control

Eggs: As also discussed above, eggs are a very important source of human salmonella infections. *Salmonella* may reside both on the inside – *S.* Enteritidis only – and outside of the egg, the latter being the more frequent. Even when contamination is a rare event it may lead to a disproportionately high number of human infections, because eggs are so frequently consumed and because raw eggs – as opposed to raw meats – are an ingredient in many dishes (mayonnaises, cold sauces, cakes, and ice creams, to name a few) and may cause infection even when partially heat treated (eggs fried on one side only or lightly scrambled eggs).

Infections occur frequently.: An EU baseline study of large laying hen holdings was conducted in 2005 and found 30% of flocks to be infected. The percentage of positive flocks in different countries varied widely, going from 0 to 80%. Infection may be introduced into the chicken houses by a number of means, and both biosecurity and cleaning in conjunction with an all-in/all-out strategy is of major importance. By far, the most important way of introducing infections, however, is introduction of infected animals. Because of the breeding pyramid production system, a small number of infected grandparent-hens will result in a large number of infected layers two generations down the line. However, targeting control measures at the top of the breeding pyramid may potentially have a major effect as have been shown in Scandinavia. In Denmark, both layers and broilers were heavily infected in the 1990s which prompted the introduction of a national top-down eradication strategy based on testing and elimination of infected birds at the top of the breeding pyramid. Today, layers and broilers are in essence salmonella free.

Postharvest strategies have also proven efficient, most notably heat decontamination of the shell and pasteurization of either the egg white, yolk, or whole eggs. The latter is done through a process of placing the eggs in water baths for several hours, which may reduce the salmonella counts within the egg by up to 7-log without changing the organoleptic properties of the egg.

Poultry: Studies from a number of countries in Europe and from North America document that broiler flocks frequently test positive for *Salmonella*, and figures ranging from 0 to 60% of flocks being infected have been reported depending on the geographic area. Commercial flocks of turkeys, geese, and ducks typically have an even higher infection rate than do broilers. Broilers may be infected with several serotypes in addition to *S.* Enteritidis and may be a source of human infection with serotypes such as *S.* Infantis, *S.* Hadar, *S.* Virchow, and *S.* Typhimurium.

The measures applying to preharvest control of salmonella in layers also applies to broilers, and controlling the infection at the top of the breeding pyramid is generally advisable. Vaccination using a live attenuated vaccine is used in some countries both for broilers and layers with good results. Vaccination does not eliminate *Salmonella* in flocks, but will increase the animals' resistance to infection and colonization and thereby serve to markedly reduce the levels of *Salmonella* in the flocks. Vaccination has been shown to have highest effectiveness if it is accompanied by hygienic measures as well. Postharvest control is important for broilers, and care should be taken to control each of the steps in the slaughter process. Several systems aiming to reduce or eliminate salmonella in the slaughtered birds or the chicken meat have been developed.

Pork: Pork meat and pork products are also an important source of human infections. An EU member state baseline study of salmonella in slaughter pigs conducted in 2007 found that 1 in 10 slaughter pigs were positive, with country prevalences ranging from 0 to 29%. The most frequently isolated serotypes were *S*. Typhimurium and *S*. Derby. An EU risk evaluation has subsequently estimated that pigs are the source of 10–20% of all human cases in the EU.

Denmark is among the few countries that have an integrated food-chain control program with focus on preharvest reduction. All herds and pigs are registered and meat juice is sampled and tested by serology at slaughter. Thereby, herds are divided into three levels of infection. Farmers with severely contaminated herds receive guidance helping them to reduce the problem, and are at the same time paid less per slaughtered pig. Also, pigs from such herds are slaughtered in a particular hygienic manner and the use of the meat is restricted.

Beef: Cattle is also a source of human infections, though probably less often. Cattle may be infected with several serotypes, but the host-adopted serotype *S*. Dublin is frequently found in cattle. This serotype does not normally cause infections in humans; although this may happen, in particular, in immunosuppressed individuals, and will then lead to bacteremia at a high rate. Both pre and postharvest measures are important for reduction of *Salmonella* infections. Movement and purchase of animals is an important route of introduction of infections and therefore also important points of control.

Unpasteurized milk is a well-documented source of human infections. Outbreaks have been described where inadequately pasteurized milk or postpasteurization contaminated milk or cheeses were found to be the source. Postprocess contamination may also occur in a range of other products, including infant food and other dry processed food products.

Other sources of infection: Fruits and vegetables have become an increasingly important source of salmonella infections. In the US, fruits and vegetables have given rise to more outbreaks than meat products in recent years. Fruits and vegetables may be contaminated in the field (by animals or by contaminated irrigation water), at harvest, processing, packing, and distribution. It appears that water used for rinsing or spraying may often be a point of contamination. Rinsing conditions should be set up so that they prevent cross-contamination and the water used should be clean. Examples of fruits and vegetables that have given rise to outbreaks recently include melons, tomatoes, lettuce of several different sorts, and sprouts. In 2008, a nationwide outbreak of *S*. Saintpaul in the US was traced back to jalapeño and serrano peppers produced in Mexico. Washing the products in the kitchens may not always eliminate the problem; thus alfalfa sprouts, which have been the source of a number of outbreaks, may be infected internally because contaminated seeds may 'incorporate' the bacteria when sprouting.

Decontamination of food by ionizing radiation has been suggested as an effective postharvest measure to improve the safety of food products. In the current European legislation, irradiation can be applied to spices and herbs. Radiation treatment at doses of 2–7 kGy – depending on condition of irradiation and the food – can effectively eliminate potentially pathogenic nonsporeforming bacteria including salmonella. If measures at the farm fail, radiation decontamination can also be applied to poultry and red meat, egg products, and fishery products. It is a unique feature of radiation decontamination that it can also be performed when the food is in a frozen state. Radiation can also be applied to seeds such as sprout seeds with only little effect on germination. However, for various reasons, radiation has currently only limited use as a postharvest measure.

Many other nonanimal foods may be the source of salmonella infections. For example, almonds have produced large outbreaks, spices may be contaminated, and several outbreaks have been caused by chocolates, which serve as efficient vehicles of infection in part because, as discussed above, the fat content of the chocolate protects the bacteria when passing through the acidic environment of the stomach. It is often difficult to attribute cases to these less usual sources of infections and also difficult to solve outbreaks caused by such sources. Owing to improved control of traditional animal sources of infection and increased global trade with exotic food products, it is expected that foods of nonanimal origin and 'unusual' vehicles will become relatively more important sources of salmonella infections in the future.

See also: Bacteria: *Salmonella* Typhi and *Salmonella* Paratyphi. Foodborne Diseases: Overview of Biological Hazards and Foodborne Diseases. Public Health Measures: Foodborne Disease Outbreak Investigation

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WHO Food Safety Programme.

Salmonella Typhi and *Salmonella* Paratyphi

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Glossary

Carrier Person infected with a pathogen who is not ill, but who is capable of transmitting the pathogen to others.
Molecular clock analysis Technique that compares the rates of random mutation among conserved housekeeping genes between related strains of microbes to estimate the time they last shared a common ancestor.
Peyer's patch Lymphoid follicles in the distal small intestine important for gastrointestinal immune surveillance and response.
Pseudogene Nucleic acid sequences that were functional in ancestral organisms, but have become modified and no longer function.
***Salmonella* pathogenicity islands** Nucleic acid sequences in *Salmonella enterica* that code for functions critical to invasion, infection, and survival in the host.
***Salmonella* serovar** A subgrouping of *Salmonella enterica* distinguished by serological testing of surface and flagellar antigens.
Type III secretion system A bacterial process that detects host eukaryotic cells and injects bioactive proteins into them.
Typhoid Vi A polysaccharide coat that surrounds most *Salmonella* Typhi and contributes to virulence.

Background

In 1881, Karl Joseph Eberth, a pathologist at the University of Zurich, used a microscope to examine sections of spleen and mesenteric lymph nodes of a patient who died of typhoid fever and published the first description of the morphological characteristics of the responsible bacteria. The bacterium was initially referred to as Eberth's *Bacillus*. The primary causative agent of typhoid fever is now referred to as *Salmonella enterica* serotype Typhi. It is more commonly referred to as *Salmonella* Typhi, but if this shorthand is used Typhi should not be italicized, because it is not a species designation. Typhi denotes a serovar of the species *S. enterica*. *Salmonella enterica* is a remarkably diverse species. Three additional serovars of *S. enterica* that also cause typhoid fever are Paratyphi A, B, and C. *Salmonella* Typhi and Paratyphi A, B, and C are collectively referred to as the typhoidal *Salmonellas*.

Characteristics

Salmonella enterica are rod-shaped, facultative anaerobic Gram-negative bacteria. Most *S. enterica* infect birds and domestic animals and zoonoses are an important pathway for human infection, but *Salmonella* Typhi and Paratyphi A have a much more restricted host range. Although it is often stated that *Salmonella* Typhi only infect humans, it can also infect other primates closely related to humans, though it is not known how frequently this occurs in nature, nor whether such infections contribute to the propagation and survival of the organism. *Salmonella* Paratyphi B and C infect other animals.

Salmonella Paratyphi A, B, and C are quite different organisms. Although *Salmonella* Paratyphi A is in a different serogroup than *Salmonella* Typhi, because of a minor difference in lipopolysaccharide sugars, they are genetically very closely related. The restricted host range of *Salmonella* Typhi apparently results from the inactivation of over 200 pseudogenes (5% of its genome). Over half of the pseudogenes are inactivated by a single frame shift or stop codon. *Salmonella* Paratyphi A also has numerous inactivated pseudogenes comprising 4% of its genome. The *Salmonella* Paratyphi A pseudogenes are less diverse than those in *Salmonella* Typhi suggesting that *Salmonella* Paratyphi A is a younger clone. *Salmonella* Paratyphi B is a remarkably diverse serovar; only some of these strains cause enteric fever. Other strains of *Salmonella* Paratyphi B, previously identified as *S. enterica* serotype Java now referred to as biotype Java, commonly infect poultry and do not typically cause typhoid fever. The *Salmonella* Paratyphi C genome is more closely related to other *S. enterica* serovars than it is to *Salmonella* Typhi.

Human infection with most *S. enterica* produces an exudative intestinal inflammation that causes diarrhea, which increases the shedding of the pathogen and so it's opportunity for ongoing transmission and survival. By contrast, in human typhoidal *Salmonella* infection, diarrhea is uncommon, occurring in only 30% of cases severe enough to be hospitalized and in $< 10\%$ of outpatients with milder illness. The typhoidal *Salmonellas* are invasive, cause systematic infection, and 1–4% of all *Salmonella* Typhi and Paratyphi A infections result in

long-term colonization of the hepatobiliary tract, especially the gallbladder. Typhoid/paratyphoid carriers can excrete the organism for decades, which provides opportunities for ongoing transmission of these host-restricted pathogens. Indeed, the evolutionary survival benefit of invasion and systematic infection for *Salmonella* Typhi and Paratyphi A likely results from the increased opportunity for hepatobiliary colonization and chronic carriage.

Like other *S. enterica*, when the typhoidal *Salmonellas* are inoculated at favorable temperatures into a nutrient-rich environment, such as cut fruit or dairy products, they reproduce exponentially.

Clinical Manifestations

Human challenge studies suggest that 10 000 *Salmonella* Typhi bacteria induce infection among 40% of human volunteers, though there is likely a wide range of infectious doses depending on the level of immunity of the person exposed, and characteristics of the food matrix where the bacteria is embedded that might protect it from stomach acids. The incubation period from exposure until onset of symptoms depends on the dose and is most commonly 7–14 days, but can be as short as 3 days and as long as 60 days. Up to 20% of infections have an incubation period >3 weeks. Typhoid fever presents with a range of severity. Among patients managed without hospitalization, high-grade fever is the cardinal symptom and no other single sign or symptom occurs among >25% of cases. Among patients with a more severe illness requiring hospitalization, most have anorexia and headache and 25–40% have vomiting, hepatomegaly, and abdominal pain. Such patients often present with a characteristic apathetic affect.

Untreated typhoid fever typically has a 4-week clinical course that can be shortened considerably with appropriate early antimicrobial therapy. Typhoid fever relapses in 5–10% of patients treated with antimicrobials. Relapse has also been observed in the absence of antimicrobial therapy.

Gastrointestinal bleeding resulting from erosion of the Peyer's patch into an intestinal vessel occurs in 10–20% of cases of typhoid fever, but blood loss is usually minor and not life threatening, detectable only through testing for fecal occult blood. Intestinal perforation, most commonly at the site of an infected Peyer's patch, occurs in 1–3% of cases. Intestinal perforation is a life-threatening complication with a case fatality rate between 10% and 45% that accounts for at least 25% of deaths from acute typhoid fever. Fluid rehydration, emergency surgery, and appropriate parenteral antibiotics can be life saving. Neuropsychiatric syndromes associated with typhoid fever include typhoid encephalopathy, meningitis, encephalomyelitis, Guillain–Barré syndrome, and cranial or peripheral neuritis.

The reported case fatality of typhoid fever varies widely. In the preantibiotic era, reported case fatality ranged from 7.5% to 19% in population-based assessments and was as high as 30–50% among hospitalized cases. More recently, case-fatality varies by location though apparently remains higher among hospitalized cases with more severe disease than among patients with more mild diseases who are treated as outpatients. However, there are very few community-based estimates of case fatality rate, and on review of available literature the estimate of global burden of typhoid assumed an average global case fatality of 1% (Crump *et al.*, 2004).

Infections with strains of *Salmonella* Typhi that are multiply drug resistant tend to cause more severe illness, are more likely to require hospitalization, and are associated with a higher case fatality ratio. Typhoid fever due to infection with *Salmonella* Paratyphi A, B, or C has a clinical presentation similar to *Salmonella* Typhi, though they tend to have a somewhat shorter incubation period and a milder clinical course.

Several studies have demonstrated an increased risk of hepatobiliary cancers among typhoid carriers. An assessment of a Scottish population-based registry compared the rate of cancers among persons who were confirmed typhoid or paratyphoid carriers to the general population of Scotland and to people who were acutely affected during a typhoid outbreak 30 years previously (Caygill *et al.*, 1994). There was no excess risk of cancer mortality among persons who had acute typhoid and who did not become typhoid carriers. By contrast, the chronic carriers (two-thirds of who carried *Salmonella* Paratyphoid serovars) had a risk of carcinoma of the gallbladder that was 167 times higher than the general population and a risk of carcinoma of the pancreas that was 8.1 times higher. The lifetime risk of gallbladder carcinoma among chronic typhoid/paratyphoid carriers was at least 6%. Patients with gallstones are at increased risk of becoming chronic typhoid carriers and are also at increased risk of gallbladder cancer. Thus, one explanation for these findings is that typhoid carriage is simply a marker of gallstone disease that does not contribute additional risk beyond the risk of gallstones. However, more recent case–control studies conducted in India, where typhoid carriage is common, have identified a higher prevalence of typhoid carriage among patients with gallbladder cancer compared with patients who have gallstones but no cancer. Although the precise molecular basis for the increased risk of carcinoma has not been identified, a clear association of gastric cancer with chronic infection of *Helicobacter pylori* confirms that chronic bacterial infection can give rise to gastrointestinal cancers. Taken together these data provide strong evidence that chronic carriage of typhoidal *Salmonellas* increases the risk of hepatobiliary cancer, especially of the gallbladder.

Pathogenesis

When nontyphoidal *Salmonellas* or other pathogens invade the gastrointestinal tract, they cause inflammatory diarrhea where the site of local invasion is characterized by neutrophilic infiltration. By contrast, typhoidal *Salmonella* intestinal invasion produces a less pronounced inflammatory response where mononuclear cells predominate and with few neutrophils. Apparently, typhoidal *Salmonellas* express toxins that downregulate the normal inflammatory host response in the intestinal mucosa. After epithelial penetration, the invading microorganisms move to the intestinal lymphoid follicles and into the reticuloendothelial system.

Macrophages engulf typhoidal *Salmonella*, but the typhoidal *Salmonellas* have an unusual ability to survive and

reproduce within macrophages. This process has been studied extensively in the mouse model of *Salmonella* Typhimurium. *Salmonella* Typhimurium is genetically closely related to *Salmonella* Typhi and Paratyphi A. *Salmonella* Typhimurium causes a typhoid syndrome in mice, but not in humans. *Salmonella* Typhimurium induces the host cell to elaborate an intracellular membrane that surrounds the bacteria and permits them to survive and replicate. This unique structure, the *Salmonella*-containing vacuole, develops an extended tubular network termed *Salmonella*-induced filaments. Within the *Salmonella*-containing vacuole, typhoidal *Salmonellas* have such high-level resistance to antimicrobial peptides, nitric oxide, and oxidative killing that even when lysosomes are fused with the *Salmonella*-containing vacuole, typhoidal *Salmonella* survive. Because they survive and multiply within mononuclear phagocytic cells, typhoidal *Salmonellas* are eventually released into the bloodstream and circulate throughout the body. The most common sites of secondary infection include the liver, spleen, bone marrow, gallbladder, and Peyer's patches of the terminal ileum.

Virulence Factors

The genetic and molecular basis of the virulence of the typhoidal *Salmonellas* is incompletely understood, but is an area of active research especially using the mouse model of *Salmonella* Typhimurium infection. *Salmonella* Typhi has several large insertions in its genome, apparently acquired by horizontal gene transfer, termed *Salmonella* pathogenicity islands that encode genes important for survival within the host. *Salmonella* pathogenicity island 1 includes genes that are important for cellular invasion of nonphagocytic cells. This invasion is mediated by a Type III secretion system that produces toxin-like virulence factors that induce a reorganization of the host cell actin cytoskeleton, prompting endocytosis of the bacteria.

Salmonella pathogenicity island 2 includes genes that are important for survival within macrophages. These genes encode a second Type III secretion system that injects over 20 effector proteins across the phagosomal membrane that subvert host functions to modify the intracellular environment. Over 900 *S. enterica* Typhimurium genes are differentially regulated in responses to the phagosomal environment. These genes encode resistance to antimicrobial peptides, nitric oxide, and oxidative killing and other incompletely characterized functions. Mutant strains of *Salmonella* Typhimurium defective in macrophage replication are not virulent in mice.

Salmonella pathogenicity island 7 is the largest genomic element present in *Salmonella* Typhi but absent in *Salmonella* Typhimurium. It contains the genes for the Vi capsule, and is present in both *Salmonella* Typhi and Paratyphi C. Clinical studies demonstrate that Vi-negative strains are less infectious and less virulent. Recognition of bacterial lipopolysaccharide by the host immune system induces a strong inflammatory response. Mouse studies suggest that the Vi capsule is able to mask *Salmonella* Typhi lipopolysaccharide from the host immune system. The presence of a very similar *Salmonella* pathogenicity island 7 in the distantly related *Salmonella* Typhi, Paratyphi C, Dublin, and *Citrobacter freundii* genome, and its absence from other serovars of *S. enterica* suggests that this gene sequence was a mobile element that was separately inserted into these diverse organisms.

Epidemiology

A formal evaluation of the global burden of typhoid fever for the year 2000 estimated that *Salmonella* Typhi infection resulted in 22 million illnesses and 220 000 deaths and *Salmonella* paratyphoid caused 5.4 million illnesses (Crump *et al.*, 2004). These estimates extrapolated incidence from 22 published studies that collected data between 1954 and 2000, which included population-based estimates of typhoid fever incidence (**Figure 1**). This estimate relies on several assumptions. It assumes that the incidence of typhoid fever is similar in places where it has not been studied compared with the places where studies were conducted. It assumes that blood cultures will identify 50% of persons who are infected with *Salmonella* Typhi. It assumes that 1% of persons who develop a clinical illness from *Salmonella* Typhi will progress to death. It assumes that the combined incidence of *Salmonella* Paratyphi A, B, and C is one-quarter the incidence of *Salmonella* Typhi. It does not account for any contribution of the typhoidal *Salmonellas* on hepatobiliary cancer mortality. There are quite limited data to support each of these assumptions, but unless better data become available, the range of estimates of the burden of typhoid fever will remain broad.

Typhoid fever occurs most commonly within communities where available water is routinely contaminated with human feces and the population density is high. The highest incidence rates of typhoid fever have been reported from urban slums in Asian megacities. Several lines of evidence suggest that the incidence of typhoid is lower in rural areas compared with urban areas. Published studies of the highest incidence of typhoid fever are consistently from urban sites. Hospital case series suggest that typhoidal *Salmonellas* are less common causes of febrile illness in rural African settings compared with urban settings in Asia (Mweu and English, 2008). Among population-based studies in rural Africa that routinely collected blood cultures from febrile children, studies measuring the incidence of *Streptococcus pneumoniae* and *Haemophilus influenza* type B noted a much lower incidence of febrile illness associated with typhoid fever than has been reported from Asian megacities. One study within Kolkata, India, noted that even within a densely populated urban slum, the areas of highest population density were at increased risk of typhoid fever (Sur *et al.*, 2009). It is an open question how much of the apparently lower incidence of typhoid fever in Africa may be due to lower population density and how much may result from differences in bacterial ecology in sub-Saharan Africa compared with South Asia.

Unlike other *S. enterica* serotypes, *Salmonella* Typhi and Paratyphi A do not live in the gastrointestinal tracts of domestic animals, so contamination of human food products by gut contents during domestic animal slaughtering is not a pathway for transmission to humans. Rather, each human infection with *Salmonella* Typhi or *Salmonella* Paratyphi A originated from a strain whose reservoir was almost certainly

Figure 1 Geographic distribution of population-based studies of typhoid fever incidence. ■ High incidence (>100 episodes/100 000 per year). ■ Medium incidence (10–100 episodes/100 000 per year). □ Low incidence (<10 episodes/100 000 per year). □ Region with high development index countries. ○ Site of contributing disease incidence study. Reproduced with permission from Crump JA, Ram PK, Gupta SK, *et al.* (2008) Analysis of data gaps pertaining to *Salmonella enterica* serotype Typhi infections in low and medium human development index countries, 1984–2005. *Epidemiology and Infection* 136(4): 436–448.

another person. Typhoidal *Salmonellas* move from the feces of an infected person to the mouth of an uninfected person. In the urban slums of Asian megacities, where the incidence of typhoid fever is highest, drinking water is routinely contaminated with human feces. When water is available through a piped network, it tends to be distributed in water distribution systems that have numerous breaks which permit cross-contamination with sewage. The water in these systems typically runs intermittently. When the water is not running, the pipes are at negative pressure relative to the surrounding environment, drawing in sewage contamination through these breaks in the pipes. In settings of high typhoid incidence, case–control studies consistently implicate drinking untreated water as a risk factor for typhoid fever.

Case–control studies also consistently implicate food as a source of typhoid fever. In low-income countries, the most likely pathway is that water contaminated with typhoidal *Salmonellas* is used to process food. Many foods provide a sufficiently nutritious environment for exponential growth of typhoidal *Salmonellas*, so that a sufficiently large dose of organisms are ingested to produce human infection. Dairy products are among the most commonly implicated foods. Because neither *Salmonella* Typhi nor *Salmonella* Paratyphi A are resident in the guts of cattle or goats, the contamination of dairy products almost certainly results from mixing contaminated water with dairy products during processing. Other foods implicated in typhoid fever include cut fruits, raw vegetables, and food sold by street vendors. Sewerage is commonly used to irrigate crops near large cities, and many of these crops may be eaten raw. In communities where typhoid fever is common, the water that is used in markets to rinse produce and cut fresh fruit is typically contaminated at least occasionally with strains of typhoidal *Salmonella*.

Nonvaccinated visitors from high-income countries generally have quite limited immunity against typhoidal *Salmonella*. Seventy percent of typhoid fever in the US is associated with foreign travel. Sporadic outbreaks of typhoid fever in high-income countries occur when a person who is either acutely infected with typhoid from recent travel or is a typhoid carrier contaminates food during preparation. An outbreak of 80 cases of typhoid fever in San Antonio, TX, USA, in 1981 occurred among patrons of a Mexican takeout restaurant who consumed barbacoa, a mixture of muscle, lips, ears, tongue, and eyes handpicked from steamed bovine head skulls by employees. One of these employees continually excreted *Salmonella* Typhi until undergoing cholecystectomy. An outbreak of 43 cases of confirmed typhoid fever occurred among guests and staff at a New York hotel in 1993 who consumed fresh orange juice that was prepared by an asymptomatic food worker who was subsequently identified as a typhoid carrier. An outbreak of 24 cases of typhoid stretching over 7 years in Terrassa, Spain, occurred among patrons of a family-run delicatessen where an elderly grandmother who was an unrecognized asymptomatic carrier of *Salmonella* Typhi helped to prepare the cannelloni once a week. Other food vehicles that have been implicated in these outbreaks include milk and other dairy products, potato salad, cooked beef, chicken salad, tossed salad, guacamole, fish, and cake. Shellfish that are exposed to human sewage have also been implicated in typhoid outbreaks.

In settings where typhoid is common, exposure to the organism is widespread, but some people are at increased risk of

illness with typhoidal *Salmonella*. Children, who have a less completely developed adaptive immune response compared with their older siblings and adults, are at increased risk of developing illness on exposure. Taking antibiotics alters gut flora and in animal studies reduces the infectious dose of *Salmonella* 100 000 fold (Bohnhoff and Miller, 1962). Case–control studies of risk factors for *Salmonella* Typhi that have included an assessment of prior antibiotic use have noted an increased risk of typhoid fever among persons who took antimicrobials in 2 weeks before onset of symptoms. As antimicrobials are available without a prescription in most low-income countries, this may be an important factor contributing to human infection and ongoing transmission of the typhoidal *Salmonellas*.

There is consistent evidence of person-to-person transmission especially for *Salmonella* Typhi. Persons with typhoid fever frequently shed the bacteria. Several case–control studies implicate having a family member or close associate with typhoid fever as a risk factor for developing typhoid fever. Case–control studies also suggest that regular handwashing with soap protects against typhoid fever. Handwashing with soap removes organisms from hands and so can interrupt transmission. In a cluster-randomized vaccine trial of Vi typhoid vaccine in Kolkata, 39% of the residents who lived in a cluster where vaccine was offered, did not receive the vaccine (Sur *et al.*, 2009). Nevertheless, these nonvaccinated persons who lived in the neighborhood cluster where 61% of their neighbors had received the vaccine, were 44% less likely to contract typhoid compared with persons living in clusters that did not receive the typhoid vaccine. This demonstrates that *Salmonella* Typhi was transmitted not just through food or water that was contaminated far outside the community, but from more local exposure.

Salmonella Typhi and Paratyphi A are organisms specifically adapted to humans, and so are dependent on humans for their propagation and survival. Molecular clock analysis suggests that *Salmonella* Typhi emerged approximately 50 000 years ago (Kidgell *et al.*, 2002), which is during Paleolithic times when human population densities were low and humans were living as hunter gatherers in relatively small extended family groups. Carriage provides a long-term reservoir for typhoidal *Salmonellas*, widespread geographical dispersion, and ongoing survival despite only occasional opportunities for transmission to new individuals and new communities. Mary Mallon, known as Typhoid Mary, was the most widely recognized typhoid carrier. She worked as a cook for several families in and around New York City in the early twentieth century. Investigators linked her to 47 typhoid cases and 3 deaths over 15 years, and suspected many more. She was forcibly isolated to protect the public health. In endemic areas today it is unclear how much transmission results from the shedding of chronic carriers and how much results from shedding associated with acute infection. However, because carriers can excrete the organisms for years, chronic carriers are certainly contributing to environmental contamination with the typhoidal *Salmonellas*.

When chloramphenicol was first introduced in 1948 all strains of *Salmonella* Typhi were universally sensitive, though drug resistance was identified within 2 years of its introduction. By 1972, chloramphenicol resistance was widespread with outbreaks in both Latin America and Asia. Chloramphenicol resistance was mediated by a plasmid that also carried resistance to sulfonamides, tetracycline, and streptomycin, though initially strains remained sensitive to ampicillin and cotrimoxazole. In the late 1980s and into the 1990s, *Salmonella* Typhi that was multiply drug-resistant to the three first-line antimicrobials – chloramphenicol, ampicillin, and cotrimoxazole – emerged in Asia, Africa, and Latin America. The drug resistance was again encoded on a single plasmid and the outbreaks were characteristically clonal. With the switch to ciprofloxacin and other fluoroquinolones as first-line drugs for the treatment of typhoid fever, the incidence of multiply drug-resistant typhoid has declined. Strains of *Salmonella* Typhi and Paratyphi A with decreased susceptibility to ciprofloxacin are being identified with increasing frequency. The ciprofloxacin resistance results from one of several single point chromosomal mutations of the DNA gyrase enzyme and is independent of the multiply drug-resistant plasmid. As noted above, patients infected with drug-resistant strains of typhoidal *Salmonella* are at increased risk of death. The history of antimicrobial resistance suggests that in the coming years the typhoidal *Salmonellas* will acquire more drug resistance and be associated with worse survival.

The epidemiology of *Salmonella* Paratyphi A, is generally similar to the epidemiology of *Salmonella* Typhi. Some studies suggest that *Salmonella* Paratyphi A infection is more common among persons who are somewhat older. There is also some suggestion that a higher dose of *Salmonella* Paratyphi A is required to cause illness compared with *Salmonella* Typhi. In some settings in South Asia, the incidence of *Salmonella* Paratyphi A is clearly increasing. In previous studies, *Salmonella* Paratyphi A was associated with as few as 5–10% of the number of typhoid fever cases, but in some settings more recently accounts for 50% of the cases. Human infection with *Salmonella* Paratyphi B and C is reported less commonly than infection with either *Salmonella* Typhi or *Salmonella* Paratyphi A.

Analytical Methods

There are many approaches to the diagnosis of typhoid fever, but none are optimal. In communities where typhoid fever is common, local clinicians often make the diagnosis based on clinical signs and symptoms. Unfortunately, this low-cost approach does not reliably differentiate typhoid fever from other common causes of fever including dengue and malaria.

The most definitive approach to diagnose typhoid is direct culture of *Salmonella* Typhi/Paratyphi A, B, or C in bone marrow, blood, or stool. Bone marrow culture is the most sensitive, because the organism is present at 10 times the concentration identified in blood and there may be less antimicrobial penetration into the bone marrow. However, bone marrow culture is uncomfortable and uncommonly performed in settings where typhoid fever is most common. A positive blood culture for *Salmonella* Typhi or Paratyphi A, B, or C provides a definitive diagnosis. In high-incidence settings, *Salmonella* Typhi is generally the most common pathogen identified in blood cultures. However, single blood cultures are only approximately 50% sensitive, a sensitivity that is reduced by the common practice of pretreatment with antimicrobials. The typhoidal *Salmonellas*

can also be cultured from stool during acute illness, but stool culture is <30% sensitive during the first 2 weeks of illness. Because culture-based diagnosis is expensive, does not reliably exclude infection, and provides results only after several days delay, in settings where typhoidal *Salmonella* is common, clinicians treat most patients with suspected typhoid fever without a definitive diagnosis.

The Widal test, an antibody-based test that has been used for over 100 years, is the most commonly used laboratory test to aid in the diagnosis of typhoid fever. The Widal test is inexpensive and available throughout typhoid-endemic areas, but is fairly insensitive, especially early in illness when a clinician is deciding whether or not to treat with antimicrobials. The Widal test is also nonspecific, especially in settings where typhoid and other *Salmonella* infections are common. Different laboratories also use different reagents and different cutoffs, further complicating interpretation of Widal test results.

Several other antibody-based tests have been developed, but none have sufficient sensitivity and specificity especially early in illness to be widely used. Some laboratories have developed polymerase chain reaction (PCR)-based diagnostics for typhoidal *Salmonella* DNA and report somewhat higher sensitivities than with culture. Different groups are currently using different primers and so the approach is not yet standardized. In addition, the cost and infrastructure required to perform PCR using currently available platforms means that it is unaffordable to low-income families at high risk of disease.

Salmonella Typhi is isolated from feces or food similar to any other *S. enterica*. A 25-g sample of feces or food is cultured in a nonselective buffered peptone broth, and then selectively enriched with a broth containing selenite. After incubation, selective enrichment broth is spread on selective agar plates for identification. Representative colonies are selected for biochemical testing and tested for agglutination against flagellar and cell surface antigens.

Control/Prevention Measures

Preventing human fecal contamination of community water supplies would dramatically reduce the burden of typhoid and paratyphoid fever. This is a difficult long-term task, but it is not impossible. Cities around the globe, beginning in the USA during the early twentieth century but more recently in Singapore and other select Asian cities, have sufficiently improved their water and sanitary infrastructure so that typhoid fever no longer presents a substantial public health problem. Upgrades to municipal water supplies so that they no longer provide a regular vehicle for enteropathogen transmission, have consistently required that the population served earn sufficient income to support the construction and ongoing maintenance of the water treatment and distribution infrastructure. This strategy is effective for middle-income countries, but has not been successfully implemented in low-income countries where the burden of typhoid fever is highest.

Improving water quality is particularly difficult when water supply is limited. When there is insufficient supply to provide piped water 24 h a day, then every day there are opportunities for contaminants to seep back into water distribution systems that are inevitably somewhat permeable to the environment. The prospect of achieving 24-h water availability is worsening because of growing demand for water and reduced supply. Currently there are only two cities in all of India that provide water 24 h a day to all of their residents. This situation is likely to worsen because of the exhaustion of fossilized groundwater sources that have been exploited to provide water to cities but require centuries or millennia to recharge; with global warming eliminating mountain ice packs as steady sources of water throughout the summer and population growth increasing water demand, countries are diverting rivers to population centers under their political authority, leaving cities in the original downstream communities increasingly water short. Thus, while efforts for a safer, steadier water supply in those communities at highest risk for typhoid fever should continue, such solutions will not come quickly. As the risk of typhoidal *Salmonella* strains that are resistant to all antibiotics is a growing public health threat, interim measures should be undertaken.

One focused context to reduce transmission risk would be improving the safety of the water that is used in food processing. Markets in settings where typhoid fever is common frequently use water to rinse and process produce. Providing water that is free of human feces to these facilities, or at least to the largest of these facilities, could substantially reduce typhoid transmission. Dairy products are consistently implicated in typhoid fever transmission, so the provision of water that is free of human feces to producers of dairy products could also be an efficient point of intervention. In countries where typhoid fever is common, there are often many small producers of dairy products. It would be more efficient to first improve the water supplying the largest producers. Sewage irrigation of crops, especially those that are consumed raw, is an efficient vehicle of enteropathogen transmission. The economic incentives to use sewage as a source of water and fertilizer and grow crops close to the large city markets are large enough so that simple recommendations and regulations are unlikely to overcome the strong financial incentives. One approach to consider would be for local authorities to restrict crops grown with sewage to those that are less likely to transmit enteropathogens, presumably those crops that are cooked before consumption and/or have a smooth surface.

Whatever attempts at interventions are undertaken, surveillance of human illness from *Salmonella* typhoid/paratyphoid would allow an ongoing assessment of the effectiveness of interventions. Specifically monitoring foodstuffs and water supplies for contamination with *Salmonella* Typhi and Paratyphi A, B, and C would be inefficient because these organisms are only one of many enteric pathogens that are prudent to restrict from the food and water supply.

Three licensed vaccines effectively prevent typhoid fever. Older whole killed vaccines were effective in reducing typhoid fever but had such frequent disabling side effects, including local pain and fever that was severe enough to require missing a day of school or work in 15% of vaccine recipients that they are no longer used. Ty21a is a live vaccine derived from a mutated *Salmonella* Typhi strain with lower pathogenicity. In clinical trials, Ty21a vaccine was approximately 60% effective in preventing typhoid fever, and is used as a vaccine for travelers from wealthy countries who are visiting typhoid-endemic areas. Vaccine trials suggest that Ty21a may provide

partial protection against *Salmonella* Paratyphi B. Because Ty21a vaccine is expensive by the standards of vaccines purchased by low-income countries and because it requires a cold chain and three doses, Ty21a has never been adopted as an ongoing government-sponsored public health intervention in a typhoid-endemic country.

Typhoid Vi vaccine is a genetically recombinant Vi capsular polysaccharide. It was developed by the US National Institutes of Health and is free intellectual property under no patent protection. In individually randomized controlled trials, Vi vaccine is 60% protective to individuals in preventing typhoid fever but, as noted above, when administered throughout communities Vi vaccine was also 44% protective to unvaccinated persons living in communities where 60% of their neighbors were vaccinated. There are several manufacturers of Vi vaccine in typhoid-endemic countries including China, Vietnam, and India. Within these settings the vaccine cost is less than $0.60 per dose. Vi vaccine has been used by local authorities as part of public health prevention programs in Vietnam, in typhoid-endemic areas of China, and in Delhi state in India. Program assessments in Vietnam and China suggest marked reduction in typhoid from these interventions.

Limitations of the Vi vaccine include that it is not effective neither against *Salmonella* Paratyphi A or B nor against *Salmonella* Typhi strains that lack the Vi antigen. Vi-negative *Salmonella* Typhi strains have been occasionally identified among patients with typhoid fever in Pakistan and India, though their global prevalence is unknown. It is theoretically possible that Vi vaccines may create sufficient evolutionary pressure so that *Salmonella* Paratyphi A and B or Vi-negative stains of *Salmonella* Typhi may fill the ecological niche of Vi-positive *Salmonella* Typhi strains, but to date there is little empiric evidence to support this hypothesis. The Vi vaccine is not immunogenic among children under the age of 2 years and, because the immunity is not T cell based, it requires revaccination at 2-year intervals. Despite these limitations, the Vi vaccine has been demonstrably effective in reducing the burden of *Salmonella* Typhi in endemic areas. The World Health Organization has endorsed the use of vaccines against typhoid fever in areas with endemic typhoid. Ongoing surveillance should assess the impact of intervention programs on disease and on any differences in circulating strains.

There are several new vaccines in development that may eventually provide even more effective approaches to prevention. A Vi vaccine that is conjugated to *Pseudomonas aureginosa* exotoxin A is immunogenic in young children and provides prolonged immunogenicity beyond 5 years. This raises the possibility of integrating typhoid vaccination into childhood vaccine programs in endemic areas and reducing or eliminating the need for periodic revaccination.

Another important approach to preventing typhoid transmission is promoting handwashing. Traditional approaches to handwashing promotion emphasized didactic health education instructing people that washing hands prevented diarrhea. More recent approaches frame handwashing promotion as a behavior change task that requires habit adoption. Newer approaches recognize the need for basic handwashing infrastructure and often employ messages encouraging handwashing using approaches that have been effective for private soap manufacturers.

Identifying and treating the 1–4% of people infected with typhoidal *Salmonella* who become chronic carriers reduces the reservoir of infection and the ongoing contamination of the environment. However, there are multiple barriers to implementing carrier identification and treatment in settings where typhoid fever is most common. In these low-income communities, typhoid fever is usually treated without confirmatory testing and so the diagnosis is both uncertain and often missed. Twenty-five percent of typhoid carriers have no clinical history of typhoid fever, and local health authorities have other higher priorities. Moreover, when the sole motivation of identifying these carriers is treating them to prevent disease in others, there is less incentive for the asymptomatic carrier to bear the inconvenience of multiple stool cultures for diagnosis and additional drug treatment. However, because carriers of typhoidal *Salmonella* are at markedly increased risk for hepatobiliary cancers, treating carriers would protect their health as well as the health of the community. Two to four weeks of high-dose antimicrobial therapy can eliminate carriage in patients without gallstones, but in the presence of gallstones surgical cholecystectomy is generally required. In New York State, in the early 20th century before the advent of antimicrobial therapy, the number of typhoid carriers progressively decreased with the spread of water treatment and sanitary infrastructure. In typhoid-endemic settings, identification and treatment of carriers is not the highest priority for typhoid control, but eradication of *Salmonella* Typhi and Paratyphi A, which is theoretically possible because of their restricted host range and the potential effectiveness of newer generation vaccines, would require reducing the reservoir sufficiently to interrupt transmission.

Research Needs

Productive areas for future research include development of a diagnostic test that is sensitive and specific early enough in illness to guide treatment and inexpensive enough to be affordable in regions where typhoid is common. The inevitable progression of antimicrobial resistance will require a robust pipeline of new antimicrobial drugs to avoid returning to the high case fatality rates that characterized typhoid fever in the preantibiotic era. Because definitive improvement in water and sanitation infrastructure in areas where typhoid is common are likely to require several decades to achieve, strategies that can reduce typhoid burden even in the setting of compromised water and sanitary infrastructure should be piloted and evaluated. For example, improving water quality in markets that sell fresh produce may be a focused cost-effective intervention that can reduce the burden of typhoid fever and other serious foodborne pathogens. Further development of typhoid fever vaccines, including those that are effective in infants, those that protect against typhoid carriage, those that provide longer term protection, and those that are effective against *Salmonella* Paratyphoid A and B would be particularly helpful. Detailed epidemiological transmission studies paired with infectious disease modeling may help to identify optimal strategies for eradicating these dangerous human pathogens.

See also: Foodborne Diseases: Overview of Biological Hazards and Foodborne Diseases. Public Health Measures: Safe Use of Wastewater for Agricultural Production; Surveillance of Foodborne Diseases. Safety of Food and Beverages: Water (Bottled Water, Drinking Water) and Ice

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BACTERIA

Shigella

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Glossary

Antibiotic A drug that destroys bacteria.
Antibody A substance produced by the immune system to fight invading organisms such as viruses.
Arthritis A disease that causes inflammation and pain in the joints.
Bloody diarrhea Diarrhea with blood in stool.
Gastroenteritis Inflammation of the stomach and intestine, with vomiting.
Hemolytic anemia Anemia due to decreased life span of erythrocytes. It is caused by excessive destruction (as in chemical poisoning, infection, or sickle-cell anemia) of red blood cells.
Proctitis A chronic inflammatory disease of the rectum which causes bloody diarrhea.
Seizure Convulsions; sudden involuntary movements of the muscles.

Family: Enterobacteriaceae

Genus: *Shigella*

Shigellosis is an acute bacterial infection of the intestinal lining caused by a group of bacteria called *Shigella*. It occurs worldwide, and tends to occur whenever war, natural calamities (e.g., earthquakes and floods), or unhygienic living conditions result in overcrowding and poor sanitation. The reported incidence of *Shigella* infections was 1780, 3.8 per 100 000 population, in 2010. It is more common in summer than in winter, especially among travelers in developing countries, day care centers, nursing homes, refugee workers, and residents of camps. Outbreaks of shigellosis are associated with poor sanitation, contaminated food and water, and overcrowding. Children aged 2–4 years are most likely to get the condition.

Shigella is a genus of gammaproteobacteria in the family Enterobacteriaceae. The genus was named more than 100 years ago after the Japanese microbiologist Kiyoshi Shiga who discovered the causative organism, *Shigella dysenteriae* type 1, of the 1896 large Japanese epidemic of dysentery.

Shigella are Gram-negative, rod-shaped, nonsporeforming, nonlactose fermenting (with the exception of *Shigella sonnei*), nonmotile because of the absence of flagellar H and capsular K antigens, urease-negative, and have translucent white or pale yellow colonies on MacConkey agar. Indole reactions are sometimes positive and sometimes negative, with the exception of *S. sonnei* which is always negative. *Shigella* not only makes adenosine-5′-triphosphate (ATP, an energy molecule) by aerobic respiration in the presence of oxygen but is also capable of switching to fermentation (facultative anaerobe). It can ferment carbohydrates with only acid production (no gas production). *Shigella* are closely related to *Escherichia coli* and *Salmonella* and share common antigens with one another and other enteric bacteria. Humans are the only host of *Shigella*, but they have also been isolated from higher primates such as monkeys and chimpanzees.

Shigella species are classified into four serogroups based on only the O antigen:

- Serogroup A: *S. dysenteriae* (13 serotypes).
- Serogroup B: *Shigella flexneri* serotypes 1 through 5 (12 including 'subtypes') *S. flexneri* (6 and 6A serotypes).
- Serogroup C: *Shigella boydii* (20 serotypes).
- Serogroup D: *S. sonnei* (1 serotype).

Groups A–C are physiologically similar, *S. sonnei* (group D) can be differentiated by biochemical assays. Three *Shigella* groups are the major disease-causing species: *S. flexneri* is the most frequently isolated species worldwide and responsible for 60% of the cases in developing countries; *S. sonnei* causes 77% of the cases in developed countries, whereas it causes only 15% of the cases in developing countries; and *S. dysenteriae* is usually the cause of epidemics of dysentery, particularly among refugee camps, whereas *S. boydii* is responsible for sporadic cases in India.

Causes of Shigellosis

Shigellosis is spread when the bacteria in feces or on soiled fingers is ingested. Poor hand-washing habits and eating contaminated food and drink may cause the condition.

- Food may become contaminated by infected food handlers who do not wash their hands with soap after using the bathroom.
- Vegetables can be contaminated if they are harvested from a field that has sewage in it.
- Shellfish and oysters can be contaminated with sewage or contaminated water.

- Flies can breed in infected feces and contaminate food.
- Drinking or ingesting contaminated water can occur while taking a bath or swimming. Water may become contaminated through a fecal source such as illegally discharged sewage or improperly sited latrines or if persons excreting *Shigella* take a bath, swim, or wash their soiled laundry in it.
- Shigellosis can also be spread through sexual intercourse, especially through anal and oral sex.

Shigellosis is often found in child care centers, nursing homes, refugee camps, and other places where conditions are crowded and sanitation is poor.

The infectivity dose is extremely low. As few as 10 *S. dysenteriae* bacilli can cause the disease, whereas 100–200 bacilli are needed for *S. sonnei* or *S. flexneri* infection. The reasons for this low-dose response are not completely clear; one possible explanation is that virulent shigellae can withstand the low pH of gastric juice.

Associated foods:

- Raw oysters and shellfish harvested from contaminated water.
- Vegetables harvested from fields contaminated with sewage.
- Salads including chicken, fruit, lettuce, macaroni, pasta, potato, shrimp, tuna, turkey, and vegetable.
- Water contaminated with sewage.

Populations that are at high risk for shigellosis include the following:

- Children under 5 years of age and their caregivers in child care centers.
- Persons in custodial institutions.
- People living in crowded conditions with poor sanitary facilities and inadequate clean water supply (e.g., refugee camps and shelters for displaced people).
- International travelers.
- Homosexual men.
- People with human immunodeficiency virus (HIV) infection.

Prevalence of Shigellosis

The global burden of shigellosis has been estimated at 165 million cases per year, of which 163 million are in developing countries. More than one million deaths occur in developing countries yearly due to *Shigella* infection. In developing countries, *S. flexneri* predominates. Epidemics of *S. dysenteriae* type 1 have occurred in Africa and Central America with case fatality rates of 5–15%. By one estimate, *Shigella* infections are responsible for 300 000 illnesses and 600 deaths per year in the USA. By another estimate, each year 450 000 Americans are infected with *Shigella*, causing 6200 hospitalizations and 70 deaths.

In general, *Shigella* is one of the most communicable and severe forms of the bacterial induced diarrheas. No group of individuals is immune to shigellosis, but certain individuals are at increased risk. Small children acquire *Shigella* at the highest rate, and persons infected with HIV experience shigellosis much more commonly than experienced by other individuals.

The following statistics relate to the incidence of shigellosis:

- 3.70 cases of shigellosis per 100 000 population in Canada, 2000.
- 2.5 new cases of shigellosis per 100 000 population were notified in Australia, 2002.
- 496 new cases of shigellosis were notified in Australia, 2002.

Symptoms and Signs

Dysentery is a severe diarrhea containing blood and mucus in the feces. Dysentery results from viral, bacterial, or protozoan infections or parasitic infestations. Amebic dysentery (amebiasis) is caused by an ameba called *Entamoeba histolytica*. Bacillary dysentery is a type of dysentery and a severe form of shigellosis caused by *S. sonnei*, *S. flexneri*, and *S. dysenteriae* characterized by blood in stool, which is the result of invasion of the mucosa by the pathogen. The most common symptoms of shigellosis are watery diarrhea, sudden fever, nausea, vomiting, abdominal pain, flatulence, tenesmus, lassitude, and prostration, 1–7 days (average 3 days) after ingestion of contaminated food or drink. The stool may contain blood, mucus, or pus. The condition may be asymptomatic in some cases or severe, especially in children. In very severe cases of shigellosis a person may have seizures, stiff neck, headache, extreme tiredness, and confusion as central nervous system symptoms, especially with *S. dysenteriae*. Shigellosis can also lead to dehydration and in rare cases other complications like kidney failure can occur. Symptoms usually last for several days, but can also last for weeks. The more severe cases of shigellosis can lead to sequelae such as reactive arthritis in 3% of cases, especially after *S. flexneri* infection.

Postinfection Excretion

Shigella can be passed into the person's stool for approximately 4 weeks even after the obvious symptoms of illness have resolved, although antibiotic treatment can reduce the excretion of *Shigella* bacteria in the stool.

Asymptomatic Carriers

Asymptomatic carriers have been incriminated in the maintenance and spread of the disease in the community. Asymptomatic carriers may shed the organism for months, although chronic carriage (>1 year) is rare. Secondary attack rates in households are high, up to 40%.

Shigella Toxins and Immune Evasion

Several virulence factors have been associated with *Shigella* spp., the most common being the ability to colonize and invade the intestinal cells. This phenomenon is mediated partially by the invasion-associated locus (*ial*), which is carried on a plasmid of 120–140 MDa, and the invasion plasmid

antigen H (*ipaH*) gene, which is present in multiple copies in both the plasmids and chromosomes of these organisms.

Another virulence factor related to *Shigella* spp. is its capacity to produce an enterotoxin called Shiga toxin (Stx) which is produced by *S. dysenteriae*. This protein enterotoxin is released by the microorganism in the intestine. The Stx acts on the vascular endothelium of the blood vessels. It is made up of an A-subunit (toxic) and a B-subunit. The B-subunit of the toxin binds to a component of the cell membrane known as Gb3 (glycolipid globotriaosylceramide) and the complex is engulfed by the target cell. When the protein is inside the cell, the A-subunit interacts with the ribosomes to inactivate them. The A-subunit of the Stx is an *N*-glycosidase that modifies the ribonucleic acid component of the ribosome to inactivate it and so brings protein synthesis to a halt, leading to death of the cell. The vascular endothelium has to continually renew itself, so this killing of cells leads to a breakdown of the lining and hemorrhage. The first response is commonly a bloody diarrhea because Stx is usually taken in with contaminated food or water. *Shigella* species have a large virulence plasmid that carries the genes necessary for invasion and colonization of the epithelial cell layer of the human gut, resulting in dysentery (bloody diarrhea). *Shigella* moves from cell-to-cell by using proteins (IcsA, a *Shigella* protein essential to intracellular movement and spread) that trigger actin polymerization in the host cell in a 'rocket' propulsion fashion. In fact, in order to evade the host's immune system, *Shigella* species avoid being presented by major histocompatibility complex (MHC) class II molecules by escaping from the endosome/phagosome after being engulfed, before it fuses with a lysosome containing enzymes that digest the bacteria and prevent it from causing harm.

Shiga-like toxin 1 and 2 (SLT-1 and 2 or Stx-1 and 2): the Stxs produced by some *E. coli* strains. Stx-1 differs from Stx by only one amino acid. Stx-2 shares 56% sequence homology with Stx-1. Both Stx-1 and 2 are encoded by a bacteriophage inserted into the chromosome. Stx-1 increases inflammatory cytokine production by human macrophages, which in turn leads to a burst of interleukin (IL)-8. This could be relevant in recruiting neutrophils to the lamina propria of the intestine in hemorrhagic colitis and accounts for elevated levels of IL-8 in serum of patients with diarrhea-associated hemolytic uremic syndrome (HUS).

Intestinal Adherence Factor

Intestinal adherence factor favors colonization *in vivo* and in animal models. This is a 97 kDa outer-membrane protein encoded by each gene on chromosomes. This codes for intimin protein, and an anti-intimin response is observed in children with HUS.

Pathology

The rectosigmoid lesions of shigellosis are characterized by proximal extension of erythema, edema, loss of vascular pattern, focal hemorrhage, and purulent exudate. Biopsy specimens from affected areas are edematous, with congestion of the capillary, focal hemorrhage, crypt hyperplasia, goblet cell depletion, mononuclear and polymorphonuclear (PMN) cell infiltration, shedding of epithelial cells and erythrocytes, and microulceration.

Initially the organisms are ingested by membranous (M) cells that are associated with lymphoid microfollicles in the colon. After transcytosis through the M cell, the bacteria are deposited into the subepithelial space where they are phagocytosed by macrophages. The macrophage phagosome is subsequently degraded, and the intracellular shigellae cause release of IL-1 that evokes an influx of PMN leukocytes. Eventually the infected macrophages undergo apoptosis (programmed cell death), and the bacteriae are released onto the basolateral surface of adjacent colonic enterocytes. In addition, PMN transmigration through the epithelium disrupts tight junctions, allowing shigellae to migrate into the subepithelial space. The bacteriae infect enterocytes by induced endocytosis, and the endocytic vacuoles are subsequently degraded. The intercellular shigellae attach to actin in the enterocyte junctional complex and then multiply and spread to contiguous enterocytes by induced actin polymerization. Ultimately, the infected enterocytes die, and the resulting necrosis of the epithelium, in conjunction with the continuing inflammatory response, constitutes the lesions of shigellosis.

Transmigration of infiltrating PMNs through the tight junctions of local epithelial cells and into the intestinal lumen allows the reverse migration of shigellae from the lumen into the subepithelial spaces. These organisms then infect the columnar epithelial cells by inducing endocytic uptake at the basolateral surface. Immediately after infection of enterocytes, intracellular shigellae lyse endocytic vacuoles and attach to the actin cytoskeleton in the area of the junctional complex. As these organisms multiply within the enterocyte cytoplasm, occasional daughter cells induce polar nucleation of filamentous actin, resulting in a 'tail' that propels the shigellae into protrusions impinging on contiguous enterocytes. Plasma membranes enveloping the organisms are again lysed, and the organisms are deposited within the contiguous host cell, resulting in intercellular bacterial spread.

The Host's Immune Response to *S. flexneri*

Innate Immunity

The most important consequence of the host's innate immune response appears to be the cytokine-induced migration of PMN cells. The transcription factor NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) is activated in *Shigella*-infected epithelial cells in a lipopolysaccharide-dependent mechanism, leading to the production and secretion of IL-8 by the infected cells. IL-8 is a potent chemoattractant for PMN cells as is the IL-1 released from apoptotic macrophages. *Shigella* is unable to escape from the phagocytic vacuole of PMN cells and is killed inside the phagosome. Recent research has implicated the human neutrophil elastase as a key host defense protein of the neutrophil capable of degrading *Shigella* virulence proteins within 10 min of *Shigella* infecting the neutrophil. PMN cells ultimately play a crucial role in controlling the *Shigella* infection, confining

extracellular bacteria to the mucosa and preventing deeper tissue invasion and systemic spread.

Another host defense mechanism directed against *Shigella* has recently been discovered. The glycoprotein, lactoferrin, present in mucosal secretions, breast milk, and phagocytic cells can impair the ability of *S. flexneri* to invade HeLa cells, exposing IpaB–IpaC complexes to protease degradation by disrupting the bacterial surface.

Cellular Immunity

Studies have shown increased T-cell activation in shigellosis patients and T-cell clones have been isolated, which proliferate in response to *S. flexneri* antigen. The cytokines induced by *Shigella* antigens in vaccine studies are suggestive of T helper (Th)1 and Th2 lymphocyte responses. Additionally, the increased susceptibility of acquired immunodeficiency syndrome patients, deficient in cluster of differentiation (CD)4^+ T-cells, to shigellosis could suggest that cell-mediated immunity can play a protective role in shigellosis.

Humoral Immunity

The humoral immune response is a major component of protective immunity to shigellosis with both systemic and mucosal responses activated against the LPS and some virulence plasmid-encoded proteins including the Ipa proteins. It appears that both the systemic and mucosal arms of the humoral response are activated as serum immunoglobulin (Ig)G, IgM, and secretory IgA (sIgA) have all been implicated in the generation of serotype-specific immunity against *S. flexneri*.

Secretory component of the sIgA binds to the mucosal coating of the epithelial cells of the intestine, forming an antibody shield over the cells. sIgA can also coat the outer membrane of *Shigella*, preventing their attachment to the mucosal surfaces, mediate antibody-dependant cell-mediated cytotoxicity, and interfere with bacterial utilization of growth factors. Also, IgA plays an important role in immunity to reinfection. Despite shigellosis generally being a localized mucosal infection, serum antibodies IgG and IgM are detected in human infections directed against the LPS and virulence plasmid antigens IgG and possibly IgM directed against the LPS have been shown to play a protective role in immunity to *Shigella* in mice studies. Serum antibodies directed against the LPS of *S. flexneri* generate serotype-specific immunity by stimulating complement to kill bacteria or mediate antibody-dependant cellular cytotoxicity in the mucosal area.

Complications from *Shigella* Infections

Complications from shigellosis can include dehydration; seizures, especially in children; bleeding per rectum; and invasion of the blood stream by the bacteria. Young children and the elderly are at the highest risk of death. The following is a list of various specific complications caused by *Shigella*.

- Proctitis and rectal prolapse: Due to inflammation of the lining of the rectum or rectal prolapse.
- Reactive arthritis (Reiter's syndrome): Approximately 3% of persons who are infected with *S. flexneri* may subsequently develop pain in the joints, irritation of the eyes, and urethritis. This condition is called Reiter's syndrome. On an average, symptoms appear 18 days after infection.

  It can last for months or years and can lead to chronic arthritis which is difficult to treat. Reiter's syndrome is a late complication of *S. flexneri* infection, especially in persons with a certain genetic predisposition, namely human leukocyte antigen (HLA)-B27. HLA-B27 is a class I surface antigen in the MHC on chromosome 6 and presents microbial antigens to T-cells.
- Toxic megacolon: In this rare complication, the colon is paralyzed and unable to pass bowel movements or gas. Symptoms of toxic megacolon include abdominal pain and swelling, fever, weakness, and disorientation. If this complication goes untreated and the colon ruptures, the patient's condition can be life threatening.
- HUS: *Shigella* rarely results in HUS, which is more commonly a complication of Stx-producing *E. coli* infections. HUS can lead to kidney failure.

Mortality/Morbidity

Although shigellosis-related mortality is rare in developed countries except among travelers, *S. dysenteriae* infection is associated with substantial morbidity and mortality rates in developing countries.

- The overall mortality rate in developed countries is less than 1%.
- In the Far East and Middle East, the mortality rates for *S. dysenteriae* infections may be as high as 20–25%, mostly among children.

Diagnosis

Clinical

Patients presenting with watery diarrhea and fever should be suspected of having shigellosis. The diarrheal stage of the infection cannot be distinguished clinically from other bacterial, viral, and protozoan infections. Dehydration with fast heart rate, low blood pressure, and abdominal tenderness are important signs. Microscopic examination of stool smears from patients with bacillary dysentery is characterized by sheets of PMN. Sigmoidoscopic examination of a shigellosis patient reveals a diffusely erythematous mucosal surface with small ulcers.

Laboratory Studies

Hematology

The total white blood cells (WBC) count reveals no consistent findings. A shift to the left (increased number of band cells) in the differential WBC count in a patient with diarrhea suggests bacillary dysentery. Leukopenia or leukemoid reactions are occasionally detected.

In HUS, anemia and thrombocytopenia occur.

Stool Culture

Watery, bloody, and mucoid stools are highly indicative of shigellosis, but the differential diagnosis should include

enteroinvasive *E. coli*, *Salmonella enteritidis*, *Yersinia enterocolitica*, *Campylobacter* spp., and *E. histolytica*.

Rectal swab or fecal specimens of the patients suspected of having shigellosis were collected in sterile screw-capped containers and immediately subjected to the following microbiological examination:

- Wet preparation by saline and eosin to exclude *E. histolytica*, *Giardia lamblia*, and other cysts or ova of parasite.
- Basic fuchsin smears to exclude *Campylobacter* spp.
- Methylene blue preparation to detect pus cells.
- Gram-stained film and motility to exclude *Vibrio*.
- Initial streaking of the blood-tinged plugs of mucus in stool specimens on the following as a primary isolation media with aerobic incubation:
  - MacConkey agar, Hektoen enteric agar, and *Salmonella–Shigella* agar: These media contain bile salts to inhibit the growth of other Gram-negative bacteria and pH indicators to differentiate lactose fermenters (coliforms) from nonlactose fermenters such as shigellae.
  - Xylose lysine deoxycholate agar is a selective growth medium used in the isolation of *Salmonella* and *Shigella* species from clinical samples and from food. It has a pH of approximately 7.4, and the indicator is phenol red. *Shigella* colonies are red because it cannot ferment sugar.
  - A liquid enrichment medium (selenite broth) may also be inoculated with the stool specimen, incubated at 37 °C and subcultured onto the selective/differential media after a short growth period. Nonlactose-fermenting colonies are streaked and stabbed into tubed slants of Kligler's iron agar or triple sugar iron agar. In these differential media, *Shigella* species produce an alkaline slant and an acid butt with no bubbles of gas in the agar. Also, nonlactose-fermenting colonies could be identified biochemically by analytical profile index (API) 10S (bioMérieux, Stoke on Trent, UK) and slide agglutination tests with antisera for serogroup and serotype confirm the identification.

Enzyme Immunoassay

An enzyme immunoassay for Stx is used to detect *S. dysenteriae* type 1 in the stool. Also, enzyme-linked immunosorbent assay using antiserum or monoclonal antibody recognizing Ipa proteins can be used to screen stools for enteroinvasive pathogens.

Rapid Techniques

Additional diagnostic tools such as gene probes and polymerase chain reaction (PCR) analysis of stool for specific genes such as *ipaH*, *virF*, or *virA* can detect cases not diagnosed by culture but are usually available in research laboratories. Also, real-time PCR was used to detect *Shigella* deoxyribonucleic acid from rectal swab specimens. Several typing methods such as serotyping, drug resistance pattern, plasmid analysis, ribotyping, and pulsed-field gel electrophoresis have been frequently used for subtyping of *Shigella*.

Food Analysis

Isolation of shigellae from foods remains a challenge. A molecular-based method (PCR) that targets a multicopy virulence gene has been developed and implemented by Food and Drug Administration. Isolation and identification of *Shigella* from foods by culture needs several days (rather than hours, as is the case with stool) depending on the food matrix and storage conditions, for example, temperature. *Shigella* species can be outgrown by the resident bacterial populations found in foods, which may reflect the usual low numbers of the organism present in foods. Another factor that reduces the chance of isolating *Shigella* from foods may be the physiological state of the pathogen at the time of analysis. Environmental conditions could affect its ability to either grow or survive in any food matrix.

Alternative Diagnoses List for Shigellosis

For diagnosis of Shigellosis the following lists of conditions have been mentioned as possible alternative diagnoses to consider during the diagnostic process for shigellosis:

- *Salmonella*
- *Campylobacter*
- Stx-producing *E. coli* (e.g., *E coli* 0157:H7)
- Amebic dysentery
- Inflammatory bowel disease
- Viral gastroenteritis
- Food poisoning
- *Y. enterocolitica*
- Cholera
- *Clostridium difficile* colitis
- Cryptosporidiosis

Treatment of *Shigella* Infections

The most commonly used antibiotics for shigellosis treatment are ampicillin (2 g day^{-1} for 5 days), trimethoprim (8 mg kg^{-1} day^{-1}), and sulfamethoxazole (40 mg kg^{-1} day^{-1}). These antibiotics will eradicate sensitive organisms quickly from the intestine, but resistance to these agents is increasing. Effective antibiotic treatment reduces the average duration of illness from approximately 5–7 days to approximately 3 days and also reduce the period of *Shigella* excretion after symptoms subside. Unfortunately, some *Shigella* bacteriae have become resistant to antibiotics and using antibiotics to treat shigellosis can actually make the germs more resistant in future. Ciprofloxacin (1 g day^{-1} for 3 days) is effective against multiple drug-resistant strains, especially in patients more than 17 years of age, to avoid the risk of cartilage damage. Persons with mild infections will usually recover quickly without antibiotic treatment. The oral rehydration treatment developed by the World Health Organization has proven effective and safe in the treatment of acute diarrhea, provided the patient is not vomiting or in shock from severe dehydration. Antidiarrheal agents such as loperamide (Imodium) or diphenoxylate with atropine (Lomotil) should be avoided. The best way to determine which antibiotic is effective is to obtain a stool culture and antibiotic sensitivity tests. Candidate shigellosis vaccines currently in advanced development include both polysaccharide conjugate and live attenuated

vaccines and mostly focus on the most frequently isolated *S. flexneri* 2a and *S. sonnei*, as well as on *S. dysenteriae* 1, because of the severity of cases. Other candidate *Shigella* vaccines such as formalin-inactivated *S. sonnei* vaccine (SsWC) was developed as an oral, killed, whole-cell vaccine at the Johns Hopkins University in Baltimore, USA and recently tested in a Phase I trial on a small number of volunteers. Similarly, Antex (USA) is developing a *Shigella*-inactivated whole-cell vaccine as well as an oral travelers' diarrhea vaccine (ActivaxTM) containing antigens from *Campylobacter*, *Shigella*, and enterotoxigenic *E. coli* (ETEC) These candidate vaccines will shortly undergo clinical testing. In China, a recombinant, live, oral, bivalent vaccine, produced by the Lanzhou Institute of Vaccines and Biological Products, is available for adults. The vaccine has approximately 60% efficacy for both *S. flexneri* and *S. sonnei*.

Preventing a *Shigella* Infection

A safe water supply important for the control of shigellosis is probably the single most important factor in areas with substandard sanitation facilities. Chlorination is another factor important in decreasing the incidence of all enteric bacterial infections. Of critical importance to the establishment of a safe water supply are the general level of sanitation in the area and establishment of an effective sewage disposal system. Frequent hand washing with soap and water after going to the bathroom and before eating is the key to prevent the spread of *Shigella* from an infected person to other person because individuals can carry *Shigella* without noticing symptoms, and *Shigella* bacteria can remain active for weeks after illness.

Basic food safety precautions and disinfection of drinking water prevents shigellosis from food and water. However, people with shigellosis should not prepare food or drinks for others until they have been shown no longer carrying the *Shigella* bacterium or if they do not have diarrhea for at least 2 days. Only treated or boiled water should be consumed while traveling, one should eat fruits peeled by oneself after washing them thoroughly, and hot food should be eaten after disinfecting the surface where food is prepared.

If a child in diapers has shigellosis, after changing the diaper the changing area should be wiped down with disinfectant such as diluted household bleach and the diapers should be put in a closed-lid garbage can, and then hands should be washed with soap and warm water. People who have shigellosis should not prepare food or pour water for others until they have been shown no longer carrying the *Shigella* bacterium. One should swim in pools maintaining a chlorine level of 0.5 ppm, and swimming beaches should have enough bathrooms near the swimming area to keep the water from becoming contaminated.

The most effective methods for controlling shigellosis are provision of safe and abundant water and effective feces disposal.

Outbreaks

Outbreaks commonly occur under conditions of crowding and poor sanitation, such as in correctional facilities, institutions for children, day care centers, mental hospitals, crowded camps, and aboard ships. If an outbreak of shigellosis (i.e., two or more cases) is suspected in a child care facility, the Epidemiology and Response Division should be notified immediately. Outbreaks of shigellosis in this situation would ordinarily be controlled by exclusion and treatment of symptomatic children and staff.

Examples of foodborne outbreaks:

1. Military campaign Operation Desert Shield, 1990: Diarrheal disease during military operation can obviously reduce the effectiveness of troops. In Operation Desert Shield, enteric pathogens were isolated from 214 US soldiers and out of those 113 cases were diagnosed with *Shigella*; *S. sonnei* was the prevalent of the four shigellae isolated. Shigellosis accounted for more time lost from military duties and was responsible for more severe morbidity than the morbidity caused by enterotoxigenic *E. coli*, the most common enteric pathogen isolated from USA troops in Saudi Arabia. The suspected source was contaminated fresh vegetable, notably lettuce. Twelve heads of lettuce were examined and enteric pathogens were isolated from all.
2. Local outbreak – moose soup in Alaska, 1991: In September 1991, the Alaska Division of Public Health was contacted about a possible gastroenteritis outbreak. In Galena, 25 people who had gathered at a local community event in which homemade foods were consumed contracted shigellosis. The implicated food was homemade moose soup. One of the five women who prepared the soup reported that she had gastroenteritis before or at the time of preparing the soup. *Shigella sonnei* was isolated from one hospitalized patient.
3. Doubletree Hotel *Shigella* Outbreak, Colorado, 2003: In September 2003, the Colorado Department of Public Health and Environment began to get reports of *Shigella* infections. Their investigation led them to the Doubletree Hotel in Westminster, CO, USA which was a common link for the infected parties. Two large groups of hotel guests were identified, a wedding party and a World War II veterans' reunion, with attendees from a number of different states. Members of both groups as well as a random sample of hotel guests were contacted and interviewed. Ten individuals were diagnosed with culture-confirmed cases of *S. sonnei* infections after eating honeydew melon served at the breakfast buffet. Three food handlers and a kitchen chef were tested positive for *Shigella*.
4. Royal Fork *Shigella* Outbreak, Washington, 2001: A total of eight culture-confirmed *S. sonnei* cases had eaten at the Royal Fork restaurant located in Mount Vernon, WA, USA, between January 13 and 20, 2001.

   The Communicable Disease Epidemiology section of the Washington State Department of Health (WDOH) found that a food worker at the Royal Fork restaurant was also infected with *S. sonnei*. The infected food worker had recently returned from Mexico. On her return, she suffered *Shigella*-like symptoms until approximately 6 January and returned to work at Royal Fork on 10 January after her symptoms had subsided. WDOH learned that the infected food worker was in charge of restocking the salad bar, and

she did not routinely wear gloves during this process. "*Shigella sonnei* was most likely transmitted to restaurant patrons by an infected food worker through the vehicle of romaine lettuce served on the salad bar."

5. Subway Restaurant *Shigella* Outbreak, Chicago, 2010: A *Shigella* outbreak at a Subway restaurant in Lombard, IL, USA, has caused at least 116 confirmed cases of *S. sonnei* and likely greater than a thousand total illnesses. The DuPage County Health Department investigators found that two employees of the defendant's Subway restaurant tested positive for the same strain of *Shigella* that caused the outbreak. These individuals had been ill with gastrointestinal symptoms related to their *Shigella* infections before the outbreak. The poor hygienic practices by the ill employees caused widespread contamination of patrons' food.
6. Other examples of shigellosis outbreaks spread by food workers: Todd *et al*. had analyzed 816 outbreaks where food workers were implicated in the spread of foodborne disease. They discussed the size, severity settings, factors contributing to outbreaks, and descriptions of different outbreak categories.

Foods Implicated in Outbreaks

Shigella is commonly transmitted by foods consumed raw, for example, lettuce or as nonprocessed ingredients such as those in a five-layer bean dip, salads (potato, tuna, shrimp, macaroni, and chicken), icing on cakes or exported raspberries, milk and dairy products, and poultry are among the foods that have been associated with shigellosis.

See also: Bacteria: *Salmonella* Typhi and *Salmonella* Paratyphi. Food Safety Assurance Systems: Personal Hygiene and Employee Health. Foodborne Diseases: Overview of Biological Hazards and Foodborne Diseases. Public Health Measures: Safe Use of Wastewater for Agricultural Production. Safety of Food and Beverages: Water (Bottled Water, Drinking Water) and Ice

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Relevant Websites

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Centers for Disease Control and Prevention (CDC).

http://www.mayoclinic.com/health/shigella/DS00719
The Mayo Clinic.

http://www.textbookofbacteriology.net/Shigella
Todar's Online Textbook of Bacteriology.

http://www.ainc-inac.gc.ca/h2o/bkg_e.html
Shigella Blog provides up-to-date news related to *Shigella* outbreaks, research, and more.

BACTERIA

Staphylococcus aureus

ECD Todd, Ewen Todd Consulting LLC, Okemos, MI, USA

Glossary

Bacteremia Bacteria entering the bloodstream through a wound, an existing infection, or through a surgical procedure or injection.

Cellulitis Spreading bacterial infection just below the skin surface.

Danger zone The temperature range in which foodborne bacteria can grow is known as the danger zone. According to the 2009 US Food and Drug Administration Food Code, the danger zone is defined as 5 and 57 °C (41 and 135 °F). However, other jurisdictions consider the danger zone between 5 and 60 °C (41 and 140 °F).

Emetic reflex center Vomiting is a complex, coordinated reflex orchestrated by the vomiting center of the brain. It responds to external signals. The central nervous system plays a critical role in the physiology of nausea and vomiting, serving as the primary site that receives and processes a variety of emetic stimuli. The central nervous system also plays a primary role in generating efferent signals that are sent to a number of organs and tissues in a process that eventually results in vomiting.

Enterotoxin (staphylococcal) A soluble exotoxin produced by some strains of *Staphylococcus*; a cause of food poisoning or intoxication.

Meningitis A serious inflammation of the meninges, the thin, membranous covering of the brain and the spinal cord.

Nasopharynx Is part of the pharynx above the soft palate that is continuous with the nasal passages. The pharynx is the membrane-lined cavity behind the nose and mouth, connecting them to the esophagus.

Osteomyelitis Is the inflammation of the bone and/or marrow due to infection by bacteria like *Salmonella* or *Staphylococcus*, caused by a complication of surgery or injury, or infection through the bloodstream.

Pulsed-field gel electrophoresis It is an electrophoretic technique in which the gel is subjected to electrical fields alternating between different angles, allowing very large DNA fragments to pass through the gel, permitting efficient separation of mixtures of such large fragments.

Sepsis It is a systemic response typically to a serious, usually localized, infection especially of bacterial origin that is usually marked by abnormal body temperature and white blood cell count, tachycardia (abnormally rapid heart rate), and tachypnea (rapid breathing).

Staphylococcal scalded skin syndrome (SSSS) It is a disease, caused by a type of bacteria, in which large sheets of skin may peel away. SSSS primarily strikes children under the age of five, particularly infants. Clusters of SSSS cases (epidemics) can occur in newborn nurseries, when staff in those nurseries accidentally pass the causative bacteria between patients. It can also strike other age groups who have weakened immune systems.

Toxic shock syndrome It is an acute infection characterized by high fever, a sunburn-like rash, vomiting, and diarrhea, followed in severe cases by shock, that is caused by a toxin-producing strain of *Staphylococcus aureus,* occurring chiefly among young menstruating women who use vaginal tampons.

Vagus nerve A nerve that supplies nerve fibers to the pharynx (throat), larynx (voice box), trachea (windpipe), lungs, heart, esophagus, and intestinal tract, as far as the transverse portion of the colon. The vagus nerve also brings sensory information back to the brain from the ear, tongue, pharynx, and larynx.

Background

Staphylococcus aureus was first identified in 1880 from pus in a knee joint abscess. Since then we know that *S. aureus* is a very frequent microorganism in humans and animals with approximately 20–50% of persons being long-term carriers (including food and hospital workers), mainly as a part of the normal skin flora and in anterior nares of the nasopharynx, but also the throat and hair. These resident bacteria do not cause disease. However, damage to the skin (such as food workers and hospital staff scrubbing hands too roughly) or other injury may allow the bacteria to overcome the natural protective mechanisms of the body, leading to infection. These include skin infections such as pimples, impetigo, boils, cellulitis, carbuncles, and abscesses. Extensive infected pimples can lead to a staphylococcal scalded skin syndrome.

These infections can be long-lasting if not treated, but they are not as serious as when the pathogen invades the body causing bacteremia and sepsis. Toxic shock syndrome and endocarditis can quickly become fatal without antibiotic treatment, but *S. aureus* can also cause pneumonia, meningitis, and osteomyelitis. It is often the cause of postsurgical

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wound infections. Many strains are resistant to antibiotics and one of the most recent concerns in hospitals is nosocomial methicillin-resistant *S. aureus* which can be fatal for immunocompromised patients.

Characteristics

Staphylococci are Gram-positive, nonspore forming, facultatively anaerobic, nonmotile, catalase-positive or negative, small, spherical bacteria from pairs to, grape-like clusters, from where the name *Staphylococcus* comes from (staphyle, meaning a bunch of grapes, and kokkos, meaning berry).

Staphylococci grow in foods as well as being present in animals with a range from 7 °C to approximately 48 °C, with its optimum at 35 °C, at a preferred pH range of 7.0–7.5, but it can grow as low as pH 4.5. Staphylococci are able to grow at low levels of water activity, for example, as low as 0.8. Thus, it is highly tolerant to salts and sugars and has historically been responsible for foodborne disease outbreaks illnesses from contaminated hams (salt) and pies (sugar). In addition, *S. aureus* does not need to grow to be a concern, because it can survive for extended periods in a dry state and subsequently contaminate wounds and foods. *S. aureus* produces a large variety of virulence factors. These include coagulases that clot plasma and coat the bacterial cell to inhibit phagocytosis; hyaluronidase that breakdown hyaluronic acid to help spreading the pathogen in tissues; deoxyribonuclease (DNase) that attacks cellular DNA; Protein A that binds IgG of the host's antibodies; the golden colored carotenoid pigment that acts as a bacterial antioxidant; and staphylococcal enterotoxins (SE) that act as superantigens capable of stimulating T-cells (at least 21 SEs have been described, with some of them proven to be emetic).

Clinical Manifestations

From a disease perspective, some staphylococcal species, mainly *S. aureus*, produce the heat-stable emetic SEs to cause gastroenteritis in humans (nausea, vomiting, abdominal cramps, and diarrhea) on ingestion of food containing these enterotoxins, often called staphylococcal food poisoning. The incubation period of illness ranges from 30 min to 8 h, but is usually within 2–4 h. The onset of symptoms depends on susceptibility of the individuals to the SEs, the amount of contaminated food eaten, the amount of toxin in the food ingested, and the general health of the patients. In severe cases, patients may present with headache, muscle cramping, severe fluid, and electrolytes loss with weakness and low blood pressure or shock. Patients usually recover within 2 days, but can take longer in severe cases, which may require hospitalization. Death from staphylococcal food poisoning is rare, although it has occurred among the elderly, infants, and severely debilitated people, or if the amount of the toxin is excessive. For instance, in 1998, approximately 8000 individuals gathered to celebrate a priest's ordination in Brazil. Within hours of food consumption, 4000 patients experienced acute gastroenteritis, and 396 required admission to hospitals, and 16 died through multisystem shock while hospitalized. The trace-back investigation implicated food preparers who were culture positive for enterotoxigenic *S. aureus* as the source of contamination. When a food is contaminated and *S. aureus* is allowed to grow, it can produce these SEs that remain active in the food even if the organism is no longer viable, such as being destroyed through cooking (some SEs can remain stable at 100 °C for 30 min). These single-chain proteins (MW 26 000–29 000) are resistant to proteolytic enzymes, such as trypsin and pepsin, allowing them to pass through the digestive tract. There are five main enterotoxin serotypes: SEA, SEB, SEC, SED, and SEE that demonstrate an emetic activity soon after ingestion, but there are several, but more rarely encountered in food. The SEs stimulate the emetic reflex center probably through the vagus nerve. The intoxication dose of SE is less than 1.0 μg of preformed enterotoxin, and there have been reports of illnesses following ingestion in the nanogram range (100–200 ng in highly sensitive people). This toxin level is reached when *S. aureus* populations typically exceed 100 000 CFU g^{-1} in food. However, the level determined on analysis of the incriminated product may not have been the highest level achieved by the pathogen because of population die-off or the food had been heated. Nevertheless, any level >1000 CFU g^{-1} is an indication of gross insanitary conditions, such as because of human contamination followed by time–temperature conditions that allow growth. Standards in foods for *S. aureus* vary, but are often ≤ 10 CFU g^{-1} for prepared foods. *S. aureus* does not compete well where there are many other microorganisms, and therefore standards or guidelines for uncooked food usually do not apply.

Epidemiology

Staphylococci are ubiquitous in the environment and they can be found in the air, dust, sewage, water, milk, and food, or on food equipment, environmental surfaces, and animals, as well as present as human colonizers. As such, staphylococci are expected to exist in any and all foods that are handled directly by humans or are of animal origin, and are impossible to prevent arriving or eradicating from food processing and service operations. Many of the 32 *Staphylococcus* species and subspecies are potentially found in foods due to environmental, human, and animal contamination. Because of this, *S. aureus* foodborne disease outbreaks are typically the third or fourth most frequent in many countries (e.g., after *Salmonella, Clostridium perfringens,* and sometimes *E. coli* O157), where such diseases are monitored and recorded; these outbreaks are typically associated today in home or foodservice prepared food. In the US, it is estimated that staphylococcal food poisoning causes approximately 241 188 illnesses, 1064 hospitalizations, and 6 deaths each year. However, this is unlikely to be completely accurate because those ill do not complain or seek medical attention because of the short duration of the illness; the investigation of an illness complaint or outbreak may be incomplete; misdiagnosis of the illness, which may be symptomatically similar to other types of rapid-onset food poisoning, such as caused by *Bacillus cereus* emetic toxin; lack of health department resources to investigate a short-term, self-limiting

illness; and inadequate collection of samples for laboratory analyses and/or improper laboratory examination.

Analytical Methods for Organisms and/or Toxins

During investigations of possible staphylococcal illnesses, *S. aureus* can be isolated from stool specimens, but unless some typing is done, these may be unrelated to the causative organism because *S. aureus* is not unusual in stools of healthy individuals. Enrichment isolation and direct plating for counts can be performed on selective media, such as mannitol-salt and Baird Parker agars and Petrifilm™ rapid *S. aureus* count plate method where numbers are expected to be >100 CFU g^{-1}. The most probable number procedure is recommended for monitoring of products expected to have smaller numbers. Phage-typing was widely used in the UK for typing isolates but used less frequently elsewhere. More recently, multilocus sequence typing , pulsed-field gel electrophoresis (PFGE), and other DNA profile methods have also been used for comparing isolates. Serological methods, typically ELISA-based, are available for detection of preformed enterotoxin in foods, and the enterotoxigenicity of *S. aureus* isolates. This is especially useful if the suspected food item has been heated in any way to destroy the pathogen.

Route of Transmission

Outbreaks usually occur today because of mistakes made by food preparers. Among these, there are first those that lead to contamination of food, such as:

- direct contact with workers with hand or arm lesions caused by *S. aureus*;
- coughing and sneezing;
- touching hair and beards;
- contact with pets;
- contact with contaminated equipment, and food- and nonfood-contact surfaces; and
- contact with sores on hands, nose wiping, and fingernails.

Foods of animal origin may also be contaminated at source, for instance, milk may carry *S. aureus* following mastitis of cows. Furthermore, there are those that lead to growth of the organism. In this regard, a common mistake which is frequently the cause of staphylococcal food poisoning is that foods that require considerable handling during preparation are kept above refrigeration temperatures for an extended period after preparation. For instance, frequently implicated foods are sliced meat, puddings, pastries, and egg sandwiches. The contaminated foods may not smell bad or look spoiled in order to produce the toxins, and thus may seem quite acceptable to consume. More specific examples include contamination of starter cultures for cheese, where the inoculated milk allows rapid growth before the curd forms and the product is too acidic with low water activity for further growth; barbecued chickens stored for many hours in the 'danger zone'; and cream-filled pies displayed in shop windows under warm ambient conditions.

Examples of Outbreaks

1. In October and November 2009, six household staphylococcal food poisoning outbreaks were notified in six French metropolitan départements. A total of 23 persons of 26 persons who had consumed cheese (attack rate 88.5%) suffered from nausea, vomiting, abdominal cramps, and diarrhea, in some cases associated with fever. The period between the ingestion of a cheese and the onset of symptoms ranged from 1.25 h to 8 h. A soft cheese made from unpasteurized cow milk was the common source of these outbreaks as all cases had eaten the same cheese (the coagulase-positive staphylococci isolates from the contaminated cheese samples showed the same SE gene pattern, the same biotype, the same PFGE profile, and also the same antibiogram). Cheese samples were available from six outbreaks and the staphylococcal food poisoning diagnosis was confirmed through (1) the high count of coagulase-positive staphylococci, (2) the detection of SE type E in the incriminated cheese type, and (3) the detection of the SEE gene in coagulase-positive staphylococci isolates from the suspected cheese samples. The SEE amount found in one of the cheese samples was 0.45 ng g^{-1}. Because, those ill ate about 200 g, the total amount of ingested SEE could be estimated to 90 ng. This was the first report of food poisoning outbreaks caused by SE type E in France.
2. In 2009, 21 persons experienced either vomiting and/or diarrhea within 12 h of eating at a Kansas restaurant in June, 5 people experienced bloody diarrhea, and 3 others sought medical care in the emergency department. The incubation period ranged from 1 h to 11 h with a median of 4 h. Several menu items were significantly associated with the illness: The cannelloni, the pasta sampler, which contains lasagna, manicotti, and cannelloni, and any chicken entree. The ingredients of the cannelloni included ground beef, spinach, mozzarella, ricotta, parmesan, and romano cheeses hand-rolled in a sheet of pasta topped with marinara sauce. An inspection of the restaurant found five critical violations: (1) inadequate cold holding temperatures, (2) refrigeration units not holding proper cold hold temperatures, (3) improper cooling of cooked foods, (4) no date markings on any refrigerated foods, and (5) open drinks in the food preparation area. Four noncritical violations were observed: (1) improper thawing, (2) blocked hand washing sink, (3) no food thermometers, and (4) improper storing of in-use utensils. A sample of cannelloni was collected during the food inspection and tested positive for *S. aureus* enterotoxin. *Staphylococcus aureus* was also isolated from the sample. No employee reported any gastrointestinal symptoms. During the inspection of the establishment, multiple food items were above proper cold holding temperatures. The cheese filling for the cannelloni was at 54 and 57 °F, raw chicken was at 79 °F, and ready to eat chicken was at 54 °F, which are all temperatures that *S. aureus* exhibits rapid growth and toxin production.
3. In December 2010, there were four outbreaks associated with the consumption of dessert items (tiramisu, cakes, cobblers, decorated cookies, tarts, pastries, and pies) from

an Illinois wholesale and retail sales bakery. Approximately 100 people reported becoming ill after consuming these at a company event, catered party, a restaurant, and a holiday party. The bakery had failed to clean and sanitize equipment in a manner that would protect against contamination of food and food-contact surfaces. Unfortunately, these problems were not resolved satisfactorily because in June 2011, multiple enterotoxigenic strains of *S. aureus* were found in environmental samples of the bakery and in the topping used to finish its cakes. The US Food and Drug Administration stated in a warning letter to the bakery that the cleaning and sanitation operations were ineffective, and that the firm was operating under insanitary conditions, which may reasonably cause contamination of food with this organism, and which may lead to toxin formation, and that foods that can support the rapid growth of undesirable microorganisms – particularly cream-filled pastries – were being held too long at unsafe temperatures.

4. The Snow Brand outbreak in Japan in June, 2000 was unusual, in that it was a large-scale incident involving commercial dairy food (low-fat milk and milk beverages made from skimmed milk powder) made by Japan's premier dairy foods company. There were 14 780 cases, but no viable organisms were found in the products, only very low levels of enterotoxin (0.05–1.6 ng ml^{-1}) in the incriminated milk products. From the source product (skimmed milk powder), SEA was detected at 4 ng g^{-1}. The reason for the contamination was defective temperature control because of a power outage that stopped the production line at the Hokkaido plant where the raw material was produced. This toxic material was sent to the next level of production line without being discarded and created toxic skimmed milk powder. Dairy products that contained the toxic material were produced and shipped from the Osaka plant and caused an outbreak throughout the Kansai region of Japan. The delay in making company and public announcements and in recalling the product allowed exacerbated the problem. Various faults were subsequently documented. When the number of bacteria in the produced skimmed milk powder exceeded safety standard, the material was considered to be safe to be reused if sterilized (which would not affect the activity of SE), and the new skimmed milk powder was produced out of the toxic material and shipped to Osaka plant. The plant manager and his staff not only did not have full knowledge about enterotoxin, but also lacked the basic knowledge that toxin generated from bacteria did not lose its toxicity by heating. There was also a lack of basic knowledge of food sanitation, and corporate standards were not strictly kept. This incident caused Snow Brand to lose consumer confidence and the sales of the largest dairy product manufacturer plummeted. The total deficit for the period of this fiscal year reached 52.9 billion yen and the company had to close two operations including the Osaka plant, and eventually merged operations with Nestlé.
5. Other species of the genus *Staphylococcus* have been rarely implicated in foodborne outbreaks. For instance, *S. intermedius*, normally considered a veterinary animal pathogen, was isolated from butter blend and margarine implicated in a 1991 outbreak. SEA was detected in both clinical and food isolates implicated in this food-related outbreak involving more than 265 cases in the western US.

Staphylococcal Enterotoxin as a Bioterrorism Agent

It has been argued that SE could be used as a biological agent either by contamination of food/water or by aerosolization and inhalation. Breathing in low doses of SE type B may cause fever, cough, difficulty in breathing, headache, and some vomiting and nausea. High doses of the toxin have a much more serious effect. However, although SEs are relatively easy to produce, there are more effective biological agents that would likely be used first.

Prevention of Staphylococcal Intoxication

Prevention of foodborne intoxication with *S. aureus* is based on prevention of contamination of the food and of prevention of growth of the organisms. Food operators should be trained in good hygienic practice, but in particular to be instructed to:

- Wash hands and under fingernails vigorously with soap and water before handling and preparing food.
- Wear hair nets and beard constraints.
- Not prepare food if they have a nose or an eye infection.
- Not prepare or serve food for others if they have wounds or skin infections on their hands or wrists.
- Wear gloves as appropriate to the operation but take care to wash hands frequently and change gloves to minimize the build-up of *S. aureus* at the warm, moist glove–skin interface.
- Prevent cross-contamination of raw and cooked food.
- Keep pets away from food preparation areas.
- Keep kitchens and food-serving areas clean and sanitized.
- If food is to be stored longer than 2 h, keep hot foods hot ($>57\,^{\circ}C/135\,^{\circ}F$) and cold foods cold ($<5\,^{\circ}C/41\,^{\circ}F$ or under).
- Store cooked food in wide, shallow containers and refrigerate as soon as possible.

See also: Food Safety Assurance Systems: Personal Hygiene and Employee Health. Foodborne Diseases: Overview of Biological Hazards and Foodborne Diseases. Public Health Measures: Health Education, Information, and Risk Communication. Risk Analysis: Food Safety Training and Health Education: Principles and Methods

Further Reading

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BACTERIA

Streptococcus

Z Hossain, Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka, Bangladesh

Glossary

Contamination The introduction or occurrence of a contaminant in food or food environment.
Foodborne disease Any disease of an infectious or toxin nature caused by or thought to be caused by consumption of food or water.
Food handler Any person who handles, prepares, or serves food, be they domestic food handlers (preparing family food) or professional food handlers, such as those working in food service establishments (cooks and waiters), retail stores, supermarkets, etc.
Hazard A biological, chemical or physical agent in, or condition of, food with the potential to cause an adverse health effect.
Incidence The rate or range of occurrence or influence of something, especially of something unwanted.
Infection The invasion and multiplication of microorganisms such as bacteria, viruses, and parasites that are not normally present within the body.
Monitoring The act of conducting a planned sequence of observations or measurements of control parameters to assess whether a critical control point is under control.
Pathogen An organism capable of causing disease.
Risk A function of the probability of an adverse health effect and the severity of that effect, consequential to a hazard(s) in food.

Background

Certain foodborne illnesses are the precursor of some potentially life-threatening diseases in humans. The occurrence of such illnesses can be partly attributed to infections with *Streptococcus* species. Streptococci are spherical, Gram-positive, extracellular bacteria, which usually exist in pairs or long chains. These microbes belong to the phylum Firmicutes and fall under the lactic acid bacteria group. The diseases caused by these bacteria include strep throat, pneumonia, scarlet fever, rheumatic fever, cellulitis, necrotizing fasciitis, and meningitis, especially neonatal meningitis. *Streptococcus* was associated with one of the first discovered diseases treatable by antibiotics. This genus of bacteria has played an inevitable role in the history of microbiology and molecular biology being the paradigm for many microbial studies around the globe. In 1880, both French biologist Louis Pasteur and George Miller Sternberg in the USA independently isolated the bacteria that caused pneumonia. The pathogen was eventually named *Streptococcus pneumoniae* in 1974. The name streptococci has derived from the Greek word *Streptos* meaning twisted or easily bent, like a chain, and was given by a Scottish doctor, Alexander Ogston, who observed a group of spheroid bacteria arranged in chains. Griffith's landmark experiment, which is recognized as the foundation of genetics, also employed *S. pneumoniae* to study the transfer of virulence from pathogenic to nonpathogenic bacterial strains. An ancient physician, Hippocrates also known as the Father of Medicine is credited for the first mentioning of the flesh-eating bacteria while describing the relative symptoms in early stages of the infection.

In 1884, these chain-forming bacteria were named *Streptococcus pyogenes* by Rosenbach. Later the discovery of serotyping of hemolytic and nonhemolytic structures by the American microbiologist Rebecca Lancefield in 1933 helped with subgrouping of α-, β-, and γ-hemolytic types of *Streptococcus* by their serotypes. Much of public attention to streptococci has resulted from the emergence of new virulent strains of these pathogens causing epidemics in recent years. Sulfonamides used to be the first treatment of choice for streptococcal diseases during 1930s. However, many of the strains of this pathogen became resistant to this treatment within a decade. The great discovery of penicillin by Alexander Fleming in 1928 led to the introduction of antibiotics in treating diseases caused by these bacteria. During World War II, penicillin was successfully used to prevent lethal outbreaks of streptococcal diseases, such as rheumatic fever.

Characteristics of the Organisms

Nomenclature/Taxonomy and Classification

The term *Streptococcus* refers to the spheroidal architecture of these microorganisms forming bead-like chains or twists. As these microorganisms can also appear in pairs, diplococci are indeed streptococci. Staphylococci are, however, segregated from this genus because of their distinct morphological and phenotypic characteristics. The genus *Streptococcus* belongs to the class Bacilli and the family Streptococcaceae while comprising an enormous number of species widely distributed in

the animal kingdom. Whereas some of these species afflict humans and animals, others are being exploited as industrially beneficial microbes.

According to the extent of hemolysis, i.e., α (partial), β (complete), and γ (no hemolysis), and surface antigen (group antigen) recognition by antibodies, streptococci are subdivided into groups consisting of one or more species. Twenty different serotypes (Groups A to V, except I and J) have been described as Lancefield groups based on the specific composition of cell-wall carbohydrates. The most significant Lancefield groups are β-hemolytic streptococci (BHS) A and B, and the α- or γ-hemolytic D. However, the typical α-hemolytic streptococci *S. pneumoniae*, *S. mutans*, and other so-called *viridans* species do not possess such group antigens, and are independent of Lancefield classification. The only group A *Streptococcus* (GAS) species that accounts for some of the major β-hemolytic streptococcal diseases is known as *S. pyogenes*. *Streptococcus agalactiae*, which is a particular problem among pregnant females and neonates, is the only member of group B *Streptococcus* (GBS). After recent reclassification, many group D streptococci (GDS) have been categorized under the genus *Enterococcus*. The only nonenterococcal GDS *Streptococcus bovis* and *Streptococcus equinus* are clinically important because of their association with human illnesses. Other BHS, which sporadically become etiological agents of human diseases, mainly include groups C and G. *Streptococcus equi* subsp. *zooepidemicus* (from *Streptococcus zooepidemicus*) and *Streptococcus dysgalactiae* subsp. *equisimilis* (from *Streptococcus equisimilis*) are two major human-specific group C *Streptococcus* (GCS) species. The only group G *Streptococcus* (GGS) occasionally prevalent in human infections is *Streptococcus canis*. The nongroupable *viridans* (from Latin *viridis* meaning *green*) streptococci, also well known as oral streptococci, embrace diverse species including two members of mutans streptococci, such as *S. mutans* and *Streptococcus sobrinus*. Current classification of these bacteria based on their 16S ribosomal ribonucleic acid (rRNA) analysis has greatly overcome common confusions arising from similar results in biochemical identification tests.

Morphology

Streptococci are coccoid bacterial cells microscopically, and stain purple (Gram-positive) when Gram staining technique is applied. They are nonmotile and non-spore forming. These cocci measure between 0.5 and 2 μm in diameter. As cellular division of *Streptococcus* spp. occurs along a single axis or plane, these bacteria grow in pairs or chains. After 18–24 h of incubation at 35–37 °C on blood agar, typically grayish-white, smooth, glossy, and translucent colonies appear with zones of α/β-hemolysis or no hemolysis. α-Hemolysis produced by many species of *Streptococcus* is characterized by a partial breakdown of the red blood cells (RBCs) in the medium, and is seen as a green zone surrounding the colony resulting from pigmentation of hemoglobin within RBCs. Although the term α-hemolysis is still used for this appearance, the property is due to oxidization of iron in hemoglobin molecules (reduction to methemoglobin), not lysis of RBCs. *Streptococcus pyogenes* or GAS forms doomed, round, and entire pinpoint

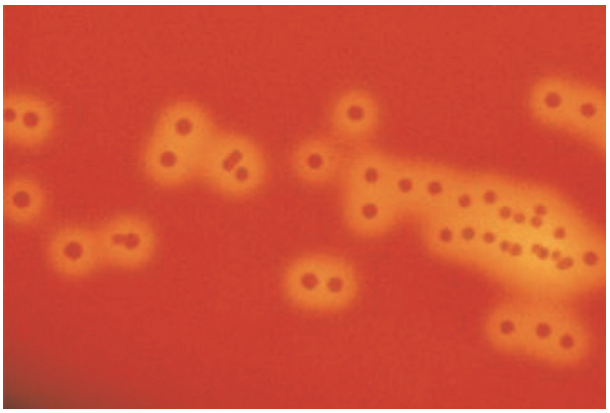

Figure 1 β-Hemolysis produced by colonies of *S. pyogenes* on blood agar.

colonies that exhibit complete hemolysis, as indicated by a clear zone surrounding the colony several times greater than the diameter of the colony itself. Some strains may produce mucoid colonies (Figure 1). *Streptococcus pyogenes* are spherical to ovoid microorganisms measuring up to 1 μm in diameter. GBS or *S. agalactiae* colonies can be flat, grayish-white or orange, mucoid, and creamy. When incubated aerobically this group of streptococci may render less obvious β-hemolysis also known as α-prime hemolysis, which is represented by a small zone of clear hemolysis surrounded by an area of partial lysis on blood agar. Colonies of some β-hemolytic GCS, such as *S. equi* are larger and more mucoid than those of most classical *Streptococcus*. Also, the human variant *S. dysgalactiae* subsp. *equisimilis* is known as the large-colony type of group C, which shares a range of phenotypic characteristics with *S. pyogenes* as well as *S. agalactiae*. However, these bacteria are normally found as nonhemolytic *in vitro*. The GDS produce usually α- or nonhemolytic colonies of 1–2 mm in diameter. Viridans group streptococci (VGS) produce tiny, α-hemolytic colonies on blood agar. Although *S. mutans* is normally found as α- or γ-hemolytic, some β-hemolytic strains have also been identified. *Streptococcus pneumoniae* (also known as pneumococci) colonies are circular with entire margins, often elevated with depressed centers. These α-hemolytic colonies are commonly mucoid on primary isolation. Cells undergo autolysis on extended incubation, and the center of colony tends to disappear. Heavily encapsulated pneumococci can have larger colonies (several millimeters in diameter) than less heavily encapsulated strains. As these bacteria are often seen as pairs of cocci, they are well regarded as diplococci. Nonetheless, some strains of *S. pneumoniae* may also be observed as single cocci or in short chains. Most streptococci generally have a rigid cell wall with typical Gram-positive peptidoglycan layer, inner plasma membrane with mesosomal vesicles, cytoplasmic ribosomes, and a nucleoid. Some strains including *S. pneumoniae* and *S. pyogenes* contain polysaccharide capsule composed of hyaluronic acid (HA). The cell wall of *S. pyogenes* possesses a group-specific C-polysaccharide and two major classes of type-specific proteins, M and T antigens. The minor classes of type-specific proteins include F, R, and M-like or M-associated protein antigens. Many streptococcal species can sometimes have pilus- or

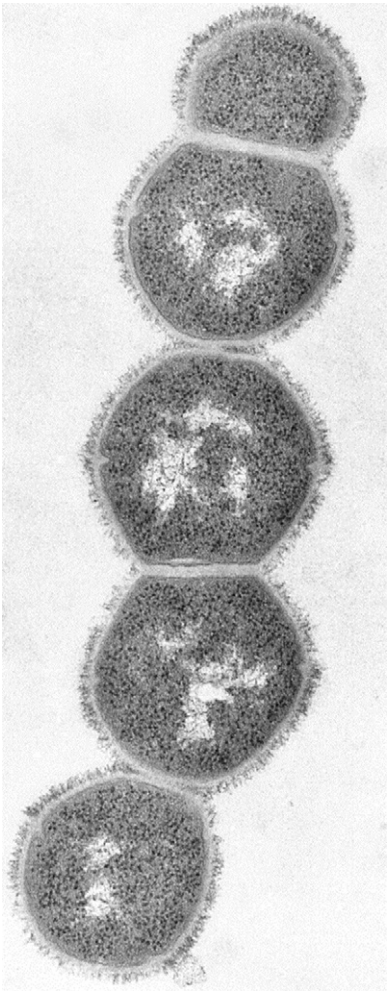

Figure 2 Electron micrograph of *S. pyogenes* with fimbriae surrounding the cell wall.

fimbria-like structures projecting from the surface of the bacteria, such as M protein-containing fimbriae of *S. pyogenes* (Figure 2). These structures are known to promote epithelial colonization and play a distinct role in virulence. For further discussion on this topic, see Section Streptococcal Toxins and Other Virulence Factors in Pathogenesis.

Metabolism and Physiology

Strains of *Streptococcus* are facultative anaerobes, but hemolytic activity of these bacteria increases in the anaerobic environment, i.e., hemolysins, which lyse the RBCs, are more stable in the absence of oxygen. They are catalase, oxidase, and nitrate negative. These bacteria depend largely on glycolysis for adenosine triphosphate (ATP) synthesis. Streptococci are chemoorganotrophic, i.e., their metabolism is fermentative producing mainly lactic acid, ethanol, and acetate from carbohydrates without the production of gas. However, *S. equinus* does not ferment lactose or grow in litmus milk. *Streptococcus pyogenes* has shown higher consumption of oxygen at thriving pH in the presence of glucose. Conversely, the percentage of glucose and galactose converting to lactic acid increases with a decrease in pH value (<6.5). Galactose is more slowly metabolized at high pH than glucose for *S. pyogenes* despite entering the pathway of glycolysis. Also, the end products of galactose fermentation vary radically from those of glucose fermentation, i.e., less lactate and more formate, acetate, and ethanol. *Streptococcus agalactiae* utilizes an aerobic pathway for glucose oxidation, and produces lactic and acetic acids, acetylmethylcarbinol, and carbon dioxide. *Streptococcus mutans*, which adheres to teeth in large numbers, is able to ferment dietary sugars into acids. These strains use an anaerobic type of energy metabolism regardless of their growth conditions. Therefore, ferrous iron is believed to boost their tolerance to oxygen-free atmosphere. Many viridans streptococcal species and certain *S. bovis* strains are able to synthesize dextrans from glucose. Streptococci require nutritionally rich media for growth. For example, they grow ideally on brain–heart infusion agar in the presence of 5–10% CO_2 (capnophiles). Growth of these nutritionally fastidious bacteria is enhanced by the addition of blood or serum. Therefore, these microorganisms are unable to synthesize many basic building blocks. Later in the growth cycle, streptococci produce hyaluronidase (Hyl), an enzyme that cleaves HA, a basic constituent of the bacterial capsule. The precise role of Hyl is still dubious, but it is assumed that the enzyme enables the bacterium to utilize host HA (found in the connective tissue) or its own capsule as a carbon (energy) source. Most streptococcal species produce leucine aminopeptidase, which hydrolyzes the substrate leucine-β-naphthylamide to form β-naphthylamine. Besides, GAS synthesize pyrrolidonyl arylamidase (PYRase) that hydrolyzes the substrate L-pyrrolidonyl-β-naphthylamide to form β-naphthylamine. *Streptococcus pyogenes* is notably sensitive to bacitracin, whereas most other groups are resistant. The GBS hydrolyze sodium hippurate. Both GAS and GBS do not usually grow on bile esculin. Strains that possess group D antigen are able to grow in the presence of 40% bile salts, and produce black precipitate by hydrolyzing esculin when grown on bile esculin. Nonenterococcal strains are intolerant to 6.5% sodium chloride (NaCl). Pneumococci are bile soluble, but susceptible to optochin. Viridans and other typical streptococci can survive in the presence of optochin. However, nearly all oral streptococci are bile insoluble, and do not grow on bile esculin. Exceptionally, *S. mutans* strains are capable of growing in a bile-supplemented medium. Most classical streptococci exhibit negative tolerance to 6.5% NaCl.

Streptococcal Toxins and Other Virulence Factors in Pathogenesis

Diverse virulence factors are associated with streptococcal pathogenicity within the host. This section will mainly focus on toxins and other pathogenic components related to GAS, being the paradigm for study of foodborne streptococcal infections. GAS strains synthesize a number of distinctive toxins and enzymes. This synthesis is thought to be mediated by lysogenic bacteriophages. Although the true mechanisms and functionality of these factors are not very clear, implication of some surface proteins in the microbial adherence to epithelial cells and colonization is quite evident. Some of the essential

virulence substances produced by GAS are exotoxins that include cytolytic toxins (streptolysins O and S) and erythrogenic or pyrogenic exotoxins (superantigens or immunomodulators). Other pathogenic cellular proteins comprise M protein family, streptokinase (fibrinolysin), deoxyribonuclease B (DNase B) or streptodornase, protein streptococcal inhibitor of complement (SIC), SpeB, F protein, and C5a peptidase (ScpA). Pyrogenic exotoxins (SpeA and SpeC) are responsible for the development of acquired hypersensitivity to streptococcal components and endotoxin shock. These superantigens are involved in nonspecific activation of T cells, and have been implicated in streptococcal toxic shock syndrome (STSS). Streptolysin S, a nonantigenic, oxygen-stable, cardiotoxic polypeptide, lyses different types of mammalian cells including RBCs. It is also associated with GAS leukotoxicity. Streptolysin O is inactive in the presence of oxygen. This cytolysin influences leukocytic functions, induces rapid antibody response, and also plays a vital role in the initiation of rheumatic fever. It is one of the key components of the bacterial β-hemolytic trait. Certain cellular components are also associated with infection and disease progression. Lipoteichoic acid (LTA), which is a major cell wall constituent of Gram-positive bacteria, is involved in adherence to epithelial cells. It is toxic to a wide range of host cells. M protein, one of the most important virulence factors for GAS incorporated in fimbriae, appears to exert an immunotoxic effect on human platelets and neutrophils. This component also prevents opsonization by the alternative complement pathway. It has been marked as the primary cause of antigenic shift and antigenic drift among GAS. More than 130 types of M protein have been recognized so far. F protein, a major adhesin of GAS, mediates adhesion to fibronectin on epithelial cells of skin and throat. ScpA is responsible for chemotactic signal destruction. SpeB, an exotoxic cysteine protease, cleaves host tissue matrix proteins (e.g., fibronectin), and is involved in modulation of fibronectin-dependent internalization of *S. pyogenes* by means of proteolysis. SIC protein interferes with complement function and inactivates antibacterial peptides. Streptokinases catalyze the transformation of plasminogen to plasmin, thereby dissolving fibrin and other proteins. DNases protect the pathogen from killing by neutrophil in extracellular traps. In GBS or *S. agalactiae*, hemolysin is a major virulence factor. Also, the polysaccharide capsule, surface-localized proteases ScpB and CspA, superoxide dismutase and LTA play crucial roles during different stages of GBS infection and in evasion of phagocytosis. The M-like protein SzP synthesized by *S. equi*, the clonal descendant of *S. zooepidemicus*, is implicated in virulence and antiphagocytosis. The sticky water-insoluble dextran molecules produced by viridans streptococci are potentially capable of adhering to heart valves by binding to the fibrin-platelet aggregate (thrombus) during an infective endocarditits. Such adherence by mutans streptococci in the mouth enhances corrosive acid production by plaque-forming bacteria leading to dental caries. HA (capsule) in many strains of streptococci is believed to evade adaptive immune response and phagocytic action of leukocytes by mimicking HA in the host connective tissue. Streptococcal Hyl that hydrolyzes the capsule is possibly implicated in the detachment of the organism from biofilms. This enzyme, as a spreading factor, is thought to play a crucial role in disseminating streptococcal virulence within the host environment.

Survival and Growth Characteristics in Food and Environment

Although the primary reservoir for *S. pyogenes* is humans, these bacteria may also be found in cattle. Humans can be infected through transmission from infected cattle, such as mastitic cows via raw or unpasteurized milk. This pathogen is capable of infecting cows to cause mastitis, and infected udder tissues can shed the bacterium into milk, where it is likely to survive for a long period. Foods that have been associated with outbreaks of disease caused by this microorganism include various dairy products, cooked sea food, and eggs. Some ready-to-eat (RTE) foods or food products, such as potato/egg/shrimp salads have been found as sources of *S. pyogenes*-associated outbreaks. These strains also survive in some vacuum-packaged RTE cheeses and meats, including beef bologna, turkey luncheon meat, and beef summer sausage, stored at low temperatures (5–8 °C) for several hours to few days. Contamination of these foods can result from handling or processing by infected individuals. These pathogens cause infections particularly when the food is left at room temperature for too long, allowing the bacteria to multiply to harmful levels. Moreover, pyogenic streptococci may multiply in raw meat at ambient temperatures. The potential duration of survival of GAS on a dry surface ranges from 3 days to 6.5 months. These microorganisms have been found to be viable approximately 18 days in ice cream, and 4 days in raw and unpasteurized milk at 15–37 °C. Also, they can survive 2–12 days in butter at room temperature. The bacteria can last for several days in cold salads at room temperature. Moisture content of certain food products, such as cottage cheese may reduce growth rate and survival period of *S. pyogenes*. This is probably due to quicker development of acidity in moisture food. Similarly, these microbes have been found dead within 5 h when inoculated in some sports drinks and fermented milk-containing beverages that had a considerably low pH (approximately 3). Therefore, acidic pH in food products drastically affects survival of these pathogens. GBS or *S. agalactiae* is found as a commensal organism in the lower gastrointestinal (GI) and vaginal tract. However, a high pH in these environments can stimulate GBS survival and biofilm production that may lead to sheer chance of a health hazard. Biofilm formation is thought to be strategy of these pathogens for prolonged survival within a given environment. Oral streptococci, particularly *S. mutans* are naturally present in the human oral microbiota. Hence, they acquire unique capacity to survive in the rapidly fluctuating harsh environment of the oral cavity. In addition, the bacteria endure exposure to a variety of antimicrobial drugs and toxic compounds during their growth. Mutans streptococci grow in biofilms on the surfaces of the teeth known as dental plaque (**Figures 3** and **4**). These microorganisms can also be competent in nutrition-limiting conditions. Quorum sensing, a microbial system of interaction with neighboring cells through small soluble signal peptides, has been revealed as a precursor for the development of competence in *S. mutans* and *S. pneumoniae*. Studies have suggested that some strains of *Streptococcus* in the environment can sometimes be inhibitory to other species of the same genus. This characteristic could be a means of survival of these bacteria under nutrient-limiting or other adverse conditions.

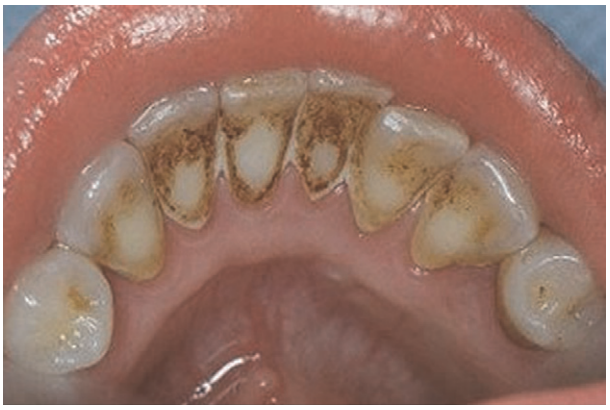

Figure 3 Greasy and sticky dental plaque on teeth surfaces.

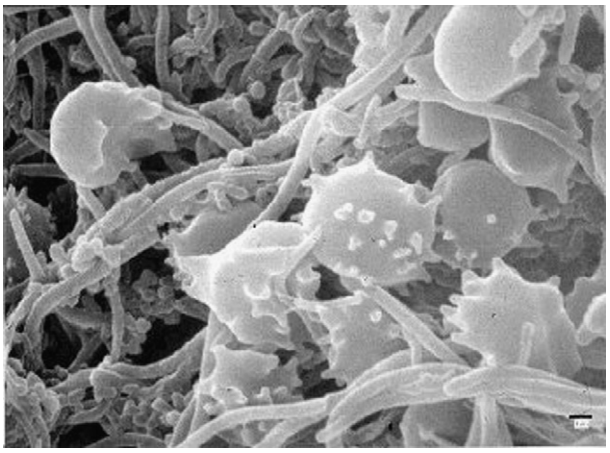

Figure 4 Scanning electron micrograph illustrating oral microbiota in dental plaque.

These organisms may persist outside the living host for months, especially in a damp environment. Streptococci do not grow at 10 °C, and the growth of majority of these strains does not usually sustain at high temperatures, such as ≥50 °C. Survival at 60 °C for 30 min with pH 9.6 differentiates group D enterococcal species from nonenterococcal strains.

Resistance to Different Factors and Survival in the GI Tract

Over the years, many streptococci have developed resistance to different host immune system as well as a variety of bactericidal compounds. Emergence of new resistant strains has received significant medical attention. Certain strains of GAS have shown resistance to a number of antibiotics, including macrolides, lincomycin, tetracyclines, chloramphenicol, and cotrimoxazole. The typically thick Gram-positive peptidoglycan layer and streptococcal encapsulation exert major influences on diverse bactericidal actions. Also, the inherent genetic plasticity of streptococci further helps them with adaptation to harsh environmental conditions. *Streptococcus mutans* cells cohabiting in dental biofilms appear resistant to various stresses induced by hosts, including low pH, high osmolarity, oxidation, exposure to organic acids, and antibiotics. The fact that certain M proteins of GAS crossreact antigenically with heart has withheld the use of GAS vaccines. More importantly, antigenic variation in these proteins between strains makes it difficult for the host to choose a common immune response to *S. pyogenes*. Hydrogen peroxide (H_2O_2) production is a phenotype of many members of the genus *Streptococcus* playing a definite role in virulence as well as antagonism within biofilm matrix. This compound has been postulated to cause direct damage to host tissues infected with *S. pyogenes*. Yet, H_2O_2-nonproducing strains of *S. pyogenes* may become resistant to intracellular killing by granulocytes. Like other commensal microflora, GBS and some strains of group C as well as viridans group have natural capacity to persist and colonize in the GI system of humans. Synthesis of certain surface proteins in *S. agalactiae* facilitates the bacteria with survival in the GI tract by interaction with proteins of the extracellular matrix. The Lmb protein is associated with binding of these organisms to human laminin. Furthermore, superoxide dismutase secreted by these bacteria has been found to reduce their susceptibility to killing by macrophages. Likewise, the relationship of protein G, a surface protein produced by GCS and GGS, with the bacterial survival in the GI tract is likely. It is thought that protein G may act as an environmental sensor for a well-adapted gene expression.

Clinical Manifestations

Among the BHS, mainly groups A and B are considered highly pathogenic to humans. Infections that do not involve food as a vehicle of transmission are not addressed in this article. The most common species of the genus potentially related to foodborne outbreaks, regardless of frequencies, are *S. pyogenes* and *S. equi* subsp. *zooepidemicus*. Of these, *S. pyogenes* primarily involves infections in the upper respiratory tract. Although some nonenterococcal group D strains, such as *S. bovis* are found in the normal flora of human GI tract, their transmission due to ingestion of food materials has not been clearly reported. Viridans streptococci, including *S. mutans* constitute part of the microflora residing in the mouth, GI tract, and vagina of healthy humans. Many of these strains can be sometimes virulent and become opportunistic etiological agents of diseases, particularly in immunodeficient or immunocompromised conditions. Consumption of food products or drinks containing saliva from carriers could literally be incriminated in some serious viridans-associated morbidity. None of the streptococcal diseases appears to occur from water sources.

Streptococcus pyogenes or GAS accounts for diverse suppurative infections after entering vulnerable human tissues. It is considered the most pathogenic bacterium in the genus *Streptococcus*. GAS is the causative agent of different acute infections, such as pharyngitis, scarlet fever, pyoderma (erysipelas and impetigo), otitis media (middle ear infection), meningitis, and sinusitis. Postinfectious complications include rheumatic fever and acute glomerulonephritis (AGN). This bacterium also functions as a host of some life-threatening invasive diseases, like necrotizing fasciitis (wound infection), myositis, STSS, puerperal fever (sepsis), and bacteremia. Some GAS infections are asymptomatic. Most symptomatic infections manifest as pharyngitis, which can occur in approximately 1–3 days after ingestion of contaminated food.

Acute Pharyngitis

Pharyngitis, also referred to as strep throat, streptococcal pharyngitis, or tonsillitis, is the most widespread *S. pyogenes*-associated disease. The disease is suppurative or purulent, but noninvasive. Infection typically involves the pharynx, including tonsils.

Signs and Symptoms

Symptoms of illness are generally mild, except otherwise complicated. The disease clinically presents with sore throat (with or without white patches/exudates), red throat (erythema), high fever, chills, runny nose (rhinorrhea), difficulty swallowing (dysphagia), painful swallowing (odynophagia), abdominal pain, nausea, vomiting, malaise or general discomfort, and headache. Inflamed tonsils followed by swelling may also occur in patients. Swollen glands (neck), a sign of tender cervical lymphadenopathy, are normally associated with prolonged pharyngitis. Coryza and cough have been found to be less implicated in foodborne streptococcal pharyngitis than the endemic airborne pharyngitis. However, submandibular nodal enlargement, a swelling of lymph nodes beneath the mandible or lower jaw, and tonsillar swelling are more likely for the former category. Clinical manifestation of foodborne pharyngitis could be relatively severe and confined to the pharynx compared to the other (Figure 5).

The symptoms usually begin to resolve in 3–5 days. However, if they sustain, scarlet fever, an acute systemic disease demonstrating the involvement of multiple sites, may develop with a deep erythematous rash on the skin and strawberry tongue. The rash is due to the synthesis of erythrogenic or pyrogenic toxins by the bacteria. It resembles typical sun burn and blanches with pressure. Initially, the rash is visible as itchy, tiny red bumps on the face, neck, and trunk. It further spreads to the chest, abdomen, and back, then all over the body with the consistency of sandpaper. The rash may look redder in the body creases and groin areas. These manifestations are usually accompanied by few or many classical symptoms of pharyngitis. In addition, the tongue may become pale and swollen, while coated with inflamed red papillae giving it an appearance like a strawberry (strawberry tongue). The fever is normally above 101 °F, and starts ceasing within 3–5 days after its onset. The illness may resolve in about a week with the help of appropriate treatment. Signs of rash will, however, take about another week to disappear. Scarlet fever has been documented quite rarely since 1940s.

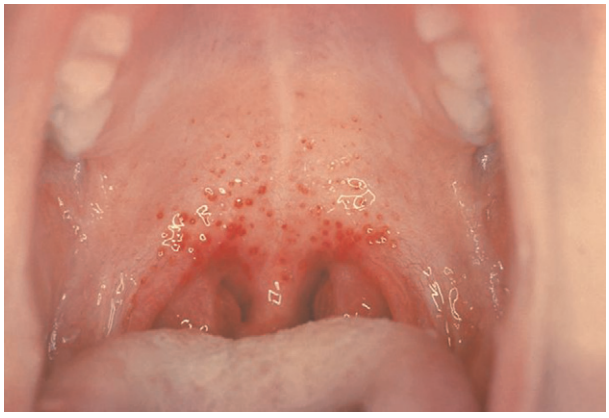

Figure 5 Acute streptococcal pharyngitis presenting with red pharynx and swollen tonsils.

Carrier Duration

Untreated pharyngitis patients usually remain infective for 7–10 days during the acute phase of illnesses. The organism can be infectious for a week thereafter unless antibiotics are used. *Streptococcus pyogenes* is capable of remaining in the body of the carrier in its asymptomatic but transmissible state for weeks or months.

Dose of Infection

Infectious dose of GAS is still unknown, but appears relatively low. Fewer than 1000 organisms are considered capable of causing a successful procedure of infection.

Sequelae/Chronic Effects and Complications

Suppurative or purulent sequelae may involve peritonsillar and paranasal abscesses. Pneumonia occurs as an occasional complication, particularly in patients with the history of preceding or concomitant viral infections. Otitis media and sinusitis are secondary to direct extension from a GAS infection in the upper respiratory tract. Acute meningitis may occasionally occur due to antecedent pharyngeal infection, particularly in children within a few hours to a few days. Bacteremia, an invasion of the bloodstream by pyogenic streptococci, may follow an upper respiratory tract infection (URTI) sporadically, but more commonly result from a pyodermal (skin) infection. Rare complications include endocarditis, a disease affecting the lining of heart chambers and the heart valves, and hematogenous spread brain abscess. The occurrence of GAS is relatively established in the skin and soft tissues. Septic arthritis, a poststreptococcal reactive arthritis, may result from bacteremic spread of *S. pyogenes* while increasing the risk of rheumatic heart disease (RHD). The presenting clinical features of such complications are generally those of the corresponding diseases.

Acute rheumatic fever (ARF) is the most frequent nonsuppurative follow-on of an uncontrolled GAS infection with potential for more serious physical conditions. It is a delayed sequel to GAS pharyngitis affecting the collagen. ARF is widely hypothesized as an aberrant immune response (autoimmune disease) because it triggers binding of the crossreactive antistreptococcal antibodies to host tissue antigens. The bacterial M protein and host human leukocyte antigen molecules play a contributing role in the autoimmune phenomena. The onset of ARF usually appears between the first and the fifth week (latent period) after an infection has been caused by *S. pyogenes*. The disease is most often characterized by the sudden occurrence of fever accompanied by an acute inflammatory course, which involves the connective tissue, blood vessels, and joints, primarily those of lower limbs. ARF usually manifests with sore throat, progressive inflammatory reaction of the joints (migratory polyarthritis), and occasional redness with defined margin on the skin (erythema marginatum). Common complaints accompanying this condition are muscle pain, swelling of joints, malaise, and unsteady gait (difficulty walking). Subcutaneous nodules may also develop. Swelling may migrate

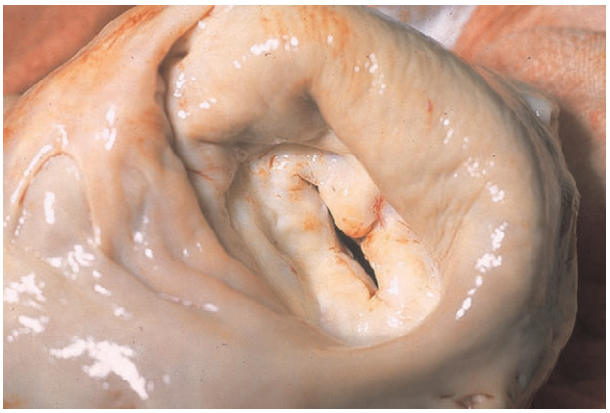

Figure 6 Narrowing (stenosis) of the mitral valve orifice as a result of acute rheumatic fever.

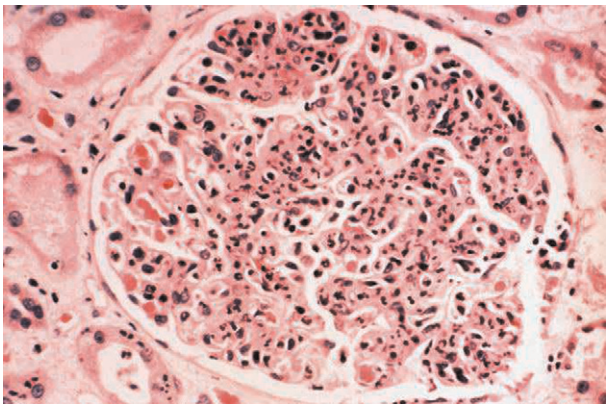

Figure 7 Photomicrograph of a glomerulus presenting APSGN with necrosis and accumulation of neutrophils.

from one joint to another. A central nervous system manifestation diagnosed by emotional instability and involuntary spastic movements (chorea) may also arise occasionally. Nervous involvement may result in fatigue, abdominal pain, vomiting, nose bleed, restlessness, difficulty breathing, tremors, and difficulty speaking. An untreated course of ARF may account for RHD referring to the inflammation of the heart (carditis) resulting in chronic valvular damage, predominantly incriminating the mitral valve (mitral stenosis). This condition may even lead to cardiac failure (Figure 6).

Another clinically significant complication of streptococcal pharyngitis is known as AGN, often referred to as acute poststreptococcal glomerulonephritis (APSGN). It is also a disease of the host immune complex as the crossreactive antibodies specific to streptococcal epitopes bind to antigens of the glomerular basement membrane. The onset of the disease usually takes place more than 1 week (approximately 10–14 days) after the streptococcal infection. AGN is marked by active inflammation and proliferation of the renal (kidney) glomeruli potentially causing damage to the basement membrane, mesangium, or capillary endothelium. The disease is clinically defined as sudden onset of smoky, dark-brown colored urine with RBCs (hematuria) and an excess of serum proteins (proteinuria), RBC casts, and depressed serum complement. A set of other manifestations include hypertension, tissue swelling (edema), and decreased glomerular filtration rate or azotemia. The scenario is often accompanied by renal salt and water retention. Patients, especially children, are also likely to suffer concurrent sore throat, diminished urine output, abdominal pain, fatigue, lethargy (drowsiness), headache, hyperventilation (increased breathing), and weight loss. Unresolved APSGN may address long-term kidney failure (Figure 7).

Risks of Misdiagnosis

ARF could be often confused with the most common rheumatic disease, rheumatoid arthritis. Also, many ARF cases go unnoticed or overlooked during the early stage of the onset of illnesses, and the patients may receive only empirical or supportive treatment, which may even worsen the prognosis of the actual disease. Similarly, if the etiology of AGN remains undefined, there are possibilities that treatment regime will not target corrective control of the underlying streptococcal infection first. Interstitial cystitis and overactive bladder syndrome are some of the undiagnosed conditions associated with AGN. Common exclusion of some potentially major causes of glomerulonephritis-related risks includes scarlet fever, bacterial endocarditits, and infection with *S. equi* subsp. *zooepidemicus*. Some of the severe gastric conditions may also be overlooked during diagnosis, such as irritable bowel disease, celiac disease, and diabetic diarrhea.

Infection with Group C Streptococci

Among the species belonging to this group, only *S. equi* subsp. *zooepidemicus* is known to cause human opportunistic infections from food sources. It is most often associated with acute group C streptococcal pharyngitis, which resembles manifestations of *S. pyogenes*-triggered pharyngitis. Major clinical presentations observed are sore throat, pharyngeal exudates, cervical lymphadenopathy, moderate fever, chills, and malaise. A number of superficial and deep clinical infections only occasionally can be attributed to this pathogen. These include endocarditis, pericarditis, cellulitis (skin infections), pneumonia, meningitis, septic arthritis, bacteremia, and septicemia. Abdominal pain may also be present in some patients. The disease is infrequently followed by a nephritic sequela (AGN), particularly if untreated. Nevertheless, GCS-induced nephritis is relatively severe.

Infection with Group G Streptococci

These bacteria are capable of colonizing the mouth, throat, intestines, genital tract, and skin of humans. *S. canis*, an example of GGS although originating from animals, can cause sporadic infections in humans through contamination of food. Infections with these organisms may also clinically present as sore throat, pharyngitis, cellulitis and sepsis. Symptoms of pharyngitis range from mild URTI with coryza to exudative pharyngitis. A great deal of manifestations normally resembles GAS infection. This condition is often accompanied by fever and lymphadenopathy. GGS may trigger a wide range of complicated infections that include bacteremia; inflammation of the connective tissue surrounding a joint (bursitis), bone and bone marrow (osteomyelitis), and lining of the abdomen (peritonitis); meningitis;

septic arthritis; and endocarditis. ARF and APSGN have not been found as implicated in chronic phases of group G infection.

It is important to note that, due to lack of a precise description of clinical manifestations and confusing biochemical identification methods relating to groups C and G streptococci, infections are most often misdiagnosed as GAS derived. Thus, it turns out to be difficult to calculate true incidences and control such infections.

Oral Streptococcal Infections

Numerous viridans streptococcal species, including *S. mutans* normally colonize in the mouth of humans while existing in harmony with other oral commensals. Certain strains of viridans category, such as *Streptococcus anginosus* or anginosus group can cause purulent infections, including brain abscess and suppurative intraabdominal abscess. Septic shock (also called α-strep shock syndrome), neonatal sepsis, meningitis, and osteomyelitis can also be triggered by these pathogens. Oral streptococci have always been considered to be of low virulence. However, some of these strains can sometimes be opportunistic, and become etiological agents of life-threatening diseases, like infective valve endocarditis by entering into the bloodstream following tooth extraction or other traumatic events of the mouth.

Disease of the Oral Health

Streptococcus mutans or other cariogenic streptococci, such as *Streptococcus sanguinis* could be potentially the causative agent of an oral infection if present in salivary substances from an infected individual mixed with food or drinks. High salivary *S. mutans* count (>105 colony forming units) is likely to pass the infectious bacteria to an unaffected person, especially children. Dental caries or tooth decay is strongly associated with *S. mutans*, which is normally present in dental plaque of humans. It is defined as demineralization and destruction of hard tissue of the teeth, enamel, dentin, and cementum. Occurrence of a dental caries largely depends on the time of the tooth surface exposure to acidic by-products fermented by the bacteria. A lesion presenting as a chalky white spot on the tooth, also known as microcavity, is the early sign of demineralization of the tooth enamel. A dull-brown lesion represents an active caries, which eventually leads to cavities or holes on the teeth and swelling on the gums. Common clinical symptoms of the disease are pain and discomfort when chewing food, difficulty in facial movement, sensitivity of tooth, jaw pain, discoloration on tooth surface, inflammation on the face, and mild fever. Dental caries can also manifest with bad breath and foul tastes. Such pyogenic oral infectious processes can be acute or chronic. During an acute carious state, the dental caries spreads laterally causing a rapid early deterioration of the pulp tissues. The pain is intense, and the dentin is light yellowish in color. There is only limited lateral spread in case of a chronic caries, and the involvement of the tooth pulp is a much slow process. Therefore, the cavity is shallow with no pulp tissue implication. Oral pain is generally not involved in this type of caries (Figure 8). Other plaque-derived forms of infection include inflammation of the gum tissue (gingivitis), gingival recession (retraction of gum tissue), bleeding gums, and more severe periodontal diseases as a result of loss of gum tissue and underlying bone.

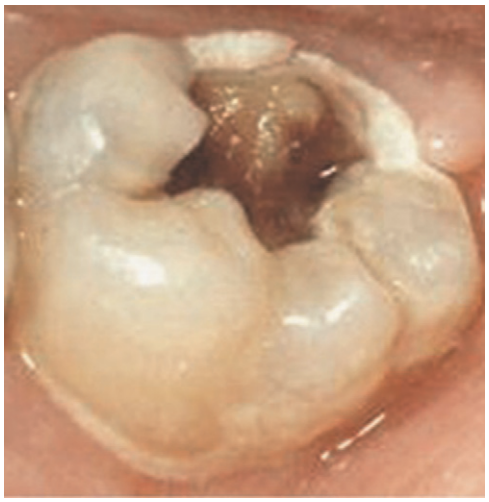

Figure 8 Acute dental caries illustrating destruction of tooth tissue while forming deep cavity on the tooth.

Prolonged untreated dental caries may confer a risk of bacteremia, which in turn triggers the likelihood of an opportunistic heart valve endocarditis. Also, highly virulent *S. mutans* strains have been proposed to be implicated in the aggravation of ulcerative colitis. Such possibilities should not be ruled out without an accurate pathological explanation.

Infective Endocarditis (IE)

The disease IE, also referred to as native valve endocarditis (NVE), occurs when VGS begin to colonize the heart valves after adherence to the valve surface, where they develop a vegetation (abnormal outgrowth). VGS-related NVE is often subacute, i.e., subacute bacterial endocarditis (SBE). IE of this nature typically affects only abnormal valves. The most common clinical presentations of SBE are low-grade fever and chills. Other complaints include malaise, weight loss, anorexia, headache, myalgia, night sweats, shortness of breath, cough, abdominal syndromes, and joint pains, especially back pain. Patients with VGS-endocardial infections may develop suppurative extracardiac complications, such as splenic abscess and septic arthritis. Severe valvular insufficiency during the course of the disease may lead to congestive heart failure. Delayed therapy accounts for additional systemic manifestations that incorporate local tissue destruction and embolic phenomena, as sterile or infected emboli, a detached intravascular mass capable of blocking the blood flow in a blood vessel. Embolic stroke may also result in neurological abnormalities. Symptoms secondary to embolism comprise acute meningitis, painless hematuria, infarction of the kidney or spleen, unilateral blindness, and myocardial infarction. Besides, IE can become a pathological basis of various immunological syndromes (secondary autoimmune effects), such as glomerulonephritis and vasculitis.

The risk of misdiagnosis of SBE is quite high. A major concern is the residual valvular damage due to a previous endocardial attack. Many of its manifestations could be muted due to indiscriminate use of antibiotics or predisposing conditions in vulnerable individuals, such as elderly or immunocompromised people.

Epidemiology

Geographical Distribution, Prevalence, and Incidence

Foodborne pharyngitis has become a mounting concern due to recent reports of severe outbreaks in different parts of the world. GAS or *S. pyogenes* is the predominant etiological agent of bacterial pharyngitis because of its highly contagious potential. Also, it is the only causative agent of the disease for which antibiotic therapy is plainly indicated. Other groups, including C, G, and F, constitute approximately 10% of all cases of this disease, but related symptoms cannot be differentiated from GAS pharyngitis. *Streptococcus pyogenes* is globally distributed, and is a frequent colonizer of the oropharynx of healthy humans, especially children. Asymptomatic colonization is less common in adults. Certain strains of this group demonstrate a predilection for the respiratory tract, but it has not been confirmed whether or not ingestion of food mostly is the portal of entry for those pathotypes. Human carriage rates have been estimated to be approximately 5–15%. *Streptococcus pyogenes* underlies acute pharyngitis in a range of 15–30% of pediatric patients. According to a recent meta-analysis, the degree of GAS positivity in throat culture has been found to be 37% in school-aged children presenting with sore throat. The prevalence of GAS in a nonspecific adult population has been assessed as 5–10%. Incidences of GAS-associated URTIs peak at approximately 5–15 years of age. Clinical presentations of these infections are variable according to different geographical settings. The disease normally appears during late winter and early spring in regions of temperate climates. Although GAS infections were the cause of serious morbidity and mortality until the middle of the twentieth century, the number of incidences considerably declined afterwards. However, recent striking resurgence of such infections and accompanying consequences has vigorously changed public attitude to the pathogenic potential of GAS. Evolution of new virulent strains of streptococci is thought be at least partly responsible for this scenario. During 1985–2002, World Health Organization (WHO) estimated over 600 million cases of symptomatic GAS pharyngitis globally each year. Rates of severe GAS infection have thrived to 3 per 100 000 population in the northern European countries. In India, there could be as many as 20–30 million cases of streptococcal pharyngitis every year. GAS foodborne pharyngitis has increasingly occurred over the past few decades. There are occasional but significant reports of foodborne GAS outbreaks in various countries. The Centers for Disease Control and Prevention have recently mentioned over 11 000 cases of foodborne streptococcal illness in the USA each year. According to recent global observations, the prevalence of acute late sequelae of *S. pyogenes* infections, i.e., rheumatic fever and AGN has shifted from temperate to tropical climates. Increased individual cases of ARF had been reported in several parts of the USA since the early 1980s, but incidences steadily declined in the 1990s with the use of penicillin as a prophylaxis. However, the episode of GAS pharyngitis has sustained in that country. It has been estimated that the incidence of ARF is 3% following epidemic pharyngitis, whereas 1 per 1000 population following sporadic pharyngitis. Acute cases of GAS pharyngitis activate RHD in 6 million individuals yearly. The prevalence of RHD has been calculated to be higher in sub-Saharan Africa (5.7/1000), in aboriginal populations of Australia and New Zealand (3.5/1000), and in southcentral Asia (2.2/1000) than in developed countries (0.5/1000). AGN has, however, appeared to be triggered by only certain nephritogenic serotypes of *S. pyogenes*. The apparent clinical attack rate for epidemic infections with nephritogenic streptococci is approximately 10%. Incidences of AGN may vary between less than 1% and 10–15%. The median annual poststreptococcal AGN incidence in children has been estimated to be 24.3 per 100 000 in less-developed countries and 2 per 100 000 in more developed countries, thereby calculating an estimate of 470 000 global cases of APSGN occurrence annually, with 97% in less-developed countries. The incidence of IE has been estimated at 2–6 cases per 100 000 general population every year. Viridans and other streptococci account for at least 45% of all cases of NVE worldwide. Viridans streptococci are the most frequent cause of SBE.

Mortality

Infections, including URTI caused by *S. pyogenes* claim at least 517 000 deaths around the world annually, of which rheumatic fever-associated issues alone cause 233 000 deaths. The death rate among those who develop invasive diseases is approximately 13%. The case fatality rate due to APSGN is relatively high in developing countries, with India and Turkey reporting approximately 2% and 0.08%, respectively.

Transmission

Streptococcus pyogenes is exclusively a human pathogen. Transmission of these bacteria from animals to humans is very rare, but human strains are more likely to infect animals (reverse zoonosis) instead. The main routes of GAS entry are oral, respiratory, and percutaneous. Food handlers, who are symptomatic or asymptomatic carriers of this bacterium, are a major source of foodborne transmission. Ingestion of unpasteurized milk, a variety of milk products, RTE foods, such as cold salads and meats contaminated with this organism, has emerged as one of the leading portals of entry of GAS. Sharing of foods, drinks, or related utensils (glasses, cups, etc.) may spread the pathogenic bacteria among individuals. *S. equi* subsp. *zooepidemicus* is a commensal microorganism of horses, but can be transmitted to humans through ingestion, aerosols, or the skin. Consumption of unpasteurized milk (often from mastitic cows) or dairy products is strongly linked to human infections with this equine pathogen. Drinking of unpasteurized milk from cows with mastitis has been associated with severe *S. equi* infections in humans. Although extremely rare, feeding of breast milk to infants may be a portal of entry for *S. agalactiae*. Human saliva, respiratory droplets, or oral blood is now addressed as a major source of foodborne infections with VGS. Prechewing or premastication of food items or herbs can be adversely implicated in the transfer of potentially virulent streptococcal strains, including *S. pyogenes* and *S. mutans*, to infants or older children. The practice of oral prewarming or precooling of food by mothers or caregivers could also be dangerous to pediatric health. Additionally, horizontal

acquisition of these bacteria can take place via food or drink containers, baby feeding nipples, and other feeding essentials.

Foodborne Outbreaks

In 2003, an outbreak of sore throat was reported in Sätila, Sweden involving 153 people, who ate sandwich-layer cakes. *Streptococcus pyogenes* isolated from patients under medical care was found to have spread from the wounds on a caterer's fingers. Two years later a case–control study assessed foodborne epidemics of tonsillopharyngitis among residents of female dormitories in Iran. When the study was conducted in a dormitory, 11 ($n=17$) throat swabs from students as well as 2 throat samples from the asymptomatic cooks tested positive for GAS. Deoxyribonucleic acid (DNA) fingerprinting revealed that the prevalence of GAS in those students was due to transmission of the identical strain from a cook. A similar occurrence was detected among inmates of a rural correctional center in New South Wales, Australia in December 1999. Curried egg rolls contaminated with GAS by food handlers were suggested to be implicated in the outbreak. Moreover, seven foodborne outbreaks caused by GAS pharyngitis have been reported in Japan since the late 1990s. An outbreak of scarlet fever due to chicken meat has occurred in China. Incidences of infections with groups C and G because of ingestion of different food products, especially unpasteurized milk or milk products have also been recorded sporadically. Consumption of fresh goat cheese contaminated with *S. equi* subsp. *zooepidemicus* has been associated with a recent outbreak in Finland. In Hong Kong, several cases have been related to group C infection resulting from eating raw or cooked pork. One large epidemic of severe acute nephritis in Brazil was linked to consumption of unpasteurized cheese, which had been contaminated with *S. equi* subsp. *zooepidemicus*. Three casualties out of 133 confirmed cases died, whereas 7 required dialysis and 96 were hospitalized. In two unrelated episodes of pharyngitis, an egg salad and a chicken salad separately have been incriminated as the sources of infection with GGS. These events resembled group A pharyngitis, except the typical sequelae.

Factors of Vulnerability

People of all ages are prone to infections with BHS in general. No age or race susceptibilities have been observed. However, school-aged children are a priority risk group. The frequency of pharyngitis in adults may be influenced by season, epidemiological background, domestic exposure to vulnerable children, and age. Elderly or immune-arrested individuals are highly susceptible to these infections. Predisposing conditions, such as skin lesions, burns, and wounds play an important role in the spread of the disease. Adults are, however, at least risk of a first attack of ARF, even if they have an undiagnosed or untreated episode of streptococcal pharyngitis. Other possible factors may comprise climate, indoor crowding, low-economic status, poor hygiene, and general attitude to prophylaxis. ARF has been determined as a particular problem among low-income communities with poor living conditions. RHD usually impacts children. Similarly, AGN is more common in children than adults. Males are twice as much susceptible to APSGN as females. Diabetes mellitus and aging are prime risk factors for adult APSGN. NVE is most likely to attack individuals with damaged heart valves or the history of endocarditis. Localized or systemic injury may also reduce host resistance. Prolonged use of high sugar-containing milk, juice, or liquid, or intake of high caries-risk diet may contribute to the occurrence of dental caries. Clinical oral health practices, including dental prophylaxis, tooth extraction, and gingival surgery, are considered a significant risk factor for NVE. Intravenous drug abuse greatly increases the option for an endocardiac attack.

Analytical Methods

First-step diagnosis of infections with streptococci depends largely on the characteristic clinical phenomena presented by the infected individual. A rapid antigen detection test (RADT) can be done to determine streptococcal pharyngitis. Laboratory detection methods initially involve isolation of *Streptococcus* strains (culture) from specimens (i.e., throat swab, blood, sputum, and food, as appropriate), and classification based on the hemolytic property. Gram staining is still considered a landmark tool for the definitive identification of the type of bacteria involved. Serological detection is performed by the precipitin test or enzyme-linked immunosorbent assay to confirm precise Lancefield group antigen. Biochemical tests are helpful in presumptive screening for phenotypic properties of the bacteria. Group A can be differentiated from other BHS by bacitracin sensitivity and PYRase production (note: other than group A, all enterococci synthesize this enzyme). Hippurate hydrolysis and Christie–Atkins–Munch-Petersen (CAMP) reaction separate group B from A. **Table 1** represents typical biochemical characteristics of different groups of streptococci. Identification of rheumatic fever and glomerulonephritis is performed by antistreptococcal antibody titers. Antistreptolysin O and anti-DNase B titers are important markers for the recognition of these late sequelae. However, age and geographic locale of the patient should also be taken into consideration while interpreting these values. Analysis of 16S rRNA patterns and polymerase chain reaction-based sequence amplification methods appear to be quite supportive in molecular diagnostics of streptococcal diseases. Rigorous investigations should be carried out to assess a possible foodborne outbreak of streptococcal pharyngitis. Certain DNA fingerprinting techniques, such as pulse-field gel electrophoresis can be a useful tool to determine primary sources of infection. Phage typing methods provide further insight into the status of specific streptococcal strains during epidemiological studies.

Control/Preventive Measures

Incidences of streptococcal pharyngitis can be reduced by avoiding common exposure to potential hazards. Improvement of personal hygiene and living standards may also slash the frequency of occurrences. Food-processing industries and food service providers must ensure aseptic packaging/

Table 1 Biochemical features of different groups of *Streptococcus*

Group	*Hemolysis*	*PYRase*	*Bacitracin*	*Hippurate*	*CAMP*	*Bile*	*Optochin*	*NaCl*
A	β	Positive	Sensitive	Negative	Negative	No growth	Resistant	Intolerant
B	β, α-prime	Negative	Resistant	Positive	Positive	No growth	Resistant	Variably intolerant
C	β, γ	Negative	Resistant	Negative	Negative	No growth	Resistant	Intolerant
D	α, γ	Negative	Resistant	Negative	Negative	Growth	Resistant	Intolerant
G	β	Negative	Resistant	Negative	Negative	No growth	Resistant	Intolerant
V	α, γ	Negative	Resistant	Negative	Negative	No growth (except *S. mutans*)	Resistant	Variably intolerant
P	α	Negative	Resistant	Negative	Negative	No growth	Sensitive	Intolerant

Abbreviations: CAMP, Christie–Atkins–Munch-Petersen; NaCl, sodium chloride; P, pneumococci; PYRase, pyrrolidonyl arylamidase; V, viridans streptococci.

handling of all food products. Regular cleaning of food contact surfaces is also necessary in mass catering facilities. Infected food handlers should be identified and restricted from access to all aspects of consumer service. Boiling or pasteurization of milk before consumption or commercial distribution is highly recommended. A negative response to all categories of uncertified RTE food products outside home may aid in significant prevention of many streptococcal outbreaks. Prechewing or prewarming/cooling of food or drinks fed to children needs to be discouraged. Mothers should relinquish kissing babies on their lips because of the extreme possibility of salivary transfer of streptococci. Acquisition of streptococcal infections in all age groups can be at least partly prevented by ceasing to share foods, such as ice cream and bottled beverages. Reduced tendency to carbohydrate-rich diet and adequate attention to oral heath may effectively decline complaints of dental caries.

Easily approachable laboratory facility will encourage people about diagnosis of GAS infections. Strict action plans for infection control and surveillance of compliance can prevent possible spread of *S. pyogenes* or other strains of streptococci within the healthcare facility. Public alertness through health education and pragmatic training to health workers as well as caregivers will support the alleviation of streptococcal disease burden. Early diagnosis, detection, and treatment of underlying conditions in patients, particularly vulnerable individuals, are likely to help minimize post-streptococcal complications and mortality.

Antimicrobial therapy has been found to be quite impressive in controlling group A infections. Penicillin, as a gold standard therapy, shows great efficacy in preventing rheumatic fever. According to the guideline by the Infectious Diseases Society of America, penicillin or amoxicillin is recommended as the preferred treatment for GAS pharyngitis. Clindamycin is suggested to those hypersensitive to penicillin. Supportive treatment of clinical symptoms benefits management of residual damages triggered by streptococcal infections. Traumatic dental procedures should be accompanied by appropriate prophylaxis. Tonsillectomy does not appear to be efficient in limiting the spread of GAS.

Development of a GAS vaccine has always been frustrating to researchers because of the widespread diversity of M protein group A serotypes. However, efforts are being made to find a suitable vaccine candidate. Several modified prototypes of GAS vaccine have recently undergone some clinical trials. WHO is developing standard protocols for monitoring the efficacy of these vaccines.

Research Avenues

Association of diverse GAS seropathotypes with multilatitudinal occurrences clearly suggests that a major antigenic shift is directly responsible for the emergence of new virulence strains. Therefore, it is important to know the underlying genetic configuration associated with such molecular diversity. Furthermore, determination of bona fide incidences of streptococcal pharyngitis due to ingestion of contaminated food is a prerequisite for the epidemiological assessment of the specific disease burden and relative nature of *Streptococcus* virulence.

See also: Bacteria: *Staphylococcus aureus*. Disciplines Associated with Food Safety: Epidemiology. Food Technologies: Pasteurization. Foodborne Diseases: Foodborne Diseases and Vulnerable Groups. Public Health Measures: Health Education, Information, and Risk Communication; Management of Food Safety in Food Service Sector. Safety of Food and Beverages: Milk and Dairy Products

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BACTERIA

Vibrio cholerae

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Glossary

Case fatality rate (CFR) CFR (case fatality rate or case fatality ratio) is the proportion of fatalities within a selected population over the course of the disease. CFR is typically stated as a percentage and signifies a measure of risk.

Cholera toxin (CT) The cholera toxin (CT) secreted by *Vibrio cholerae* is an oligomeric complex composed of six protein subunits: a single enzymatic A subunit and five receptor binding B subunits.

GM-1 ganglioside GM1 is a prototype ganglioside (monosialotetrahexosylganglioside) and its oligosaccharide groups extend beyond the surfaces of the intestinal cell membrane. These carbohydrates act as specific receptors for cholera toxin secreted by *V. cholerae*.

Horizontal gene transfer Transfer of genes (also known as lateral gene transfer) between bacterial cells through transformation, conjugation, transduction etc., which can be detected by analysing G + C contents, codon usage, amino acid usage, and gene position.

Lateral flow system A type of capillary based sandwich immunochromatographic assay with built-in controls for the rapid detection of a pathogen or antibodies from liquid clinical specimens used for medical diagnostics.

Ligated rabbit ileal loop An *in vivo* technique used in the confirmation of enterotoxins produced by diarrheagenic bacteria. The sterile culture supernatant from the test organisms is inoculated into a ligated segment of ileum may induce the onset of intestinal fluid accumulation as early as 4–18 h due to elevated cyclic adenosine monophosphate.

Quorum sensing A system of stimulus and response that can be directly correlated to the population density of *V. cholerae* or any other bacteria. *V. cholerae* generally use quorum sensing to coordinate gene expression according to the cell density either in the gut or in the aquatic environment.

Rugose *V. cholerae* The rugose variant forms of *V. cholerae* have corrugated colonies, well-developed biofilms and exhibit increased levels of resistance to osmotic and oxidative stresses. *V. cholerae* can undergo phenotypic variation in response to environmental stresses, resulting in rugose and smooth colonial variants phase.

Toxigenic *V. cholerae* *V. cholerae* strains that produce cholerae toxin and the infection caused by these strains are characterized by a severe watery diarrhea due to the effect of this toxin. Generally, *V. cholerae* O1, O139 and some of the non-O1 and non-O139 strains belongs to this group.

Introduction

Toxigenic *Vibrio cholerae* that produces cholera toxin is responsible for the deadly disease 'cholera,' which is coded as 001 in the International Classification of the Diseases. *Vibrio cholerae* is well-known as a waterborne pathogen and is also increasingly recognized as the cause of foodborne infections. Its close association with marine fauna and flora leads to transmission of diarrhea through seafood. Like other foodborne pathogens, transmission of *V. cholerae* occurs through secondary contamination coupled with its long-term survival in foods. In the US, the estimated illness caused by toxigenic *V. cholerae* is approximately 54 per year and the foodborne transmission is 90%. The ecological fitness and change in the genomic constitution by horizontal gene transfer makes the organism robust to meet the challenges posed by the environment as well the host's defense mechanisms. Successive appearance of classical and El Tor biotypes, emergence of a novel serogroup O139, and acquisition of some of the classical biotype features by the recent El Tor strains are the important events in the changing epidemiology of cholera. Improper control measures and deteriorating status of drinking water, sanitation, and hygiene in many developing countries will perpetuate the disease cholera.

This article will focus on the clinical microbiology, epidemiology, pathogenesis, and molecular aspects of *V. cholerae*.

Historical Outline

Toxigenic *V. cholerae* has been associated with cholera, a devastating diarrheal disease which occurs in the form of epidemics and pandemics. There is no clear indication when and where the disease cholera originated. Based on the disease symptoms it appears that cholera was rampant in many parts of the world around the sixteenth century. The period from 1817–1961 was described as the history of the seven pandemics (Table 1) and each pandemic affected almost all the countries in the world. The duration of each pandemic was

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Table 1 Details of seven cholera pandemics

Pandemic	*Period*	*Number of years prevailed*	*Approximate number of affected countries*
1	1816–26	11	21
2	1829–51	23	36
3	1852–60	9	37
4	1863–75	13	46
5	1881–96	16	24
6	1899–23	25	24
7	1961–till date	>49	Ongoing

not uniform, as the pandemicity was based on the intensity rather than the extent of time. The classical biotype was involved in sixth and seventh pandemics. Although strains of the El Tor biotype caused sporadic infections and cholera epidemics as early as 1910, it was not until 1961 that this biotype was recognized as the causative agent of seventh cholera pandemic. Though *V. cholerae* was first visualized by Pacini in 1854 and then by Koch in 1884, the virulence properties of this pathogen remained unknown for many years. In 1959, S.N. De first showed that the enterotoxin produced by *V. cholerae* evokes fluid accumulation when cell-free filtrates are tested in ligated rabbit ileal loops. Later this toxin was purified to homogeneity and named as choler toxin (CT).

A novel *V. cholerae* serogroup O139, synonym Bengal, was identified in 1992 from the Indian subcontinent which spread to other Asian countries in the following years. Emergence of O139 serogroup was considered as the beginning of eighth pandemic, but due to its slow disappearance from late-1990s in many countries, this view was not considered. Extension of El Tor biotype from the Indian subcontinent and Haiti to the continents of Africa and subsequently South America makes the seventh pandemic both temporally and spatially the longest and most widely spread pandemic of cholera.

Taxonomy and Characteristics of *V. cholerae*

Vibrios belong to the large bacterial class *Gammaproteobacteria* within the phylum *Proteobacteria*. The family *Vibrionaceae* consist of seven genera and the genus *Vibrio* has more than 50 species of which 11 are of clinical importance including *V. cholerae*. The somatic O antigen is heat-stable and is composed of an amino acid sugar D-perosamine (4-amino-4,6-dideoxy-D-mannose) in which the amino groups are acylated by 3-deoxy L-glycero-tetronic acid. The different O groups are referred to as serogroups. For serogrouping, only the somatic (O) antigen is used, as the flagellar (H) antigen is homogenous in all the *Vibrio* species. The two serogroups, O1 and O139, are associated with epidemic cholera and linked to its ability to produce CT, the main toxin responsible for the disease. Based on the three antigenic factors designated A, B, and C, the O1 serogroup is divided into three serotypes; Inaba, Ogawa, and Hikojima. Ogawa strains produce the A and B antigens and a small amount of C, whereas Inaba strains produce only the A and C antigens. The rare Hikojima subtype contains all three factors, thereby reacting with both Inaba and Ogawa antisera. Inaba strains are mutant of the Ogawa O antigen encoding gene *rfbT*, and the conversion from Ogawa to Inaba may be due to the antiOgawa immune selective pressure.

Vibrio cholerae O1 is classified into two biotypes, classical and El Tor. The characteristics used to distinguish the biotypes are hemolysis, agglutination of chicken erythrocytes, Voges–Proskauer reaction, inhibition by polymyxin B (50-U), susceptibility to classical IV, and El Tor V phages. After the emergence of the O139 serogroup, the isolates that were identified as *V. cholerae* on the basis of biochemical tests but that were negative for O1 and O139 serogroups are referred to as non-O1, non-O139 strains. The non-O1 *V. cholerae* was previously referred to as noncholera vibrios or non-agglutinable vibrios. On the basis of the differences in lipopolysaccharide somatic antigens, this large group has been divided into more than 200 serogroups. Phage typing of *V. cholerae* O1 and O139 are not much in use as this assay is confined only to the Reference centers.

Clinical Symptoms of *V. cholerae* Infection

Human volunteer studies demonstrated that 10^3–10^4 of *V. cholerae* O1 administered with sodium bicarbonate buffer (to neutralize the stomach acidity) develop diarrhea and the lower inocula correlated with a longer incubation period and decreased stool volumes. Food has a buffering capacity comparable to that of sodium bicarbonate, thereby increasing the chances of infection when contaminated food/water is consumed. Patients infected with CT-producing *V. cholerae* (mainly O1/O139 serogroups) develop the most severe clinical manifestations of the disease, termed cholera gravis. Depending on the inoculum size, the incubation period of the infection varies between few hours up to 5 days. The main symptoms include vomiting, profuse effortless watery diarrhea (500–1000 ml h^{-1}), anorexia and abdominal cramps due to hypokalemia at the acute stage. This stage may rapidly lead to tachycardia, hypotension, and vascular collapse due to dehydration. The other external symptoms include weak peripheral pulses, poor skin turgor, sunken eyes, wrinkled hands and feet. Signs of dehydration can be detected with higher plasma protein concentration, hematocrit, serum creatinine, urea nitrogen, plasma specific gravity, severe acidosis manifested by depression of blood pH and plasma bicarbonate and an increased serum anion gap. Owing to prolonged circulatory collapse, ischemic renal tubular necrosis may also be seen. Children may develop hypoglycemia with coma and convulsions.

The nontoxigenic *V. cholerae*, mostly belong to the serogroups other than O1 and O139, but cause milder diarrhea commonly known as gastroenteritis. The non-O1 non-O139 strains are also associated with invasive extraintestinal disease, septicemia, formation of ascites with generalized abdominal tenderness and high white blood cell count (WBC) with polymorphonucleocytes. Compared with patients with nonbacteremic infections, patients with non-O1, non-O139 bacteremia are more likely to have cirrhosis and thrombocytopenia.

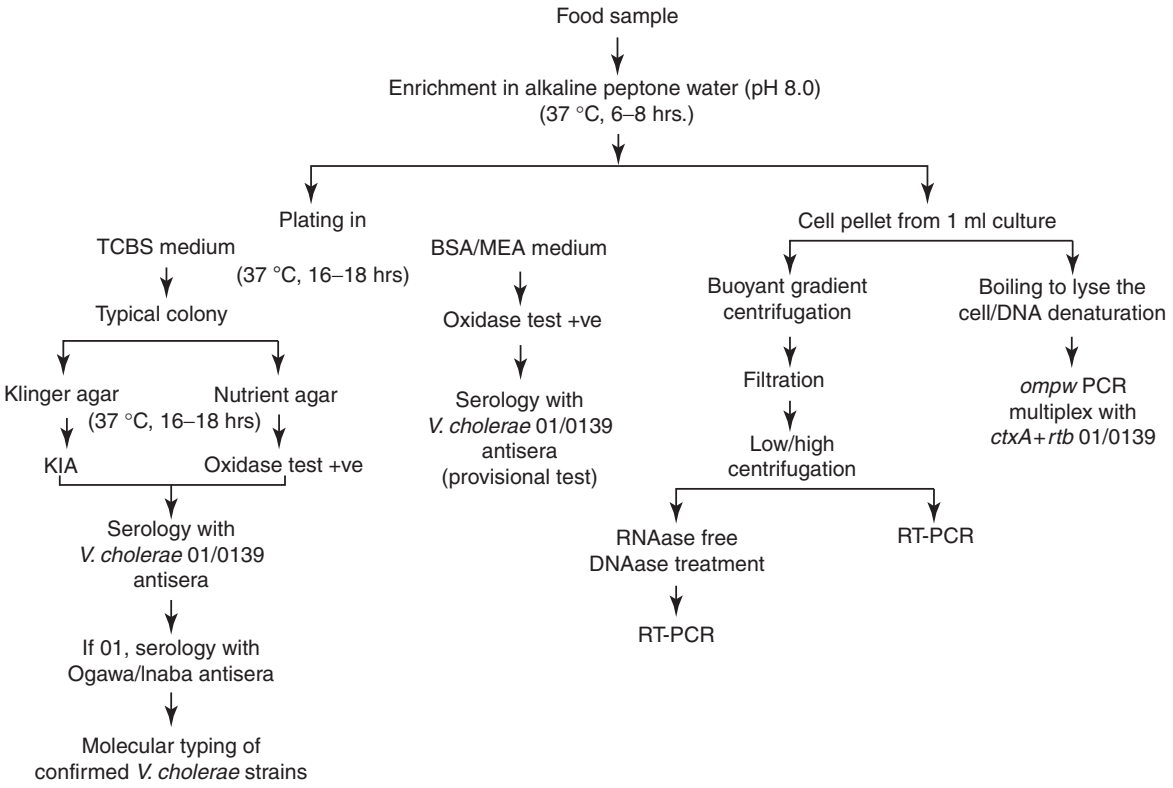

Figure 1 Flow chart showing the isolation and identification of *V. cholerae* from foods using culture and molecualr methods. BSA, bile salt agar medium; KIA, Klinger iron agar; MEA, meat-extract agar; PCR, polymerase chain reaction; RT-PCR, real-time polymerase chain reaction; TCBS, thiosulphate-citrate-bile salts-sucrose.

Detection Methods

Culture Methods

A stepwise method for the isolation and identification of *V. cholerae* is shown in Figure 1. Unlike clinical stool specimens, the concentration of *V. cholerae* in food samples may not be very high and hence an enrichment step is essential. Alkaline peptone water (APW) is the most commonly used enrichment broth with alkaline pH (8.4–9.2). Usually, enrichment lasts for 6–8 h at 37 °C, as excess incubation in APW may result in overgrowth of other organisms. If the plating cannot be done within this stipulated time, a secondary enrichment in APW is necessary to inhibit the growth of background organisms. The most commonly used plating medium for *V. cholerae* is thiosulfate-citrate-bile salts-sucrose (TCBS) agar, which is available from several commercial sources. The sucrose fermenting *V. cholerae* isolates are readily detected on this medium as large, golden yellow, smooth colonies (Figure 2). For the presumptive identification, nonselective media such as bile-salt agar (BSA) and meat-extract agar (MEA) can be used in parallel with the TCBS agar. But these nonselective media are not commercially available. Selective media that are being used for the isolation of members of the family *Enterobacteriaceae* are not suitable for isolation of *V. cholerae*.

For routine identification of suspected colonies, few biochemical tests are mandatory. Either conventional tests or commercial systems can be adopted for identification and the tests should be very simple, rapid, and specific. A crucial test for distinguishing *V. cholerae* from members of the *Enterobacteriaceae* is the oxidase test, in which, *V. cholerae* gives a positive response. Fresh colonies obtained directly from BSA or MEA agar can be used for oxidase test and growth from TCBS agar should not be used. Suspected *V. cholerae* colonies from the isolation plate can also be tested in the Kligler iron agar (KIA)

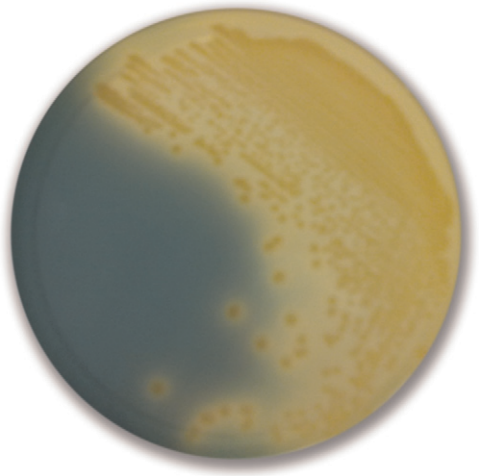

Figure 2 Typical sucrose fermenting colonies of *V. cholerae* in TCBS medium after 16 h of growth at 37 °C.

medium for confirmation. Typical *V. cholerae* yielding an alkaline slant over acid but with no gas or H_2S are then tested for oxidase activity. The key confirmation of *V. cholerae* O1/O139 is agglutination with antisera raised against the respective serogroup. Confirmed O1 strains should be tested with Ogawa and Inaba antisera for completion of the serological testing. Satisfactory serological results can be obtained from colonies picked from nonselective media or KIA medium.

Immunological Methods

Several direct methods are available based on antigen–antibody reactions for the detection of *V. cholerae* O1 and O139 either by using stool specimens or enriched cultures of food samples. Fluorescent antibody technique, coagglutination tests, and lateral flow-based detection systems for the detection of O1 or O139 are now commercially available. Cholera SMART kit (New Horizons Diagnostics Corp, USA) and Crystal VC (Span Diagnostics, India) are colloidal gold-based lateral flow immunoassay for the detection of *V. cholerae* O1/O139 strain within 10–20 min. At times, it is crucial to confirm production of CT by *V. cholerae*. With tissue culture assay employing Chinese hamster ovary or Y-1 adrenal cells elongation cells caused by the action of CT which can then be confirmed by neutralization of the activity using antiCT antibody. Enzyme-linked immunosorbent assay (ELISA) using purified ganglioside M-1 (GM1) as the capture molecule or a highly sensitive bead ELISA, which uses polystyrene beads coated with antiCT antibody as the solid phase are being used in many reference laboratories. A latex agglutination for the detection of CT is less time-consuming than the ELISA, with excellent sensitivity and specificity. For the detection of *V. cholerae* O1 from foods, an enzyme immunoassay has been formulated with specific rabbit antiserum, immobilized target bacterial cells and beta-D-galactosidase-labeled goat antirabbit immunoglobulin G as tracer. Animal models are also available for confirmation of CT-producing strains of *V. cholerae*, but these models are prohibitive as one cannot accommodate many strains in the assay in addition to the animal ethical issues.

Molecular Methods

For screening larger number of isolates, deoxyribonucleic acid (DNA) and oligonucleotide probes are useful as it reduces the labor and screening time. The molecular approach confirms *V. cholerae* as pathogenic strains if the DNA probes are targeted toward virulence genes such as CT encoding gene (*ctx*). Molecular methods are routinely used in food industry because majority of the *V. cholerae* strains isolated from environmental and food are nonpathogens as they lack recommended virulence marker genes. Polymerase chain reaction (PCR) technique has also been used to detect *ctx* or other virulence gene sequences. Several PCR methods are now available using multiplex format with virulence genes along with species-specific targets such as *ompW* and biotype-specific *tcpA* or *hlyA*. Detection of these genes directly from the stool or food samples is not advisable as the sample may contain substances that can be inhibitory to the PCR. To overcome this hindrance, extraction of DNA/RNA (ribonucleic acid) from the samples is recommended following quick extraction protocols, which are now available in the form of silica column or quick spin purification kits. PCR can amplify target DNA from both viable and nonviable cells of *V. cholerae*. Detection of pathogens in clinical and food samples is important to ensure that positive test results are associated with viable bacteria. Positive results caused by dead cells may lead to misguided decisions concerning the effectiveness of treatment and destruction of the suspected foods.

Combination of multiplex PCR with a colorimetric microwell plate sandwich hybridization assay using phosphorylated and biotinlylated oligonucleotide probes are specific and sensitive for the detection of the microbial pathogens in shellfish. Immobilized oligonucleotide array that targets mutation regions of the 23S rRNA gene or amplified PCR products are being used for the identification of bacteria causing foodborne infections including *V. cholerae*. Similarly, DNA microarray-based identification system in combination with multiplex PCR targeting *ompU*, *toxR*, *tcpI*, and *hlyA* is also available for the detection of *V. cholerae*.

Real-time PCR (qPCR) assay that identify the *ctxA* or any other marker gene from viable toxigenic *V. cholerae* can also be adopted as an alternative method for standard culture methods. Before real-time quantitative PCR, buoyant density gradient centrifugation followed by filtration and low- and high-speed centrifugation is recommended to separate bacteria from complex food materials as well as to remove compounds that inhibit rapid detection methods. Loop mediated isothermal amplification PCR assay that was designed with five primers targeting *ompW* seems rapid and specific in detection of *V. cholerae*. Unlike normal PCR, there is no cycling process involved in this technique. Detection of viable but nonculturable (VBNC) state of *V. cholerae* O1 is difficult with the existing molecular methods. The transcriptome-based RT-PCR analysis detects increase in the expression mRNA of VC0230 (iron(III) adenosine-triphosphate-binding cassette (ABC) transporter), VC1212 (*polB*), VC2132 (*fliG*), and VC2187 (*flaC*) in the VBNC state. Thus, these genes appear to be suitable markers for the detection of *V. cholerae* VBNC.

Virulence Features and Pathogenicity of *V. cholerae*

CT is directly responsible for the characteristic symptoms of cholera. The pathogenesis of cholera begins with colonization of toxigenic *V. cholerae* in the upper intestine and secretion of CT. This toxin is composed of two types of subunits, a 56-kDa oligomer composed of five identical 'light' B subunits ('B' for binding) responsible for receptor binding and a single 'heavy' 28-kDa toxic active A subunit ('A' for active toxin). Monosialoganglioside GM1: (Gal(β1–3)GalNac(β1–4)(NeuAc(α2–3)Gal(β1–4)Glc)-ceramide acts as a cell membrane receptor for CT. First, the CT binds to the GM1 receptors on host cells through its pentamer B subunit followed by translocation of A subunit to the cytosol of the target cell. The A subunit catalyzes the adenosine diphosphate (ADP)-ribosylation of the host G protein Gsα, which in turn activates host cell adenylate cyclase. This is accomplished by elevating cAMP levels in intestinal cells through the activation of a G-protein (Gsα) that controls host cell adenylate cyclase activity. The cyclic adenosine

monophosphate (cAMP) that accumulates in target cells activates protein kinases, which in turn phosphorylate membrane proteins one of which is the cystic fibrosis transmembrane conductance regulator chlorine and bicarbonate conductance channel. Active Cl^- and HCO_3^- transport into the lumen of the intestine produces an osmotic movement of water out of the tissues resulting profuse secretory diarrhea.

V. cholerae non-O1, non-O139 serogroups (otherwise known as noncholera vibrios are ubiquitous in the aquatic environment and fauna living therein. The reported asymptomatic carriage rate is approximately 4% among persons involved in high-risk activities and their contribution in several outbreaks have been published in many findings. Unlike O1 and O139, the non-O1, non-O139 serogroups does not appear to be a single virulence mechanism similar to the heterogeneity seen among diarrheagenic *Escherichia coli*. The pathogenic mechanisms of non-O1, non-O139 serogroups are different from that of O1 and O139 serogroups as they lack the *ctx* gene cassettes but heat-stable enterotoxin (Stn), El Tor-like hemolysin (Hly) plays a role in human pathogenesis. Clinical strains of *V. cholerae* rarely carry the heat-stable enterotoxin encoding genes (*stn/sto*) but sequence type (ST) positive strains are detected in high proportions with environmental *V. cholerae* O1, O139, and non-O1/non-O139 strains. Type three secretion system is one of the other possible virulence factors commonly found in non-O1, non-O139 serogroups (30–40%). Prevalence of putative accessory virulence genes (*mshA*, *hlyA*, and *RTX*) both in the clinical O1/O139 serogroups as well as non-O1, non-O139 serogroups supports a hypothesis that these genes impart increased environmental fitness. Hemagglutinin/protease and mannose-sensitive hemagglutinin are the other possible virulence factors among non-O1, non-O139 vibrios. In several instances, it was shown that the non-O1, non-O139 serogroups carry *ctx* and associated with cholera-like diarrhea. Considering the importance of non-O1/non-O139 *V. cholerae* isolated from the aquatic environment or food samples they should be screened for the presence of *ctxA*, *stn/sto*, *tcpA*, and other virulence marker genes for the human health risk assessment.

Risk Factors and Reservoirs

Several risk factors have been identified for the prevalence and spread of cholera, which include population displacement and refugee crisis, heavy rain and floods, traditional funeral rituals and feasts, water storage in large mouthed containers, ice made up of contaminated water, lack of previous disease exposure in endemic regions, asymptomatic carriers/food handlebars, etc. *Vibrio cholerae* is ubiquitous in the water and sediments of various aquatic water bodies including costal, estuarine, and freshwater systems. Because of its widespread distribution, foods are easily contaminated at various levels of food preparation. The distribution of non-O1, non-O139 vibrios is very common in these environments. Isolation of O1 and O139 from the environments is reported during epidemic and interepidemic periods due to fecal contamination. Overall, the most commonly noted risk factor for cholera outbreaks was transmission through foods that account for 32 and 71% in South America and East Asia, respectively. In several reports, seafood is responsible for many sporadic cases and epidemics of *V. cholerae* infections and strains are generally devoid of *ctx*. The exoskeleton chitin of copepods, shrimps, and crabs are made up of mucopolysaccharide, a preferential substrate of *V. cholerae* that enhances adherence through their production of chitinase.

Survival Strategies

Quorum sensing helps the bacteria for cross communication and alters their gene expression in concordance with several ecological factors. In several findings it was shown that quorum sensing is important in the infectious cycle of *V. cholerae* in humans and passage through acid barriers in the stomach. The colonized cells of *V. cholerae* O1 on shrimp carapace showed remarkable resistance to the effects of high temperatures, low pH, and desiccation conditions. This increased resistance to extreme environmental conditions of *V. cholerae* O1 may have significant implications on food safety and contamination of edible parts of shrimps. It has been suggested that its environmental persistence is associated with conversion of *V. cholerae* into VBNC state or rugose type and these forms of *V. cholerae* enhance their survival in many adverse conditions. It is noteworthy to mention here that the rugose variants can survive in the presence of high concentrations of chlorine and other disinfectants.

Epidemiology Cholera with Reference to Contamination of Foods

In cholera endemic countries, the case fatality rate (CFR) remains $<5\%$ but in some African countries the CFR is approximately 50% during peak outbreak periods. In many cholera outbreak investigations, water was recognized as the primary source for transmission. In the past 60 years, outbreaks of cholera have been documented with consumption of contaminated food. Transmission of cholera may vary from place to place, influenced by local customs and practices. Molluscan and crustacean seafood are generally contaminated in its natural environment at the time of harvest or during preparation. Food items initially free from *V. cholerae* may become contaminated when mixed with water, other contaminated food, or through foodhandlers.

In Guatemala, the 1991 cholera epidemic was significantly associated with contaminated street-vended food items. Foodborne outbreaks of cholera occur mostly in developing countries and have potential cause of large morbidity and mortality that require considerable public health and acute care resources. Storing contaminated meals at ambient temperatures, the common practice in most of the developing countries, allows the growth of *V. cholerae*. Many epidemiological studies have shown that food plays an important role in the transmission of *V. cholerae*, and different foods have been incriminated in many epidemic outbreaks of cholera.

The Guinea-Bissau cholera epidemic in 1994 that resulted in 15 878 reported cases and 306 deaths was strongly associated with eating at a funeral with a nondisinfected corpse. A huge cholera epidemic in western Kenya in 1997 with 14 275 cholera admissions and 547 deaths was due to use of contaminated water from Lake Victoria or from a stream, sharing food with a person with watery diarrhea, and

attending funeral feasts. Ingestion of undercooked, contaminated fish has long been known to be associated with cholera transmission. Household epidemiological studies indicate that cholera infection is more likely to occur through patient's/carrier's hands rather than by consumption of contaminated foods. Undercooked seafood continues to account for most US cholera cases. In majority of the cases, *V. cholerae* non-O1, non-O139 is involved in the seafood associated cholera-like diarrheal infection and asymptomatically infected foodhandlers play a great role in the transmission of cholera. Travelers to epidemic countries may be at increased risk of contracting cholera if they ingest contaminated food or water. It has been estimated that approximately 0.2 cases per 100 000 European and North American travelers suffer from cholera without any fatalities. Violations of retail food establishment rules and regulations, and underutilization of safer, post-harvest processed shellfish resulted in significant increase in the incidence of *V. cholerae* mediated infections in the USA.

Molecular Typing Methods

Molecular typing has become an essential component to compliment epidemiological data, and hence many methods have been established for the identification of DNA finger prints of *V. cholerae*. Following are some of the currently used methods in the molecular epidemiology of cholera.

PCR-Based Typing

Though PCR is used for the detection of several virulence encoding genes, this assay is also employed for strain typing in which DNA fragments can be amplified (100–>35 kb) even if the template DNA is in minute quantity. Several PCR methods such as random amplification of polymorphic DNA (RAPD) with the use of single oligo, amplified fragment length polymorphism (AFLP) technique with two sets of restriction enzyme-primer combinations, enterobacterial repetitive intergenic consensus sequence that targets arrangements of conserved sequences in most of the bacteria are used in typing *V. cholerae*. The advantage of such PCRs is that they can be used in laboratories with minimal facility. Several studies performed with RAPD-PCR using *V. cholerae* O1 has shown the genetic dissimilarity in many Asian and African countries. The AFLP analysis supported that a single clone of pathogenic *V. cholerae* has caused several cholera outbreaks in Asia, Africa, and Latin America during the seventh pandemic. Generally, PCR assays are rapid and simple means of typing strains for epidemiological studies. However, consistency of the results of PCR is subject to various experimental parameters such as concentrations of the template DNA, Taq polymerase enzyme, annealing temperature, and number of PCR cycles.

Mobile Genetic Elements

Mobile genetic elements (MGE) such as plasmids, integrons, and integrating conjugative elements are used in molecular typing of *V. cholerae*. This is not a routine fingerprinting method, but it gives information on movement of antimicrobial resistance genes in *V. cholerae* strains as they are mostly located in MGEs. Conjugative plasmid IncC was found to be responsible for multidrug resistance in *V. cholerae* O1 associated with many cholera outbreaks in Africa. Integrons, the gene capture and expression systems and integrating conjugative elements which are chromosomal self-transmissible MGEs are specifically detected in El Tor strains indicating they may be considered as a recent evolutionary trend.

CTX Prophage and CT Genotypes

Analysis of CT prophage is important to determine the evolution of toxigenic strains of *V. cholerae*. The CT encoding gene (*ctxAB*) reside in the genome of a lysogenic filamentous phage known as CTXΦ, which is located on a 4.5 Kb 'core region' of the CTX element (Figure 3). Adjacent to the core is the RS2 region encoding open reading frame (ORF) *rstR*. Based on the allelic types, the *rstR* is classified as $rstR^{class}$, $rstR^{ET}$, and $rstR^{calc}$, respectively for classical, El Tor, and O139 strains. Using *ctx* restriction fragment length polymorphism (RFLP), the structure, organization, and location of the CTX prophages can be determined. Identification of distinct strains of *V. cholerae* O1 belongs to biotype classical and El Tor, and O139 demonstrates the evolutionary significance and clonal dynamicity of the pathogen (Figure 3).

RFLP, the structure, organization, and location of the CTX prophages were identified in many *V. cholerae* O1 as well O139 strains isolated from different geographical regions. The unique clonal nature of the US Gulf Coast *V. cholerae* O1 was identified with 6–7 kb *Hin*dIII restriction fragments that contained *ctx* gene. Unlike classical or El Tor vibrios, the assembly of *ctx* in O139 strains differed with three or more copies of this gene. The CTX genetic element was different in *V. cholerae* O139 strains that resurged in Calcutta and Bangladesh in 1996, China, from 1993–99. Of recent *V. cholerae* O1 Inaba from India, presence of CTX prophage was detected in a single site of the chromosome with at least two RS elements. Interestingly, *V. cholerae* O1 El Tor isolated from Mozambique carried a classical type (CTX^{class}) prophage and the genomic

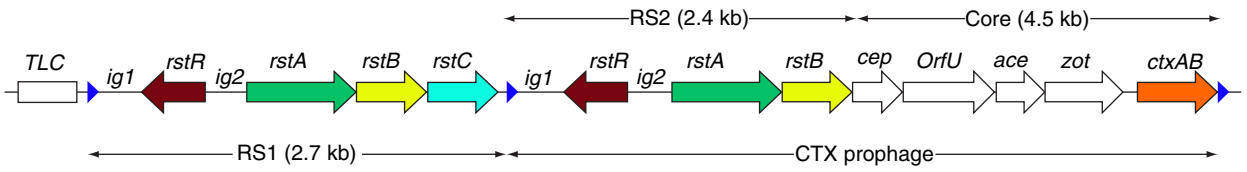

Figure 3 Typical genetic structure and arrays of CTX prophage in *V. cholerae*. Small black triangles in between the genome sequence indicate the repeat regions flanking the integrated phage DNA.

analysis of CTX prophage together with chromosomal phage integration sites showed that these strains carried two copies of prophages located in the small chromosome in tandem.

DNA sequencing of *ctxB* of *V. cholerae* O1 and O139 strains identified several CT genotypes. The CT genotype 1 was found in classical biotype worldwide and El Tor biotype strains associated with the US Gulf Coast. The El Tor strains from Australia belong to CT genotype 2 and 3 was common in the seventh pandemic El Tor strains including the Latin American epidemic strains. Emergence of El Tor strains having CT genotype 1 was reported from India, Bangladesh, Vietnam, and also from few African countries. The epidemiological significance of this trend is related to several recent cholera outbreaks with increased severity of the illness and also for a tendency for the outbreak to become protracted. Identification of new CT genotypes such as 4–6 in *V. cholerae* O139 strains in also considered as a novel genetic trend.

Ribotyping

This method exploits the DNA polymorphism of rRNA genes (*rrn*) in the chromosome of *V. cholerae* after digestion with *Bgl*I. Universal probe generated from the *E. coli* (pkK3535) RNA is used to screen the restriction patterns of bacterial DNA. The *rrn* operons and their flanking regions cause ribotype variation in *V. cholerae* O1 due to recombinational events. This typing method has identified 7 and 20 ribotypes among classical and El Tor biotypes, respectively. Analysis of *V. cholerae* O139 strains isolated in India and Bangladesh revealed four different ribotypes. *Vibrio cholerae* O1strains isolated after the emergence of O139 and reemerged O139 strains in India and Bangladesh showed newer ribotypes. This change in the ribotype profiles was correlated well with the new antimicrobial resistance patterns, which indicated successive replacement of different clones of *V. cholerae* O1/O139 in these regions.

Multilocus Sequence Typing

In multilocus sequence typing (MLST), the genetic variations of *V. cholerae* at several housekeeping genes are indexed after nucleotide amplification and sequencing. MLST data is highly suitable for software based analysis and hence can be adopted in the long-term epidemiological studies. However, standardized methods are not adopted in the MLST for the universal use, as the target genes varies from 3 to 26.

Results of split decomposition analysis of three housekeeping genes, *mdh*, *dnaE*, and *recA* showed that widespread recombination plays an important role in the emergence of toxigenic strains of *V. cholerae* O1. With the Argentinean *V. cholerae* O1 isolates, six distinct genetic lineages identified among seven housekeeping loci. The *gyrB*, *pgm*, and *recA*-based MLST analysis performed better than pulse-field gel electrophoresis (PFGE) as there was clear clustering of epidemic serogroups. MLST analysis using nine genetic loci showed that the Mozambique isolates that harbored classical CTX prophage had the ST identical to that of El Tor N16961, a representative of the current seventh cholera pandemic. However, MLST of *V. cholerae* is incoherent as many investigations did not follow the specific set of genes in the analysis.

Variable Number of Tandem Repeat (VNTR) Loci

In many housekeeping genes, the DNA regions known as VNTR are cataloged on the basis of their repeat unit. VNTR is otherwise called simple sequence repeats . This repetitive DNA contains monomeric sequences and arranged in a head-to-tail configuration. These repeat loci are highly conserved and hence the discrimination power is more compared with that of MLST. Five VNTR loci of *V. cholerae* strains collected between 1992 and 2007 from different areas in India showed that each VNTR locus was highly variable, with 5–19 alleles. The eBURST (based upon related sequence types) analysis of sequence types revealed four large groups of genetically related isolates with two groups containing O139 serogroup and the other two groups including O1strains. VNTR also helped to track the spread of specific genotypes across time and space. Genetic relatedness of *V. cholerae* collected from 2004–05 from Bangladesh showed minimal overlap in VNTR patterns between the two communities that was consistent with sequential, small outbreaks from local sources. VNTR-analysis of nontoxigenic *V. cholerae* that caused outbreak in Rostov region in 2005 showed that they differed from previous outbreaks and formed separate group with strains isolated from patients, carriers, and environment. Phylogenetic analysis of the combined VNTR data also showed a clear discrimination between the clinical O1 and O139 strains and the environmental isolates.

PFGE

PFGE has proven to be highly effective molecular typing technique for different foodborne bacterial pathogens. Database for PFGE patterns for various foodborne pathogens have been established, and being used by many PulseNet groups all over the world. PFGE was shown to be useful for the identification of spread of specific clones of many pathogens. In the PFGE, suspected sources of the pathogens have been identified, followed by implementation of timely interventions to prevent further spread of the pathogens. In the US and other countries, considerable reduction of foodborne infections was observed after the inception of PulseNet program. International PFGE typing protocol for *V. cholerae* was established for generation and submission of subtype patterns to the database.

Most of the published works shows in-house PFGE typing scheme and cannot be compared with data generated by others due to variation in several parameters. However, in the PFGE, various parameters are controllable including the electrophoretic programs. PFGE patterns of representative *V. cholerae* O1, El Tor strains from Australia, Peru, Romania, and the US were different from Asian countries, such as Bangladesh, India, and Thailand, indicating a close genetic relationship or clonal origin of the isolates in the same geographical region. In India, Thailand, Iran, South Africa, clonality of *V. cholerae* O1 tends to differ during each cholera outbreak.

In Italy and Albania, cholera epidemic occurred during 1994 after more than a decade. PFGE analysis indicated that the strains from both the countries belonged to the same clone that was part of the larger global spread of epidemic ribotype 6 strains, which started in southern Asia in 1990. PFGE-type patterns of Peruvian *V. cholerae* O1 strains isolated during 1991–95 suggest that genetic changes are occurring in Latin American cholera epidemic, more frequently than previously reported. *Vibrio cholerae* O1 strains that appeared in India after the O139 appearance had new pulsotypes in which the H type was the predominant one. Pulsotypes A–C dominated before 1992 and F type was common among the O139 serogroup. Pulsotype H was stable for a long time in India and was associated with several cholera outbreaks since 1993. This trend was the same in Bangladesh and Thailand, though the pulsotype nomenclature was different. Pulsotype IV, which was a new clone introduced after 1993 from overseas was frequently present in both domestic and imported cases from 1994 to 1997 in Aichi, Japan.

PFGE was used to identify the clonality and spread of *V. cholerae* O1 in Kenya. The PFGE profiles of Iranian *V. cholerae* O1strains were similar to that of Pakistan, Nepal, and India, suggesting the dissemination of common clones in this region. Nontoxigenic *V. cholerae* O1 strains isolated between 1998 and 2000 from Mexico differed in the PFGE patterns compared with Latin America and US Gulf Coast clones. The outbreak associated nontoxigenic *V. cholerae* O1 strains from India had more resemblance with O139 serogroup rather than classical or El Tor *V. cholerae*. The El Tor hybrid strains from Mozambique that appeared during 2004–05 are different from the Bangladeshi hybrid strains and overall the El Tor hybrid strains differed markedly from conventional classical and El Tor strains. The PFGE patterns of toxigenic O139 strains isolated from turtles in Sichuan, China, during 2004 were identical with the patterns of strains that appeared in the outbreaks, thereby indicating the sources of infection causing these outbreaks.

Prevention and Control Measures

As death occurs in 50–70% of the untreated severe cholera cases, adequate rehydration therapy in mandatory. In severe cases, rehydration can be accomplished by intravenous infusion of fluid followed by oral rehydration with oral rehydration solution (ORS). Various modifications to the standard ORS have been successfully made that include hypoosmolar or hyperosmolar solutions, rice-based ORS, zinc supplementation, and the use of amino acids, including glycine, alanine, and glutamine. The precise rates of fluid administration should be adjusted according to the patient's state of hydration and volume of the stool. Antimicrobial agents such as tetracycline and fluoroquinolones are effective in reducing the volume of the stool and duration of the diarrhea.

Preservation of foods from contamination of *V. cholerae* without spoiling their natural flavor, esthetic, and nutritive values is always a big challenge. Some of the normal practices such as addition of lime juice to foods thereby reducing pH toward acidic kills most *V. cholerae*. Many modern techniques such as high pressure processing, cold temperatures, ionizing irradiation, exposure to chlorine or iodophor, etc. decontaminates most of the vibrios. Preventive measure such as restricting the consumption of oysters during summer months, cleanliness of cutting boards, microbial monitoring of shellfish/shrimp growing areas, postharvest practices including depuration and relaying in offshore waters can reduce *V. cholerae* mediated infections.

WHO's Strategic Advisory Group of Experts on immunization recently recommended that cholera vaccination should be considered in endemic areas targeting the higher-risk population to reduce the disease burden. However, this vaccination should not interrupt the provision of other priority health interventions to control and prevent cholera outbreaks.

Two oral cholera vaccines are now available i.e., Dukoral and Shanchol and their characteristics and other details are described in Table 2. mORCVAX vaccine is basically identical to that of Shanchol, except it was made by a different

Table 2 Available oral cholera vaccines and their characteristics

Cholera vaccine	*Characteristics*	*Vaccine schedule*
Dukoral[a]	Vaccine: Monovalant vaccine with formalin and heat-killed whole cells of (1×10^{11} bacteria). *Vibrio cholerae* O1 consisting of both Ogawa and Inaba serotypes and classical and El Tor biotypes along with recombinant cholera toxin B subunit (rCTB) 1 mg Usage: Given with bicarbonate buffer, 150 ml for adults and 75 ml for children aged 2–5 years to protect the CTB Expected protection: After 7 days	2 doses $\geq$7 days apart 1 booster dose for every 2 years for adults and children $\geq$6 years. For children 2–5 years, 1 booster is recommended for every 6 months
Shanchol/mORCVAX[b]	Vaccine: Bi vaccine with formalin and heat-killed whole cells of *V. cholerae* O1 consisting of both Ogawa and Inaba serotypes and classical and El Tor biotypes along with the O139 serogroup, without cholera toxin B subunit Usage: Can be given directly without bicarbonate including children $\geq$1 year Expected protection: After 7 days	Two liquid doses 14 days apart for individuals aged $\geq$1 year with booster dose after 2 years 1 booster dose for every 2 years for adults and children $\geq$6 years. Booster dose for children for every 6 months is not required

[a]Not licensed for children aged $<$2 years.
[b]Identical vaccines in terms of *V. cholerae* strains different manufacturers using different methods.

manufacturer with different methods. The protective efficacy (PE) of Dukoral cholera vaccine was more than 80% with two doses of vaccine irrespective of the age groups. Because CTB is structurally and functionally similar to the heat-labile toxin produced by enterotoxigenic *E. coli* (ETEC), Dukoral vaccine gave 67% protection against ETEC, which is another enteric pathogen commonly found in developing countries. The PE of Shancol vaccine for all age groups after two doses was 66% and the overall effectiveness after 3–5 years was 50%. Overall, vaccination can be considered as an additional preventive tool by the health authorities to prevent cholera outbreaks and its spread to newer areas.

Information regarding cholera outbreaks and characteristics of the strains would help many clinicians and laboratory works to prevent its spread and proper management of cholera. Though official notification of cholera outbreaks by WHO Member States is mandatory under the International Health Regulations, the reporting by many countries is incomplete due to political and economical reasons that also include tourism and food export. The online forum, Program for Monitoring Emerging Diseases supported by the International Society for Infectious Diseases complements the WHO cholera reports and provides detailed information on subnational, monthly, and temporal distribution of cholera cases.

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BACTERIA

Vibrio parahaemolyticus

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Glossary

High pressure processing A nonthermal process using water under very high hydrostatic pressure to produce packaged foods that are safer, longer lasting, more natural, and better tasting.

Kanagawa phenomenon (KP) A well-defined β-type hemolysis produced by *Vibrio parahaemolyticus* on Wagatsuma agar medium. KP considered as a marker for virulence of clinical isolates of *V. parahaemolyticus* and has been related to the production of the thermostable direct hemolysin. KP is a useful marker to distinguish pathogenic from nonpathogenic strains.

Pandemic strain of *Vibrio parahaemolyticus* Genetically defined strains with specific changes in *toxRS* region commonly seen in serovars O3:K6, O4:K68, O1:K25, and O1:KUT.

Pulsed-field gel electrophoresis (PFGE) A kind of gel electrophoresis with the voltage periodically switched among three directions; one that runs through the central axis of the gel and two that run at an angle of 120° either side. The pulse times are equal for each direction resulting in a net forward migration of the large fragments of DNA. PFGE is a gold standard technique for genotyping of pathogenic organisms in epidemiological studies.

Postharvest processing After harvesting, common food-processing technologies such as quick freezing, heat–cool pasteurization, and high hydrostatic pressure may be used to reduce pathogenic/spoilage bacteria in the oysters to nondetectable levels.

Ribotyping A standard technique for genotyping of pathogenic bacteria in epidemiological studies exploiting variation in the highly conserved structural rRNA molecules (16S, 23S, and 5S) and their copy numbers.

Thermostable direct hemolysin (TDH) TDH has been considered a major virulence factor of *Vibrio parahaemolyticus.* It is a protein toxin, which has been reported to show intestinal toxicity, cardiotoxicity, hemolytic activity, and cytolethal activity. TDH is known for its intractable property of being reactivated by heating at high temperatures.

TDH-related hemolysin (TRH) In *Vibrio parahaemolyticus*, TRH is regarded as an important virulence factor that has an amino acid sequence that is approximately 67% homologous with thermostable direct hemolysin. Also a hemolytic toxin, TRH is produced by KP-negative *V. parahaemolyticus* and is suspected of playing an important role in the diarrhea caused by this organism.

Type III secretion system (TTSS) TTSS is a protein appendage of several Gram-negative bacteria that mediate interactions with their hosts and allows bacteria to deliver protein effectors across eukaryotic cellular membranes. In pathogenic *V. parahaemolyticus*, TTSS delivers a variety of effectors directly into the host cytosol, allowing them to manipulate host cellular processes and subvert them for their benefit.

Introduction

Though commonly found as an autochthons halophilic bacterium in marine environments, *Vibrio parahaemolyticus* is known as an agent of gastroenteritis associated with the consumption of contaminated seafood. This bacterium was first identified in Japan in 1950 by Tsunesaburo Fujino from a foodborne outbreak due to the consumption of semidried sardines. Emergence of a novel clone of O3:K6 in 1995 and its subsequent spread to many countries causing several diarrheal outbreaks marked the first pandemic of *V. parahaemolyticus* infection. The global spread of this pandemic strain along with its genetically related serovars have been related to the consumption of seafood. Association of water temperature in the epidemiology of *V. parahaemolyticus* infections caused by this pathogen has been reported in many temperate regions, but no such correlation has been found in tropical countries.

Mechanisms of virulence in *V. parahaemolyticus* are well established. Strains of *V. parahaemolyticus* from environmental sources generally lack virulence gene markers. Pathogenesis of *V. parahaemolyticus* is conventionally associated with the production of thermostable direct hemolysin (TDH), which is responsible for the β-hemolysis of human erythrocytes in a special Wagatsuma agar medium. The reaction is known as the 'Kanagawa phenomenon (KP).' Other putative virulence factors are the polysaccharide capsule, TDH-related haemolysins (TRH), cytotoxins, and adherence factors. However, virulence

factors, such as TDH, TRH, or both, are associated with strains that cause infectious diarrhea. Production of TDH is associated with KP. Type III secretion system (TTSS) is a protein appendage found in several Gram-negative bacteria and help the bacteria to infect. In *V. parahaemolyticus*, two sets of genes, for TTSS1 and TTSS2, were identified on the large and small chromosomes, respectively. TTSS1 showed it to be involved in the cytotoxicity to HeLa cells, whereas TTSS2 has a role in enterotoxicity in a rabbit model. Several molecular typing techniques such as polymerase chain reaction (PCR), deoxyribonucleic acid (DNA) fingerprinting using ribotyping, and pulsed-field gel electrophoresis (PFGE) have been successfully introduced for rapid detection and spread of this pathogen from clinical specimens and suspected food samples. Several techniques have been in use for the elimination of *V. parahaemolyticus* from seafood at the industrial level. This article gives an understanding on the epidemiology, virulence features, use of different detection and typing methods, and control of *V. parahaemolyticus* in seafoods.

Taxonomy and Characteristics of *V. parahaemolyticus*

Vibrios belong to the large bacterial class Gammaproteobacteria within the phylum *Proteobacteria*. The family *Vibrionaceae* consists of 7 genera, and the genus *Vibrio* has more than 50 species, of which 11 are of clinical importance. *Vibrio parahaemolyticus* is a Gram-negative straight or curved rod-shaped facultative anaerobic bacterium, which is motile by means of a single polar flagellum. They do not form endospores or microcysts and are oxidase-positive, utilize D-glucose as a sole or main source of carbon and energy. Like other vibrios, *V. parahaemolyticus* is a halophile (salt requiring) and sodium ions stimulate their growth. This *Vibrio* can grow well from neutral to alkaline pH, therefore the pH values of both selective and enrichment media are generally adjusted from 8–8.8. pH less than 7.0 are lethal to this organism. Serotyping scheme for subspecies identification of this pathogen is well established with the combination of somatic (O) and capsular (K) antigens and serotyping is done using commercially available antisera that include 11 different O antigens and 71 different K types. Phage typing of *V. parahaemolyticus* is not in use.

Pandemic Strains

An abnormal increase in the *V. parahaemolyticus* O3:K6 mediated diarrheal cases were recorded in February 1995 at the Infectious Diseases Hospital in Kolkata, West Bengal, India. After this report, genetically identical O3:K6 were reported from sporadic cases/foodborne outbreaks from Bangladesh, Chile, France, Japan, Korea, Laos, Mozambique, Peru, Russia, Spain, Taiwan, Thailand, and the USA. Owing to its fast spreading nature to several countries, this serovar was labeled as 'pandemic strain.' Almost during the same period, other serovars, such as O4:K68, O1:K25, and O1:KUT (untypeable K antigen) that had identical molecular characteristics, such as the presence of *tdh*, new *toxRS* gene allele, and PFGE profile similar to that of the O3:K6 serovar were identified. In Peru, apart from the pandemic serovars O3:K6, O3:K68, O3:K58, and OUT (untypeable):K6 also possessed the pandemic markers. One of the O3:K6 strains was isolated in 1996, indicating that the pandemic strain was present in Peru almost during the same time that it caused the first outbreak in Kolkata in February 1996. The emergence of pandemic strains might be due to the serotype conversion from O3:K6 to O4:K68 involving recombination event with a region much larger than the O- and K antigen-encoding gene clusters. In addition, pathogenicity islands and mobile elements are also likely involved in the evolution of pandemic strains of *V. parahaemolyticus*.

DNA microarray-based analysis indicated that approximately 86% of the genes from the whole genome sequenced pandemic strain RIMD2210633 were conserved in all the strains. At least 65 genes over 11 loci were specifically identified in the pandemic strains compared with any of the nonpandemic strains, suggesting that the difference between pandemic and nonpandemic strains is not due to a simple or single genetic event. Only the genes in the 80-kb pathogenicity island (Vp-PAI) on chromosome II, including two *tdh* genes and a set of genes for TTSS were detected in the KP-positive pathogenic strains. Pandemic strains have four specific genomic islands, which are considered as potential factors of the pandemicity. When the origin and function of 24 genes in the insertion element VP-PAI-1 were analyzed, two were found only in *Vibrio vulnificus* and *Shewanella* sp. The 8 kb genomic segment with the two genes showed synteny. These two genes are associated with cold adaptation in *Shewanella* sp., which can grow even at 4 °C.

Clinical Symptoms of *V. parahaemolyticus* Infection

Gastroenteritis, wound infections, and septicemia are the major clinical syndromes caused by *V. parahaemolyticus*. Gastroenteritis symptoms include diarrhea, abdominal cramps, nausea, vomiting, headache, and low-grade fever. Sometimes the diarrhea may be bloody, with stools described as 'meat washed' because the stool is reddish in color. The illness is generally self-limiting and lasts for approximately 3 days in immunocompetent patients. The incubation period for *V. parahaemolyticus* infection may vary from 4–96 h. *Vibrio parahaemolyticus* causes though rarely necrotizing fasciitis, especially in patients with underlying disease, such as chronic renal failure, diabetes mellitus, and malnutrition. Early diagnosis and appropriate antibiotic therapy are essential to save the patient's life, because clinical evolution can be fulminant and mortality rates are high (43%). Multidrug resistance is not reported in *V. parahaemolyticus* and the organism is susceptible to most of the antimicrobials used in the treatment of diarrhea.

Detection Methods

Isolation and identification of *V. parahaemolyticus* from clinical specimens are well established. Isolation of this pathogen from fishery products and other food samples is often hampered by the lack of standardized methods. Presently, the conventional assay system is supplemented with highly

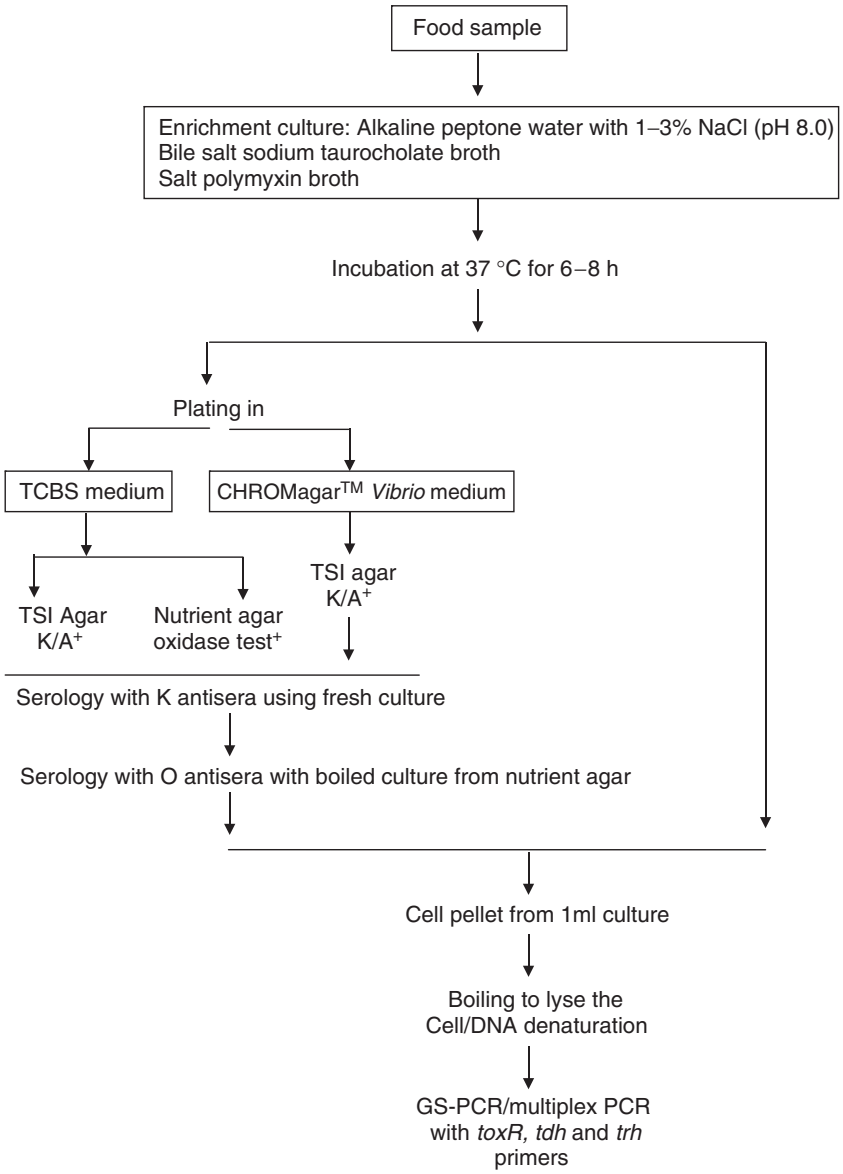

Figure 1 Flowchart showing a stepwise procedure for isolation of *V. parahaemolyticus*.

sensitive immunological and molecular detection methods for the specific identification of *V. parahaemolyticus*.

Culture Methods

Methods for the isolation and identification of *V. parahaemolyticus* are shown in Figure 1. The density of *V. parahaemolyticus* in food samples may not be very high and hence an enrichment step is essential. Alkaline peptone water (APW) with pH of 8.0–8.7 supplemented with 1–3% NaCl is most suitable for the initial enrichment. Usually, enrichment is for 6–8 h at 37 °C, as excess incubation in APW may result in overgrowth of other organisms. If plating cannot be done within this stipulated time, a secondary enrichment in APW is necessary to inhibit the growth of background organisms. Other broth media such as bile salt sodium taurocholate (ST) broth and salt polymyxin broth (SPB) are also useful for enriching *V. parahaemolyticus* from seafood. Use of ST broth was shown to give better results than APW for isolating toxigenic *V. parahaemolyticus* from seafood. The most commonly used plating medium for *V. parahaemolyticus* and *Vibrio cholerae* is thiosulfate–citrate–bile salts–sucrose (TCBS) agar, which is available from several commercial sources. The nonsucrose fermenting *V. parahaemolyticus* strains are readily detected in this medium as large dark sticky green colonies (Figure 2). Compact Dry VP comprising peptone, NaCl, bile salts, antibiotics, chromogenic substrates, and polysaccharide gum as a cold water-soluble gelling is a ready-to-use culture medium for enumeration of *V. parahaemolyticus*. Blue–green colonies can be counted after 18–20 h of incubation at 35 °C. Better results were reported when two-step enrichment, one in APW followed by SPB in which CHROMagar *Vibrio* was used as the selective medium.

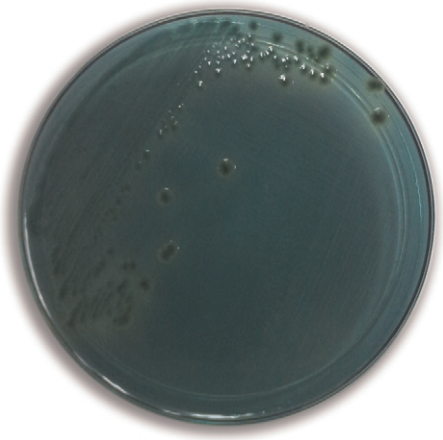

Figure 2 Sucrose nonfermenting pure culture of *V. parahaemolyticus* in TCBS medium.

For routine identification of suspected colonies, biochemical tests are mandatory. Either conventional tests or commercial systems can be adopted for identification and the tests should be very simple, rapid, and specific. API 20E and both of the Vitek cards, correctly identify more than 90% of the *V. parahaemolyticus* strains. However, extreme care must be taken in the interpretation of results with commercially available identification systems. It was suggested to increase the NaCl concentration while performing the biochemical tests to get reliable results. Generally, *V. parahaemolyticus* is negative for urease production. However, *trh* or both *tdh*- and *trh*-positive strains invariably produce urease. For KP test, fresh cultures of *V. parahaemolyticus* should be used with commercially available Wagatsuma medium (Eiken, Japan) containing 5% washed human erythrocytes, followed by incubation at 37 °C for 24 h. Positive strains will exhibit *β*-hemolytic zones around the growth. Those with a very narrow hemolytic zone are said to be KP indeterminate. For serotyping, antisera against O and K (poly and monovalent) antisera are available commercially (Denka Seiken Co., Ltd., Tokyo, Japan).

Immunological Methods

Antipolar flagellin monoclonal antibodies (mAbs) are useful for the rapid and selective isolation, concentration, and detection of *V. parahaemolyticus* cells from environmental sources. Cowan I *Staphylococcus aureus* cells coated at low concentration of mAb against *V. parahaemolyticus* polar flagellin specifically coagglutinated with the homologous antigen in 30 s, but not with other vibrios. In an immunomagnetic separation protocol, anti-H mAb exhibited 35–45% binding activity against 10^2–10^3 *V. parahaemolyticus* cells. TDH produced in the culture broth by *V. parahaemolyticus* strains can be detected by using a commercial kit (KAP-RPLA; Denka Seiken) that was based on the reversed-phase latex agglutination reaction with rabbit anti-TDH immunoglobulin G.

Molecular Methods

Pathogenicity of *V. parahaemolyticus* is related to hemolysins such as TDH, TRH, and thermolabile hemolysin. Direct-PCR targeting *tdh* and *trh* after sample enrichment in APW are the most successful methods for the detection of *V. parahaemolyticus*. The other gene targets such as *toxR*, *gyrB*, *tlh*, and the chromosomal fragment pR72H were evaluated for their accurate use in the PCR assay. The target gene *toxR* achieved the highest performance (100%) followed by *tlh* (91%). PCR assays targeting the *gyrB* and pR72H fragment were less reliable and, in some cases, difficult to interpret. PCR based on the detection of *toxR* can identify both pathogenic and nonpathogenic strains whereas *tdh* and *trh* help in the confirmation of pathogenic strains. Other genes such as *rpoA*, *pntA*, and *vpm* were also found to be specific, sensitive, and rapid for the detection of *V. parahaemolyticus* by PCR.

The loop-mediated isothermal amplification (LAMP) assay has shown to be sensitive and rapid in detecting many target genes such as *tdh*, *trh1*, *trh2*, *rpoD*, and *toxR* in less than 2 h. Based on the target gene, the sensitivity of the LAMP assay ranges from 1 to 450 CFU, that is more than 10-fold compared to conventional PCR assay. PCR analysis can be converted into most probable number (MPN) format targeting a stable marker gene for the quick quantification of *V. parahaemolyticus* from food samples. Real-time PCR for simultaneous detection of the species-specific *tlh* and the pathogenicity markers genes such as *tdh* and *trh* are recommended for the identification of *V. parahaemolyticus* from oysters. To eliminate false-negative reporting, an internal amplification control (IAC) has been incorporated to ensure the PCR integrity. This assay was specific and sensitive with < 10 CFU per reaction of pathogenic *V. parahaemolyticus* with a background of $> 10^4$ CFU per reaction of total *V. parahaemolyticus* and can be used in the MPN format. Real-time reverse transcription-PCR method was employed to determine the viable but nonculturable (VBNC) *V. parahaemolyticus* targeting virulence genes coding for the TDH and TTSS2 (VPA1354, VPA1346, and VPA1342) along with the housekeeping genes such as *rpoS*, *pvsA*, *fur*, and *pvuA* for quantification of expression. Combination of multiplex PCR and DNA–DNA hybridization on a microarray with *tlh*, *tdh*, and *fla* gene amplicons and their specific primers, respectively was also successfully tested using chemiluminescence with avidin-conjugated alkaline phosphatase.

Culture-based techniques for the identification of *V. parahaemolyticus* from environmental samples may be misleading as most of the bacterial cells will remain in a state called VBNC. A fluorescence visualization technique, called recognition of individual gene fluorescence *in situ* hybridization (RING-FISH), which targets *tlh* has been useful in the identification of *V. parahaemolyticus*. When coupled with the Kogure method to distinguish viable from dead cells, RING-FISH probe reliably enumerated total, viable *V. parahaemolyticus*.

Based on the phylogenetic difference in the 23S ribosomal RNA (rRNA) gene of various *Vibrio* species, specific DNA probes have been designed and assessed. Reverse line blot assay showed that these probes specifically detected different pathogenic and nonpathogenic vibrios from the marine environments. DNA diagnostic chips with type-specific PCR primers labeled with biotin and oligonucleotide probes have been successfully used in the rapid identification of *V. parahaemolyticus* and other foodborne pathogens in a 96-well format. Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) has become a

powerful tool for rapidly characterizing bacterial species. Whole-cell MALDI-TOF MS-based proteometric characterization was useful for the rapid identification of *V. parahaemolyticus*. Closely related species like *Vibrio alginolyticus, V. cholerae,* and *Vibrio mimicus* could be differentiated with other signatures of species-identifying biomarker ion such as *rpoB* gene. Construction of phylogenetic trees from MALDI-TOF MS and *rpoB* sequences revealed a very good congruence between both methods.

Rapid multiplexed bead-based mesofluidic system (BMS) distinguishes many foodborne pathogenic bacteria, including *V. parahaemolyticus*. BMS is based on the utilization of isothiocyanate-modified microbeads, which are immobilized with specific amino-modified oligonucleotide probes and placed in polydimethylsiloxane microchannels. When pumped into microchannels, the PCR products will hybridize with the oligonucleotide-modified beads and hybridization signals could be detected using a conventional microarray scanner. The detection procedure can be performed in less than 30 min with high sensitivity and specificity with 500–6000 CFU per ml.

Risk Factors and Reservoirs

Bivalves feed by filtering enormous volumes of water. In this process, it accumulates many marine microbes including pathogenic bacteria and viruses. Many investigations show that presence of *V. parahaemolyticus* and other vibrios from 20 to 38% of the bivalves sampled irrespective of the place and quality of the water. Studies on the seasonal incidence of *V. parahaemolyticus* in Mobile Bay oysters, AL, USA, showed their densities ranging from <10–12 000 per g and were associated with higher water temperatures. Water temperature is inversely related to the prevalence of diverse serotypes of pathogenic strains. In the Great Kwa River estuary Calabar, Nigeria, a trimodal peak in *Vibrio* counts was noticed with 13.6% isolation rates of *V. parahaemolyticus* during the warmer periods. After harvesting, storing oysters at ambient temperatures ($\sim$26 °C) leads to proliferation of *V. parahaemolyticus* to approximately 13- to 26-fold within 24 h with increase in the detection levels up to 40%.

The emergence and spread of *V. parahaemolyticus* pandemic strain in Peru in 1997 was due to El Niño events. This pattern was similar to the previously reported onset of cholera epidemic in 1991. Thus, the movement of oceanic waters seems to be one of the driving forces for the spread of *Vibrio*-associated diseases. El Niño is also suspected for geographic dispersion of pandemic strains in Antofagasta, Chile, and other South American coastal regions. *V. parahaemolyticus* densities in oysters varied seasonally and were found to be positively correlated with water temperature, turbidity, and dissolved oxygen.

Reports on the incidence of *V. parahaemolyticus* in African regions are few. In Limbe and Tiko, Cameroon, of the 236 shrimps examined, 55 (44%) were contaminated with *V. parahaemolyticus*. Gastroenteritis caused by *V. parahaemolyticus* has been associated with foods prepared with seaweeds. Studies carried out in Kii Channel, Japan, showed that seaweeds supported diverse *V. parahaemolyticus* throughout the year and its abundance in seaweeds was at least 50 times higher during summer. In Khanh Hoa province of Vietnam, the observed risk factor for *V. parahaemolyticus* infections was high socioeconomic status indicting its typical foodborne nature rather than its associating with hygiene and sanitation.

Survival Strategies

Growth kinetics of nonpathogenic *V. parahaemolyticus* was more rapid than that of pathogenic *V. parahaemolyticus*, regardless of tested model medium. This may be the reason why the nonpathogenic strains are abundant in the natural environments and bivalves. In shellfish growing areas, densities of *V. parahaemolyticus* is correlated significantly with water temperature than with salinity. In tropical regions, the MPN values of *V. parahaemolyticus* increases in accordance with rise in water temperature. This phenomenon has been reported in many places including southern coasts of Sao Paulo State, Brazil. Studies conducted in Mississippi indicated that there is an intraseasonal variation in the densities of total and pathogenic *V. parahaemolyticus* in oysters and overlying waters during summer. Regression analyses indicated significant associations ($p<.001$) between total *V. parahaemolyticus* densities with salinity and turbidity of the water and in oysters. Algal-derived organic matter and temperature can influence the abundance of different *Vibrio* spp. In microcosm studies, it was proved that the cyanobacterial-derived organic matter represented an important factor regulating growth and abundance of *V. parahaemolyticus* in brackish waters.

VBNC concept has been defined in 1982, which demonstrate the existence of bacteria that lost their capability to reproduce in culture, but stay live, keeping their metabolic activity intact. However, bacteria in VBNC state can multiply and cause infection under suitable conditions. Specific proteins expressed in the VBNC induction/VBNC state or strongly down-regulated in the starved cells were identified. The upregulated proteins are known to be associated with transcription (two homologs of α-subunit DNA-directed ribonucleic acid (RNA) polymerase and phosphoribosylaminoimidazole carboxamide formyltransferase/inosine monophosphate (IMP) cyclohydrolase), translation (ribosomal protein S1, two homologs of elongation factor thermo unstable (EF-TU), and elongation factor guanosine (EF-G), adenosine triphosphate (ATP) synthase (F1α-subunit), gluconeogenesis-related metabolism (dihydrolipoamide acetyltransferase and glyceraldehyde 3-phosphate dehydrogenase), antioxidants (two homologs of peroxiredoxins and AhpC/Tsa family), and a conserved hypothetical protein with unknown function. These proteins may play important roles in the induction or maintenance of VBNC *V. parahaemolyticus*. Concomitant cold and carbon starvation treatment of *V. parahaemolyticus* leads to VBNC state and these stresses are also associated with the expression of cytoskeleton genes such as *mreB*, *minE*, and *ftsZ*.

Owing to its halophilic attribute, *V. parahaemolyticus* is distributed in estuarine and coastal ecosystems all over the world. It has the ability to grow in fluctuating saline environments with the mechanisms of osmoadaptation through two putative compatible solute synthesis systems (encoded by *ectABC* and *betABI*) and six putative compatible

solute transporters (encoded by four *bcct* loci and two *proVWX* loci). To master the hyperosmotic stress of saline environments, *V. parahaemolyticus* not only accumulate osmoprotectants through uptake or endogenous synthesis of compatible solutes, but also remodel its profiles of outer membrane protein to restore its cell membrane. Lysine decarboxylase plays a role in the adaptive acid tolerance response in *V. parahaemolyticus*. Transcriptional analyses of lysine decarboxylase gene operon *cadA* and *cadB* revealed enhanced acid induction by external lysine.

Incidence and Epidemiology of *V. parahaemolyticus* Infection

In Kenya, acute gastroenteritis associated with increase in fish consumption has been reported since 1951 and the incidence of *V. parahaemolyticus* has increased due to foodborne outbreaks. Among vibrios, the proportion of *V. parahaemolyticus* was found to be high (13.6%) in shellfish from the Great Kwa River estuary, Calabar, Nigeria. Shrimps harvested from the coastal waters of Limbe and Tiko, South West Cameroon, were contaminated with *V. parahaemolyticus* (44%), which is in parallel to its incidence (12%) associated with diarrhea. Rural wastewater treatment facilities in the Eastern Cape province of South Africa were reported as potential sources of vibrios including *V. parahaemolyticus* (23.1%). During 2004–05 in Dakar, Senegal, along with *V. cholerae* and *V. parahaemolyticus*, gastroenteritis was also identified due to consumption of contaminated food. In Osaka and Kansai Airport Quarantine Station, *V. parahaemolyticus* was detected among 29 587 (7%) overseas travelers suffering from diarrhea during 1979–95, which was higher than the expected enterotoxigenic *Escherichia coli* level (5.4%).

In February 1996, *V. parahaemolyticus* serovar O3:K6 was first detected in Kolkata that accounted for 50–80% of the acute diarrheal cases. Subsequent to this report, several outbreaks and sporadic cases were reported from many parts of the world. Emergence of this new clone in 1996 was the first documented pandemic spread of *V. parahaemolyticus*. Subsequent to the emergence of O3:K6, other serovars, such as O4:K68, O1:K25, and O1:KUT (untypeable), that had genetic characteristics identical to that of the O3:K6 serotype were recognized. Pandemic serovars O3:K6 and O4:K68 were identified from hospitalized diarrhea patients in Beira, Mozambique, from January to May 2004, which were closely related to the Asian pandemic strains. The other serovars such as O3:K58, O4:K13, O3:KUT, and O8:K41 had different characteristics.

Vibrio parahaemolyticus causing an epidemic in Antofagasta, Chile, was first reported between November 1997 and April 1998 due to consumption of shellfish. O3:K6 strain was detected in southern Chile in 2004, and 8000 seafood-related diarrhea cases were identified. Serovar O3:K6 caused outbreaks in Puerto Montt from 2004 to 2006 with 3600 cases in 2005. In de los Lagos, 3725 diarrheal cases were reported during 2004–05. Cases steadily decreased to a total of 477 in 2007 with 40% were associated with a pandemic strain of a different serovar (O3:K59). In the summer of 2008, diarrheal cases again increased from 477 to 1143 and 98% of the clinical cases were associated with the pandemic O3:K6 serovar.

Bacterial foodborne disease surveillance detected 1082 outbreaks in China during 2005–08 mainly due to *V. parahaemolyticus* infections. Majority of the strains (79%) were identified as pandemic O3:K6. The other pandemic serovars encountered in this study were O1:KUT, O1:K25, O1:K26, and O4:K68 along with new servoars O1:K36, O3:K25, and O3:K68. In Hangzhou, 13 food poisoning outbreaks during 2000–02 were caused by O3:K6 in 11 episodes (84.6%) and O4:K8 with other two outbreaks (15.4%). From 2001 to 2002, 23 different serovars were identified in a case-control study of expatriates and Thai adults. The pandemic strains belonged to three recognized serovars (O3:K6, O1:K25, and O1:KUT) along with a new serovar, O3:K46, which was closely related to the pandemic strains. In Songkhla Province, Thailand, pandemic *V. parahaemolyticus* was constantly high ($>60\%$) from 2000–03 and decreased in 2004–05.

During 1999–2000, large outbreaks of *V. parahaemolyticus* infections were reported in the coast of Galicia in north-western Spain. Environmental investigation has shown that the overall incidence of *V. parahaemolyticus* was 12.5% primarily in areas of reduced salinity close to freshwater discharge points. Characterization of these strains resulted in the identification of same serotypes and identical DNA profiles indistinguishable from the clinical source. In 2005, pandemic *V. parahaemolyticus* O3:K6 strain was introduced in France through contaminated seafood. Recurrent presence of pandemic serovars O3:K6 and O1:KUT were reported in Italy during 2008.

Molecular Typing Methods

PCR-Based Typing

PCR-based typing has good discriminative ability and can be used as a rapid means of comparing *V. parahaemolyticus* strains for epidemiological investigation. The random amplification of polymorphic DNA (RAPD) with the use of single shot primer has identified genetic variability within *V. parahaemolyticus* strains from clinical and seafood samples. Strains from Chinese seafood samples harboring *tdh* and *trh* were separated into distinct groups. Fingerprinting of *V. parahaemolyticus* from sporadic diarrhea patients and seafood in Hangzhou with enterobacterial repetitive intergenic consensus sequence (ERIC)-PCR, and RAPD showed that almost all the O3:K6 strains from food poisoning and sporadic diarrhea patients were close to each other, and there were difference between O3:K6 strains from clinical and O3:KUT strains from environment. The pandemic (O3:K6) and nonpandemic seorvars (O3:KUT) from Brazil were separated by the RAPD-PCR into two amplification patterns. The O3:K6 strains isolated from clinical sources in Bangladesh, Taiwan, Laos, Japan, Thailand, Korea, and the USA between 1997 and 1998 showed identical banding profile in an arbitrarily primed PCR. In addition, the other serovars such as O4:K68 and O1:KUT were also identified as the same clone. Cluster analysis of amplicons generated using primers specific for conserved ribosomal gene spacer sequence (RS), repetitive extragenic palindromic sequence (REP), and ERIC indicated that these methods apparently differentiated *V. parahaemolyticus* strains and grouped them into 15, 27, and 27 patterns, with discrimination

indexes of 0.91, 0.97, and 0.98, respectively. REP-PCR is preferable to ERIC-PCR because of the greater reproducibility of the fingerprints, whereas RS-PCR may be a practical method because it generates fewer amplification bands and patterns than the alternatives.

Ribotyping

Ribotyping exploits the DNA polymorphism of rRNA genes (*rrn*) in the chromosome of *V. parahaemolyticus*. Universal probe generated from the *E. coli* RNA is used to screen the restriction patterns of genomic DNA of the test strains. *V. parahaemolyticus* has 11 copies of ribosomal operons and the intergenomic *rrs* recombination is very high in this species. Pandemic O3:K6, O4:K68, and O1:KUT serovars that emerged after 1996 from different countries were indistinguishable in the ribotyping analysis, whereas the O3:K6 and O1:KUT serovars isolated before 1995 had different profiles. This result also indicated that the O4:K68 and O1:KUT strains most likely originated from the pandemic O3:K6 clone with different serovar background. Ribotyping of *Eco*RI and *Pst*I profiles of *V. parahaemolyticus* O3:K6 from two US outbreaks of oyster-associated gastroenteritis in 1998 with the use of a Qualicon riboprinter showed involvement of a single strain closely related to the Asian clone. In contrast, multiple strains were involved in the Texas outbreak and genetically distinct from the north-eastern and Asian clone.

Multilocus Sequence Typing (MLST)

MLST of *V. parahaemolyticus* with housekeeping genes from both the chromosomes has been applied to *V. parahaemolyticus* strains. Several sequence types were identified, which are available in the database (http://pubmlst.org/vparahaemolyticus). In the eBURST (based upon related sequence types) analysis, three major clonal complexes were identified with strains originating from the Pacific and Gulf coasts of USA and the third clonal complex consisted of strains of pandemic clone all over the world. MLST of *V. parahaemolyticus* associated with foodborne illness in Florida has identified 13 novel sequence types and 7–9 novel alleles for each locus. MLST diversity appears primarily due to frequent recombination rather than mutation, with the estimated ratios of 2.5:1 and 8.8:1 by allele and site, respectively.

Variable Number of Tandem Repeat (VNTR) Loci

In housekeeping genes, regions known as VNTR are classified on the basis of their repeat units. These repeat loci are highly conserved and hence the discrimination power is more compared to that of MLST. VNTR-based fingerprinting discriminated clinical O3:K6 strains from different outbreaks and sporadic cases in Tokyo during 1996–2003. Multiple-locus VNTR analysis (MLVA) displayed between 2 and 15 alleles at each of 8 loci and shown to have high resolution and reproducibility for typing of *V. parahaemolyticus*. VNTR assay was applied to clinical and environmental *V. parahaemolyticus* strains from Chile with 24 potential loci on both chromosomes. In this analysis, 59 MLVA groups were identified, providing differentiation among very closely related strains.

Pulse-Field Gel Electrophoresis (PFGE)

PulseNet is an International molecular subtyping network for foodborne disease surveillance closely monitored by the Centers for Disease Control (CDC, USA). In this network, the member laboratory performs molecular subtyping of pathogens using a standardized method PFGE after comprehensive testing process allowing the sharing of data with other members as well as with the CDC. A standard PFGE protocol is available for subtyping *V. parahaemolyticus* for use in PulseNet activities. Comparative analysis of the PFGE profiles generated by different laboratories demonstrated that the protocol is both reliable and reproducible.

Phylogenetic analysis of *Not*I PFGE profiles showed that the 1998 O3:K6 and O4:K68 strains from India and Thailand formed a cluster with 78–91% similarity indicating a close genetic relationship between the two different serovars. In 1999, *V. parahaemolyticus* caused a large outbreak associated with raw oyster consumption in Galicia, Spain. O4:K11 and O4:KUT serovars were identified in this outbreak, which were not related to the pandemic serovars. Norwegian environmental trh^+ and/or tdh^+ strains were different from prototype trh^-/tdh^- strains. However, the clinical strains of O3:K6 and O3:KUT were identical or related to a pandemic reference strain. Large epidemics of diarrhea associated with seafood consumption and *V. parahaemolyticus* occurred for the first time during 2004–05 in Puerto Montt, Chile. PFGE analysis confirmed that the epidemics were caused by the O3:K6 pandemic serovar in this region. Pandemic serovars of *V. parahaemolyticus* O3:K6 and O4:K68 spread to Beira, Mozambique, in 2004–05. In the PFGE, these strains were closely related to the pandemic strain that emerged in Asia in 1996. The O3:K58, O4:K13, O3:KUT, and O8:K41 strains showed unique characteristics different from the pandemic clone. The *Sfi*I PFGE patterns of *V. parahaemolyticus* strains from Taiwan showed 115 different patterns grouped into 13 types with dissimilarity value of <15. Type I consisted exclusively the pandemic O3:K6 strains and genetically closely related strains. *V. parahaemolyticus* associated diarrheal outbreaks were reported from Shenzhen, China, in 2007–08. In these outbreaks, O3:K6, O4:K8, and O1:KUT serovars accounted for 67.9%, 7.5%, and 6.1%, respectively. Identical PFGE patterns were recorded from six locations.

Pathogenesis

Pathogenic *V. parahaemolyticus* are known to produce TDH, TRH, or both. TDH exists as a tetramer in solution and possesses β-hemolytic activity. TRH is further differentiated into TRH1 and TRH2 on the basis of genetic and phenotypic differences. Almost all the clinical *V. parahaemolyticus* strains display β-type hemolysis on Wagatsuma agar and thus are known as KP^+ strains, which has been considered an important marker to distinguish pathogenic strains from nonpathogenic ones. TDH has been considered as a major virulence factor of gastroenteritis because it has biological activities, including cytotoxic and enterotoxic activities. Five variants of the *tdh* genes have been identified, of which *tdh*1 and *tdh*2 are associated with KP, and the rest are negative for KP. These *tdh* variants share 97% sequence identity. The *trh*

gene shares approximately 68% sequence identity with the *tdh* gene. The *trh* sequence variants can be separated into two groups: the *trh*1 and *trh*2 that share 84% sequence identity.

Vibrio parahaemolyticus has two sets of TTSS genes on chromosomes 1 and 2 (TTSS1 and TTSS2, respectively). TTSS1 is responsible for cytotoxicity, whereas TTSS2 is a major contributor to enterotoxicity and is present only in KP^+ strains. Tissue culture assay and animal models with nonfunctional mutant TTSSs *V. parahaemolyticus* strain harboring *tdh*, showed that TTSS1 alone was involved in cytotoxic activities in all the cell lines. TTSS1 and TDH played a significant role in lethal activity in a murine infection model. The TTSS1 induces rapid cellular death that initiates with acute autophagy. TTSS2 and TDH had cytotoxic effects on a limited number of cell lines. *In vitro* studies demonstrate that TTSS1 genes are positively regulated by ExsA and negatively regulated by ExsD.

TTSS translocon complex is composed of several associated proteins, which form a translocation channel through the host cell plasma membrane and deliver effector proteins into host cells. In *V. parahaemolyticus* there is only one gene (the VPA1362 gene) in the TTSS2 region that is homologous to other translocon protein genes. VPA1362 (designated VopB2) and VPA1361 (designated VopD2) are the TTSS2-dependent secretion proteins. Functional analysis of these proteins showed that they are essential for TTSS2-dependent cytotoxicity, for the translocation of one of the TTSS2 effector proteins (VopT), and for the contact-dependent activity of pore formation in infected cells *in vitro* and enterotoxicity *in vivo*. VPA1327 (*vopT*), a gene encoded within the proximity of TTSS2, is partly responsible for cytotoxic effect in Caco-2 and HCT-8 cells. VopT is an adenosine diphosphate (ADP)-ribosyltransferase effector protein secreted via TTSS that has approximately 45% and 44% identity with the ADP-ribosyltransferase domain of ExoT and ExoS, respectively. TTSS2 was found to be necessary not only for the secretion, but also for the translocation of the VopT into host cells.

The genes for TTSS2 have not been found in *trh*-positive (KP^-) strains, which are also pathogenic for humans. However, in a *trh*-positive *V. parahaemolyticus* strain, a novel TTSS was identified, which was found strongly associated with enterotoxicity in animal experiments. Phylogenetically, this novel TTSS is closely related to TTSS2 of KP^+ *V. parahaemolyticus* though it belongs to a distinctly different lineage.

Vibrio parahaemolyticus induces a rapid remodeling of macrophage actin and activates RhoB guanosine triphosphatase (GTPase). This effect was identified on TTSS1 that regulated translocation effector protein VP1686 into the macrophages. This remodeling of actin is shown to be necessary for increased bacterial uptake and triggering an 'eat-me-and-die' signal to the host, that is, initiation of apoptosis in macrophages. The ability of *V. parahaemolyticus* to inhibit Rho family GTPases also causes cytoskeletal disruption in HeLa cells. The inhibition of Rho family GTPase activation was identified both in clinical and environmental strains and was dependent on TTSS1. In addition, Rho inhibition was accompanied by a shift in the total actin pool to its monomeric form. These phenotypes were not found in Vp1686 deletion mutants indicating that the inhibiting actin polymerization may be a downstream effect of Vp1686-dependent GTPase inhibition.

Norepinephrine (NE) is released when a host of physiological changes are activated by a stressful event. NE controls the functions of the gastrointestinal tract, but its role in the pathogenicity of enteropathogens is not clear. NE stimulated the cytotoxic activity of *V. parahaemolyticus* in Caco-2 cells and the transcription of the TTSS1-related genes *vscQ* and *vscU*. In rat ileal loop model, the enterotoxicity of *V. parahaemolyticus* was increased by NE through interaction with $\alpha(1)$-adrenergic receptors. These results indicate that $\alpha(1)$-adrenergic receptors on the intestinal epithelium appear to interact with *V. parahaemolyticus* enterotoxicity.

In many Gram-negative bacteria, the type 2 secretion system (T2SS) is responsible for the transport of a diversity of proteins from the periplasm across the outer membrane into the extracellular space. Cholera toxin is one of the T2SS secreted proteins in *V. cholerae*. The T2SS consists of three subassemblies, one of which is the inner membrane complex, which contains multiple copies of five proteins, including the bitopic membrane protein EpsL. The 2.3 Å resolution crystal structure of the periplasmic domain of EpsL from *V. parahaemolyticus* showed 56% identical in sequence to its homolog in *V. cholerae*. Capsular polysaccharide (CPS) aids in adherence to epithelial cells and plays an important role in the initiation of pathogenesis of *V. parahaemolyticus*. The role of CPS in adherence was established by testing the opaque (with CPS) and translucent (with less CPS) colonies, purified CPS, and anti-CPS antibodies. *V. parahaemolyticus* extracellular zinc metalloprotease (VPM) is a putative virulence factor for host infection. It is synthesized from the *vpm* gene as a polypeptide of 814 amino acids with an estimated molecular mass of 89 833 Da, containing a HEXXH consensus motif.

Decontamination and Other Preventive Measures

Vibrio parahaemolyticus is a common cause of shellfish-related gastroenteritis all over the world. Several effective decontamination and other preventive measures are introduced for food safety to obliterate this pathogen. Because the infective dose of *V. parahaemolyticus* is $>10^4$ cells per serving of oysters or other foods, quantification of this pathogen is mandatory at the industrial level. Postharvest processing (PHP) greatly reduce exposure of *V. parahaemolyticus* and other vibrios to oysters and pose a much lower risk of illness to consumers. PHPs such as on-board and dockside icing of oysters are less effective in reducing the levels of *V. parahaemolyticus* in oysters.

The National Shellfish Sanitation Program's (NSSP) PHP for *V. parahaemolyticus* recommends flash freezing, followed by storage at $-21\,^{\circ}C$. In some studies it was demonstrated that storing Pacific oysters at $-10\,^{\circ}C$ was more effective in inactivating *V. parahaemolyticus* than was storing at -20 or $-30\,^{\circ}C$, as the levels of *V. parahaemolyticus* in oysters were reduced after 6 months by 4.55, 4.13, and 2.53 log MPN per g, respectively. In shell stock and shucked oysters, *V. parahaemolyticus* decreased from 5.46 to 0.38 log MPN/g after 75 days of storage at $-30\,^{\circ}C$, and at $-18\,^{\circ}C$ no viable cells were detected after 60 days.

Extended depuration at $15\,^{\circ}C$ for 96 h increased reductions of *V. parahaemolyticus* in American oyster (*Crassostrea virginica*) than at $22\,^{\circ}C$. HPP conditions (pressure level, time, and

temperature) are useful to achieve a 5-log reduction of *V. parahaemolyticus* in live oysters. The recommended pressure treatment processes are $\geq$350 MPa for 2 min at temperatures between 1 and 35 °C and $\geq$300 MPa for 2 min at 40 °C. Treatment with X-ray could control pathogenic bacteria and extend the shelf life of oysters. X-ray (1–5 kGy) significantly ($p < .05$) reduced *V. parahaemolyticus* and inherent microflora on oysters to less than detectable limit (<1.0 log CFU per g). When tested with HPP, *V. parahaemolyticus* O3:K6 was more resistant to pressure than other serovars. In oysters, this serovar required a pressure of 300 MPa for 180 s for a 5-log reduction.

Chlorine dioxide (ClO_2) is considered to be a safe and effective disinfectant and is routinely applied for treatment of drinking water and seafood. Bioaccumulated *V. parahaemolyticus* in different oyster tissues could be disinfected completely after 6 h of treatment with 20 mg l^{-1} of ClO_2. After ClO_2 treatment, the shelf life of oysters was extended to at least 12 days at 4 °C. Pulsed low direct current or alternating low-amperage electric treatments (LAETs) of saline solutions exerts superior inactivation efficacy against *V. parahaemolyticus* owing to the generation of available chlorine. LAET at 263 mA for 100 ms eliminated all *V. parahaemolyticus* cells due to substantial structural damage at the cellular level. Treatment of electrolyzed oxidizing (EO) water (pH 2.7, chlorine 40 ppm, oxidation–reduction potential 1151 mV) inactivates *V. parahaemolyticus*. Rinsing the food contact surfaces with EO water or soaking cutting boards in EO water for up to 5 min could reduce cross contamination of *V. parahaemolyticus* during food preparation. The bactericidal activity of weakly acidic electrolyzed water (WAEW), a type of EO water was effective against *V. parahaemolyticus* when treated with sodium hypochlorite (NaClO), solution containing 35 mg l^{-1} available chlorine concentration (ACC) or WAEW containing 35 mg l^{-1} ACC. WAEW maintains bactericidal activities against *V. parahaemolyticus* under open storage conditions even after 5 weeks and could be used as a postharvest treatment to reduce *Vibrio* contamination in oysters. However, it is recommended that treatment should be limited to 4–6 h to avoid death of oysters.

In challenge studies, feeding shrimps with diets containing the potential probiotics showed its effectiveness by reducing disease caused by *V. parahaemolyticus*. Outer membrane protein antigens such as OmpW, OmpV, OmpU, and OmpK from *V. parahaemolyticus* are immunogenic during *in vivo* infection of fishes and could be the efficient vaccine targets in aquaculture. The US Food and Drug Administration has published a *V. parahaemolyticus* risk assessment for consumption of raw oysters that predicts densities of *V. parahaemolyticus* based on water temperature. This recommendation is further evaluated with the use of remotely sensed parameters for predicting incidence and risk associated with *V. parahaemolyticus* in Gulf Coast oysters. Using the remotely sensed surface temperature data as a basis for risk management/predictions, variations in *V. parahaemolyticus* density in oysters can be predicted.

See also: Foodborne Diseases: Overview of Biological Hazards and Foodborne Diseases; Prevalence of Foodborne Diseases in Western Pacific Region. Safety of Food and Beverages: Seafood

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Vibrio vulnificus

I Karunasagar, Food and Agriculture Organization of the United Nations, Rome, Italy

Glossary

Benthic fish Fish that live at the lowest level of an aquatic body such as sediment surface or subsurface.
Oligonucleotide Short single-stranded DNA or RNA molecules.
Open reading frame (ORF) A sequence of nucleotides in a reading frame (set of consecutive non-overlapping triplets coding for amino acids) and contains a start codon but does not contain a stop codon.
Plankton Small organisms living in a water column.
Plasmid A small DNA molecule that can replicate independently and is outside the chromosome in a cell.
Restriction enzyme An enzyme that cuts DNA at specific nucleotide sequences called recognition sites.

Background

In 1976, researchers at the Centers for Disease Control (CDC) in the USA carried out taxonomic studies on a group of halophilic *Vibrio* spp. isolated from blood cultures that fermented lactose and identified these as new species designated *Vibrio vulnificus* (wound inflicting). This organism can cause wound infections and septicemia that could be fatal in susceptible individuals.

Characteristics of the Pathogen

Vibrio vulnificus is a Gram-negative curved rod-shaped organism inhabiting warm water estuarine environments all over the world. The organism is motile by polar flagellum and can be distinguished from other members of the genus *Vibrio* by its ability to ferment lactose. *Vibrio vulnificus* is characterized by oxidase positive reaction, sensitivity to 10 μg O129, ability to ferment lactose, cellobiose; inability to ferment sucrose and arabinose; positive for ornithine and lysine decarboxylase, negative for arginine decarboxylase, capable of growth at 6% NaCl, but no growth at 8% NaCl. Presently three biotypes are recognized based on a combination of phenotypic, serologic, and host range characters. Biotype 1 strains are indole positive, serologically diverse and are associated with human infections. Biotype 2 strains are indole negative and considered mainly as eel pathogens, but may also be opportunistic human pathogens, being associated with infections in eel handlers. This biotype has three serotypes and strains associated with eel and human infections belong to serotype E. Biotype 3 has five atypical biochemical characters, is genetically clonal and has been isolated from some patients in Israel with wound infection or septicemia. This biotype has not been associated with foodborne infections and reported infections are limited to persons handling tilapia fish.

The virulence of this organism is presumably related to multiple factors such as presence of a polysaccharide capsule, ability to obtain iron from transferrin, ability to produce extracellular enzymes and exotoxin. Pathogens ingested through food encounter highly acidic gastric environment as the first host defense. Exposure of *V. vulnificus* to low acid environments leads to increased expression of lysine decarboxylase, leading to breakdown of lysine to cadavarine, which functions as acid neutralizer and as a superoxide radical scavenger that contributes to oxidative stress tolerance. The capsule has an important role in resistance to host defenses like phagocytosis and nonencapsulated transposon mutants have four times higher lethal dose compared to the wild-type strains. However, most freshly isolated environmental strains are capsulated, irrespective of their virulence to mice. Antibodies specific to capsule are detected in patients with *V. vulnificus* infections. Iron acquisition system plays an important role in virulence of *V. vulnificus*. Mouse lethal doses are greater than 10^6, but drop down to 10^5-fold lower in iron dextran treated mice. Individuals with underlying chronic liver diseases have a higher susceptibility to *V. vulnificus* infections and generally such conditions are associated with impaired iron metabolism. Most iron in human serum is bound to transferrin making it unavailable to invading pathogens and *V. vulnificus* has multiple systems of iron acquisition such as ability to produce both catechol (vulnibactin) and hydroxamate siderophores. The *V. vulnificus* hemolysin has cytotoxicity to a variety of mammalian cell lines and has 60–65% amino acid homology with non-O1 *Vibrio cholerae* hemolysin and *V. cholerae* ElT or hemolysin. Mice injected with *V. vulnificus* hemolysin display lesions similar to that noticed in infected humans. *rtxA1* gene homologous to *rtxA* toxin gene of *V. cholerae* has been identified in *V. vulnificus* and studies in mutants suggest that this toxin could play a role in causing cell necrosis and facilitating invasion of *V. vulnificus* into blood stream by crossing the intestinal epithelium. Enzymes such as

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Table 1 Methods used for differentiation of clinical and environmental strains of *V. vulnificus*

Method	*Findings*
Sequencing of 492 bp fragment of 16S rRNA gene	Presence of *Alu*I cleavage sites after nt 202 and 244; *Hae*III sites after nt 168 and 372. Most environmental strains have additional *Alu*I site after nt 140 and most clinical strains have additional *Hae*III site after nt 147
Multilocus sequence typing based on six housekeeping genes	Strains divided into Lineage I consisting exclusively of Biotype 1; Lineage II consisting of Biotypes 1 and 2. Most clinical isolates are clustered in Lineage I
PCR based on 700 bp sequence surrounding 200 bp region found in most clinical isolates	Two groups based on the sequence of the portion of open reading frame (ORF) VV0401 in this 700 bp region – C-type (clinical strains) and E-type (environmental strains)

lecithinase, lipase, caseinolytic protease, and DNase are produced by most environmental and clinical strains.

Most of the virulence associated factors are present in over 95% of environmental strains. This has led to further studies on various molecular typing methods to discriminate environmental and clinical strains (Table 1). Differences in the sequence of 492bp fragment of 16S rRNA gene of *V. vulnificus* strains have been recorded. This fragment has cleavage site for the restriction enzyme *Alu*I after nucleotides 202 and 244, and *Hae*III cleavage sites after nucleotides 168 and 372. Most environmental strains have an additional *Alu*I cleavage site after nucleotide 140 and most clinical strains have an additional *Hae*III cleavage site after nucleotide 147. Analysis of clinical and environmental strains using multilocus sequence typing of six housekeeping genes has revealed that two main lineages can be recognized with Lineage I consisting exclusively of Biotype 1 isolates and Lineage II consisting of isolates belonging to Biotype 1 and all isolates of Biotype 2. The proportion of clinical isolates in Lineage 1 is much higher than those in Lineage II and a 33kb genomic island is unique to Lineage I. Partial alignment of the sequence of an open reading frame (ORF) VV0401 referred to as virulence coregulated gene or *vcg* indicates that clinical and environmental strains cluster into two distinct groups, C-type (90% clinical strains) and E-type (93% environmental strains). Polymerase chain reaction (PCR) based on sequence variations in this region can distinguish the two groups of strains. Examination of sequence data for eight genes including virulence associated and housekeeping genes in multiple strains confirms that the C-genotype and E-genotypes can be distinguished.

The complete genome sequence of *V. vulnificus* YJ016 isolated from a case of primary septicemia is available. The genome includes two chromosomes of approximately 3377 and 1857 kbp size and a plasmid of 48 kbp size. Most of the genes coding for putative virulence factors are present in the small chromosome.

Ecology

Vibrio vulnificus is widely distributed in warm coastal environments throughout the world, but detailed ecological studies have been done only in a few countries. This organism can be isolated from coastal marine and estuarine waters, sediment, plankton, various shellfish (both molluscan and crustacean), and finfish species. The abundance varies considerably and is greatly influenced by temperature and salinity. In North America, higher densities are observed in mid-Atlantic, Chesapeake Bay, and Gulf coast waters, where temperatures are warmer throughout the year, whereas densities are lower in Pacific, Canadian, and North Atlantic waters. The lowest temperature at which *V. vulnificus* has been isolated varies geographically, being 8 °C at Chesapeake Bay and <12.5 °C in Gulf coast of the USA. The organism survives in sediment during winter. In warm tropical environments, as in India, salinity is the major factor affecting the abundance of this organism. Effect of salinity can be seen even in temperate waters. In US waters, numbers of *V. vulnificus* were high at salinity between 5‰ and 25‰, but dropped by 58–88% at salinities over 30‰. *Vibrio vulnifucus* can colonize plankton and fish gut. *Vibrio vulnificus* produces chitinase, which might help the organism to colonize zooplankton. 14.1% aquatic birds in Japan are positive for *V. vulnificus*.

The levels of *V. vulnificus* in the USA Gulf coast oysters could be approximately 10^4 cfu g^{-1} during summer months and usually less than 10 per g during winter, and in Indian oysters, 10^3 cfu g^{-1} could be detected when salinities were lower than 25‰. Although the levels in oysters could be 100 times higher than in seawater, numbers exceeding 10^6 per g have been reported from the intestines of benthic fish inhabiting oyster reefs. In Japan, highest densities of *V. vulnificus* ($10^3 \sim >10^6$ MPN 10 g^{-1}) were observed from fish in Lake Shinji. There is no correlation between the prevalence or occurrence of *V. vulnificus* and fecal contamination of waters, hence fecal coliforms/*Escherichia coli* cannot be used as an indicator organism for this pathogen.

Survival in the Environment and Food

Vibrio vulnificus can enter into viable non-culturable (VBNC) phase under conditions of low nutrient or temperature. In the VBNC state, *V. vulnificus* cells are small (0.3 μm) cocci and on resuscitation, they regain rod-shaped (3 μm × 0.7 μm) morphology. Changes in fatty acid composition of cell membrane in *V. vulnificus* in response to lower temperatures has been recorded, with proportional increase in unsaturated fatty acids. Temperature shift from 35 to 5 °C resulted in VBNC state, however, when cells were subjected to 15 °C before downward shift, *V. vulnificus* cells remained viable. *Vibrio vulnificus* cells prestarved for 24 h before exposure to 5 °C remained culturable, but cells starved for the same period at

5 °C entered VBNC. VBNC *V. vulnificus* cannot be resuscitated after temperature upshift to 22 °C in nutrient rich broth, but can be revived in media supplemented with pyruvate or catalase and this has been attributed to increased sensitivity of cells in VBNC state to hydrogen peroxide. 10^5 VBNC cells injected intraperitoneally were lethal to mice.

Vibrio vulnificus does not grow in oysters at temperatures below 13 °C and prolonged refrigeration could lead to reduction in numbers. Although some investigators noted that levels in refrigerated shellfish became nondetectable (<3 per g) in 14–21 days, others observed survival in artificially contaminated oysters for 14 days at 2 °C, suggesting that refrigeration cannot be relied upon for elimination of this pathogen in oysters. The rate of decline in refrigerated oyster shellstock has been estimated to be 0.041 log unit per day. 4–5 $\log_{10}$ reduction in numbers of natural *V. vulnificus* population in oysters occur when frozen to −40 °C and stored for 3 weeks. However cold adaptation at 15 °C may reduce the effectiveness of freezing. A combination of vacuum packaging and freezing can bring down *V. vulnificus* counts by 3–4 $\log_{10}$ units in 7 days and though numbers continue to decline till day 7, complete elimination cannot be achieved.

Vibrio vulnificus is sensitive to temperature with 6 $\log_{10}$ reduction in numbers occurring when subjected to 50 °C for 5 min in shucked oyster meat. Natural population of *V. vulnificus* (4.3×10^3 cfu g^{-1}) can be reduced to nondetectable levels by exposing them to 50 °C for 10 min. D-values at 47 °C are 3.44–3.66 min for opaque colonies and 3.18–3.38 min for translucent colonies. *Vibrio vulnificus* cells are inactivated at pH 2.0. *Vibrio vulnificus* is sensitive to ionizing radiation and irradiation doses of 1.0 kGy applied on whole shell oysters can reduce the cell numbers from 10^7 cfu g^{-1} to undetectable levels. Hydrostatic pressure of 250 MPa for 120 s reduced *V. vulinificus* >5 $\log_{10}$ units in oysters.

Vibrio vulnificus resides inside various tissues of oysters, hence depuration is ineffective in elimination of this pathogen, but relaying oysters in high-salinity (>30‰) waters for 17–49 days caused a decrease in population from 10^3 cfu g^{-1} to <10 MPN g^{-1}.

Clinical Manifestations

Vibrio vulnificus infections may manifest as wound infections or as primary septicemia. Foodborne infections result in primary septicemia in susceptible individuals and in most instances, these are sporadic. Primary septicemia is a systemic illness in a patient who has no wound infection preceding illness and is characterized by fever and shock and presence of *V. vulnificus* in blood or other sterile site. The incubation period ranges from 7 h to 10 days, with symptoms appearing in 36 h in most cases. Most patients present with sudden onset of fever and chills, generally accompanied with nausea, vomiting, abdominal pain, and hypotension (systolic pressure <85 mm). In over 60% cases, secondary lesions appear, mostly on the legs that often develop into necrotizing fasciitis or vasculitis that may require surgical debridement or amputation. *Vibrio vulnificus* can be isolated from blood and cutaneous lesions. Primary septicemia due to *V. vulnificus* is generally associated with consumption of raw or undercooked molluscan shellfish like oysters. The disease rarely (<5%) occurs in healthy individuals, and liver disease including cirrhosis due to alcohol consumption is a risk factor for *V. vulnificus* infection. Other predisposing factors are diabetes, gastrointestinal disorders (ulcer, surgery), hematological conditions, and immunocompromised condition associated with cancer, therapy with immunosuppressive drugs. The disease has a high fatality rate (approximately 50%), which is the highest among foodborne pathogens. However, the attack rate is low with one illness occurring per 10 000 meals of raw US Gulf coast oysters (containing *V. vulnificus*) served to the highest risk population, i.e., people with liver diseases. Epidemiological data suggests that men are more susceptible than women to *V. vulnificus* infection. Experimental studies with rats suggest the estrogen may play a role in protection, because mortality rate was 82% in normal male rats, 21% in normal female rats, and 75% in ovariectomized female rats.

It is estimated that approximately 100 cases of primary septicemia due to *V. vulnificus* occurs per year in the USA and nearly all the cases have been associated with consumption of raw oysters harvested from Gulf Coast with 90% of cases occurring during April–November. The Korean CDC estimates 40–70 confirmed cases per year and this high rate is suspected to be due to consumption of raw seafood or higher prevalence of predisposing factors. However, in Japan, it is estimated that 12–24 cases occur per year; and in Taiwan, there was a peak occurrence in 2000 with 26 cases per million populations. Records of infection are also available from India, Thailand, Europe, and Australia, but cases are rare. Interestingly, in Japan, oysters are not the primary source, because raw oysters are eaten only in winter and most infections occur during June–November with a peak in July. A mud shrimp *Upogebia major* was the common agent associated with *V. vulnificus* infections.

Vibrio vulnificus may also be associated with wound infections and 69% of these are related to occupational exposures among oyster shuckers and fishermen. Preexisting wounds are generally infected and inflammation is one of the first symptoms that may progress into cellulites and necrosis. Patients may become septicemic and experience fever, chills, mental state changes, and hypotension, but the fatality rate (20–30%) is lower than that for primary septicemia. Occasionally, *V. vulnificus* may also be associated with gastroenteritis. In such cases, the organism is isolated from stools only and illness is characterized by abdominal cramps, vomiting, or diarrhea. Of 422 *V. vulnificus* infections reported in the USA during 1988–96, 45% were wound infections, 43% primary septicemia, and 5% gastroenteritis.

As *V. vulnificus* is common in warm coastal waters, it has been suggested that climate change may widen the geographical range of infections. The outbreak in fish handlers in Israel in 1996 has been linked to warm summer that year. Cases in Europe are rare and are limited to wound infections.

Antibiotic therapy is important for both primary septicemia and wound infections. Tetracycline has been the most effective and in some cases, this antibiotic has been used in combination with third generation cephalosporin or gentamycin or chloramphenicol.

Methods for Detection and Enumeration in Foods

Several methods have been described for isolation and identification of *V. vulnificus* from foods and the environment. Currently, there are no internationally validated methods. The method described in US FDA *Bacteriological Analytical Manual* (BAM) for enumeration of *V. vulnificus* involves most probable number (MPN) analysis using alkaline peptone water, plating the tubes showing growth onto modified cellobiose-polymyxin-B colistin (mCPC) agar, identification of typical colonies by biochemical tests or by colony hybridization using enzyme-labeled DNA probe. Though traditionally, thiosulphate citrate bile salt sucrose (TCBS) agar has been used for isolation of pathogenic *Vibrio* spp., a number of studies have indicated that the selectivity of this medium is not adequate for isolation of *V. vulnificus* from environmental samples. Cellobiose-polymyxin-B colistin (CPC) agar has colistin and polymyxin as selective agents and cellobiose to differentiate *V. vulnificus* from non-cellobiose fermenting Vibrios like *V. cholerae*. Selectivity is further improved by incubation at 40 °C. This was modified (mCPC) by reducing the concentration of colistin from 1.4×10^6 to 4×10^5 U l^{-1}. Other selective agars described for *V. vulnificus* include *Vibrio vulnificus* agar (VV agar), sodium dodecyl sulfate-polymyxin B-sucrose (SPS) agar, *Vibrio vulnificus* medium (VVM) with or without colistin (VVMc), and *Vibrio vulnificus* enumeration (VVE) agar. Some investigators reported higher isolation rate using alkaline peptone water with polymyxin for enrichment and cellobiose-colistin (CC) agar for selective plating. Figure 1 summarizes the steps involved in conventional culture-based isolation or enumeration of *V. vulnificus*. The temperature of incubation varies with the selective agar used. Colony color depends on carbohydrate used. In media containing sucrose (TCBS, SPS), the colonies appear green or bluish green and in media containing cellobiose (CPC, mCPC, CC, VVM), the colonies appear yellow. In VV agar containing salicin, the colonies are gray with dark center and in VVE containing cellobiose, X-Gal (5-bromo-4-chloroindoxyl-β-D-galactopyranoside), the colonies are bluish green. Identification of colonies coming up on selective agars by biochemical tests could be a challenge due to phenotypic plasticity, but systems such as API20E or Biolog have been used. Isolates can also be identified by hybridization with digoxigenin-labeled DNA probe binding to the cytolysin gene *vvhA* or by PCR amplification of *vvhA* gene.

Alternate method described for isolation/enumeration of *V. vulnificus* involves direct plating of samples on nonselective agar, VV agar, followed by colony lifts on whatman filter paper, lysis of colonies, fixing of DNA, and hybridization with alkaline phosphatase-labeled probes binding to *V. vulnificus* cytolysin/hemolysin (*vvhA*) gene. Digoxigenin-labeled *vvhA* probes can also be used. Nitro-blue tetrazolium/5-bromo-4-chloro-3'Indolyl phosphate (NBT/BCIP) substrate is used for color development. For isolation of colonies reacting with the probe, the filter is aligned with the plate the colonies from the area reacting with probe are picked up, isolated by streaking on mCPC or CC agars and confirmed by PCR or probe hybridization (Figure 2). Results with MPN and direct probe hybridization methods have been found to be comparable,

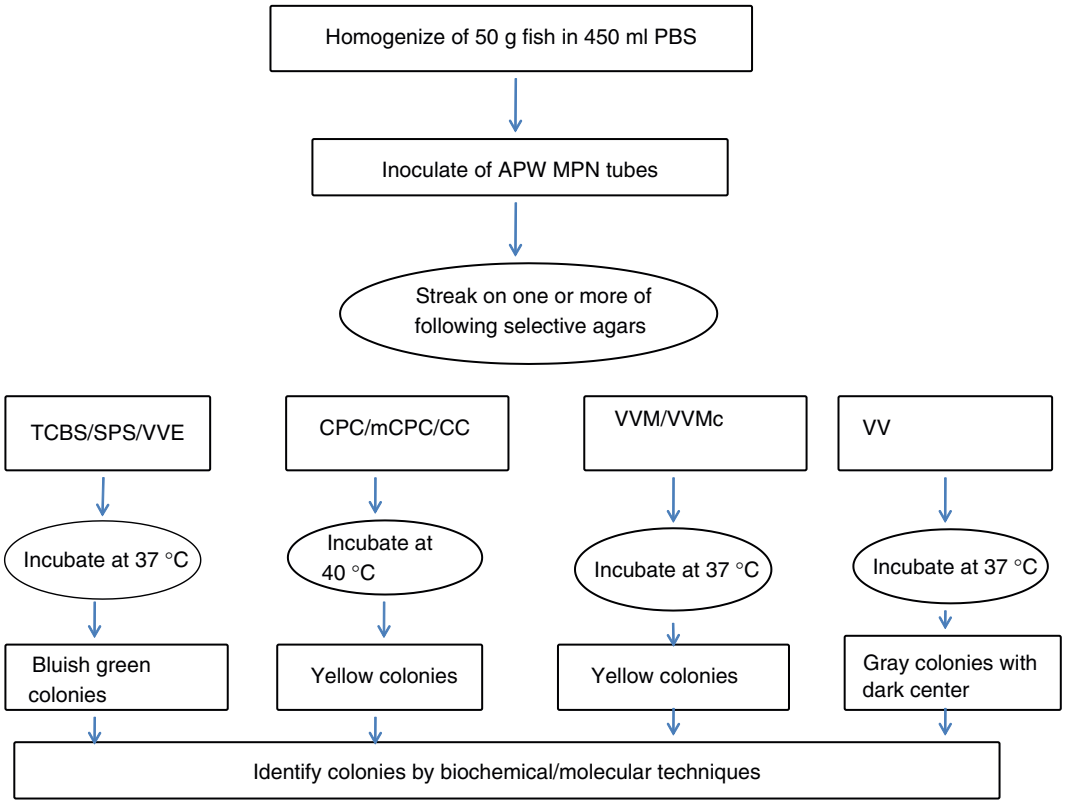

Figure 1 Steps used in conventional method for isolation/enumeration of *V. vulnificus* in seafood.

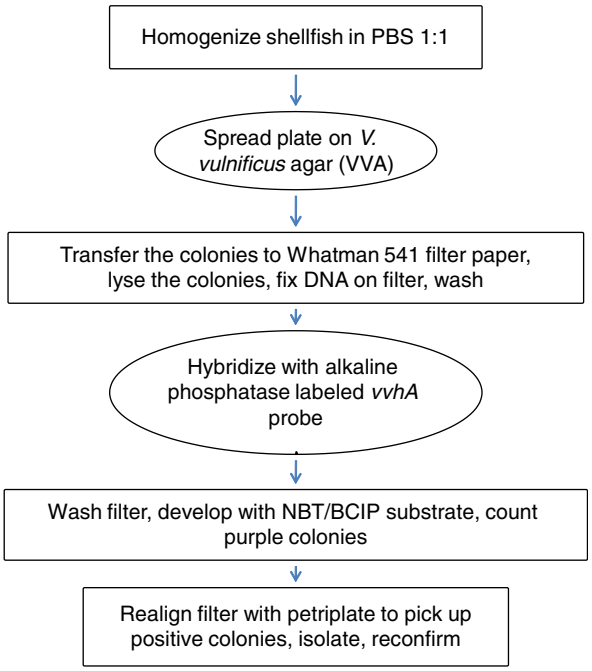

Figure 2 Steps used in enumeration of *V. vulnificus* by colony hybridization technique.

capable of detecting as few as 10 viable cells per 100 g. Digoxigenin-labeled probes binding to *vvhA* gene or 16S rRNA gene in conjunction with selective differential medium VVM could also be used.

A number of PCR-based assays have been used for detection of *V. vulnificus* in seafood and the target for PCR amplification has been various regions of *vvhA* gene or 16S rRNA/23S rRNA gene or gyrB gene. One of the problems in applying PCR for detection of *V. vulnificus* in seafood directly is the inhibition of reaction by seafood matrix. Generally, DNA extraction protocols are used to minimize this problem. Another problem is the inability to distinguish between dead and viable cells. Nested PCR using 23S rRNA gene universal external primers and *V. vulnificus* specific internal primers had a sensitivity of detecting 120 cells of *V. vulnificus* in artificially inoculated samples without enrichment in 24 h. Nested PCR amplifying *vvhA* gene could detect as low as 1 cfu g^{-1} of seafood, when guanidinium isothiocyanate method is used for extraction of DNA. PCR can be used in conjunction with MPN technique for enumeration of *V. vulnificus*. Multiplex PCR for detection of *V. vulnificus* with other pathogens such as *Salmonella* Typhimurium, *V. cholerae*, *Vibrio parahaemolyticus*, and *E. coli*. In oyster homogenates seeded with pathogens, 10–100 cfu g^{-1} could be detected with two rounds of PCR. Combination of PCR and microtitre plate sandwich hybridization has been used to detect multiple pathogens like *V. vulnificus*, *V. cholerae*, *V. parahaemolyticus*, and *S.* Typhimurium. Amplified target DNA was hybridized with capture probes in microtitre plates and detection was achieved by hybridization with a biotinylated oligonucleotide probe internal to amplified DNA. In oyster homogenates seeded with *V. vulnificus*, detection limits were 100 cfu g^{-1}.

Recently, there have been several reports on the application of real-time PCR for quantification of pathogens associated with foods. Real-time PCR assay based on TaqMan probe as well as SYBR green dye have been used for detection and quantification of *V. vulnificus*. Sensitivity of the assay based on *vvhA* gene fragment amplification was 100 cells per g in oyster homogenates or seawater. Enrichment for 5 h increased the sensitivity of detection to one cell. Results of enumeration of *V. vulnificus* by real-time PCR performed on MPN tubes after 6 h enrichment showed 98% agreement with that obtained by plating the MPN enrichments followed by confirmation of colonies by colony hybridization but real-time PCR gave faster results. Loop mediated isothermal amplification (LAMP) is another technology that is gaining popularity in food safety testing laboratories, because this can be performed and the results read without expensive equipment. LAMP assay using primers binding to *vvhA* of *V. vulnificus*, had a detection limit of 10^7 cfu g^{-1} with oysters and 7 cfu g^{-1} after 5 h enrichment.

Risk Assessment

The FAO/WHO risk assessment for *V. vulnificus* in raw oysters modified the US FDA *V. parahaemolyticus* risk assessment model to assess the risk of *V. vulnificus* primary septicemia in the USA, because quantitative data for *V. vulnificus* levels in oysters, at the point of consumption and for the susceptible population available for the USA. This risk assessment used data obtained by weekly analysis of *V. vulnificus* levels in oysters from four Gulf States conducted during 1994–95. Since presently, it is not possible to specifically identify pathogenic strains, all strains were considered equally virulent. Postharvest practices (duration oysters in harvest vessel in water, time to first refrigeration) used in the model were based on surveys conducted in Gulf Coast. *Vibrio vulnificus* growth in oysters, survival during refrigeration, and levels at consumption were estimated based on data from studies in the US Gulf Coast. The model predicted that the mean *V. vulnificus* levels in oysters would be 5.7×10^4 per g in summer and 8.0×10^1 per g in winter. At a serving size of 196 g, the ingested dose would be 1.1×10^7 *V. vulnificus* in summer and 1.6×10^4 in winter. US FDA data on prevalence of risk factors in US population and oyster consumption data from surveys was used in the model. The dose response relationship was modeled by estimating the exposure per eating occasion and number of eating occasions for oyster associated *V. vulnificus* cases reported to US CDC during 1995–2001. The model predicted 0.5, 11.5, 12.2, and 8 illnesses for winter (January–March), spring (April–June), summer (July–September), and autumn (October–December) seasons, respectively. When compared with epidemiological data, the number of reported cases (average for 1995–2001 was 0.6 in winter, 9.6 in spring, 13.5 in summer, and 7.4 in autumn) were within the 90% confidence limit predicted by the model.

The risk assessment also predicted the reductions in illness that could be achieved by postharvest treatments to reduce *V. vulnificus* levels to target values such as 3, 30, or 300 per g. In the USA, there are three validated methods to achieve endpoint criterion of <3 MPN g^{-1} *V. vulnificus* and these include mild heat treatment (50 °C), freezing with extended frozen

storage, and high hydrostatic pressure. If all oysters are treated to achieve target level of 3 per g, the model predicted that the number of cases could be reduced from current 32 reported cases per year to one case every six years. If the target is shifted to 30 or 300 per g, then the predicted cases would increase to 1.2 and 7.7 cases per year, respectively. At a time to refrigeration range of 0–20 h, the predicted illness ranged from 17.7 to 59.3 cases, suggesting that immediate cooling of oysters alone is not adequate to achieve substantial reduction in the number of *V. vulnificus* illnesses. As *V. vulnificus* levels in oysters harvested from waters with a salinity of >30‰ is greatly reduced, it is predicted that if all oysters are harvested from waters at salinity of >30‰, irrespective of the water temperature, *V. vulnificus* illness would be <1 case per year. Relaying oysters to high-salinity waters (>32‰) has been shown to reduce *V. vulnificus* levels by 3–4 log units (<10 per g) within 2 weeks.

Control Measures

Cooling of oysters after harvest is very important for preventing multiplication of *V. vulnificus*. Multiplication of *V. vulnificus* does not occur at temperatures below 13 °C, hence this is the target temperature for cooling oysters for control of this pathogen. Levels of this pathogen in shellstock oysters held without refrigeration for 3.5, 7.0, 10.5, and 14 h increased 0.75, 1.3, 1.74, and 1.94 log units, respectively. According to the US regulations, when water temperature exceeds 27 °C, commercial shellfish should be refrigerated within 10 h of harvest. There are additional control plans for shellstock associated with two or more *V. vulnificus* illnesses. Temperature control should be applied within 14 h when water temperature is between 18 and 23 °C; within 12 h if water temperature is 23–28 °C; within 6 h if water temperature exceeds 28 °C.

In the USA, freezing combined with frozen storage is accepted as a control measure for *V. vulnificus*, but the process should be validated and hazard analysis critical control point (HACCP) compliant. High hydrostatic pressure is another technology that is permitted in the USA for postharvest treatment of oysters. To obtain a 5 $\log_{10}$ reduction of *V. vulnificus* levels, a treatment of 250 megapascal (MPa) for 120 s is required.

Commercial heat shock process is used in some parts of the USA as a processing aid for oysters. The process involves submerging chilled oysters in potable water at a temperature of 67 °C for approximately 5 min depending on oyster size and condition. Heat shocked oysters are cooled by spraying with potable water for 1 min before shucking and washing. 2–4 $\log_{10}$ reduction in *V. vulnificus* counts can be achieved by heat shock process. Low temperature pasteurization (50 °C for 10 min) reduced *V. vulnificus* counts from 10^5 MPN g^{-1} to undetectable levels in inoculated shellstock oysters.

Based on FAO/WHO risk assessment, Codex Committee on Food Hygiene developed a Code of Hygienic Practice for control of *Vibrio* spp. in seafood with an annex on control measures for *V. parahaemolyticus* and *V. vulnificus* in bivalve molluscs. This Code recommends assessment of the need for control measures based on (1) number of sporadic illness associated with bivalve molluscs in the area, (2) water temperature at harvest, air temperature, and harvest and postharvest practices, and (3) water salinity at harvest. Because there is a wide geographical variation in prevalence and levels of *V. vulnificus* in bivalves, control measures that have been validated and appropriate for the region may be adopted by the competent authority having jurisdiction and implemented under HACCP system. Validation of control measure should be carried out in accordance with the Codex Guidelines for the validation of food safety control measures (CAC/GL 69-2008).

Because only a certain section of population is susceptible to *V. vulnificus*, health education could be a useful tool for preventing infections. The US FDA has a health education kit. The Interstate Shellfish Sanitation Program has developed education materials and fact sheets to inform public about the link between *V. vulnificus* infections and diabetes, liver disease, and other immunocompromised conditions.

See also: Bacteria: Other Vibrios; *Vibrio cholerae*; *Vibrio parahaemolyticus*. Safety of Food and Beverages: Seafood

Further Reading

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BACTERIA

Other Vibrios

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Glossary

AIDS Acquired immunodeficiency syndrome is a disease of the human immune system caused by the human immunodeficiency virus (HIV), which causes severe damage to the immune system.
Bacteremia The presence of viable bacteria in the circulating blood.
Cholecystitis Acute cholecystitis is a sudden inflammation of the gallbladder that causes severe abdominal pain.
Cytolysin The substance or antibody elaborated by microorganisms, plants, or animals that is specifically toxic to individual cells, in many cases causing their dissolution through lysis.
DNAse A deoxyribonuclease (DNAse) is a type of nuclease enzyme that catalyses the hydrolytic cleavage of phosphodiester linkages in the DNA backbone. DNases either can cleave only residues at the ends of DNA molecules or cleave anywhere along the DNA chain.
Fulminant Any event or process that occurs suddenly and quickly, and is intense and severe to the point of lethality.
Microbiome The totality of microbes, their genetic elements (genomes), and environmental interactions in a defined environment, for example, the gut.
QPCR-MPN Quantitative real-time polymerase chain reaction (QPCR) is a laboratory technique based on the polymerase chain reaction, which is used to amplify and simultaneously quantify a targeted DNA molecule. For one or more specific sequences in a DNA sample, QPCR enables quantification, which facilitates the determination of most probable number (MPN) enumeration.
rRNA Ribosomal ribonucleic acid, the RNA component of the ribosome, performs critical functions in the ribosome that allows protein synthesis to occur. The genes that encode rRNAs evolve (i.e. change sequence over time) in a very unique manner that makes them excellent 'markers' to trace evolutionary history and powerful tools to identifying species from sequence data.
Vibrovax Vaccine is used for the prevention of infertility and abortion in cattle caused by vibriosis.

Background

Noncholera *Vibrio* infections are caused by Gram-negative bacteria belonging to the same family, Vibrionaceae as those that cause cholera. Different species of *Vibrio* (except *Vibrio cholerae* O1 and O139) along with *V. cholerae* non-O1–non-O139 are collectively grouped as 'other vibrios.' These salt-tolerant Gram-negative bacilli are commonly found in warm coastal waters and infection can result from direct contact with contaminated seawater or ingestion of contaminated seafood.

Vibrio species are part of the normal flora of marine habitats and many of them are serious pathogens in fish and shellfish in marine aquaculture worldwide. Members of the genus *Vibrio* are autochthonous bacterial flora in the aquatic ecosystem and quite a few of them are associated with infections in humans and aquatic animals. Human infection follows either direct contact with aquatic environment or indirectly through contaminated food and water. While transmission of *Vibrio* infections in developing countries is by and large water- and foodborne *Vibrio* infections tend to occur more frequently in developed countries. Further, the magnitude of foodborne infections in developing countries is still incompletely understood due to lack of robust scientific information.

Characteristics of the Organism

The family Vibrionaceae currently consists of seven genera, namely, *Enterovibrio*, *Salinivibrio*, *Enhydrobacter*, *Listonella*, *Allomonas*, *Photobacterium*, and *Vibrio*.

At present, the genus *Vibrio* has 48 species that share the following characteristics: they are Gram negative, curved or straight rod, oxidase positive (except *Vibrio metschnikovii*), reduce nitrate to nitrite, susceptible to vibriostatic compound O129, motile by polar flagella, and facultatively anaerobic. Vibrios reach high densities in marine waters especially during the summer months. Although found primarily in marine ecosystems, some species (*V. cholerae* non-O1 and *Vibrio mimicus*) can live in fresh water. Halophilic vibrios require Na^+ for growth. Different abiotic factors like temperature, salinity, and pH of the water bodies are considered to be the factors that regulate their abundance and diversity. Among the currently described *Vibrio* species, 12 are known to be associated with infections in humans. *Vibrio parahaemolyticus* is the most common noncholera *Vibrio* associated with human infections, whereas *Vibrio vulnificus* is less common but more lethal. Other *Vibrio* species that occasionally cause human infections include: *Vibrio fluvialis*,

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Vibrio furnissii, *Vibrio hollisae*, *Vibrio alginolyticus*, and *Vibrio cincinnatiensis*.

Different types of relationships like mutualistic association between *Vibrio fischeri* and fish; symbiotic association of *V. fischeri* with sepiolid squids, chitinous shellfish; and parasitic relationships of some vibrios typically affecting fish, frogs, and eels are known. In temperate climates, abundance of *Vibrio* vary seasonally as in the summer, vibrios can easily be isolated from water, suspended particulate matter, plankton, algae, sediment, fish, shellfish, and benthic marine environment while during winter months, they decline markedly in number and are found overwintering in sediments. Many of the *Vibrio* species have the capacity to produce biofilm, or surface-associated communities, which helps in the attachment of bacterial cells to host or environmental surfaces and in bacterial survival. Through the formation of biofilms, vibrios enter into a viable but noncultivable state.

Clinical Manifestation

Noncholera *Vibrio* infections are largely classified into two distinct groups: halophilic or nonhalophilic, depending on their requirement of sodium chloride for growth. Some species of noncholera vibrios are usually associated with foodborne gastroenteritis, soft-tissue infections or systemic infections such as meningitis, septicemia, cholecystitis, cellulitis, ear infection, and a variety of other wound infections.

Soft-tissue infections may result from direct inoculation or from localization after sepsis. Intense swelling and compromised blood supply necessitate a combined medical and surgical approach for treatment.

Gastroenteritis is the most common syndrome and is characterized by the acute onset of watery stools and crampy abdominal pain. Approximately half of those afflicted will have low-grade fever, headache, and chills; approximately 30% will have vomiting. Spontaneous recovery follows in 2–5 days. Wound infection is especially severe in people with liver disease or who are immune compromised.

Vibrio causes primary bacteremia, skin, and soft-tissue infections. The mortality can be up to 50% in septic patients. Sometimes, *V. vulnificus* can be isolated from the sputum without obvious signs of pneumonia, with the manifestations like erythema and painful swelling to hemorrhagic and necrotizing fasciitis.

Pathogenesis

Vibrio vulnificus is capable of causing severe and often fatal infections in susceptible individuals. It causes two distinct disease syndromes, a primary septicemia and necrotizing wound infections.

The presence of thermostable direct hemolysin (TDH) and TDH-related hemolysin (TRH) in *V. parahaemolyticus* remains closely associated with the severity of diarrheal illness. TDH and TRH have various biological activities including hemolytic activity, cardiotoxicity, and enterotoxicity. Type-III secretion system (TTSS) has been implicated to be involved in cytotoxicity to host cells and is instrumental for multifaceted host cell infection by induction of autophagy, cell rounding, and cell lysis.

Vibrio necrotizing soft-tissue infection is characterized by rapid progressing soft-tissue necrosis and fulminant septicemia in the at-risk host. Fulminant disease may quickly follow ingestion of raw oysters or clams in patients with hepatic cirrhosis, even when the liver disease is fully compensated. Different species like *V. vulnificus*, *V. hollisae*, *Vibrio carchariae*, *V. alginolyticus*, and *Vibrio damsela* cause serious wound infections. They can cross the intestinal mucosa barrier and produce different types of infections. Apart from enteric and wound infections, vibrios, along with other bacteria such as *Shewanella putrefaciens*, *Photobacterium phosphoreum*, and *Pseudomonas spp.* are likely sources of tetrodotoxin in pufferfish poisonings and probably other sources of tetrodotoxin in poisonous marine animals like the blue-ringed octopus and some sea snails.

Virulence Factor

Serogroups like *V. cholerae* non-O1, non-O139, O141, O75, O37, O10, O12, O6, and O14 have been reported to cause outbreaks of cholera-like illness. The variation in virulence of *V. cholerae* non-O1 and non-O139 isolates or host resistance factors potentially allow some strains to cause mild to severe gastroenteritis, whereas others may be involved in bloodstream invasion or deeper body sites.

Vibrio parahaemolyticus and *V. vulnificus* are serious human pathogens. *Vibrio parahaemolyticus* causes gastroenteritis in which the hemolysins, TDH and/or TRH, play a crucial role. Along with TDH/TRH, two TTSS of *V. parahaemolyticus* also plays a role in the pathogenicity of the bacterium. Other toxins, proteases, cytolysins, and pili may also play a role as virulence factors in both *V. parahaemolyticus* and *V. vulnificus*. The virulence of environmental strains of *V. vulnificus* appears to be indistinguishable from that of clinical isolates. A capsular polysaccharide is the primary virulence factor in *V. vulnificus* pathogenesis. *Vibrio vulnificus* secretes a cytotoxin RtxA which enables them to make their pathogenicity. Other vibrios (Table 1) have been sporadically found to be associated with human infections.

Few recent studies revealed that virulence genes could also be present among strains in the environment and acquisition of such genes might have taken place in the aquatic environment. Most of the pathogenic genes acquired by *Vibrio* spp. are either due to acquisition of mobile genetic elements or by phage infection.

Other Infections

In marine and estuarine environments, fish fauna are infected with species belonging to the genus *Vibrio*. *Vibrio* species that have been associated with disease in fish are *Vibrio anguillarum*, *Vibrio ordalii*, *V. damsela*, *V. carchariae*, *V. vulnificus*, *V. alginolyticus*, and *Vibrio salmonicida*. *Vibrio shiloi* and *Vibrio coralliilyticus* are pathogens implicated to bleach corals, with an extent of pathogenicity through adhesion, penetration, and

Table 1 Human infections caused by other *Vibrio* species

Vibrio *species*	*Clinical manifestation*	*Source of infection*	*Virulence factor*
V. cholerae non-O1 and non-O139	Gastroenteritis	Water and food	Hemolysin
V. parahaemolyticus	Gastroenteritis, wound infection, and bacteraemia	Shellfish and seawater	Cytotoxin and hemolysin
V. vulnificus	Wound infection, bacteraemia, and cellulites	Shellfish and seawater	Cytotoxin, hemolysin, lipopolysaccharide, capsular lipopolysaccharide, metalloprotease, and siderophore.
V. fluvialis	Gastroenteritis, fever, wound infection, and bacteraemia	Seafood	Cytotoxin
V. furnissii	Gastroenteritis	Seawater and seafood	Protease and hemolytic
V. mimicus	Gastroenteritis, wound infection, bacteraemia, and ear infections	Fresh water and seafood	Hemolysin (homologous to El Tor)
V. hollisae	Gastroenteritis, wound infection, bacteraemia, and septicemia	Shellfish and seafood	Hemolysin
V. alginolyticus	Wound infection, otitis, ear infection, diarrhea in AIDS patients, conjunctivitis, and posttraumatic intracranial infection	Seawater	Elastases, collagenases, DNAses, chondroitinases, gelatinases, lechitinases, and keratinases
V. damsela	Wound infection	Seawater	Cytolysin
V. metschnikovii	Bacteraemia	–	Cytolysin
V. cincinnatiensis	Bacteraemia and meningitis	–	Hemolysin
V. carchariae	Wound (shark bite)	Seawater	Hemolysin and cytotoxin

AIDS, Acquired immunodeficiency syndrome; DNAse, deoxyribonuclease.

multiplication within the coral tissues. *Vibrio fortis*, *Vibrio campbellii*, *Vibrio harveyi* are responsible in coral bleaching. *Vibrio harveyi* is a primary opportunistic pathogen of marine animal viz. oysters, lobsters, the common snook, prawns, turbot, barramundi, milkfish, and seahorses. *Vibrio fetus* causes abortion and/or infertility in sheep. Bacterial diseases have been reported in penaeid shrimp culture caused by 14 species of *Vibrio*.

Epidemiology

Although most *Vibrio* infections are reported from coastal areas, reports of gastrointestinal and wound infections in humans have also been documented from inland areas far from the sea coasts of Asia, Africa, Europe, Australia, and North and South America because of travel and shipment of contaminated food. Most of these infections are caused either by *V. cholerae* non-O1 or by *V. vulnificus*. Gastroenteritis caused by the noncholera *Vibrio* occurs in sporadic or common infection after consumption of raw, improperly prepared, or recontaminated seafood. All types of seafood products may be involved including oysters, mussels, clams, shrimp, and crabs, all of which are available almost universally. The risk of infection with *Vibrio* spp. is highest in filter-feeding bivalves as they concentrate bacteria in nature from the surrounding water. Contact with contaminated water is a risk factor mainly for wound infections and may involve the direct immersion or splashing of water in or onto open wounds or mucous membranes or entry through fish hook wounds, as well as less obvious routes such as cuts by broken shells or shark and other fish bites. Most pathogenic vibrios isolated from the environment exhibit wide strain-to-strain variation in virulence, except *V. vulnificus* (virulence of environmental strains are indistinguishable from clinical isolates). Animal-associated *Vibrio* microbiomes are increasingly documented as an influential source of *Vibrio* infection. In temperate and subtropic waters, *Vibrio* spp. show their greater consistency in the formation of different microbiomes with zooplankton, crabs, and mussels with higher variation among individuals, which suggests the evolution of a greater degree of host specificity. On the contrary, in planktonic (micro) habitats the microbiome seems to be an open system for vibrios in which high rates of immigration can take place.

Analytical Methods

Detection of pathogens either in sources (transmission vehicle) or at the site of infection is essential for ensuring the safety of different risk-prone groups. Advances in biotechnology have resulted in the development of rapid methods reducing the analysis time. Two major categories of rapid methods include immunological or antibody-based assays and genetic-based assays such as the polymerase chain reaction (PCR). Immunological identification and confirmation tests include enzyme immunoassay and enzyme-linked immunosorbent assay for pathogenic vibrios. Most of the genetic identifications are based on species-specific genes or 16S ribosomal ribonucleic

acid (rRNA). Recently QPCR-MPN (quantitative multiplex PCR) technique has been developed to minimize the work load of bacterial quantification through conventional MPN method. Molecular methods for identification and confirmation include simplex PCR, multiplex PCR, and loop-mediated amplification. PCR technique is widely used for efficient detection of foodborne as well as waterborne pathogens because of speed and specificity. However, PCR methods have focused mostly on species-specific detection. Different advanced approaches like multiple-locus variable-number tandem-repeat analysis, direct genome restriction enzyme analysis, matrix-assisted laser desorption ionization-time of flight mass spectrometry (MS), PCR-electrospray ionization-MS, capillary electrophoresis (resulting in a high-resolution and high-throughput), etc., genotyping methods help to understand the clonal relationship between the clinical as well as environmental *Vibrio* isolates. Light Upon eXtension real-time PCR assays are in use to observe the species-specific polymorphisms and rapid differentiation of the major pathogenic *Vibrio* species.

Control/Preventive Measures

People, both healthy and ill, including immunocompromised individuals, should be informed about the risk of eating raw seafood like oysters or clams and should be instructed not to eat them in a raw state. Consumption of well-cooked oysters and clams should be encouraged.

Knowledge about the resistance and growth characteristic of *Vibrio* species is more important to enable food manufacturers and consumers to avoid people getting contaminated by this foodborne pathogen in different parts of world. A set of regulations covering both foods in general and specific food stuffs also ensure that food has not been rendered injurious to health. Although foodborne infections are usually encountered in smaller numbers in tropical countries, pathogenic foodborne vibrios have been recognized as a reportable disease in the temperate countries wherein the practice of consumption of seafood is quite common.

As it is well known that most of the *Vibrio* species are naturally occurring, sanitation or other public health controls cannot delimit the contamination of oysters. As a result of which, California, Florida, and Louisiana in the USA enacted regulations in 1990 requiring restaurants and other establishments that serve or sell oysters to warn prospective purchasers about possible ill effects by using tagging, labeling, records retention, etc., which will help in rapid identification of the event of disease episodes.

Vaccination Research Needs

Vibrio is a major cause of many infectious diseases and a global threat to human health, aquaculture, and animal. Prevention by vaccination is the most efficient and economical way of preventing such infections, but one of the persistent challenges to prevent *Vibrio* infections and transmissions is the existence of closely related species and genera and the lack of efficient polyvalent vaccines against them. To date no such vaccination program has been initiated. Different studies targeting eels, fishes, and mice, showed a good immune response after vaccination. Moreover, Vibrovax has been used to vaccinate the cattle against vibriosis. A detailed study is required for generating the effective vaccine against other *Vibrio* infections.

See also: Bacteria: *Vibrio cholerae*; *Vibrio parahaemolyticus*; *Vibrio vulnificus*. Safety of Food and Beverages: Seafood

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Yersinia enterocolitica and *Yersinia pseudotuberculosis*

ECD Todd, Ewen Todd Consulting LLC, Okemos, MI, USA

Glossary

Chitterlings (chitlins) Intestines of a pig, although cattle and other animals' intestines are similarly used, that have been prepared as food. In various countries across the world, such food is prepared and eaten either as part of a daily diet or at special events, holidays, or religious festivities. In the US, chitlins are an African American culinary tradition and a Southern culinary tradition sometimes called 'soul food' cooking.

Cirrhosis A chronic degenerative disease in which normal liver cells are damaged and are then replaced by scar tissue.

Diabetes mellitus A condition in which the pancreas no longer produces enough insulin or cells stop responding to the insulin that is produced, so that glucose in the blood cannot be absorbed into the cells of the body. Symptoms include frequent urination, lethargy, excessive thirst, and hunger.

Endocarditis Inflammation of the lining of the heart and its valves.

Erythema nodosum A skin disorder characterized by painful red nodules appearing mostly on the shins, and occasionally on the arms and face.

Eukaryotic Pertaining to a single celled or multicellular organism whose cells contain a distinct membrane bound nucleus. Eukaryotes include protoctists, fungi, plants, and animals.

Facultative anaerobe A microorganism that is capable of aerobic respiration in the presence of oxygen or fermentation in the absence of oxygen.

Fecal–oral route A means of spreading pathogenic microorganisms from feces produced by an infected host, usually via contaminated hands, objects, food, or water to the mouth of a recipient host.

Foci Origin or center of a disseminated disease. A small group of cells occurring in an organ and distinguishable, either in appearance or histochemically, from the surrounding tissue.

Glomerulonephritis Acute glomerulonephritis is an inflammatory disease of both kidneys, predominantly affecting children from age 2–12. Chronic glomerulonephritis can develop over a period of 10–20 years and is most often associated with other systemic disease including diabetes, malaria, hepatitis, or systemic lupus erythematosus.

Hemochromatosis An inherited blood disorder that causes the body to retain excessive amounts of iron, which can lead to serious health consequences, most notably cirrhosis of the liver.

Hypoacidity Decreased acidity (in the stomach).

Kawasaki disease An autoimmune disease in which the medium-sized blood vessels throughout the body become inflamed and affects the skin, mouth, and lymph nodes. It is most common among children less than five years of age of Japanese and Korean descent.

Lymphadenitis Inflammation of a lymph node. It is often a complication of a bacterial infection of a wound, although it can also be caused by viruses or other disease agents.

Modulate The functional and morphological fluctuation of cells in response to changing environmental conditions.

Multiple locus variable number tandem repeat analysis A laboratory tool designed to recognize tandem repeats and other qualities in a genome (in this case bacterial) to provide a high-resolution deoxyribonucleic acid (DNA) fingerprint for the purpose of identification.

Plasmid A circular, double stranded unit of DNA that replicates within a cell independently of the chromosomal DNA. Plasmids are most often found in bacteria and are used in recombinant DNA research to transfer genes between cells.

Polymerase chain reaction (PCR) primer A strand of nucleic acid that serves as a starting point for DNA synthesis. In PCR, primers are used to determine the DNA fragment to be amplified by the PCR process.

Pulsed field gel electrophoresis An electrophoretic technique in which the gel is subjected to electrical fields alternating between different angles, allowing very large DNA fragments to pass through the gel, and hence permitting efficient separation of mixtures of such large fragments.

Reactive arthritis An autoimmune condition that develops in response to an infection in another part of the body (cross-reactivity). A bacterial infection causes the arthritis, but by the time the patient presents with symptoms, the causative agent is no longer present.

Uveitis Serious form of eye inflammation.

Voges–Proskauer A diagnostic test to help identify bacterial species. Specifically, it is used to detect acetoin in a bacterial broth culture. The test is performed by adding alpha-naphthol and potassium hydroxide

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to the Voges–Proskauer broth which has been inoculated with bacteria. A cherry red color indicates a positive result, whereas a yellow–brown color indicates a negative result.

Background

The genus *Yersinia* belongs to the Enterobacteriaceae family, and of the 11 species, only 3 are known to be pathogenic to humans, the plague bacillus, *Yersinia pestis*, and two that cause gastroenteritis: *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*. Actually, *Y. pestis* is more genetically related to *Y. pseudotuberculosis* and less so to *Y. enterocolitica*. Genetic analysis of *Y. pestis* revealed it to be a clone of *Y. pseudotuberculosis*, which evolved sometime between 1500 and 20 000 years ago. *Yersinia enterocolitica* has between 10% and 30% DNA homology with the Enterobacteriaceae family and is only 50% related to *Y. pseudotuberculosis* and *Y. pestis*. *Yersinia enterocolitica* was first reported in 1934 and the first recognized description of five human isolates occurred in 1939. The causative bacillus of pseudotuberculosis was earlier described in 1883 as Bacille de Malassez et Vignal and was later renamed twice, i.e., as *Bordetella* and *Pasteurella pseudotuberculosis* before the current name *Y. pseudotuberculosis* was established in the 1960s. *Yersinia pseudotuberculosis* is the least common of the three main *Yersinia* species that cause infections in humans. *Yersinia pseudotuberculosis* primarily causes zoonotic infections in various hosts, including domestic and sylvatic animals and birds but has been associated with foodborne infection in humans, and a few outbreaks of *Y. pseudotuberculosis* infections in humans have been reported (see Section 'Examples of Outbreaks'). The most susceptible populations for these diseases and potential complications are the very young (<10 years), the debilitated, the very old, and people undergoing immunosuppressive therapy. Those most susceptible to postenteritis arthritis are people with human leukocyte antigen (HLA)-B27 (or related antigens such as B7). Because *Y. pestis* is not a foodborne pathogen, it will not be further discussed.

Characteristics of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*

Yersinia spp. are small, rod-shaped, facultatively anaerobic, intracellular Gram-negative bacteria that tend to be motile at room temperature but nonmotile at 37 °C. Members of this species usually range from 0.5 to 0.8 μm by 1–3 μm in size. *Yersinia enterocolitica* and *Y. pseudotuberculosis* can grow over a pH range of 4–10, generally with an optimum pH of 7.6. They tolerate alkaline conditions very well, compared with acidic conditions (although that depends on the kind of acid used, environmental temperature, composition of the medium, and growth phase of the bacteria). Both of these are psychrotrophic with the ability to grow at temperatures <4 °C, but their optimum growth temperature is 28–30 °C. The generation time varies by incubation temperature: 34 min at 30 °C, 60 min at 22 °C, and 5 h at 7 °C.

Yersinia enterocolitica has six biogroups that may be present in feces, sputum, and mesenteric lymph nodes and infected wounds. For *Y. enterocolitica* there are 54 serogroups, but O:3, O:5, O:8, O:9, and O:27 are usually the most prevalent for human infections. More specifically, the pathogenic *Y. enterocolitica* serotypes/biotypes are O:3/4 and 3 variant Voges–Proskauer (VP) negative, O:5, 27/2, O:8/1b, and O:9/2 have been reported worldwide. In Japan, O:3/3 variant VP negative is the most frequent cause of human yersiniosis. In the US, despite declining incidences of serotype O:8/1b infections, O:3/4 and O:5, 27/2 infections are on the increase. In Europe, Serotype O:3 and O:9 infections account for more than 90% of *Y. enterocolitica* isolates from patients. For *Y. pseudotuberculosis*, there are 15 serogroups; serogroups O:1 and O:2 are each divided into subtypes a, b, and c, and serotypes O:4 and O:5 are each divided into subtypes a and b, for a total of 21 serotypes.

Virulence and Pathogenesis

Both pathogens harbor virulence genes that affect pathogenesis. High pathogenicity *Y. enterocolitica* biotype 1A and *Y. pseudotuberculosis* 1 (III) have the virulence plasmid (pYV) and high pathogenicity island (HPI). These include an outer membrane protein, *Yersinia* adhesion A (YadA), and the genetic suite comprising the type III secretory system. This process usually is facilitated by *Yersinia* outer proteins (Yops) proteins, which contribute to the ability of the cells to resist phagocytosis by causing disruption of the phagocytes. Pathogenicity of *Y. pseudotuberculosis* is determined by the HPI and the *Y. pseudotuberculosis*-derived mitogen, a superantigen. The presence of the three virulence factors varies among *Y. pseudotuberculosis* isolates.

Although *Y. pestis* is considered a bioterrorism agent, this does not apply to *Y. enterocolitica* or *pseudotuberculosis*. Nevertheless, because of the similarity between *Y. pestis* and *Y. pseudotuberculosis*, anyone isolating and working with this genus should be meticulous about the proper culturing, labeling, and transporting of these organisms.

Clinical Manifestations

The clinical manifestations may be similar for both species. These are enteroinvasive and/or destructive strains which typically invade the colon and terminal ileum, multiply within the mucous cells, neutralize the macrophages, and colonize the Peyerian glands or patches. They also elaborate enterotoxins responsible for the gastroenteritis symptoms. The infective dose for humans is high, estimated to be in a wide range of 10^4–10^9 organisms for both pathogens. However, the infective dose may be lower depending on the type of infecting strain and certain host factors, for example, in people with gastric hypoacidity.

Infection with *Y. enterocolitica* can cause a variety of symptoms depending on the age of the person infected. Infection with *Y. enterocolitica* occurs most often in young children, and the infection is more common in the winter.

Common symptoms in children are fever, abdominal pain, and diarrhea, which is often bloody. Diarrhea occurs in approximately 80% of cases; abdominal pain and fever are the most reliable symptoms. Symptoms typically develop 4–7 days (reported 1–11 days range) after exposure and may last 1–3 weeks or longer, even up to several months. In older children and adults, right sided abdominal pain and fever may be the predominant symptoms and may be confused with appendicitis. Yersiniosis usually resolves itself without antibiotic treatment unless the disease is unusually severe or there are ongoing complications. In a small proportion of cases, bacteremia can occur. However, fatal cases are rare. If septicemia or other invasive diseases occur, antibiotic therapy, typically with gentamicin or cefotaxime (doxycycline and ciprofloxacin), is administered. Complications can occur in a small proportion of cases because of the infecting strain type and/or the presence of the specific human immunologic leukocyte antigen, HLA-B27. Sequelae include reactive arthritis, glomerulonephritis, endocarditis, erythema nodosum (which occurs predominantly in women), uveitis, and thyroid disorders. The frequency of the arthritic conditions is approximately 2–3%, and these may occur even in the absence of obvious gastrointestinal symptoms. Performance of unnecessary appendectomies also may be considered a major complication of yersiniosis, as one of the main symptoms of the disease is abdominal pain in the lower right quadrant. However, it has been argued that both *Y. enterocolitica* and *Y. pseudotuberculosis* are important causes of granulomatous appendicitis, and *Yersinia* infections may mimic Crohn's disease.

Yersinia pseudotuberculosis infection in humans usually leads to a gastroenteritis (although diarrhea is less frequent than in yersiniosis) characterized by a selflimited mesenteric lymphadenitis that mimics appendicitis. *Yersinia pseudotuberculosis* infects the intestinal tract, liver, spleen, and lymph nodes and causes these tissues to become inflamed. In Japan, *Y. pseudotuberculosis* was implicated in the etiology of Kawasaki disease. *Yersinia pseudotuberculosis* invades mammalian cells and survives intracellularly; the primary virulence factor is a plasmid-encoded protein that causes increased invasiveness. Postinfectious complications include erythema nodosum and reactive arthritis (particularly the O:3 strain). The arthritic phase of the disease can last up to six months. Thus, a major triad for *Y pseudotuberculosis* infection includes fever, abdominal pain, and rash, and later joint or back pain. *Yersinia pseudotuberculosis* infects eukaryotic hosts through a type III secretion system which involves the transport of proteins through the bacterial envelope and past the host cell plasma membrane. The secretion is part of a highly regulated system that is indirectly modulated by temperature changes and by a calcium dependency. The secretion system, regulatory pathways, and toxic proteins are all encoded on the virulence plasmid. It is relatively resistant to the nonspecific human immune response. Blood infections caused by *Y. pseudotuberculosis* may be rare but Kaasch *et al*. (2012) documented 72 of them since 1911,with 29 occurring in the past decade (2001–2011), and found that the most frequently described predisposing factors were diabetes mellitus in 21 patients (30%), cirrhosis in 16 patients (22%), and hemochromatosis in 6 patients (8%). Foci at distant sites were reported in 15 cases (21%). Overall, 26 patients (36%) died from the bloodstream infection. Thus, the mortality of invasive *Y. pseudotuberculosis* infection is comparable to other bloodstream infections.

Analytical Methods

As yersiniosis or pseudotuberculosis are diseases that are not routinely looked for in gastrointestinal cases, analysis of stool specimens must be requested by the epidemiologist or other investigators for the respective *Yersinia* species when either of these two are suspected as causes of these cases. The organism can also be recovered from other sites including the throat, lymph nodes, joint fluid, urine, bile, and blood. Yersiniosis may be misdiagnosed as Crohn's disease (regional enteritis) or appendicitis. Diagnosis of yersiniosis begins with isolation of the organism from the human host's feces, blood, or vomit, and sometimes at the time of appendectomy. Confirmation of a foodborne infection occurs with the isolation of *Y. enterocolitica* or *Y. pseudotuberculosis* from both the human host and the ingested food, followed by biochemical and serological identification. Molecular subtyping may also be useful for determining the source of the pathogen.

Yersinia enterocolitica or *Y. pseudotuberculosis* in patients with acute gastroenteritis can be readily isolated via conventional bacteriological media designed to isolate *Yersinia*. It is much more challenging to isolate these pathogens in asymptomatic carriers or from foods. Because *Yersinia* is able to resist weak alkaline treatment, potassium hydroxide (KOH) is used to select the organism while suppressing background flora such as *Pseudomonas, Proteus,* and *Serratia*. It is reported that *Y. enterocolitica* serotypes O:3, O:5, 27, O:8, and O:9 and *Y. pseudotuberculosis* serotype 5a strains in the artificially contaminated pork samples showed comparatively high resistance to KOH, and all *Yersinia* strains were recovered from the pork samples contaminated with more than 10^2 CFU g^{-1} after direct KOH treatment, without enrichment. However, food samples with lower contamination levels require an enrichment procedure for successful recovery of *Yersinia*. For these, cold and selective enrichment methods based on phosphate buffered saline and possibly mannitol, sorbitol, or bile salts, have been successful, for example, 25 g sample of the food mixed with 225 ml of peptone sorbitol bile broth for 10 days at 10 °C. Generally, cold enrichment yields higher recovery rates of pathogenic *Y. enterocolitica* than selective enrichment containing antibiotics, and an effective selective enrichment system for *Y. pseudotuberculosis* has yet to be developed. *Yersinia enterocolitica* can be presumptively identified in 36–48 h using biochemical testing or API 20E or Vitek GNI. Because many *Y. enterocolitica* isolated from nonhuman sources are not considered pathogenic, it is important to distinguish these isolates from pathogenic *Yersinia* species. Serology is used to identify the biotype (based on biochemical analysis) and serogroup (O antigen). Sera from acute or convalescent patients can also be titered against the suspect serotype of *Yersinia* spp. Polymerase chain reaction (PCR) based assays have been developed to target virulence genes on both the chromosome and plasmid and can be used to rapidly confirm the pathogenicity of the isolate. Several PCR primer sets are directed to either the plasmid-borne genes, for example, virF or yadA or the chromosomally located loci such as ail.

Because these pathogens are not normally looked for in stools or other clinical specimens in most countries, clinicians should consider both *Y. pseudotuberculosis* and *Y. enterocolitica* as a cause of gastroenteritis and pseudoappendicitis and request appropriate microbiological testing for patients with suspected cases.

Epidemiology

Both yersiniosis and pseudotuberculosis can be spread from animals to humans by contact with infected animals and their feces; human-to-human transmission also can occur (pathogens can be present in stool weeks after the clinical symptoms have ceased, and the bacterium is passed from the stools or soiled fingers of one person to the mouth of another person). However, consumption of contaminated foods is the most frequent means of infection. Because pigs are the principal reservoir for virulent strains of *Y. enterocolitica*, pork products are most at risk of causing yersiniosis, such as home slaughtered pork and chitterlings. In these cases, contact with the raw meat or organs contaminated with pathogen plus lack of hand hygiene has led to infections. For instance, an outbreak associated with chocolate milk occurred after chocolate was hand mixed in an open vat with bare hands at a school cafeteria. Bulk milk tanks and untreated water sources have been found to be contaminated with *Y. enterocolitica*. This explains why milk and tofu packed in unchlorinated spring water have been vehicles of outbreaks of yersiniosis in the US. Yersiniosis is far more common in Scandinavia and Japan than it is in the US. Different biotypes of *Y. enterocolitica* have been associated with infections around the world, with the most common biotype being 4/O:3.

The EFSA Panel on Biological Hazards (BIOHAZ) (2007) concluded that the best and most reliable indicator of *Y. enterocolitica* pathogenicity is the biotype as the various biotypes are either pathogenic or nonpathogenic. The serotype is not a reliable marker of *Y. enterocolitica* pathogenicity because several serotypes are common to both pathogenic and nonpathogenic strains. Strains of biotype 4 (serotype O:3) and biotype 2 (serotypes O:9) are commonly associated with human infections in Europe. Biotype 4 predominates in most Member States. However, biotype 2 might predominate in a few other Member States. These biotypes are seldom reported to be isolated from the environment. Animals (pigs and cattle) are the main reservoir and human cases are typically sporadic. Even though the number of reported human cases of yersiniosis in the EU has been decreasing since 2005, yersiniosis was the third most common zoonoses reported in the EU in 2009. A total of 12 647 single samples and batch based samples were included in the present analysis of *Yersinia* in food from 2004 to 2009 in Member States. Pathogenic biotype 2 (serotype O:9, O:5, 27) was reported from pig meat in Germany, Portugal, and the UK, and serotype O:3 was reported from pig meat in Germany. This O:3 serotype might belong to biotype 4, which is the most common human pathogenic biotype in the EU. Serotype O:5, belonging to nonpathogenic biotype A1, was reported from raw cow's milk, bovine meat, and sheep meat (UK).

Although *Y. pseudotuberculosis* infections are distributed worldwide, particularly in northern Europe, epidemiological information on this pathogen is not as well defined, and cases of *Y. pseudotuberculosis* are reported less frequently than infections from *Y. enterocolitica*. For instance, little is known about the incidence and epidemiology of *Y. pseudotuberculosis* in the US. It was first recognized in that country in 1938 and has rarely been identified since then. During 1996–2007, 1903 *Yersinia* infections were reported in the US FoodNet sites. Of these, 1471 (77%) had species information available. Most of the isolates were *Y. enterocolitica* (1355; 92%); compared with 18 (1%) *Y. pseudotuberculosis*. The average annual incidence of *Y. pseudotuberculosis* infections was 0.04 cases per 1 000 000 persons, with most cases reported from California and Oregon.

Both pathogens have a history of causing a higher incidence of disease in cooler months and in temperate climates. In many cases and even epidemics, the source of infection remains unknown, and there may be more reservoirs and implicated food sources yet to be determined. One example of this is an outbreak in Turku, Finland in winter 1981–1982 with 19 cases of infection caused by *Y. pseudotuberculosis* serotype 3. Postinfection complications developed in 10 of 19 patients, but the presence of antigen HLA-B27 was not a determining factor. In spite of active screening of the respective families and environments of the patients, no transmitting factor was found, and the precise source of the infection was never identified. However, vegetables including carrots were the most likely common source because they were grown, sold, or eaten by the 19 cases. From succeeding outbreaks in Finland, where carrots and lettuce were implicated, raw vegetables could have been the vehicle for the pathogen (see Section 'Examples of Outbreaks'). In a later study in France, *Y. pseudotuberculosis* infections increased in that country during the winter of 2004–2005 in the absence of epidemiologic links between patients or strains. This increase represents transient amplification of a pathogen endemic to the area and may be related to increased prevalence of the pathogen in rodent reservoirs.

Because both of these species cause gastroenteritis, they are transmitted through the fecal–oral route and have been found in the gut of a variety of wild and domestic animals. The major reservoir for *Y. enterocolitica* strains that cause human illness is the tonsils of pigs. Rodents that inhabit pig abattoir may also be carriers of virulent types of *Yersinia*. Other, mostly nonpathogenic, strains of these species are found in many other animals including rodents, rabbits, beaver, sheep, cattle, horses, dogs, and cats. Nonpathogenic strains of *Y. enterocolitica* have also been found in surveys of a wide variety of environmental sources, such as frogs, fleas, flies, soil, ponds, lakes, meats (other than pork), oysters, fish, crabs, and raw milk. Although the prevalence of this organism in soil, water, and animals offers many opportunities for nonpathogenic strains of *Yersinia* to enter the food supply, raw or undercooked pork products have drawn the greatest attention as a source of the pathogenic strains of *Y. enterocolitica*. *Yersinia enterocolitica* can grow easily at refrigeration temperature in contaminated vacuum-packed meat, boiled eggs, boiled fish, oysters, raw shrimp, cooked crab meat, pasteurized liquid eggs, pasteurized whole milk,

cottage cheese, and tofu. It persists longer in cooked foods than in raw foods due to increased nutrient availability. The risk of contracting an infection from these pathogens would be most likely from poor sanitation by food handlers, handling of raw pork, and inadequate cooking. Prolonged refrigerated storage of contaminated foods may be a contributing factor in cases of foodborne disease. Hunters may be at risk by eviscerating and dressing carcasses, because sudden death of nine deer in a deer farm were caused by *Y. pseudotuberculosis* serotype O:3 found with virulence factors for invasion and colonization of host intestine and lung.

Examples of Outbreaks

Yersinia enterocolitica

Yersinia enterocolitica foodborne outbreaks have occurred in Australia, Finland, Japan, Norway, the US, and Brazil. There were two foodborne outbreaks in China in 1980s; one was caused by beef contamination in Lanzhou of Gansu Province in 1986 with 109 patients with diarrhea caused by *Y. enterocolitica* O:3 infection. The second occurred in a school in Shenyang, Liaoning Province, with 352 students having diarrhea caused by *Y. enterocolitica* O:8 infection. *Yersinia enterocolitica* is a concern worldwide with foodborne infections having been reported in hundreds of countries. The incidence of yersiniosis is lower than the better known foodborne pathogens partly because yersiniosis is of rare occurrence in nontemperate regions, and routine surveillance for *Yersinia* spp. in patients and foods is limited or nonexistent in many countries. Vehicles either confirmed or suspected have included liquid milk, both pasteurized and unpasteurized, powdered milk, pork products, and tofu and bean sprouts contaminated through spring or well water. Mishandling of the vehicles was often cited in reports, such as infected workers contaminating reconstituted powdered milk and chocolate milk.

1. 50 persons were infected with *Y. enterocolitica* after eating contaminated tofu (soybean curd) in Washington State in the winter of 1981–1982. The majority had gastroenteritis; two had appendectomies and one had a partial colectomy. *Yersinia enterocolitica* serotype O:8 was isolated from the patients and the tofu. Two of thirteen workers were culture positive for the *Yersinia*, although they denied illness. The plant had no hand washing facilities; only pit privies were available and a badly contaminated, untreated surface water source (so-called spring water) that was positive for the *Yersinia*. The workers packed the tofu in this water with their bare hands before shipment.
2. In July 1981, an outbreak of gastroenteritis occurred at a summer diet camp. Of the 455 campers and staff, 35% developed an illness characterized by abdominal pain, fever, diarrhea, and/or nausea and vomiting. Seven persons were hospitalized, five of whom had appendectomies. *Yersinia enterocolitica* serogroup 0:8 was isolated from 37 (54%) of 69 persons examined, including the camp cook and three assistants. An epidemiologic investigation demonstrated that illness was associated with consumption of reconstituted powdered milk and/or chow mein. The epidemic strain was subsequently isolated from milk, the milk dispenser, and leftover chow mein. Information obtained during the investigation suggested that the *Yersinia* had been introduced by a food handler during food preparation procedures.
3. Yersiniosis, a notifiable disease in Norway, is the fourth most common cause of acute bacterial enteritis registered by the Norwegian Surveillance System for Communicable Diseases. Approximately 30 domestic cases are reported annually, and fecal specimens from patients who have acute gastroenteritis are routinely tested for the presence of *Y. enterocolitica*. Presumptive *Y. enterocolitica* O:3 and O:9 isolates are sent by primary laboratories to the National Reference Laboratory (NRL) at the Norwegian Institute of Public Health, where they are routinely verified, serotyped against a range of O antisera, biotyped if relevant, and tested for *Yersinia* virulence plasmid (pYV). If the strains are pathogenic, they are typed by use of multiple locus variable number tandem repeat analysis (MLVA). In March 2011, a multidisciplinary investigation was initiated after the NRL received five isolates of *Y. enterocolitica* O:9 from humans in disparate areas of the country. All had an identical MLVA profile, which had not been previously seen in Norway. The identified 21 case patients, mostly ill between 7 February and 20 March, resided in 10 geographically dispersed municipalities throughout the country. Therefore, a widely distributed product was probably the vehicle of infection, and the cases were epidemiologically associated with ingestion of ready-to-eat commercially prepared salad mix. The likely source of contamination for the outbreak, radicchio rosso, was imported from another European country. Few European countries regularly type *Y. enterocolitica*, which might explain why international requests for information produced no similar reports from other countries. The Norwegian company voluntarily withdrew all salad mixes containing radicchio rosso from the market, and since then no new outbreak cases were reported.

Yersinia pseudotuberculosis

Outbreaks of *Y. pseudotuberculosis* have been reported in the Northern Hemisphere, including Canada, Japan, Finland, and Russia. However, only in a few of the outbreaks has the vector or source of the infection been identified, and most published reports have originated from Finland. Milk and fresh produce, such as iceberg lettuce and carrots, have been implicated by epidemiologic investigations and some laboratory analysis as a source of infection, but mechanisms of contamination of such produce are as yet unclear. To date, no foodborne outbreaks caused by *Y. pseudotuberculosis* have been reported in the US, but it may be a matter of misdiagnosis that this has not yet happened.

1. Three outbreaks of *Y. pseudotuberculosis* infection were documented from 1982 to 1984 in Okayama Prefecture, Japan, and in two of these, untreated water seemed to be vehicle. In outbreak A, *Y. pseudotuberculosis*, a causal organism, was detected in 16 patients (serotype 5A). The incubation period of the infection was long (2–20 days).

Outbreak B and C occurred in remote mountain areas. In outbreak B, *Y. pseudotuberculosis* was detected in the feces of 35 out of 276 people (serotype 4B in 34 stools, 2C and 4B in one stool). In outbreak C, 12 children became sick; one of the 4 patients whose stools were examined showed an organism belonging to serotype 4B. The inhabitants in the area of outbreak B and C took unchlorinated mountain stream and well water for drinking. *Yersinia pseudotuberculosis* was detected in 3 out of 51 water samples (serotype 4A in one, 6 in 2 samples) and 2 out of 57 wild animal fecal samples (serotype 2C in 2 samples) in B area, and 4 out of 33 water samples (serotype 4A in 2, 4B in 2 samples) in C area.

2. In Finland, in October 1998, the number of *Y. pseudotuberculosis* serotype O:3 infections increased markedly. A case-control study revealed 47 case patients. One of these with bacteremia died, and five underwent appendectomies. Iceberg lettuce was implicated as the vehicle of a widespread outbreak. Four lunch cafeterias that had served iceberg lettuce were associated with clusters of case patients. The lettuce was traced back to originating farms. Without laboratory-based surveillance and serotype analysis this outbreak would not have been recognized and brought to a halt. Cases of yersiniosis, which appear to be sporadic, may be part of unrecognized outbreaks caused by contaminated fresh produce.
3. A single brand of homogenized milk was associated with an outbreak of *Y. pseudotuberculosis*, serotype O:1B, in British Columbia. The case-control study identified the same brand of homogenized milk, pork, and fruit juice as possible risk factors for illness. All case-patient stool isolates were genetic matches. A consumer sample of the brand of homogenized milk implicated by case patients was found to be contaminated with the outbreak strain of the *Yersinia*. An assessment of the consumer's home could not exclude the possibility of cross-contamination of the milk within the consumer's home. The traceback investigation found that this milk originated from a single plant; milk obtained from the plant showed no contamination. The precise cause of this outbreak was never determined. The incidence of postinfectious complications and the effect of antibiotic use on the clinical course were followed up for four months after the outbreak. The most common postinfectious symptoms were rash (8/59) and joint pain (7/59). Microbiological analysis, at follow-up, revealed 0/36 stools positive for *Y. pseudotuberculosis*. There was no significant difference in the frequency of postinfectious symptoms between cases who had or had not taken antibiotics. The postinfectious pathogenicity of *Y. pseudotuberculosis* serotype lb is lower than that documented for other serotypes.
4. In Finland, in 2004, 53 school children suffered from a *Y. pseudotuberculosis* outbreak case after they ate in a school cafeteria in March in which carrots were the suspected food vehicle. The kitchen had received all vegetables from a fresh food processing plant with produce supplied from two farms. Samples were taken from the carrot peeling line, carrot peeling leftovers, grated carrots, and other vegetable processing lines at the plant. Carrots originated from only two farms, which were inspected, and samples were obtained for bacteriological examination. Small mammals at the farms were caught in carrot fields and investigated microbiologically to identify the reservoir of *Y. pseudotuberculosis*. This pathogen was isolated from the carrot peeling line in the fresh food processing plant, from spoiled carrots, fluid draining from spoiled carrots, and a pooled sample of common shrew (*Sorex araneus*) intestines from one of the farms. The serotype was subtype O:1b and all 22 isolates from human and environmental samples had an identical or similar pulsed field gel electrophoresis pattern. The shrews may have been picked up with carrots by harvesting machinery and ended up dead in wooden storage frames with the carrots. If carrots become contaminated from the shrews, long storage at cold temperatures would favor growth of *Y. pseudotuberculosis*. After the outbreak, the Finnish Food Safety Authority recommended controlling contamination at the farm level by removing spoiled carrots and paying attention to any subsequent spoilage during handling procedures. Wild animals have been suspected as the reservoir of *Y. pseudotuberculosis*. The vole population had a cyclical peak in western Finland in 2001–2002 and declined until the spring 2003. Two field voles (*Microtus agrestis*) and two common shrews (*Sorex araneus*) were caught in the surrounding fields of the implicated farm in June 2004. The pooled intestinal sample of the shrews was positive for *Y. pseudotuberculosis* but not the pooled intestinal sample of the voles. This small study seems to implicate carnivorous shrews rather than typically herbivorous voles, but their link to carrots was not determined.

A second large outbreak of *Y. pseudotuberculosis* involving carrots occurred in 2006. *Yersinia pseudotuberculosis* O:1 infection affected more than 400 children from 23 schools and 5 day-care centers in two municipalities in southern Finland in August–September, 2006. A retrospective cohort study conducted in a large school center showed that the outbreak was strongly associated with the consumption of grated carrots served at a school lunch. The risk of illness increased with the amount of carrots eaten. Poor quality carrots grown the previous year had been delivered to the school kitchens in the two municipalities affected. In the patients' and the environmental samples collected from the carrot distributor's storage facility, identical serotypes and genotypes of *Y. pseudotuberculosis* were found, but the original source and the mechanism of the contamination of the carrots remained unclear. Domestic carrots for human consumption are usually consumed by the end of March next year, but some large farms have been able to provide carrots even up to July of the following year after harvesting, and during this long storage time, some carrots become spoiled and liquefied. In the current investigation, *Y. pseudotuberculosis* was detected in the fluid from spoiled carrots after one week's enrichment, indicating there were high levels of bacteria in the fluid. Outbreaks of *Y. pseudotuberculosis* linked to fresh produce have been detected repeatedly in Finland. To prevent future outbreaks, instructions in improved hygiene practices on the handling of raw carrots have been issued to farmers, vegetable processing plants, and institutional kitchens.

Unless epidemiologists and physicians are aware that *Y. pseudotuberculosis* could be a cause of gastroenteritis and

know which diagnostic test to order, *Y. pseudotuberculosis* infections will continue to go largely undiagnosed.

Control and Preventive Measures

To prevent yersiniosis and pseudotuberculosis, consumers should be educated to:

1. Avoid eating raw or undercooked pork or drinking unpasteurized milk or nonpotable water. Those at the highest risk of infection (very young and < 10 years, the very old, persons undergoing immunosuppressive therapy, and persons most susceptible to postenteritis arthritis) should be particularly vigilant about drinks and foods consumed and exposure to raw pork.
2. Wash hands with soap and water before eating and preparing food, after contact with animals, carcasses, and after handling raw pork. Because wild animals may be carriers of *Yersinia* spp., those dressing game meat should be especially careful after contact with intestinal contents and be meticulous about subsequent hygiene.
3. Clean hands and fingernails scrupulously with soap and water after handling raw chitterlings, or any raw pork product, before touching infants or their toys, bottles, or pacifiers. Someone other than the food handler should care for children while these foods are being prepared.
4. Prevent cross-contamination in the kitchen, for example, use separate cutting boards for meat and other foods, and carefully clean all cutting boards, countertops, and utensils with soap and hot water, especially after preparing raw meat.
5. Dispose of hog feces and offal in a sanitary manner.

See also: Disciplines Associated with Food Safety: Food Microbiology. Food Safety Assurance Systems: Personal Hygiene and Employee Health. Foodborne Diseases: Overview of Biological Hazards and Foodborne Diseases. Public Health Measures: Health Education, Information, and Risk Communication; Surveillance of Foodborne Diseases. Safety of Food and Beverages: Meat and Meat Products; Milk and Dairy Products

Further Reading

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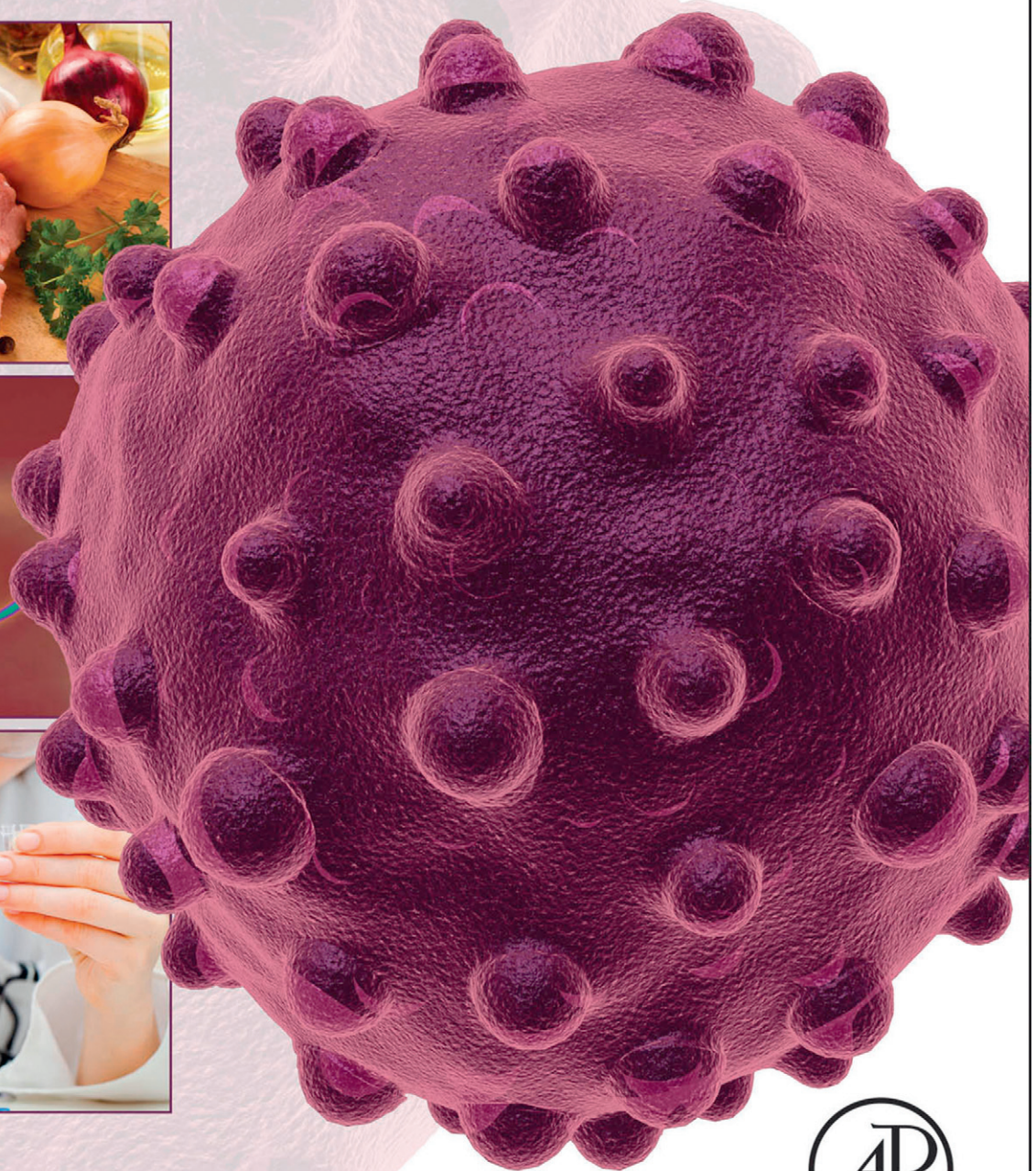
FOSA

Encyclopedia of Food Safety

ENCYCLOPEDIA OF FOOD SAFETY

Edited by **Yasmine Motarjemi, Gerald Moy, Ewen Todd**



ENCYCLOPEDIA OF
FOOD SAFETY

VOLUME 2

ENCYCLOPEDIA OF
FOOD SAFETY

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VOLUME 2

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PREFACE

Why an Encyclopedia on Food Safety?

With the world's growing population, the provision of a safe, nutritious, and wholesome food supply has become a major challenge. To achieve this, effective risk management based on sound science and unbiased information is required by all stakeholders, including the food industry, governments, and consumers themselves. In addition, the globalization of the food supply requires the harmonization of policies and standards based on a common understanding of food safety among authorities in countries around the world.

Furthermore, reports of food safety incidents and foodborne disease outbreaks in one country are disseminated almost instantaneously through the 24/7 news cycle to consumers in other countries all over the world. Consequently, food safety managers in government and industry are sometimes called on to respond to queries from politicians, the media, and the general public even before they may be aware of the problem. Taking effective intervention measures and communicating the basis of their decisions and actions are essential for maintaining confidence in the safety of the food supply.

In all the above circumstances, sound scientific information is the key to effectively and efficiently assess, manage, and communicate on food safety risks. Yet, professionals and other specialists working in this multidisciplinary field are finding it increasingly difficult to keep up with developments outside their immediate areas of expertise. The time and staff needed to provide this information are beyond the resources of most individuals and organizations. Therefore, a single source of concise, reliable, and authoritative information on food safety has, more than ever, become a necessity.

This is the role that the Encyclopedia on Food Safety sought to fulfill by gathering all of the world's knowledge and expertise covering the entire spectrum of food safety topics into one comprehensive reference work. This was done with the objective of facilitating the work of those working in the field of food safety and related fields, such as nutrition, food science and technology, and environment. The Encyclopedia also provides a platform for experts to share their state-of-the art expertise and experience with the rest of the food safety community. Furthermore, the Encyclopedia's online feature is designed for rapid search and retrieval of relevant information.

Who Will Benefit from the Food Safety Encyclopedia?

The Encyclopedia will be useful for professionals and other specialists working in, but not limited to, the following institutions:

- Regulatory and enforcement agencies.
- Food industry.
- Trade and industry organizations.
- Audit and certification bodies.
- Academic institutions.
- Private and governmental scientific and research institutions.
- International and nongovernmental organizations with an interest in food.

What Does the Encyclopedia of Food Safety Contain?

With some 280 articles, the Encyclopedia provides comprehensive coverage a broad range of food safety topics, which may be grouped under the following general categories:

- History and basic sciences that support food safety.
- Foodborne diseases, including surveillance and investigation.
- Foodborne hazards, including microbiological and chemical agents.
- Substances added to food, both directly and indirectly.
- Food technologies, including the latest developments.
- Food commodities, including their potential hazards and controls.
- Food safety management systems, including their elements and the roles of stakeholders.

In developing the Encyclopedia, the editors and members of the Editorial Advisory Board have aimed to ensure that the Encyclopedia provides:

- Contributions by the foremost authorities in their fields.
- Unbiased and concise overviews on a multitude of food safety subjects.
- References for further information
- Specialized and general definitions for food safety terminology.

While the editors have made every effort to ensure that the Encyclopedia reflects the most complete and up-to-date information available, new scientific findings, and advances in food safety occur continuously. In undertaking a project of this scale and with the inevitably delays that occur during production, the editors acknowledge that some topics may have been omitted or insufficiently addressed. Therefore, the feedback of readers to point out any such errors or oversights will be greatly appreciated and will facilitate the development of future editions.

Acknowledgments

The lead editors would like to thank the Editorial Advisory Board members, section editors, and particularly, the authors who have generously contributed their time and talent to the development of this Encyclopedia. We are indebted to the Elsevier secretariat, which has assisted in the production of this work since its inception. Finally, a special note of thanks goes to our families whose patience and support are greatly appreciated.

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DEDICATION

This Encyclopedia is dedicated to our children, our grandchildren, and all the world's future generations who we hope will enjoy the benefits of a safe and nutritious food supply, produced with fair management of people working in industry and ethical treatment of animals.

FOREWORD I

Today's food system is one of the humanity's great achievements. It includes millions of commercial actors all over the world who produce, process, transport, store, market, and serve food that feeds billions of people daily. The complexity, diversity, and scope of the food system are almost beyond comprehension – ranging from small producers and processors serving local communities to vast global enterprises producing food for millions and managing extended international supply chains – all aimed at meeting high consumer expectations for safe, nutritious, and affordable food.

For all of its successes, the food system is full of challenges. Food insecurity and hunger remain major problems worldwide, and, for those with ready access to the foods of their choice, it is too easy to choose products high in salt, fat, and added sugar. Food safety – the task of avoiding chemical and microbiological contamination of food that can make people sick – is another persistent and dynamic challenge. In fact, new products in the marketplace, new patterns of production and supply, new consumer behaviors and new bacterial and chemical hazards – coupled with high consumer expectations – conspire to make food safety one of the central challenges of today's food system.

People working in the food system know this. Prominent illness outbreaks and contamination incidents take a toll on the public's health and cause a loss of confidence that can steer consumers away from healthy foods, like fresh fruits and vegetables, and impose big economic losses on food producers and processors. And the food system is responding with a heightened awareness of food safety at all levels of the food system and tremendous effort across the system to improve food safety. Much progress is being made.

One of the most important food safety developments of the last quarter century has been the emergence of a widely shared, science-based understanding of foodborne illness, its causes, and how it can be prevented. This begins with the understanding that the current burden of foodborne illness is unacceptable because it is largely preventable. It is preventable if we see food safety as a food system issue and recognize that microbiological and chemical hazards can enter the food supply at any point in the system along the pathway from the farm through processing, transport, storage, and retail sale. Likewise, opportunities to minimize hazards and help prevent food safety problems exist throughout the system, which means that everyone in the system shares responsibility for the safety of the food we eat.

Fulfilling this responsibility requires that we understand as much as we can about food safety hazards and their causes, devise the appropriate, science-based preventive controls for particular hazards and food production settings, monitor their effectiveness, and adjust the controls as needed based on experience. In short, progress on food safety depends fundamentally on a strong base of knowledge and continuous learning to systematically prevent food safety problems. And participants across the global food safety community are actively seeking and applying the knowledge needed to produce safe food and meet high consumer expectations.

This food safety encyclopedia provides a comprehensive overview of what we know about food safety hazards and control measures. We have more to learn, but the knowledge compiled in this encyclopedia demonstrates that we know a lot and that what we know can help empower participants in today's food system to fulfill their food safety responsibility. Although the food safety challenge is global and continuing, and may seem daunting, it can be met if all who share responsibility for food safety take advantage of the knowledge we have, participate in continuous learning, and place first priority every day on protecting the safety of food. That will be good for the food system – and for the consumers it serves.

Michael R Taylor
Deputy Commissioner for Foods and Veterinary Medicine, US Food and Drug Administration (FDA), Silver Spring, MD, USA

FOREWORD II

Food is one of the most basic requirements to sustain life. However, the safety of food and water cannot be taken for granted. Owing to both manmade and natural processes, an array of chemical and microbiological disease-causing agents find their way into food through multiple routes. When contaminated, such food can endanger or even destroy life. Therefore, from time immemorial, humankind has waged a constant battle against foodborne disease. Over many centuries of human development, people invented technologies that helped them fighting this battle, such as cooking, smoking, sun drying, canning, and freezing, to mention but a few. But like any scientific advance, some of these technologies presented their own food safety issues.

In a number of holy books, religious proscriptions for handling food contributed to food safety. In addition, many centuries ago, some governments already recognized that they had responsibilities in this domain and many laws were enacted to ensure the purity of certain foods. But it was only at the end of the nineteenth century, following scientific developments in the field microbiology and other areas of food science, that 'modern' food regulatory activities started.

In 1948, the availability, accessibility, and affordability of food were recognized as a basic human right by the United Nations in its Universal Declaration of Human Rights (Article 25, 1948). Implicit in this concept is the assumption that the food is first and foremost safe to consume, i.e., absence of health damaging properties. It is therefore not surprising that in the same year, the World Health Organization (WHO) was established as a specialized agency of the United Nations with a broad health mandate that included the specific responsibility to "develop, establish and promote international standards with respect to food…". Subsequently in 1963, WHO together with the Food and Agriculture Organization of the United Nations established an intergovernmental body to develop international standards for food – the Codex Alimentarius Commission. Today Codex stands as a major achievement in the promotion of food safety worldwide with an extensive collection of health and safety recommendations for food that are international recognized and referenced by the World Trade Organization and its member countries.

Thirty years ago, in 1983, WHO, again jointly with FAO, convened an Expert Committee on Food Safety to review the global food safety situation and provide guidance for governments, the food industry and consumers on how to cope with the inherent hazards and risks of our food supply. Based on available data and evidence at the time, the committee concluded that "illness due to contaminated food is perhaps the most widespread health problem in the contemporary world and an important cause of reduced economic productivity."

Unfortunately, this rather alarming statement appears to be still true today. Recent studies examining the morbidity and mortality of foodborne diseases have confirmed the significant public health burden posed by these diseases. In developed countries, one-quarter to one-third of the population are made ill each year because of foodborne diseases. In the developing world, the burden is much more severe. For example, diarrheal diseases are now estimated to cause 2.43 million deaths a year. According to WHO statistics, this is the second leading cause of mortality in low-income countries and kills more people than human immunodeficiency virus infection/acquired immunodeficiency syndrome (HIV/AIDS), malaria, or tuberculosis.

In addition, the large number of food safety crises, which occur with increasing frequency, is contributing to the growing public demand for better health protection from contaminated food. This has prompted governments to strengthen their food safety legislation, improve capacities and infrastructure, and tighten control measures. Examples of governmental measures include the creation of European Food Safety Agency by the European Union in 2002, the Food Safety Modernization Act in the USA of 2011 and, most recently, 2013, the commitment of the Premier of the People's Republic of China, Mr. Li Keqiang, to act with an 'iron fist' to improve food safety.

These positive developments are, unfortunately, contrasted by the fact that in many other countries, mostly developing countries, food safety does not receive the attention it deserves. In this regard, the medical profession and public health community appear to be slow in accepting the role that contaminated food plays in the epidemiology of diarrhea, particularly in infants and young children. The treatment of hospitalized cases and outpatients is rarely seen as an opportunity for educating patients and their families on why foodborne diseases occur and how they can be prevented. Two publications published in WHO's Bulletin in 1993 and 2003 urged the health sector to take steps to correct this oversight. Yet even today progress has been disappointing. For example, in the 2009, United Nations Children's Fund (UNICEF) and WHO published a document entitled 'Diarrhea: Why children are still dying and what can be done,' that again overlooked food safety as one of the most important interventions for these diseases. Consequently, in a recent publication in a prestigious *Medical Journal of Gastroenterology*, the issue had to be raised again and omission corrected. It can only be hoped that the public health and donor communities will eventually adopt a more holistic approach for the prevention of diarrheal diseases, which includes essential food safety interventions.

It is for this and many other reasons that I enthusiastically welcome the initiative of Elsevier to publish this Encyclopedia of Food Safety under the editorial leadership of Drs. Yasmine Motarjemi and Gerald Moy (my former WHO colleagues) as well as Dr Ewen Todd, a world renowned expert in food safety. The laudable collaboration and support of the Editorial Advisory Board, Section Coordinators, and the many authors who have freely devoted their time to advance the cause of food safety through the development of this Encyclopedia is also acknowledged.

With such a collection of information, whoever needs first-hand, reliable, and authoritative information on food safety does not need to consult various books, periodicals, or

websites. All of what is presently known in this domain can be found in this comprehensive work. In particular, the Encyclopedia will be useful for decision-makers, managers, officials, and scientists working in government, the food industry, academia, and nongovernmental organizations.

This Encyclopedia may be particularly important for colleagues in developing countries to not only improve food safety for their people but also convince politicians and other policy makers of the pivotal role of food safety in health and development. Without this awareness, the ultimate goal of safe food for all cannot be achieved.

F Käferstein
Former Director, Food Safety and Food Aid Programme (1980–98), World Health Organization, Wiesbaden, Germany

Further Reading

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EDITOR-IN-CHIEF

Yasmine Motarjemi holds a Masters degree in Food Science and Technology from the University of Languedoc, Montpellier, France (1978) and a Doctoral degree in Food Engineering from the University of Lund, Sweden (1988).

After her research and academic career at the University of Lund, in 1990, she joined the World Health Organization in Geneva as Senior Scientist. In WHO, she was responsible for the surveillance and prevention of foodborne illnesses (including education of professional food handlers and consumers), the development of the food safety assurance systems (e.g., Hazard Analysis and Critical Control Point system), and for assistance to the WHO Member States in strengthening their national food safety programme. She also contributed to the development of the risk analysis process. She has served in the Secretariat of various sessions of the Codex Alimentarius Commission and its Committees.

From 2000 to 2011, she held the position of Assistant Vice President in Nestlé where she worked as the Corporate Food Safety Manager. In this capacity, she has, among others, developed the Nestlé Food Safety Management system and managed various emerging food safety issues and crises.

She is the author, co-author, or editor of numerous peer-reviewed articles, books, training manuals, and other publications. Her latest books are Food Safety Management: A Practical Guide for the Food Industry (Elsevier 2014) and Invisible Things (original in French under the title: Les Invisibles), a book on food safety for children.

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Dr Gerald G Moy, since his retirement from the World Health Organization in 2008, is a scientific adviser with Food Safety Consultants International working with numerous governments and international organizations. His expertise includes assessment of national food safety programs, risk assessment of chemical hazards in food, total diet studies, food safety during mass gatherings, and food defense. Dr Moy is the editor-in-chief for recently published book Total Diet Studies (Springer) and is currently preparing a chapter on food and water protection for a book on key public health considerations during mass gatherings. He serves on the International Scientific Advisory Committee of the China National Center for Food Safety Risk Assessment, the Technical Advisory Group of the World Food Program Technical Advisory Group, and the WHO International Virtual Advisory Group on Mass Gatherings. He is the author of numerous book chapters, articles, and publications and serves on the editorial boards for several food safety journals.

He received his BS in chemistry from the University of Wisconsin and his PhD in physical organic chemistry from Oregon State University, followed by a post-doctoral fellowship in biophysics at the University of New Mexico. He is a Fellow in International Academy of Food Science and Technology and a recipient of the 2009 Great Wall Friendship Award for his contributions to food safety during the Beijing Olympics.

Ewen CD Todd is the President of Ewen Todd Consulting and the former Professor in the Department of Advertising, Public Relations and Retailing, and he is also the Adjunct Professor in the Departments of Food Science and Human Nutrition and Large Animal Clinical Sciences at Michigan State University (MSU). He was former directors of the Food Safety Policy Center and the National Food Safety and Toxicology Center at MSU. At both these centers, Dr. Todd coordinated research in microbiology, toxicology, epidemiology, risk assessment, social science, and policy in the area of food safety, distance education programs, and outreach in the community. Previously, he was in the Bureau of Microbial Hazards, Health Products and Food Branch, Health Canada, Ottawa where he was a research scientist for 33 years working on methods development for pathogens in foods, foodborne disease investigation and reporting, costs and surveillance of disease, illnesses caused by seafood toxins, and risk assessment of foodborne pathogens. He also helped develop risk management strategies for the Department including producing videos and pamphlets on food safety education. Some of his recent research has been working on *Listeria* and *E. coli* O157 transfer coefficient and modeling projects, hygiene in child care centers, schools, and elder care facilities. He has also collaborated with government agencies and academia in Spain, Kuwait, Saudi Arabia, Lebanon, Cambodia, Korea, Japan, and China on food safety issues, and is an expert witness in legal suits involving food safety. He has published extensively on many different aspects of food safety, including 11 recent papers on food workers and hand hygiene. He is active in the International Association for Food Protection (IAFP) and other organizations, and speaks and organizes symposia at national and international meetings. He is the associate editor for the Journal of Food Science and is a frequent reviewer of manuscripts submitted to several different scientific journals.

He has received the Government of Canada Distinctive Service Award for extraordinary teamwork and support to the Science and Technology Community; Recipient of the Excellence in Science Award for 1998 by Health Canada; Deputy Minister's Award of Team Excellence for the work done in promoting the Fight BAC! Campaign in Canada; the Professional Institute of the Public Service of Canada Gold Medal for Pure and Applied Science; and he is Fellow of the American Association for the Advancement of Science, the IAFP, and the MSU University Outreach and Engagement. He is also an honorary life member of the IAFP. He is a graduate of Glasgow University with a BSc in Bacteriology and a PhD in bacterial systematics.

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HOW TO USE THE ENCYCLOPEDIA

The material in this encyclopedia is organized into five broad sections, presented in four volumes. The sections consist of:

1. **History, science, and methods**: this section includes papers which help in the understanding of basic sciences underpinning food safety and foodborne diseases and their historical development.
2. **Hazards and diseases**: this section addresses the features of major foodborne hazards be they chemical, microbial, parasitical or physical and their health consequence.
3. **Food technologies**: this section explains the various food technologies and aspects related to their safety, or risks in their application.
4. **Foods, materials, and risks**: similarly, in this section, various groups of food products are described in terms of their risks and measures needed to ensure their safety.
5. **Food safety management**: finally, in this part, the building blocks of food safety management in the private and public sector are explained. The role of major international organizations is also reported.

To help realize the full potential of the material in the Encyclopedia the authors have provided five features to help you find the topic of your choice: a preface giving an overview of the encyclopedia and its objectives, a contents list by subject; an alphabetical contents list; cross-references to other articles; and a full subject index.

1 Contents List by Subject

Your first point of reference will probably be the contents list by subject. This list appears at the front of each volume, and groups the entries under subject headings describing the broad themes of quaternary science. This will enable the reader to make quick connections between entries and to locate the entry of interest. Under each main section heading, you will find several subject areas and under each subject area is a list of those entries that covers aspects of that subject, together with the volume and page numbers on which these entries may be found.

2 Alphabetical Contents List

The alphabetical contents list, which also appears at the front of each volume, lists the entries in the alphabetical order. This list provides both the volume number and the page number of each entry. On the opening page of an entry a contents list is provided so that the full details of any articles within the entry are immediately available.

3 Cross-references

All of the entries in the Encyclopedia have been extensively cross-references. The cross-references, which appear at the end of the entry, serve three different functions:

i. To indicate if a topic is discussed in greater detail elsewhere.
ii. To draw the reader's attention to parallel discussions in other entries.
iii. To indicate the material that broadens the discussion.

Example

The following list of cross-references appear at the end of the entry Characteristics of Foodborne Hazard and Diseases | Drug Resistant Pathogens.

> *See also*: Bacteria: *Listeria monocytogenes*; *Salmonella* Non-Typhi; *Salmonella* Typhi and *Salmonella* Paratyphi; Shiga Toxin-Producing *Escherichia coli* and Other Pathogenic *Escherichia coli*; *Shigella*; *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*. Characteristics of Foodborne Hazard and Diseases: Cost of Foodborne Diseases. Food Safety Assurance Systems: Cleaning and Disinfection. Public Health Measures: Food Safety in Hospitals and Other Healthcare Settings; Surveillance of Foodborne Diseases

Here you will find examples of all three functions of the cross-reference list: a topic discussed in greater detail elsewhere (e.g., *Listeria monocytogenes*; *Salmonella* Non-Typhi; *Salmonella* Typhi, and *Salmonella* Paratyphi; Shiga Toxin-Producing *Escherichia coli*), parallel discussion in other entries (e.g., Other Pathogenic *Escherichia coli*), and reference to entries that broaden the discussion (e.g., Food Safety Assurance Systems: Cleaning and Disinfection. Public Health Measures: Food Safety in Hospitals and Other Healthcare Settings).

4 Index

The index provides you with the page number where the material is located. The index entries differentiate between materials that is a whole entry, is part of an entry, or is data presented in a figure or a table. Detailed notes are provided on the opening page of the index.

5 Contributors

A full list of contributors is listed at the beginning of each volume.

GLOSSARY OF SELECTED TERMS

This Glossary of Selected Terms is a partial list of definitions for terms commonly used in the area of food safety. The terms selected are those that are important for communication among the various disciplines or are often subject to misunderstanding. Most of the definitions are taken from those recommended by international organizations or given by the authors contributing to this Encyclopedia. In cases where there are different definitions for a term, the Glossary presents the definition that is most consistent with usage by the majority of authors. Note that in some instances, slight differences between general definitions in this Glossary and those appearing in the individual articles may occur as the result of the specific context of the articles.

Acceptable daily intake The estimate of the amount of a substance in food or drinking water, expressed on a body weight basis, that can be ingested daily over a lifetime without appreciable health risk to the consumer.

Acute reference dose The estimate of the amount of a substance in food or drinking water, expressed on a body mass basis, that can be ingested in a period of 24 h or less without appreciable health risk to the consumer.

Adulteration (economic) A fraudulent action which is intended to omit a valuable constituent or substitute another substance, in whole or in part, for a valuable constituent; conceal damage or inferiority in any manner; or add any substance to increase its bulk or weight, reduce its quality or strength, or make it appear bigger or of greater value than it is (Note that in the US, adulterated food is generally defined as impure, unsafe, or unwholesome food.).

Antiseptic A substance that inhibits the growth and development of microorganisms. For practical purposes, antiseptics are routinely thought of as topical agents, for application to skin, mucous membranes, and inanimate objects, although a formal definition includes agents which are used internally, such as the urinary tract antiseptics.

As low as reasonably achievable A risk management approach that aims to keep exposure to a substance at the lowest level that is realistically achievable.

Asymptomatic shedder A person who does not exhibit the symptoms of an illness but excrete the pathogen (*see also* carrier).

Benchmark Reference point or standard against which performance or achievements can be assessed. A benchmark refers to the performance that has been achieved in the recent past by other comparable organizations, or what can be reasonably inferred to have been achieved in the circumstances.

Biomarkers Indicators of changes or events in human biological systems. Biomarkers of exposure refer to cellular, biochemical, or molecular measures that are obtained from biological media such as human tissues, cells or fluids, and are indicative of exposure to a substance. Biomarkers of effect refer to biological changes that represent an alteration in endogenous body constituents (e.g., depression of cholinesterase levels as an indicator of exposure to pesticides).

Carrier A person or animal that harbors a specific infectious agent without discernible clinical disease and serves as a potential source of infection. The carrier state may exist in an individual with an infection that is unapparent throughout its course (commonly known as healthy or asymptomatic carrier), or during the incubation period, convalescence and postconvalescence of an individual with a clinically recognizable disease (commonly known as an incubatory or convalescent carrier). Under either circumstance the carrier state may be of short or long duration (temporary or transient carrier, or chronic carrier) (*see also* asymptomatic shedder).

Case-fatality rate Usually expressed as the percentage of persons diagnosed as having a specified disease who die as a result of that illness within a given period. This term is most frequently applied to a specific outbreak of acute disease in which all patients have been followed for an adequate period of time to include all attributable deaths. The case-fatality rate must be clearly differentiated from the mortality rate (Compare with mortality rate).

Colony-forming unit A measure of viable bacterial or fungal cells in which a colony represents an aggregate of cells derived from a single progenitor cell.

Contaminant Any biological, chemical, or physical agent not intentionally added to food, which is present in food as a result of the production, manufacture, processing, preparation, transport, or holding of such food (Compare with hazard).

Control (noun) The state wherein correct procedures are being followed and critical criteria are being met.

Control (verb) To take all necessary actions to ensure and maintain compliance with criteria established in the Hazard analysis and critical control point system (HACCP) plan.

Control measure Any action and activity that can be used to prevent or eliminate a food safety hazard or reduce it to an acceptable level.

Corrective action Any action to be taken when the results of monitoring at the Critical Control Point (CCP) indicate a loss of control.

Crisis A predicted or unpredicted event which represents an immediate or future significant threat to an organization, its employees, consumers, and the public at large.

Critical control point A step at which control can be applied and is essential to prevent or eliminate a food safety hazard or reduce it to an acceptable level.

Critical limit (CL) A criterion which separates acceptability from unacceptability.

Detergent A chemical used to remove grease, dirt and food, such as washing-up liquid.

Disability adjusted life year (DALY) A metric used to express a health gap that extends the concept of potential years of life lost due to premature death to include equivalent years of 'healthy' life lost by virtue of being in states of poor health or disability. The DALY combines in one measure the time lived with disability and the time lost due to premature mortality. One DALY can be thought of as one lost year of 'healthy' life and the burden of disease as a measurement of

the gap between current health status and an ideal situation where everyone lives into old age free of disease and disability.

Disinfectant A chemical agent or a process that destroys, neutralizes, or inhibits the growth of pathogenic microorganisms (*see also* sanitizer).

Dose–response assessment The determination of the relationship between the magnitude of exposure (dose) to a chemical, biological, or physical agent and the severity and/or frequency of associated adverse health effects (response) in the exposed organism, system, or (sub) population in reaction to the agent.

Endotoxin A toxin present in intact bacterial cells and released when bacteria die or the cells are disrupted. A notable endotoxin is lipopolysaccharide, which is a major constituent of the outer cell membrane of Gram-negative bacteria and can cause toxic effect on lysis of bacteria. The term 'endotoxin' is to be differentiated from 'exotoxin', which is a toxin secreted in the surrounding medium and environment of the bacterial cell.

Enterotoxin A cytotoxin produced by bacteria that is specific for the mucous membrane of the intestine and causes diarrhea and/or vomiting associated with foodborne disease. Many infectious microorganisms produce enterotoxins in the gut, but some are produced external to the host (*see also* exotoxin and endotoxin).

Exotoxin A toxin that is secreted by bacteria. There are many different types of exotoxins. They can be released into the susceptible host (after infection and growth) or into the environment, including food (after contamination and growth). Those released into the intestines are typically heat labile (but some *E. coli* strains can produce both heat labile (HL) and heat stable (HS) toxins). *Clostridium perfringens* produces a HL enterotoxin after completion of sporulation in the host's intestines. *Staphyloccocus aureus* and *Bacillus cereus* enterotoxins produced in food are HS and cause vomiting and diarrhea, whereas toxins of *Clostridium botulinum* toxin, also produced in food, are HL and cause systemic neurological symptoms (*see also* exotoxin and endotoxin).

Epidemic The occurrence in a community or region of a group of illnesses which are similar in nature and clearly in excess of normal expectancy, and derived from a common or from a propagated source (Compare with pandemic).

Equivalence The situation where the application of two different food safety management measures lead to the same, or equivalent, public health outcomes.

Equivalence of sanitary measures (import–export of food) Equivalence is the state wherein sanitary measures applied in an exporting country, though different from the measures applied in an importing country, achieve, as demonstrated by the exporting country, the importing country's appropriate level of sanitary protection.

Exposure assessment The qualitative and/or quantitative evaluation of the likely ingestion of a biological, chemical, or physical agent in food as well as exposures from other sources if relevant.

Fecal–oral route A means of spreading pathogenic microorganisms from feces produced by an infected host to another host, usually via the mouth; for example, contact between contaminated hands or objects and the mouth.

Flow diagram A systematic representation of the sequence of steps or operations used in the production or manufacture of a particular food item.

Food Any substance, whether processed, semiprocessed, or raw, which is intended for human consumption, and includes drink, chewing gum, and any substance which has been used in the manufacture, preparation or treatment of 'food' but does not include cosmetics or tobacco or substances used only as drugs.

Food additive Any substance not normally consumed as a food by itself and not normally used as a typical ingredient of the food, whether or not it has nutritive value, the intentional addition of which to food for a technological (including organoleptic) purpose in the manufacture, processing, preparation, treatment, packing, packaging, transport, or holding of such food results, or may be reasonably expected to result, (directly or indirectly) in it or its byproducts becoming a component of or otherwise affecting the characteristics of such foods.

Food allergy A form of food intolerance in which there is evidence of an abnormal immunological reaction to the food (Compare with food intolerance).

Food establishment Any building or area in which food is handled and the surroundings under the control of the same management.

Food handler Any person who handles, prepares, or serves food, be they domestic food handlers, such as preparing family food, or professional food handlers, such as those working in food service establishments (cooks and waiters), retail stores, supermarkets, etc. (*see also* food worker).

Food hygiene All conditions and measures necessary to ensure the safety and suitability of food at all stages of the food chain.

Food industry The term includes primary manufacturing and processing industry as well as some other establishments involved in the food chain.

Food intolerance A reproducible, unpleasant reaction to a food or food ingredient, including reactions due to immunological effects, biochemical factors, such as enzyme deficiencies and anaphylactic reactions that often include histamine release (Compare with food allergy).

Food poisoning (or acute foodborne intoxication) A disease caused by a toxin or a chemical in food with symptoms usually appearing within 24 h after ingesting the agent. This term is commonly misused as a synonym for foodborne disease, which covers both infections and intoxications.

Food safety Assurance that food will not cause harm to the consumer, when it is prepared and/or eaten according to its intended use (Compare with food suitability and food hygiene).

Food safety hazard A biological, chemical, or physical agent in, or condition* of, food with the potential to cause an adverse health effect (Compare with contaminant). *Refers also to property of a food.

Food safety objective (FSO) The maximum frequency and/or concentration of a hazard in a food at the time of consumption that provides or contributes to the appropriate level of protection (ALOP) (Compare with Performance objective).

Food suitability Assurance that food is acceptable for human consumption according to its intended use (Compare with food safety and food hygiene).
Food worker Individuals who harvest, process, prepare and serve food, i.e., across the whole food chain to retail/foodservice; it is broader than that of a food handler, who typically works in foodservice establishments typically foodservice; however, the two terms are used interchangeably in the literature (*see also* food handler).
Foodborne disease Any disease of an infectious or toxic nature caused by or thought to be caused by consumption of contaminated food, including drink.
Foodborne pathogens Disease-causing microorganisms that are transmitted to humans via foods.
Generally recognized as safe Status of a substance that is generally recognized, among qualified experts, as having been adequately shown to be safe under the conditions of its intended use (used mainly in the USA).
Genomics The study of an organism via decoding the entire genetic sequence of the organism.
Genetic modification A process of altering the genetic makeup of an organism by techniques of modern biotechnology.
Genetically modified organism (GMO) AGMO or genetically engineered organism is an organism whose genetic material has been altered using genetic engineering techniques.
Good animal husbandry practice A system of management controls that need to be adopted at the level of primary producers to ensure production of food of animal origin that is safe for human consumption and acceptable for trade in the world market.
Good hygienic practice A system of management controls that need to be adopted at production, processing, storage, distribution, and preparation to ensure safety and suitability of products of consumption.
Good laboratory practice A system of management controls for laboratories and research organizations to ensure the quality, integrity, consistency, and reliability of results.
HACCP plan A document prepared in accordance with the principles of HACCP to ensure control of hazards which are significant for food safety in the segment of the food chain under consideration (see also HACCP).
Hazard A biological, chemical, or physical agent in, or condition*; of, food with the potential to cause an adverse health effect (Compare with contaminant). *Refers also to a property of a food.
Hazard analysis The process of collecting and evaluating information on hazards and conditions leading to their presence to decide which are significant for food safety and therefore should be addressed in the HACCP plan.
Hazard analysis and critical control point system A preventive system which identifies, evaluates, and controls hazards which are significant for food safety.
Hazard characterization The qualitative and/or quantitative evaluation of the nature of the adverse health effects associated with a biological, chemical, or physical agent which may be present in food. For a chemical agent, a dose–response assessment should be performed. For a biological or physical agent, a dose–response assessment should be performed if the data are obtainable.
Hazard identification The identification of the type and nature of adverse effects that a biological, chemical, or physical agent in food is capable of causing in an exposed population.
Incidence rate The number of new cases of a condition arising in a defined group within a given period or the number of new infections per unit of person–time at risk (Compare with prevalence).
In vitro In an artificial environment outside the living organism.
In vivo Within a living organism.
Lethal dose 50% The dose of a substance that would be expected to kill half of a population of exposed organisms.
Margin of exposure Ratio of the no-observed-adverse-effect level or benchmark dose lower confidence limit for the critical effect to the theoretical, predicted, or estimated exposure dose or concentration.
Maximum residue limit The maximum concentration of residues resulting from the use of a pesticide or veterinary drug that is acceptable in or on a food.
Minimum infective dose The lowest number of microorganisms required to cause an infection in the host.
Monitoring (CCP) The act of conducting a planned sequence of observations or measurements of control parameters to assess whether a CCP is under control.
Monitoring (general) Continuous or repeated observation, measurement and evaluation of health, and/or environmental or technical data for defined purposes, according to prearranged schedules in space and time, using comparable methods for sensing and data collection.
Morbidity rate An expression of the number of illnesses in a population at risk over a given period of time (usually one year).
Mortality rate An expression of the number of deaths in a population at risk over a given period of time (usually one year).
Nanomaterials Materials engineered at the nanoscale to have novel functionality or properties. Such properties will typically, but not exclusively, be demonstrated in the size range 1–100 nm, but this size range should be considered approximate.
Nanoparticles Particles with one or more external dimensions in the range 1–100 nm, but this size range should be considered approximate.
Nanotechnology The manipulation or materials at the nano level.
Notifiable disease A disease that must, by law or by ministerial decree, be reported to a government authority.
Outbreak A group of at least two cases of a single illness that are demonstrated by epidemiological investigation to have a single common exposure or source.
Pandemic Epidemic occurring over a very wide area, crossing international boundaries (often more than one continent) and usually affecting a large number of people.
Pasteurization A process involving heat treatment at a prescribed time–temperature combination to kill vegetative forms of pathogens that may be present, while causing minimal changes in the composition, flavor, and nutritive value of food. However, with advances and the development

of new food technologies, the term is sometimes used for nonthermal technologies leading to the same effect.

Pathogen An organism capable of causing disease.

Pathogenesis The course of a disease from its origin to its manifestation; more specifically it refers to the cellular events and reactions, and other pathologic mechanisms occurring in the development of the disease.

Pathogenicity Ability of a microorganism to cause disease in a host (Compare with virulence).

Performance criterion The effect in frequency and/or concentration of a hazard in a food that must be achieved by the application of one or more control measures to provide or contribute to a performance objective (PO) or an FSO.

Performance objective The maximum frequency and/or concentration of a hazard in a food at a specified step in the food chain before the time of consumption that provides or contributes to an FSO or ALOP, as applicable (Compare with Food Safety Objective).

Pesticide Any substance intended for preventing, destroying, attracting, repelling, or controlling any pest including unwanted species of plants or animals during the production; storage; transport; and distribution and processing of food, agricultural commodities, or animal feeds or which may be administered to animals for the control of ectoparasites. The term includes insecticides, herbicides, fungicides, rodenticides and algicides as well as plant growth regulators, defoliants, desiccants, and agents for thinning fruit or preventing the premature fall of fruit.

Prerequisite program Practices and conditions needed prior to and during the implementation of HACCP and which are essential to food safety.

Prevalence The number of persons in a population who have a disease at a specified point in time or over a specified period of time (Compare with incidence rate).

Primary production Those initial steps in the food chain up to and including, for example, harvesting, slaughter, milking, and fishing.

Processing aid Any substance or material, not including apparatus or utensils, and not consumed as a food ingredient by itself, intentionally used in the processing of raw materials, foods, or its ingredients, to fulfill a certain technological purpose during treatment or processing and which may result in the nonintentional but unavoidable presence of residues or derivatives in the final product.

Processing contaminant Undesirable contaminants that are formed during the treatment of food as a result of the interaction of their natural components or their ingredients.

Provisional maximum tolerable daily intake (PMTDI) The health-based reference value used for contaminants with no cumulative properties. Its value represents permissible human exposure as a result of the natural occurrence of the substance in food and in drinking water. In the case of trace elements that are both essential nutrients and unavoidable constituents of food, a range is expressed, the lower value representing the level of essentiality and the upper value the PMTDI.

Provisional tolerable monthly intake The health-based reference value used for a food contaminant with cumulative properties that has a very long half-life in the human body. Its value represents permissible human monthly exposure to a contaminant unavoidably associated with otherwise wholesome and nutritious foods.

Provisional tolerable weekly intake The health-based reference value used for food contaminants such as heavy metals with cumulative properties. Its value represents permissible human weekly exposure to those contaminants unavoidably associated with the consumption of otherwise wholesome and nutritious foods.

Quality management Quality management includes all the activities that organization use to direct, control, and coordinate quality. These activities include formulating a quality policy and setting quality objectives. They also include quality planning, quality control, quality assurance, and quality improvements.

Recommended dietary allowance The average daily dietary nutrient intake level that is sufficient to meet the nutrient requirements of nearly all (97–98%) healthy subjects in a particular life stage and gender group.

Reservoir An animal species that specifically harbors an infectious agent over long periods, often without harm to the host.

Risk A function of the probability of an adverse health effect and the severity of that effect, consequential to a hazard(s) in food.

Risk analysis A process of decision making (usually government) for managing food safety, consisting of three components: risk assessment, risk management, and risk communication.

Risk assessment A scientifically based process for evaluating risks associated with foodborne hazards, consisting of the following steps: (i) hazard identification, (ii) hazard characterization, (iii) exposure assessment, and (iv) risk characterization.

Risk assessment policy Documented guidelines on the choice of options and associated judgments for their application at appropriate decision points in the risk assessment such that the scientific integrity of the process is maintained.

Risk characterization The qualitative and/or quantitative estimation, including attendant uncertainties, of the probability of occurrence and severity of known or potential adverse health effects in a given population based on hazard identification, hazard characterization, and exposure assessment.

Risk communication The interactive exchange of information and opinions throughout the risk analysis process concerning risk, risk-related factors, and risk perceptions, among risk assessors, risk managers, consumers, industry, the academic community, and other interested parties, including the explanation of risk assessment findings and the basis of risk management decisions.

Risk estimate The quantitative estimation of risk resulting from risk characterization.

Risk management The process, distinct from risk assessment, of weighing policy alternatives, in consultation with all interested parties, considering risk assessment and other factors relevant for the health protection of consumers and for the promotion of fair trade practices, and, if needed, selecting appropriate prevention and control options.

Risk manager A person or an organization (usually government) with the authority to decide on the acceptability of risk and, if necessary, measures needed for their management.

Risk profile The description of the food safety problem and its context.

Safe (food) A level of risk that is deemed to be acceptable by some standard. The question of safety always involves the question of to whom the risk is acceptable, and by what criteria that party judges it so.

Sanitizer Type of antimicrobial (disinfectant) that kills or irreversibly inactivates microorganisms present on a surface, especially designed for use on food-processing equipment. The US Environmental Protection Agency further defines a sanitizer as providing at least 99.9% reductions of all microorganisms on a surface (*see also* disinfectant).

Shelf-life The predicted time at which a product will change from acceptable to unacceptable quality. It is influenced by factors such as raw ingredient quality, processing conditions, packaging practices, and storage conditions. Typically, shelf-life is determined by a combination of microbial, sensory, and chemical methods. 'Shelf-life' can be expressed on food labels by a variety of dates, including 'expiry', 'use by', 'sell by', 'best before', and 'consume by', depending on the applicable legislation.

Step (HACCP) A point, procedure, operation, or stage in the food chain including raw materials, from primary production to final consumption.

Strain An isolate of the same type of microorganism possessing different properties.

Surveillance The systematic, ongoing collection, collation, and analysis of data on specific diseases in a defined population, to guide public health decisions.

Surveillance (active) Public health surveillance that regularly reaches out to diagnostic laboratories or to clinicians to actively collect reports of specific diagnoses of infections.

Surveillance (passive) Public health surveillance that collects reports of specific diagnoses from clinicians or diagnostic laboratories, which they are required or requested to submit because of notifiable diseases regulations.

Time–temperature abuse A situation where food has not been cooked for long enough or at a sufficient high temperature to reduce contaminants to safe levels, or food has been stored for a time or at a temperature that permits bacteria to proliferate.

Traceability/product tracing The ability to follow, forward as well as backward, the movement of a food through specified stage(s) of production, processing, and distribution.

Uncertainty In risk assessment, imperfect knowledge concerning the present or future state of an organism, system, or (sub) population under consideration.

Validation (analytical methods) Practice undertaken to substantiate or confirm methods or procedures perform as expected and in a reliable manner and consistently meet expectations.

Validation (control measures) Obtaining evidence that a control measure or combination of control measures, if properly implemented, is capable of controlling the hazard to a specified outcome.

Validation (HACCP) Obtaining evidence that the elements of the HACCP plan are effective.

Variability Heterogeneity of values over time, space, or different members of a population. Variability implies real differences among members of that population.

Verification (general) The application of methods, procedures, tests, and other evaluations, in addition to monitoring and to determine whether a control measure is or has been operating as intended.

Verification (HACCP) The application of methods, procedures, tests, and other evaluations, in addition to monitoring and to determine compliance with the HACCP plan.

Veterinary drug Any substance applied or administered to any food-producing animal, such as meat or milk producing animals, poultry, fish, or bees, whether used for therapeutic, prophylactic, or diagnostic purposes, or for modification of physiological functions or behavior.

Virulence The degree of pathogenicity of a microorganism as indicated by case-fatality rates and/or its ability to invade the tissues of the host; the competence of any infectious agent to produce pathologic effects. The virulence of a microorganism is a measure of the severity of the disease it causes (Compare with pathogenicity).

Waterborne disease A disease resulting from the contamination of water either by pathogenic viruses, bacteria or protozoa, or by chemical substances. These agents are directly transmitted to people when the water is used for drinking, preparing food, recreation, or other domestic purposes.

Withdrawal period (veterinary drugs) The interval between the time of the last administration of a veterinary drug and the time of the collection of edible tissue or products from a treated animal that ensures the concentration of residues in food comply with the maximum residue limit for the drug.

Zoonosis A disease that can be passed directly or indirectly from animals, whether wild or domesticated, to humans. Also called zoonotic disease.

ABBREVIATIONS OF TECHNICAL TERMS

This is a nonexhaustive list of commonly used abbreviations in the area of food safety.

ADI	Acceptable daily intake.
ADME	Absorption, distribution, metabolism, and excretion.
AI	Adequate intake.
ALARA	As low as reasonably achievable.
ALOP	Appropriate level of protection.
ARfD	Acute reference dose.
BMD	Benchmark dose.
BMDL	Benchmark dose at lower confidence limit.
CCP	Critical control point.
CFR	Case fatality rate.
CFU	Colony forming unit.
CIP	Cleaning in place.
DALY	Disability adjusted life year.
DGGE	Denaturing gradient gel electrophoresis.
DNA	Deoxyribonucleic acid.
EAR	Estimated average requirement.
ED_{50}	Effective dose 50%.
ELISA	Enzyme linked immunosorbent assay.
EMRL	Extraneous maximum residue limit.
FSO	Food safety objective.
GAHP	Good animal husbandry practice.
GAP	Good agricultural practice.
GHP	Good hygienic practice.
GAqP	Good aquacultural practice.
GC	Gas chromatography.
GC–MS	Gas chromatography–mass spectrometry.
GHP	Good hygienic practice.
GLP	Good laboratory practice.
GM	Genetically modified.
GMO	Genetically modified organism.
GMP	Good manufacturing practice.
GPVD	Good practice in the use of veterinary drugs.
GRAS	Generally recognized as safe.
HAB	Harmful algal bloom.
HACCP	Hazard analysis and critical control point.
HPLC	High performance liquid chromatography.
HPLC–MS	High performance liquid chromatography–mass spectrometry.
HPP	High pressure processing.
HTST	High temperature short time.
HUS	Hemolytic uremic syndrome.
IEDI	International estimated daily intake.
IESTI	International estimated short term Intake.
LD_{50}	Lethal dose 50%.
LOAEL	Lowest observed adverse effect level.
LOD	Limit of detection.
LOQ	Limit of quantitation.
MFFB	Moisture on a fat free bases.
ML	Maximum level.
MLST	Multilocus sequence typing.
MLVA	Multiple locus variable number tandem repeat analysis.
MOE	Margin of exposure.
MRL	Maximum residue limit.
mRNA	Messenger ribonucleic acid.
MS	Mass spectrometry.
NEDI	National estimated daily intake.
NOAEL	No observed adverse effect level.
NOEL	No observed effect level.
OPRP	Operational prerequisite programme.
PC	Performance criterion.
PCR	Polymerase chain reaction.
PDCA	Plan do check act.
PEF	Pulsed electric fields.
PFGE	Pulsed field gel electrophoresis.
PMTDI	Provisional maximum tolerable daily intake.
PO	Performance objective.
PRP	Prerequisite program.
PrP	Protease resistant protein.
PTMI	Provisional tolerable monthly intake.
PTWI	Provisional tolerable weekly intake.
QPS	Qualified presumption of safety.
RDA	Recommended dietary allowance.
RNA	Ribonucleic acid.
SMEs	Small- and medium-sized enterprises.
SOP	Standard operating procedure.
SPS Agreement	Agreement on the Application of Sanitary and Phytosanitary Measures.
TBT Agreement	Agreement on Technical Barriers to Trade.
TDI	Tolerable daily intake.
TDS	Total diet study.
TEF	Toxic equivalency factor.
TEQ	Toxic equivalence.
TMDI	Theoretical maximum daily intake.
TSE	Transmissible spongiform encephalopathy.
UHT	Ultra high temperature.
UL	Upper limit.
UV	Ultra violet.

PRIONS AND AGENTS OF TSES

Contents

Bovine Spongiform Encephalopathy in Cattle

D Matthews, United Kingdom

Glossary

Oral ID_{50} The infectious dose, administered by mouth, that is predicted to kill 50% of animals inoculated. Note that the figure quoted for bovine spongiform encephalopathy (BSE) is not an absolute, as it is dependent on the concentration of infectivity in the starting material. Nevertheless, it is sufficient to demonstrate that the amount would be undetectable in contaminated raw ingredients or finished feed and difficult to prevent unless extreme measures are adopted.

Peyer's patches Peyer's patches are nodules or collections of lymphoid tissue found within the wall of the intestine, especially in the young. They can become continuous and prominent, particularly within the lower small intestine (ileum). In ruminants, they are prominent before maturity but eventually regress in adulthood.

Phenotype The biological concept of phenotype is defined as the detectable characteristics displayed by an organism resulting from its interaction with the infected host and its environment or the sum of the observable characteristics embracing the entire physical, biochemical, and physiological make up of an organism. Disease phenotype can be defined as the characteristic clinical signs and pathological changes of a particular form of a disease entity or group, enabling it to be characterized and distinguished by such criteria from other forms of the disease. The frequent detection of infected, but clinically healthy, individuals through surveillance frequently prevents the inclusion of criteria such as clinical signs and pathology in consideration of phenotype. In such circumstances, the term is used more loosely in describing other characteristics, such as patterns of banding obtained by biochemical tests, or the secondary characteristics produced when laboratory rodents are inoculated.

Prion The term prion was originally coined to refer to an infectious protein, and at that time conflicted with those that believed that diseases previously known as transmissible spongiform encephalopathies (TSEs) were caused by viruses, or were at least associated with viruses. Although abnormal prion protein is still the only marker consistently associated with disease, it is still not possible to exclude the possibility that it does not act alone. The term is now accepted generically as a substitute for the infectious agent(s) that causes TSEs, acknowledges the role of prion protein in the transmissibility and pathogenesis of the diseases in which protease-resistant protein PrP^{Sc} is detected, but accepts that it is not yet possible to exclude a role for other molecules in conferring infectivity on prion protein.

PrP^{Sc} PrP stands for protease-resistant protein, the protein that is associated with prion diseases, and the product of the PrP gene (*PRNP*). It can be found in many tissues of healthy individuals although its function remains unclear. In a healthy individual, it is normally designated at PrP^{C}, where the C stands for cellular. PrP^{Sc} denotes the disease-specific isoform of PrP, that is folded differently, with a greater proportion of β sheet, and as a result is more resistant to digestion by proteases both within the body and in immunological tests. The Sc arises from its first identification in scrapie in sheep and rodent models, but it is often used generically to refer to the abnormal isoform of PrP^{C} in all affected species. At times, it is referred to as PrP^{res} to denote the protease-resistant property of the pathological protein, PrP^{d} for disease-specific PrP (particularly when detected by methods, such as immunohistochemistry, that do not rely on the use of proteases), or specifically in the case of BSE as PrP^{BSE}. In some instances, such as the World Health Organization (WHO) categorization of infectious tissues, the term PrP^{TSE} is used.

Strain Because it is not possible to isolate individual prions and to extract DNA or other agent/strain-specific molecules other than PrP (which is host-derived), science cannot categorize isolates into strains or species/subspecies by means of methods that are appropriate for bacteria or viruses. The characterization of isolates as different from each other relies, therefore, on a combination of approaches. At one time there was reliance on the inoculation of laboratory rodents, followed by a description

of the ensuing disease, or pathology, such as attack rate, incubation period, and lesion profile of vacuolation produced within the brain. More recently, molecular tools that measure and characterize prion protein extracted from host tissues are also used, but remain relatively crude. Most depend on the characterization of the products of enzyme digestion and their comparison with defined strains from either laboratory rodents or from naturally infected animals or humans. As a consequence, the process of confirming that two isolates are different, identical, or similar is slow and now involves both biochemical and bioassay approaches. Genetically modified rodents may also be used as they can speed up the process and sometimes facilitate infection that would otherwise fail in unmodified animals. Given the current state of knowledge and the logistical difficulties of processing many isolates, the categorization of isolates into strains remains relatively crude.

Transmissible spongiform encephalopathy (TSE) Before the adoption of prion for the categorization of diseases such as scrapie, BSE, chronic wasting disease, and Creutzfeldt–Jakob disease, they were grouped under the term TSEs. The term describes the pathology of the diseases in the brain of affected individuals, but acknowledges that each can be experimentally transmitted to laboratory rodents. The association of prion protein with these diseases has resulted in the more common reference to prion diseases, rather than TSEs, although the term remains both accurate and appropriate.

Introduction

Bovine spongiform encephalopathy (BSE) is a disease of domesticated cattle, first recorded in November 1986 in the United Kingdom (UK), but subsequently recognized in another 26 countries. It is a disease of the nervous system and is spread between cattle by the consumption of contaminated feed. The epidemic also spread to other countries via exported contaminated feed constituents and infected live cattle. Retrospective investigations suggest that clinical cases of BSE were first seen on British farms early in 1985, but modeling studies indicate a likely origin at least a decade earlier.

Although it was the subject of some international concern soon after its first recognition, a larger worldwide crisis of confidence in food safety was triggered almost 10 years later. This followed the recognition in 1996 that BSE had transmitted to humans, giving rise to a disease that was referred to as variant Creutzfeldt–Jakob disease (vCJD).

Where this article focuses on events in the UK, this is solely because the size of the epidemic in that country enabled the consequences of exposure of both cattle and humans to be studied more comprehensively than elsewhere. The principles described remain sound in all at-risk countries, although the degree of risk faced by cattle and humans will vary according to the extent of exposure of cattle and the nature of controls introduced.

Nature of the Agent

The first diagnosis of BSE was made after the detection of brain lesions similar to those described for a disease of sheep and goats called scrapie. The lesions include vacuolation of neurons identified by the histological examination of fixed tissue sections stained with hematoxylin and eosin. Subsequent studies demonstrated that BSE could be transmitted experimentally to laboratory rodents by inoculation of brain tissue from affected cattle. Consequently, BSE was classified alongside scrapie as a transmissible spongiform encephalopathy (TSE). Chronic wasting disease (CWD) of cervids in North America is another member of this group of disorders.

TSEs are now more commonly referred to as prion diseases. The 'prion hypothesis' is the favored hypothesis for the nature of the infectious agent and mechanisms of transmission. It has been proved difficult to demonstrate the involvement of alternative putative infectious agents, such as viruses or nonviral nucleic acid. Common to the various diseases is the presence in the central nervous system (CNS), and occasionally in other tissues, of an abnormal isoform (prion protein, PrP^{Sc}) of a host-encoded protein (PrP^{C}). When PrP, which is encoded by the PrP gene (*PRNP*), is viewed three-dimensionally, more than 40% of the molecule is organized into α-helix. It contains no β-sheets, but conversion to PrP^{Sc} involves refolding into an isoform that comprises both β-sheets and α-helices, with the former being predominant. PrP^{Sc} is prone to aggregation and accumulation in affected tissues. Prion diseases are part of a wider group of disorders in which abnormalities of protein folding occur, but natural transmissibility has so far only been demonstrated for the prion diseases.

The prion hypothesis ascribes infectivity to protein alone, with transmissibility between individuals being influenced by the degree of homology between their PrP gene sequences. Significant differences can prevent transmissibility, whereas small differences result in greater difficulty of transmission and longer incubation periods. This variability in susceptibility to transmission between species is normally referred to as the species barrier. Although PrP^{Sc} is the only protein that appears to be associated with infectivity, leading to its use as a marker for infection, there are instances where it is undetectable despite the presence of significant amounts of infectivity. This may simply reflect the lack of sensitivity of the tools used to detect PrP^{Sc}, but such instances fuel debate about the potential involvement of another factor in conferring the infectious state.

In the absence of a clearly defined pathogen, there are significant challenges in characterizing isolates of the infectious agent from animals or humans. Biological characteristics of disease produced by inoculation of experimental animals, mostly rodents, have been used to define different 'strains' of isolates. Molecular tools are overtaking these traditional bioassay approaches, which are too expensive and slow for routine use. Cell cultures remain of limited use at present, as no model can support replication of most natural

isolates. Available molecular diagnostics, involving western immunoblotting and enzyme-linked immunosorbent assay (ELISA) in particular, remain rather unsophisticated. Their dependence on the visualization or quantification of PrP after enzymatic degradation makes it difficult to standardize and control methods to facilitate comparison of results obtained in different laboratories. These tools cannot, in isolation, definitively identify a strain or subtype, or confirm an association between BSE in cattle and prion disease in other species. Nevertheless, with the introduction of immunoblotting into the diagnostic armory for surveillance, two additional phenotypes of BSE have been recognized in cattle. Commonly referred to as atypical BSE, they have been classified as H-type and L-type on the basis of their banding pattern on western blot. 'H' and 'L' denote high and low positions for the unglycosylated band relative to classical BSE, which is referred to as C-BSE for the sake of consistency. Such variants are however rare.

Irrespective of the components of the BSE pathogen, it is, along with other prions, relatively resistant to normal approaches to decontamination, disinfection, or sterilization. In the context of food safety, it is clearly not possible to rely on cooking to eliminate infectivity. Regulatory approaches rightly aim to exclude infectious tissues from the food chain.

Epidemiology

The visible face of BSE in the UK was a large epidemic of clinically affected animals. Almost 200 000 such animals were identified by the end of 2009. By 2003, however, it was estimated that more than 4 million cattle had actually been infected. The majority died or were slaughtered for human consumption, without being recognized as infected. Epidemics in all other countries have been significantly smaller. In some, the risk of a large long-term epidemic was small for a variety of reasons: exposure levels may have been low; factors required for the escalation of infectivity levels in feed were absent; or, based on British experience, it was possible to introduce control measures sooner than was possible in the UK.

Early epidemiological investigations identified one factor in common for all cases detected. They had all consumed commercial feed that contained meat-and-bone meal (MBM) derived from ruminants (cattle or sheep). This was a commonly used ingredient in feed for dairy cattle in the UK before 1988. In common with many countries, animals that died on farm were usually rendered (cooked), and the residual solid material, MBM, was considered to be a valuable source of protein. Waste material from animals slaughtered for human consumption also entered the rendering system. Before the recognition of BSE, the safety of MBM as an ingredient of animal feed had focused largely on potential contamination with salmonella species. With the benefit of hindsight, it is clear that rendering systems were at that time unable to inactivate BSE infectivity, and the absence of any species barrier between cattle meant that transmission was relatively easy. Although the exact origin of the first case of BSE still remains in doubt, it is clear that the majority of cattle that became infected did so when fed MBM derived from cattle.

Although sheep scrapie is known to transmit naturally from mother to lamb (maternal transmission) and from sheep to sheep (horizontal transmission), there remains no evidence that BSE transmits horizontally, and evidence of maternal transmission is slender and improbable. Also, there is no suggestion that the disease is transmitted via germ plasm (semen, ova, and embryos).

The cycle of infection, therefore, relied on infectious tissues from cattle being rendered and converted into MBM, followed by subsequent consumption by more cattle, especially in their first year of life (Figure 1). This cycle has not been demonstrated for atypical BSE, primarily because cases have been detected in insufficient numbers to enable detailed epidemiological investigations. It is postulated that they may represent a natural scenario of low prevalence spontaneous disease, which may indeed have become the origin of the C-BSE epidemic. If truly spontaneous, it is possible that the total removal of regulatory controls may enable BSE to return, or an escalation of atypical BSE, especially in countries where

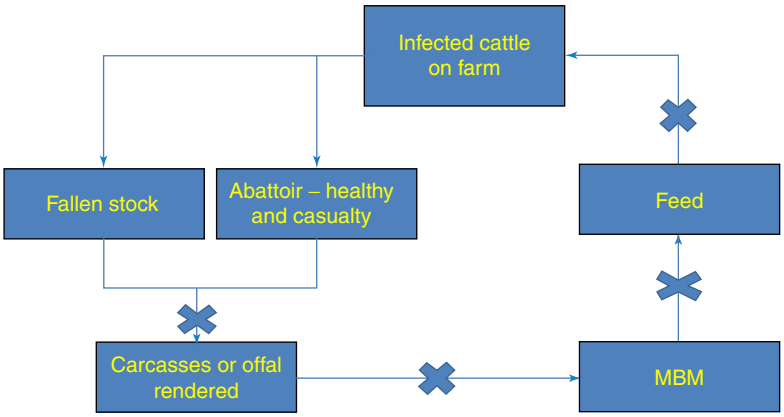

Figure 1 The epidemiology of BSE. Crosses denote points at which the cycle of re-infection needs to be broken. It is normally insufficient to rely on breaking the cycle at a single point, but this decision may be influenced by estimates of risk. It is essential to ensure that accidental cross-contamination does not perpetuate risk despite prohibition on the inclusion of raw material or by-product in products at the next stage of the cycle.

rendering standards have not been modified to inactivate the agent. It should however be stressed that at the time of writing, the oral transmission of atypical BSE to cattle has not been demonstrated. Research to investigate this possibility remains in progress.

The initial assumptions on which the control of BSE was based were, therefore, shown to be correct. The disease can be eradicated by breaking the cycle of infection via feed. With hindsight, however, it is clear that breaking the cycle is also difficult and requires the rigorous application of regulations and auditing of compliance. Failure to prevent cross-contamination of feed with traces of bovine-derived infectivity led to the infection of many cattle after the use of ruminant MBM was banned. The initial UK feed ban was still very successful, and it is clear that even the application of incomplete measures in many countries did significantly reduce the likelihood of infection. Although it has been demonstrated that calves can become infected by consuming as little as a milligram of BSE-infected brain tissue, the oral ID_{50} is now estimated to be 0.16 g. The significance of cross-contamination of feed with such a low infectious dose during the production, storage, and transportation of feed ingredients was not anticipated, and led to incomplete compliance with feed controls. Draconian risk-management measures are justified by the need to eliminate such feed-borne exposure sooner rather than later.

Exported MBM also proved to be the conduit by which BSE spread to many other countries, some before BSE was even recognized in the UK. Infected cattle were also exported. When infected imported or indigenous cattle died, they initiated epidemics in the importing countries. The timescales from the initiation of recycling of infectivity to the implementation of control measures were usually shorter than in the UK, thus preventing the weight of infectivity in cattle feed from reaching the levels experienced in the UK. Additionally, unlike the UK, in many countries MBM was only an infrequent component of cattle feed.

Early in the course of the epidemic in the UK, the recognition of TSEs in animal species in which such diseases had not previously been recorded strengthened concerns that BSE was capable of spreading to nonruminant species. In domestic cats, feline spongiform encephalopathy (FSE) was recognized in 1990, and also occurred in exotic Felidae, including cheetah, lion, tiger, ocelot, puma, Asian golden cat, and leopard cat. Additional ruminant species were also affected and included nyala, greater kudu, and three species of oryx, eland, ankole cattle, and bison. Most of the cases in ruminants and felids, other than domestic cats, were in zoological collections in the UK. Although the exotic felids would most probably have consumed raw infected tissues from cattle, domestic felids and all ruminants are more likely to have consumed MBM incorporated into their feed.

Pathogenesis

Because the infectious agent cannot be visualized, even by microscopic examination, the pathogenesis or course of the disease from infection to death has been proved difficult to study. Naturally infected animals were examined early in the British BSE epidemic, but the examination of tissues from animals killed at clinical end stage cannot determine the behavior and distribution of the agent in the body during the incubation period of several years. Quantification of risk associated with the consumption of tissues, therefore, relied on the examination of materials derived from experimentally infected calves, sequentially slaughtered during the incubation period.

The gold standard for determination of the presence of infectivity was the inoculation of inbred laboratory rodents. Bioassay in cattle, and in genetically modified mice, have more recently offered increased assay sensitivity. The timescales involved in awaiting incubation in cattle, followed by incubation to negative end points in the assay model, have been long (up to 14 years). The high cost of such studies has also limited the number of assays such that negative results must be interpreted with caution. At times, the categorization of a tissue as noninfectious may arise from the inoculation of a single sample from a single animal. This does not devalue the result, particularly if interpreted alongside the additional negative results from other tissues. Together, they build up a body of evidence that enables confident classification into risk categories, recognizing that negative transmissions cannot be guaranteed to represent the total absence of infectivity. All assay models have limits to their power to detect infectivity.

Once BSE infectivity is ingested, replication of the agent is first detectable, both by bioassay and by immunochemical detection of PrP, in the Peyer's patches (PPs) of the lower small intestine. Most cattle appear from epidemiological studies to become infected in calf-hood, which partly reflects early exposure through feed, but may also be dependent on the presence of prominent and numerous PPs in the ileum of the young animals. These regress naturally when mature.

The next organ in which significant infectivity can be detected is the CNS, appearing almost simultaneously in the brain stem and spinal cord. Thereafter, as infectivity levels rise exponentially and disperse within the CNS (including the eye), there also appears to be gradual retrograde spread into the peripheral nervous system (PNS), including the dorsal root ganglia (DRG), trigeminal ganglion, and peripheral nerve trunks (facial, optic, sciatic, phrenic). DRG lie within the vertebral column, close to the spinal cord, and form the interface between the CNS and motor and sensory peripheral nerve trunks.

The route by which infectivity migrates from the intestine to CNS remains uncertain, but most probably involves transport via the autonomic nerves (vagus and splanchnic) and their associated ganglia. These nerves are, therefore, infected early in the pathogenesis, during preclinical stages of disease. The adrenal gland with its rich sympathetic innervations contains infectivity at or close to clinical onset.

Although infectivity has not been detected in the thymus or spleen, two of the original tissues designated as potential risks to consumers, or indeed in lymph nodes, traces have been detected in palatine tonsils of experimentally infected cattle. Additionally, single equivocal results from sternal bone marrow collected close to the onset of clinical disease in experimentally infected cattle and from pooled nictitating membranes from naturally infected cattle have not been replicated. Consequently, although infectivity has been demonstrated to circulate in blood in scrapie-infected sheep, it is not

possible to conclusively demonstrate a role for hematogenous spread through the body in BSE-infected cattle. Indeed, blood is accepted as a safe commodity as long as there is no risk of contamination with brain tissue at slaughter.

The only additional tissue in which there is published evidence of transmission is muscle. This result, from a single naturally infected and clinically affected cow, was based on evidence of transmission to only one of 10 inoculated transgenic mice expressing the bovine PrP gene. This transgenic mouse, estimated to be even more susceptible than a calf to BSE, otherwise produced results that were consistent with all other bioassays of bovine tissues. Consequently, muscle is not considered to be infected, although the presence of peripheral nerves within muscle masses gives rise to potential risk if consumed after the onset of clinical disease.

Arising from the analysis of pathogenesis is a general theme that in the early stages of infection, the greatest risk is associated with the intestine and particularly the ileum. In the later stages of incubation, intestinal infectivity levels are exceeded by CNS infectivity, which reach maximal levels after onset of clinical disease. Bioassay results have demonstrated that infectivity can be present marginally before the CNS tests positive with a postmortem test. It is not possible to specify when the CNS will become infected in any individual animal. There is considerable variation in incubation period, with naturally infected and clinically affected cattle in the UK ranging from 20 months to 22 years of age when identified. Incubations will have been marginally shorter than lifespan. In experimentally infected cattle, incubation period is generally dose-related, but even within a single dose group it can be highly variable.

The assumption that risk is primarily associated with CNS from cattle aged 30 months or more, as used in defining regulations for the removal of specified risk materials (SRMs), derives from the pragmatic interpretation of epidemiological data, taking into account the decline of risk of infection in young calves by the time the rule was first adopted in the UK in 1996. It is however supported by subsequent evidence from

Table 1 Tissues in which infectivity is confirmed or presumed to be present

Tissue	*When infectious*	*Designated SRMs*
Higher levels of infectivity		
Brain and spinal cord	Late incubation – shortly before onset of clinical disease	Yes
Eye (retina) and optic nerve	After onset of clinical disease	Yes – may be incorporated into definition of skull
Trigeminal ganglion	After onset of clinical disease	Yes – may be incorporated into definition of skull
Spinal ganglia	After onset of clinical disease	Yes – removed with vertebral column
Lower levels of infectivity		
Ileum (lower small intestine)	Probably throughout incubation, but with infectivity levels greatest in first two years of incubation	Yes
Gastrointestinal tract excluding the ileum	Probably throughout incubation, but with infectivity levels greatest in first two years of life. Infectivity levels are much lower than detected in the ileum. Not yet classified by WHO as infectious	In EU, still assumed to be infected, and, partly for ease of risk management, entire intestine is defined as SRM. Other countries restrict definition to ileum
Peripheral nerve trunks Facial Sciatic Phrenic	After onset of clinical disease	No – but likely to be partially removed during carcass dressing/deboning and jointing of meat
Autonomic nerves and ganglia, vagus/splanchnic nerves	Probably from early stages of incubation, and up to clinical onset	No – partial removal likely during carcass dressing
Tonsil	Probably for significant part of incubation period, but only demonstrated in experimentally infected cattle	Yes
Bone marrow – equivocal result	Not clear, but possibly close to onset of clinical disease	No
Third eyelid (nictitating membrane) – equivocal result	Not clear – may be a rare event	No – but will be removed along with skull and eyes
Muscle (equivocal result)	Not clear – evidence is unconvincing, has not yet been repeated	No
Not detected but designated as SRMs		
Thymus	No infectivity detected	Yes originally, but now delisted
Spleen	No infectivity detected	Yes originally, but now delisted
Lymph nodes	No infectivity detected	No – but visible lymph nodes removed along with major peripheral nerves during deboning process

Note: Infectivity levels and risk levels are not synonymous. Many factors determine the degree of risk to consumers, and will include not only the quantity of tissue to which they are exposed, but also the stage of incubation of the source animal (early or late), and the route of exposure. For example, oral exposure is regarded as relatively inefficient in comparison with direct inoculation into the brain or blood stream.

calves experimentally exposed to low doses of infectivity, to simulate the most likely dose received naturally, and provides a considerable margin of safety to consumers.

Tissues in which infectivity or PrP^{Sc} have definitely been detected are summarized in Table 1, which is based on data collated and tabulated on behalf of the World Health Organization (WHO).

Controls

The principles of risk management for animals and humans were, and indeed still are, similar. They involve a precautionary approach, based on known science, but taking into account the need for feasible regulatory measures. The aim is to eliminate or minimize the risk of exposure by mouth, the only routes by which BSE is considered to be naturally transmitted to cattle and humans.

The initial steps were taken before there was any understanding of the pathogenesis of BSE, and were based on what was already known about sheep scrapie. With the benefit of hindsight, this proved to be appropriate. Measures were, however, tempered by the recognition of uncertainty regarding the science of BSE, necessitating a balance between the maintenance of viable food production and allied industries and reduction of putative risk. Excessive regulation, unsupported by scientific evidence, could have generated resistance to the implementation of controls, and put lives at risk.

Protection of Animal Health

Because cattle became infected through the consumption of ruminant-derived MBM, the key control was the prevention of the recycling of ruminant protein. Therefore, the first feed ban in the UK prohibited the feeding of ruminant-derived MBM to ruminants. The World Organisation for Animal Health (OIE) continues to retain this position, insisting that the mechanism of implementation and enforcement of such a prohibition is an issue for individual countries.

In time it became clear that some cattle were still becoming infected in the UK even if born after the introduction of the ruminant-to-ruminant feed ban. In part, this arose because ruminant-derived MBM still contained some infectivity (not all infected tissue had been identified and excluded at that time), and the use of such material in feed for pigs and poultry was still permitted. Rendering standards had not been changed at this point to maximize the inactivation of infectivity in source tissues. These changes are described below.

The presence of contaminated MBM in facilities and equipment that produced or stored feed for ruminants led to a real risk of cross-contamination. Furthermore, it was not possible to test the feed for evidence of random contamination, and uncertainty remained with respect to verification of the species of origin of feed ingredients. No tests introduced to audit feed production systems actually attempt to identify infectivity. They simply aim to detect the presence of tissues derived from prohibited species.

As a result, from 1994 the use of any mammalian-derived MBM (MMBM – to include porcine protein) in ruminant feed was prohibited in the European Union (EU). The continuing evidence for the role of cross-contamination in premises manufacturing animal feed led to an even wider prohibition in the UK in 1996. From this point, MMBM was excluded from all feed for farmed animals, including fish and horses, and indeed also for pets where there was a risk of contamination of feed intended for ruminants. Feed prohibitions were similarly extended in the EU at the beginning of 2001, although the prohibition referred to processed animal protein rather than MMBM. Minor exclusions are permitted, such as the feeding of milk or blood meal.

Three additional measures reinforce the protection of cattle, namely the slaughter and destruction of clinically affected cases, a prohibition on the use of high-risk tissues (SRMs) for animal feed (although now encompassed in rules for their segregation and destruction) and improved rendering standards. Because clinically affected cattle represent the greatest source of infectivity, in particular within the CNS, their exclusion from the rendering system will have reduced the infectious load entering the process.

SRMs were originally defined for the protection of human health, and were removed from healthy animals at slaughter. Their removal from animal feed, including animals that die on farm from which SRMs are not removed, protects animal health by also reducing the infectious load entering the rendering and animal feed chains. Although retrospective audits and advancement of the underlying science confirmed that some infectivity continued to enter the rendering plants in the UK in the early 1990s, the absence of cases of FSE in cats born in the UK after the first exclusion of SRMs from animal feed demonstrates the effectiveness of the measure.

Research conducted in the EU into the effectiveness of rendering processes in inactivating BSE, and scrapie demonstrated that most could not guarantee the elimination of infectivity. This led to changes in EU regulations, both in terms of processing standards (e.g., higher temperatures) and the segregation of raw materials into risk categories. The end result, a regulation that prescribes processes for the handling of all wastes, demands the most rigorous standards for tissues and carcasses that potentially carry a risk of transmitting TSEs. Animal by-products, other than SRMs, arising from animals that are healthy and fit for human consumption are subject to less rigorous processing standards. In non-EU countries, rendering processing standards have not necessarily been adjusted, however, particularly where risk is perceived as being low.

In summary, the removal, segregation, and processing of SRMs, or carcasses containing SRMs, reinforce the wider feed prohibition in order to minimize the risk of continued exposures and infection of cattle through the cross-contamination of feed. Protection of animal health inevitably delivers a reduction in risk for humans, albeit over a longer time frame. With the incubation period averaging 5 years in the UK, and frequently much longer elsewhere, it is usually necessary to wait many years before controls visibly reduce the prevalence of disease. Subtle effects may be seen sooner, but a decline in case numbers is likely to take 5–6 years. This is why it is recommended that preventive measures are implemented before an epidemic is detected in countries classified as being at risk. The consequences of the introduction of controls, in the

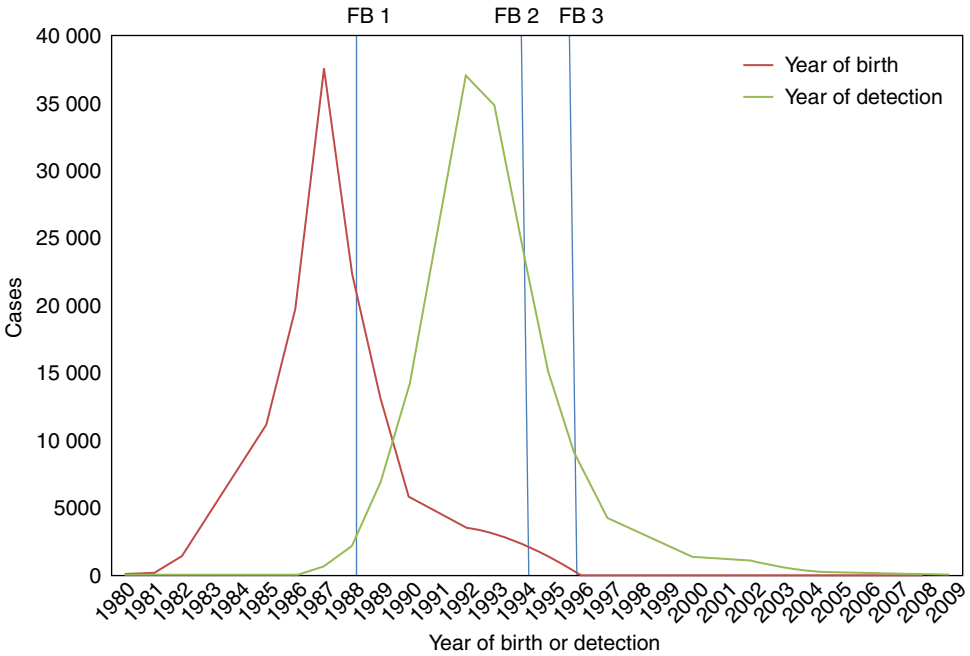

Figure 2 Graphic of British epidemic, showing by date of birth and date of confirmation – with feed bans superimposed, based upon data supplied by the Veterinary Laboratories Agency, United Kingdom. The two lines represent all cases detected, predominantly as clinical cases, the first plotted by date of birth (infection will have occurred shortly afterwards), the second by date of detection. The second peak includes animals for which no accurate date of birth was available, and therefore represents more animals than in the first peak, but this does not detract from the trends depicted, and their relationship to feed bans. The interval between peaks represents the mean incubation period of 5 years. FB1: first feed ban – ruminant protein to ruminants; FB2: second feed ban – mammalian MBM to ruminants; FB3: third feed ban – MMBM to all livestock.

form of graphic representation of the UK epidemic, on which the different feed bans are superimposed, are provided in Figure 2.

Protection of Human Health

The eradication of BSE offers the greatest protection to consumers, but interim measures are necessary to mitigate risk of exposure until this is achieved.

The most obvious protective measure is the exclusion of clinically affected cases of BSE from the human food chain. These represent the greatest risk because infectivity levels in the brain and spinal cord are at maximal levels. It is important, however, to appreciate that the clinical signs of BSE can be very subtle in their early stages, and will not always guarantee detection and exclusion of the affected animal from the food chain.

By late 1989, it proved possible to define and introduce additional controls in the UK that recognized the fact that infected cattle, destined to be consumed by humans, remained alive but undetectable on British farms. They could not be tested while alive to determine whether they were infected, and this remains unchanged at the time of writing. Based on knowledge of the pathogenesis of sheep scrapie, a short list of tissues was identified that appeared to represent the greatest risk to consumers if eaten. At the time, there were no data about the actual risk associated with these tissues in cattle, nor evidence that humans were susceptible to BSE, but they were excluded from the human food chain on a precautionary basis.

With time, research into BSE has confirmed the potentially infectious status of some of these tissues (brain, spinal cord, intestine), but did not confirm the categorization of others (thymus, spleen). Additional tissues have been added to the list of SRMs on the basis of new research findings, but it is interesting that results arising late in the EU epidemic, when prevalence of infection was low, and consumer concerns reduced, did not trigger the extension of the list of SRMs. For example, peripheral nerves have never been classified as SRMs, but are thought only to become infectious at about the time of clinical onset. With respect to the likely impact of such tissues on human exposure levels, it is necessary to appreciate that the amounts of infectivity estimated to be present in nerves are substantially less than in the brain and spinal cord.

There continues to be some international disagreement in the definition of SRMs, with the EU adopting a more precautionary approach than elsewhere. This is primarily because of the scale of the crisis in consumer confidence that occurred in Europe in 1996. Consumer risk was inevitably greater in the EU by virtue of the large number of infected cattle present, but relaxation of regulations is anticipated as risk falls to acceptable levels. It is, however, more difficult to relax regulations than to introduce them, because the relaxation inevitably increases risk to consumers even though the residual risk may still be extremely low. SRM measures in Europe, North America, and Japan as at January 2009 are summarized in Table 2. Some SRMs, such as skull and vertebral column,

Table 2 A summary of bovine-derived SRMs in Europe, North America, and Japan as at January 2009

	European Union and Switzerland
Cattle	
Skull (including brain and eyes)	>12 months
Tonsils	All ages
Spinal cord	>12 months
Vertebral column (including dorsal root ganglia – DRG – but excluding vertebrae of the tail and the transverse processes of lumbar and thoracic vertebrae)	>30 months
Intestines and mesentery	All ages
	USA
Cattle	
Skull (including brain, eyes, and trigeminal ganglia)	>30 months
Tonsils	All ages
Spinal cord	>30 months
Vertebral column (including dorsal root ganglia – DRG – but excluding vertebrae of the tail and the transverse processes of lumbar and thoracic vertebrae, and wings of sacrum)	>30 months
Distal ileum	All ages
	Canada
Cattle	
Skull (including brain, eyes, and trigeminal ganglia)	>30 months
Tonsils	>30 months
Spinal cord	>30 months
Dorsal Root Ganglia (Vertebral column, excluding vertebrae of the tail and the transverse processes of lumbar and thoracic vertebrae, and wings of sacrum, is not defined in law as SRM, but removal from the human food chain is ensured by administrative action through meat hygiene controls.)	>30 months
Distal ileum	All ages
	Japan
Cattle	
Head (including brain, eyes, and tonsils, but excluding tongue and cheek meat)	All ages
Spinal cord	All ages
Vertebral column (including dorsal root ganglia)	All ages
Distal ileum	All ages

are not inherently infected. They are designated as SRMs because they are closely associated with the brain and spinal cord, and it is assumed that they are either contaminated during the slaughter and carcass dressing process or that it is impossible to completely separate the CNS tissues from the bones encasing them.

There were two further examples of protective measures that were driven by potential contamination of raw materials during the process of slaughter and meat production, rather than the inherent presence of infectivity. These affected procedures for the stunning of cattle and the production of mechanically separated meat. When cattle were stunned before slaughter with a mechanism that injected air into the skull, particles of brain tissue were subsequently detected in the blood stream. This meant that any tissues exposed to that blood could potentially be contaminated. Blood itself has not been shown to be infectious. The prohibition of the use of such methods minimized the risk of accidental contamination of muscle and other organs.

Mechanically separated meat, however, was a process by which the vertebral column and some other bones were subjected to further treatment after the primary dressing of the carcass. This process mechanically stripped residual meat from the bone. Any residual infectivity attached to the vertebral column after carcass splitting, via retained DRG, incomplete removal of spinal cord, or contamination of the cut surface caused by the splitting saw, presented a risk that the stripped meat could be contaminated. Controls were, therefore, necessary to prohibit the use of bovine bones in the manufacture of such products.

It is interesting that no cases of vCJD have been identified in the UK in individuals that were born after the introduction of the first SRM prohibition. Although with hindsight it is now known that this measure did not remove all infectivity from the food chain, it appears to have had a significant effect in reducing human exposures and likelihood of infection.

Two further measures were perceived to have possible additional protective effect for humans. First, milk from clinically affected cattle was excluded from the human food chain (UK only). Although infectivity has not been detected in milk from BSE-affected cattle, it has been demonstrated in sheep with scrapie. Second, the identification of birth or feed cohorts of affected cattle, and their exclusion from the food chain, was adopted internationally. Birth or feed cohorts represent animals that were born or reared at about the same time as an infected animal, on the same farm, and were potentially exposed to the same feed source. They clearly represent a greater risk than the population at large, but with the exception of cattle born in the UK before the first feed ban came into force in 1988, the numbers of infected animals actually detected in cohorts has been extremely low.

A final protective measure, not specifically introduced to reduce the risk for BSE, now included in international BSE-related guidelines for trade in beef includes the removal of visible lymph nodes and nerves during the process of dressing carcasses following slaughter. This applies particularly to deboned beef, where preparation of the meat for sale, storage, and transit has involved the removal of such tissues for many years. Infectivity has never been detected in bovine regional carcass lymph nodes, or in bones, but such a measure provides the final level of protection should clinically affected cattle be inadvertently slaughtered.

The introduction of postmortem testing of healthy cattle of more than 30 months of age in Europe, and later in Japan even in younger cattle, was frequently portrayed and perceived as a risk reduction measure. There is no doubt that it increased consumer confidence, but in reality, SRM removal ensured that the vast majority of infectivity was removed from the food chain anyway. Furthermore, SRM removal protected consumers from instances where infectivity was present in the brain, but the postmortem test result was negative. Following research into the time-course of detection of PrP^{Sc} by

postmortem tests on experimentally infected cattle, it has been estimated that postmortem testing would only detect approximately 50% of infected animals at 1.7 months before onset of clinical signs. Detection would fall close to zero at 3 months before onset of disease, or 97% of the incubation period. In most countries, where epidemics are likely to be small and infectivity levels in cattle feed low, incubation periods are likely to be very long. Therefore, infected cattle that are slaughtered while still healthy are most unlikely to be detected by postmortem testing.

At the time of writing, investigations into cases of vCJD have not identified any route of infection directly from cattle to humans other than through contaminated food produced before the introduction of regulatory controls.

Specific Food Products

To the consumer, the relationship between a food product and a specific source tissue may not always be obvious. This is particularly so where a manufacturing process is involved. Additionally, the pooling of materials from different animals and regions compromises traceability. Regulatory authorities have used the evolving knowledge of the pathogenesis of BSE to ensure that food, whether processed or unprocessed, is safe by excluding certain tissues from the food chain, but their impact on certain commodities may not be obvious. In view of their widespread use, it is appropriate to discuss certain commodities and their safety in a little more detail.

It is important to remember that safety is assured by two measures: the exclusion of infectivity by the removal of tissues in which the presence of infectivity is suspected or confirmed and the prevention of contamination with infected tissues during the manufacturing process. At no time have authorities accepted the principle that food can be made safe solely by the application of specific processing standards. Heating, or cooking, has never been accepted as sufficient to remove infectivity, and even in the production of animal feed, it is only expected to reduce infectivity levels rather than guarantee elimination.

The most obvious commodity that receives minimal processing before consumption is milk. The initial exclusion of milk from clinically affected cows from the human food chain in the UK was precautionary. It presumed that because sheep scrapie appeared to transmit from ewe to lamb, this may have been via milk. There is now sound scientific support for such transmission in sheep, but none for transmission of BSE via bovine milk. For this reason the OIE, supported by the WHO, lists bovine milk as a commodity that can be traded safely irrespective of the BSE status of the country of origin.

Gelatine is an animal by-product that is incorporated into a wide range of food and pharmaceutical products. It is derived from two source tissues, hides or bones, and the extraction of gelatine involves rigorous chemical processes. Infectivity has not been detected in hides. Gelatine derived from skin or hide is, therefore, considered to be free of risk. Although infectivity has not been detected in bone, the close association of the skull and spinal (vertebral) column with the brain and spinal cord means that the bones are inevitably contaminated with CNS tissue during the process of carcass dressing. Despite the rigor of the process for extracting gelatine from bones, it has not been possible to conclusively demonstrate that it is free of infectivity when derived experimentally from raw materials that contained BSE infectivity. In part, this is because of the limitations of sensitivity of the assay methods. The outcome of extensive consideration by expert committees is a requirement to exclude bovine skull and vertebral column from the manufacturing process in BSE-affected countries.

Tallow is a product that most consumers are unaware of, but is an essential ingredient of much processed food and of pharmaceuticals. It is produced by rendering tissues resulting in three by-products, namely water, fat, and protein. The water is driven off as steam, the fat is separated from the protein, and the latter is further processed into MBM. The separated fat is processed further to produce a range of end products or derivatives. There are several categories of tallow determined in part by the nature of the raw materials and by the type of rendering process used. Tallow intended for use in food products would be derived from animals and raw materials already inspected and passed fit for human consumption. Although there is no published evidence that infectivity is found in tallow after rendering, there is both unpublished data and scientific opinion that suggest that such a risk cannot be totally ignored. As for gelatine, this subject has been debated extensively, concluding that any residual infectivity is likely to be associated with traces of protein that may remain in the tallow. Consequently, trade rules focus on establishing maximal levels for insoluble impurities in tallow if it is allowed to be traded freely. Levels of contamination above this maximum require the application of additional conditions to the production process, which may include the exclusion of certain SRMs.

It is worth stressing, however, that the residual risks estimated to be associated with gelatine and tallow are extremely low. Furthermore, these commodities are not inherently risky. If there is no BSE in the cattle from which source tissues are derived, then there will be no scope for infectivity to be present in the end product unless contaminated from another source after production.

Diagnosis

At the time of writing, there is no live animal test for BSE. Claims that tests can detect BSE *in vivo* must be treated with caution unless accompanied by evidence of thorough evaluation by a third party.

Clinical signs alone are insufficient to enable detection of the majority of infected animals. In most countries that have experienced BSE, such cases are too rare to reinforce diagnostic skills, and only the most extreme clinical signs are recognized.

In accordance with rules established by the OIE, BSE should be legally notifiable. This ensures that suspicion of disease is reported to national authorities, and that tests used to confirm a diagnosis are appropriate. The accepted approaches for postmortem diagnosis are established in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial animals, and accommodate the likely range of technical facilities that may be present in member countries.

BSE Risk – Categorization of Countries

Traditional approaches to international trade in animals and animal products require the declaration of health status of source countries, zones, farms, or animals. This may involve the testing of individual animals or herds. Because of the difficulties encountered in identifying BSE-infected animals while still alive, national and international organizations have adopted alternative approaches to define BSE-related risk. Additionally, it is accepted that the risk mitigation measures that need to be introduced in a country should be driven by the estimated risk within it, as determined by independent scrutiny of national data. In other words, countries that represent a low BSE risk require fewer control measures and smaller surveillance programs than those with significant risk. A proportionate response to risk is considered appropriate.

Although with other diseases the incidence of disease is normally an appropriate indicator of risk, the recognition that clinically affected cases of BSE are difficult to detect and that they represent only a small proportion of infected animals challenges this traditional approach to categorization. The European Commission was advised by its former Scientific Steering Committee (SSC) to apply a comprehensive qualitative risk-assessment approach, based initially on likelihood of exposure from the UK through the importation of MBM, and potential for further amplification of infectivity after importation. This approach proved to be very successful, and was reinforced by the later results of active surveillance. Infected cattle were detected in several countries that previously claimed freedom from disease, and which may have exported BSE-infected cattle or contaminated products before indigenous cases were identified.

The SSC methodology was eventually updated by the European Food Safety Authority (EFSA), and took into account changes adopted in the OIE rules for categorization and surveillance. It is important to recognize the fact that statements of BSE-related risk are most robust where countries have been categorized as being of negligible risk or controlled risk. Remaining countries, which are of undetermined risk, may not have been exposed to BSE. It would be wrong, therefore, to presume that their inclusion within this broad category is an indicator of risk, but the absence of external evaluation of risk means that statements of safety must be interpreted with caution.

Future categorization will take into account the establishment of surveillance programs that target the most appropriate population of cattle (potential clinical cases in particular), but also recognize the infrastructure in individual countries. A one-size-fits-all program is not possible, other than in the establishment of a target for each to achieve in order to facilitate categorization. Such programs are intended to detect trends in prevalence of infection, as a means of monitoring the effectiveness of controls.

Conclusions

There are two reasons for anticipating that BSE will eventually assume a more appropriate position in the spectrum of foodborne zoonoses to which consumers are exposed. First, however incomplete, the research conducted over the past 20 or more years indicates that irrespective of disease incidence in a source country, the distribution of infectivity within the body of infected cattle lends itself to practical approaches to risk reduction. Second, the natural experiment conducted in the UK before the introduction of control measures, when exposure levels were high, demonstrates that exposure of both animals and humans can be controlled.

The steep and continued decline in prevalence of BSE in cattle following the introduction of the feed ban illustrates this statement, albeit with a time lag that is determined by the long incubation period. In addition, the absence of vCJD cases in humans born after the first ban on consumption of SRM in 1989 will, if sustained, confirm the effectiveness of excluding targeted high-risk tissues in protecting consumers. Although the consequences of infection are fatal, the likelihood of infection after the initial SRM regulation was low relative to the quantity of infectivity that entered the food chain beforehand. Evidence for transmission between humans, for example via blood transfusions, does not undermine such a statement.

The first announcement of the likely transmission of BSE to humans prompted unprecedented concerns about human safety. Uncertainty prompted prognostications of doomsday scenarios, with millions of human deaths. They also failed to recognize the fact that the human cases arose from historical exposure, before the introduction of protective measures. Global events drove the establishment of international protective measures to unprecedented levels, but the process of relaxation has begun and may conclude with residual, but focused, measures that may remain in place in perpetuity. Countries that have taken the trouble to determine their status *vis-à-vis* BSE risk should be congratulated, and others encouraged to transparently eliminate any uncertainty surrounding the status of their national herds.

See also: Analytical methods: Transmissible Spongiform Encephalopathy Diagnosis. Prions and Agents of TSEs: Creutzfeldt–Jakob Disease

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PRIONS AND AGENTS OF TSES

Creutzfeldt–Jakob Disease

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Glossary

Creutzfeldt–Jakob disease The most important human prion disease.

Mechanically recovered meat A material derived by a pressure system applied to portions of the carcass of animals which is used as an additive in food products.

Prion diseases Diseases caused by an infectious form of a misfolded host protein.

Sporadic Creutzfeldt–Jakob disease The commonest form of human prion disease which is of unknown etiology and occurs worldwide.

Variant Creutzfeldt–Jakob disease A form of Creutzfeldt–Jakob disease causally linked to bovine spongiform encephalopathy in cattle.

Characteristics

Creutzfeldt–Jakob disease (CJD) is the most important human form of transmissible spongiform encephalopathy or prion disease and is unique biologically as it may occur as a sporadic, hereditary, or transmissible disease. There are a number of subtypes of CJD, classified in part according to etiology (Table 1), but all forms of CJD are progressive and fatal neurological diseases, with no effective treatment for the underlying disease process. Prion diseases, including CJD, pose particular challenges to public health because the causal agents are relatively resistant to decontamination or sterilization, and infectivity is present, asymptomatically, in peripheral tissues as well as the central nervous system (CNS). Human prion diseases are associated with extended incubation periods, measured in years or even decades, and there is no available *in vivo* test to identify individuals who are incubating the disease. The identification of variant CJD (vCJD) as a zoonosis, causally linked to bovine spongiform encephalopathy (BSE), has had significant implications for the food industry because of the hypothesis that the transmission from cattle to humans was through dietary exposure to BSE infectivity in the food chain.

Epidemiology

Sporadic CJD (sCJD) occurs worldwide with a relatively standard mortality rate of 1–1.5 cases per million per annum and the temporal and geographical distribution of cases within countries appears random, with no localized aggregation of cases. This evidence, together with the failure to identify any consistent risk factor for the development of disease, has led to the hypothesis that sCJD is not related to acquired environmental infection, but develops following the chance, spontaneous transformation of prion protein in the brain to the disease-associated self-replicating form. This hypothesis is now widely accepted and is compatible with the occurrence of sCJD in countries, such as Australia, in which animal prion diseases have not been identified. The implication is that sCJD is not a zoonosis and therefore has no implications for food safety.

Hereditary forms of human prion disease, including genetic CJD, Gerstmann–Straussler syndrome (GSS), and fatal familial insomnia (FFI), are dominantly inherited and associated with point or insertional mutations of the human prion protein gene (*PRNP*). The frequency of these disorders varies by country, for reasons that are unclear, except that in Libyan-born Israelis and localized areas of Slovakia there may be relative genetic isolation. The occurrence of a genetic disease that is nevertheless infectious, as confirmed by laboratory transmission studies, is a paradox, but the occurrence of cases within branches of pedigrees that are separate in time and space argues against genetic susceptibility to an acquired infection. The favored hypothesis is that the *PRNP* mutations lead to instability in the brain prion protein, which is more likely to adopt the disease-associated form and eventually result in disease.

Iatrogenic CJD is caused by the transmission of infection from person to person in the course of medical or surgical treatment. Although such transmission has rarely occurred through contaminated neurosurgical instruments or corneal grafts, the numerically most significant mechanism of transmission has been through human pituitary derived hormones, in particular human growth hormone (hGH), and human dura mater grafts, with more than 400 cases in total worldwide. Pituitary glands or dura mater obtained at postmortem from sCJD patients must presumptively have been included in the production process and cross-contamination resulted in dissemination of infection. Importantly, these transmissions

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Table 1 Human prion diseases

Disease	*Geographical distribution*	*Etiology*
sCJD	Worldwide	Unknown
vCJD	Predominantly UK, smaller numbers in France, Ireland, Italy, Netherlands, Portugal, Spain, USA, Canada, Saudi Arabia, Japan	BSE
Genetic CJD/GSS/FFI	Worldwide but higher incidence in Slovakia, Italy, Israel	Mutation in *PRNP* gene
Iatrogenic CJD	Variable; hGH cases most frequent in France and UK. Dura mater most frequent in Japan. Four cases of transfusion transmission of vCJD in the UK	CJD-infected pituitary hormones, dura mater grafts, neurosurgical instruments, stereotactic EEG, corneal grafts, blood

have involved CNS tissues with high levels of infectivity and there is no evidence, to date, of transmission of sporadic (or hereditary) CJD through general surgical instruments or peripheral tissue transfer. In vCJD, there are higher levels of infectivity in peripheral tissue, including the lymphoreticular system, and there have been four instances of transmission of infection from person to person through blood transfusion, including three clinical cases and one pre- or subclinical infection, in which abnormal prion protein was identified in the spleen of an individual who died of a nonneurological disease.

vCJD was first identified in 1996 in the UK and was thought to be a novel human prion disease as the clinical and pathological phenotype had not been observed previously. It was suggested that this disease might be a zoonosis owing to the occurrence of this disease in a country with a potentially novel risk factor for prion disease, BSE, and the absence, at that time, of cases with a similar phenotype in some other European countries which had established systematic surveillance systems for CJD, namely France, Germany, Italy, the Netherlands, and Slovakia. The hypothesis that BSE infection had been transmitted to humans through infectivity in the human food chain raised concerns that there might be a large epidemic of vCJD as the human population had potentially been exposed to large quantities of BSE-infected material. However, the number of observed cases of variant CJD in the UK has been relatively limited (**Figure 1**) and the epidemic curve peaked in 2000 and has subsequently declined.

Since 1996, cases of vCJD have been identified in a number of other countries ($n = 10$), mainly in Europe. There is an international agreement that cases of vCJD will be attributed to the country of residence at the time of disease onset, even if the causal exposure may have taken place in another country at a time of prior residence. **Table 2** provides data on the number of cases of vCJD by country and includes the number of cases linked to blood transfusion and the number of cases by country, with a history of residence in the UK for a period of at least 6 months. These cases are presumed to have been exposed to BSE infection in the UK and to have later developed clinical disease in the country of residence. There is some evidence that the vCJD cases outside the UK, with no history of having lived in the UK, may have been infected through exposure to infection through BSE-implicated exports from the UK, rather than indigenous BSE. The source of infection in the cases from Saudi Arabia is unknown as BSE has not been identified in Saudi Arabia, and UK imports were limited. The overall trend in the number of vCJD cases outside

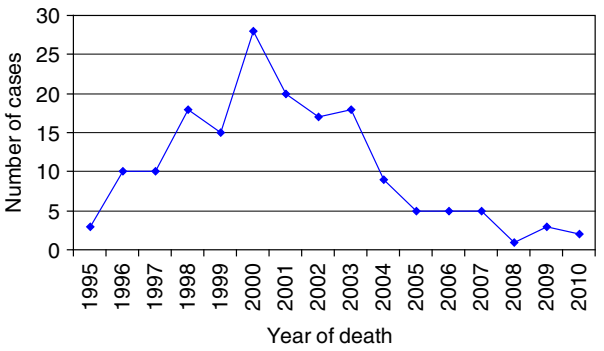

Figure 1 Deaths from vCJD in the UK.

the UK is in decline, most notably in France, which has the second largest number of cases ($n = 25$), but it is still possible that further countries exposed to indigenous BSE or with a history of UK imports will identify cases of vCJD.

The incubation period in vCJD is unknown because the timing of the causal exposure cannot be identified in any individual case. However, mathematical modeling of the epidemic suggests that the mean incubation period is approximately 15 years and this is consistent with estimates of the incubation period derived from the known periods of residence in the UK and the timing of disease onset in non-UK cases. The infectious dose and the route of exposure are important determinants of incubation periods in prion disease and the interval between blood transfusion and disease onset in transfusion transmitted cases is between 5 and 8 years, shorter than by oral exposure, probably because the intravenous route of exposure to infection is more efficient.

One characteristic that distinguishes vCJD from sCJD is a significantly earlier age at death with mean ages at death of 29 and 66 years, respectively. The reason for this disparity is unknown but hypotheses include age-related susceptibility to infection and variation in dietary exposure to the BSE agent by age cohort. Fitting the vCJD epidemic curve to estimated BSE exposure patterns requires an age-related susceptibility factor, but variation in exposure to BSE by age is likely to be a codeterminant of age at onset of the disease. The incidence of vCJD in the UK is approximately double in the north of the UK in comparison with the south and one analysis showed a relationship between the regional incidence of vCJD and regional differences in past intake of foodstuffs potentially contaminated with high levels of BSE infectivity, including food containing bovine-derived mechanically recovered meat (MRM).

Table 2 Variant CJD cases worldwide (July 2010)

Country	*Total number of primary cases (number alive)*	*Total number of secondary cases: blood transfusion (number alive)*	*Residence in UK more than 6 months during the period 1980–96*
UK	170 (4)	3 (0)	173
France	25 (0)	–	1
Republic of Ireland	4 (0)	–	2
Italy	2 (1)	–	0
USA	3 (0)[a]	–	2
Canada	1 (0)	–	1
Saudi Arabia	1 (0)	–	0
Japan	1 (0)[b]	–	0
Netherlands	3 (0)	–	0
Portugal	2 (0)	–	0
Spain	5 (0)	–	0

[a]The third US patient with vCJD was born and raised in Saudi Arabia and has lived permanently in the US since late 2005. According to the US case-report, the patient was most likely infected as a child when living in Saudi Arabia.
[b]The case from Japan had resided in the UK for 24 days in the period 1980–96.

The main risk factors for developing vCJD are young age, a history of residence in the UK and a specific genotype, methionine homozygous at codon 129 of the human prion protein gene (*PRNP*). Codon 129 of *PRNP* is a polymorphic region, with genotypes at this locus varying in the normal population between methionine homozygous (MM), valine homozygous (VV), and heterozygous (MV) (Figure 2). Variation at this site is known to influence susceptibility to sporadic and iatrogenic CJD, but in vCJD to date, all definite and probable cases with available genetic analysis have been MM homozygous. The biological mechanism underlying this observation is uncertain, but homology of the prion protein gene may facilitate prion conversion and all bovines are MM at the equivalent site in the bovine prion protein gene.

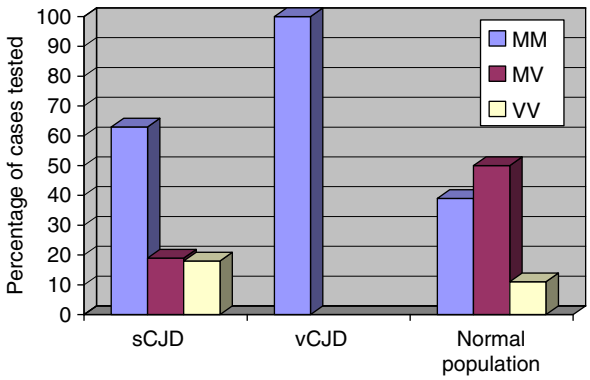

Figure 2 Codon 129 distribution in sporadic CJD, variant CJD, and the general Caucasian population.

Evidence of a Link Between BSE and vCJD

The first indication of a causal link between BSE and vCJD was the occurrence of a new human prion disease in the UK, where the human population had been exposed to a potential novel risk for prion disease in the form of BSE. Although cases of vCJD have subsequently been identified in a number of other countries, the highest incidence of vCJD is in the UK, the country with the highest incidence of BSE, and other countries with cases of vCJD have either indigenous BSE epidemics or a history of importing bovines or bovine products from the UK. As indicated above, there are also a small number of cases that were exposed to BSE in the UK and developed vCJD years later in their country of residence. The possibility that vCJD was identified as a result of improved ascertainment of an already existing disease is unlikely, as no previous case with a similar pathology has been identified despite rigorous review of pathology archives in the UK and other European countries. Furthermore, only small numbers of cases have been identified outside the UK despite systematic surveillance in many countries, including a coordinated surveillance program for CJD in Europe. It would be a remarkable coincidence if vCJD was a rare indigenous disease and the isolated cases identified in countries such as the USA and Canada had a history of residence in the UK as a matter of chance. Recent laboratory transmission studies have demonstrated that the agent characteristics in terms of incubation periods and anatomical distribution of brain lesions are virtually identical in cases of vCJD from the Netherlands, France, Italy, the USA, and the UK.

The hypothesis that the infectious agent or prion in vCJD is derived from BSE infection has been supported by a number of laboratory studies. The biochemical properties of the prion protein on Western blot analysis are identical in BSE and vCJD, and distinct from other forms of human prion disease. The characteristic florid plaques that are deposited in the brain in vCJD are also present in a primate model after intracerebral inoculation of BSE. Most compelling are the results of transmission studies in laboratory rodent models, including wild-type and transgenic mice. In these studies, the incubation periods and neuropathological lesions are remarkably similar following intracerebral inoculation of BSE and vCJD, whereas the results following inoculation of sCJD are clearly distinct. The conclusion of these studies is that the agent causing vCJD is the same as the agent causing BSE.

If BSE is the cause of vCJD, a critical question is the mechanism of transmission of infection from cattle to humans. Human exposure to the BSE agent could potentially occur through a range of mechanisms, for example, through dietary contamination, bovine-sourced medications, or through occupation, such as working in an abattoir. Searching for a potential causal exposure is complicated by the long incubation period and the necessity to obtain information on relevant risk factors from the relatives of patients, because the nature of the neurological illness often precludes direct interview with cases. Additional information has been sought from medical and general practitioner records, which contain details of past surgical procedures and medical treatments, including vaccination history. A case-control study of risk factors for vCJD, including 136 cases from the UK, found that frequent consumption of beef and beef products were thought likely to contain mechanically recovered or head meat, or both, including burgers and meat pies, and was associated with increased risk for vCJD. No other risk factors were identified and there was no link with prior occupation or animal exposure, or with medical or surgical exposures. This is consistent with dietary exposure to BSE as the mechanism of human exposure to BSE, but the results are compromised by the potential for reporting bias. However, no other plausible mechanism of exposure has been identified and the UK population was exposed to large quantities of BSE infection in the human food chain during the 1980s and to a lesser extent during the early 1990s.

The possibility of secondary transmission of vCJD through organ or tissue transplant, or through the reuse of contaminated surgical instruments, has been an important concern for public health. To date, there is no evidence of this form of transmission, but the potential protracted incubation periods following relatively low-dose exposure by a peripheral route indicate that it may be many years before this type of transmission can be excluded.

Clinical Features and Diagnosis of CJD

All forms of CJD present with a progressive neurological disorder, but the clinical and investigative features vary by subtype. Diagnostic criteria for the various forms of human prion disease have been formulated and partially validated.

sCJD characteristically presents with a rapidly evolving dementing illness, often associated with cerebellar ataxia and myoclonic involuntary movements of the limbs and trunk. The mean survival from symptom onset to death is only 4 months, which distinguishes sCJD from other, more common, forms of dementia. The mean age at death is 65 years, but there is a wide range in age at onset of symptoms and sCJD has been identified in individuals in their teens and in their nineties. Age alone cannot distinguish sCJD from other forms of human prion disease. The clinical and pathological phenotype varies according to the codon 129 genotype and the biochemical form of prion protein deposited in the brain, of which there are two main forms, Type 1 and Type 2, distinguished by their mobility on Western blot analysis. The typical, rapidly progressive, form of sCJD is associated with an MM or MV genotype and Type 1 prion protein, whereas atypical forms of sCJD with a relatively prolonged survival, atypical neuropathological features, or a younger age at onset of symptoms are associated with the other combinations of genotype and protein type, MM2, MV2, VV1, or VV2. The typical MM1 or MV1 cases account for approximately 80% of sCJD cases and the other forms are rare.

Hereditary forms of human prion disease vary in clinical and neuropathological features according to the underlying mutation. Some forms of hereditary CJD have a phenotype that is very similar to sCJD, for example, cases associated with the mutation at codon 200 of *PRNP*, but other forms of hereditary human prion disease have distinctive features, including cases of GSS, which present with cerebellar ataxia and FFI which presents with insomnia and autonomic disturbance. The genotype at codon 129 influences the clinical presentation, notably with the codon 178 mutation in which an MM genotype is associated with FFI and a VV genotype with an illness similar to sCJD. However, there is marked variation in clinical phenotype both between and within families which is unexplained and not determined solely by genetic influences. Overall, patients with hereditary forms of human prion disease develop symptoms approximately 5 years earlier than sCJD and the duration of illness is also longer.

In iatrogenic CJD, the clinical features are determined in part by the route of exposure to infection. Iatrogenic CJD with a peripheral route of exposure to infection, for example, cases related to prior treatment with human pituitary growth hormone, which was given by injection, present with a cerebellar syndrome and there may be little evidence of cognitive impairment. Cases caused by the introduction of infection directly in to the CNS, for example, through contaminated neurosurgical instruments, present with features akin to sCJD. In cases related to human dura mater grafts there is variation in clinical presentation, although many of these cases have features similar to sCJD. Homozygosity at codon 129 of *PRNP* is a risk factor for the development of iatrogenic CJD.

vCJD has a clinical presentation which is different from other forms of human prion disease, consistent with the hypothesis that this condition is caused by a distinct strain of infectious agent. In addition to the relatively early age of disease onset mentioned above (mean 29 years), the first symptoms are psychiatric rather than neurological, with early depression , anxiety, and sometimes delusions for a mean of 6 months before the development of ataxia, involuntary movements including chorea or myoclonus, and progressive cognitive impairment. The terminal stages are similar to sCJD and the mean survival is 14 months from symptom onset. The nonspecific initial psychiatric features make early diagnosis very difficult and indicators include persistent painful sensory symptoms, gait ataxia, or frank cognitive decline. All tested definite or probable cases of vCJD internationally have been MM homozygous at codon 129 of *PRNP*.

Diagnosis of CJD

A number of specialist investigations are useful in supporting the diagnosis of CJD, but their utility varies according to the disease subtype. As with other neurological disorders, diagnosis depends on suspecting the relevant condition and

arranging and interpreting the results of investigations in the clinical context.

In sCJD, the most useful investigations are the electroencephalogram (EEG), which shows periodic complexes at a frequency of approximately $1\,s^{-1}$ in approximately 50% of cases and immunoassay for 14-3-3 protein in the cerebrospinal fluid (CSF) which is positive in approximately 80% of cases. Recently high signal in the caudate or putamen on MRI brain scan has been found to have a relatively high sensitivity (75%) and specificity for the diagnosis of sCJD, and has been included in the diagnostic criteria for case classification. These criteria include a combination of suggestive clinical features and the results of EEG, CSF, and MRI brain scan to allow classification of cases as definite, probable, or possible. A definite diagnosis requires the identification of the cardinal neuropathological features of sCJD (spongiform change, neuronal loss, and astrocytic gliosis) at postmortem or rarely brain biopsy. Most authorities report definite and probable cases of sCJD as there is significant diagnostic uncertainty in cases classified as possible sCJD.

The diagnosis in hereditary forms of human prion disease depends on either obtaining a family history of CJD or other human prion disease in a relative or relatives of the index patient or on identifying a disease-associated mutation on sequencing of *PRNP*. Only approximately one-third of mutation-associated cases have a positive family history and *PRNP* sequencing is essential for an accurate diagnosis if hereditary CJD is suspected, although routine genetic analysis in all cases of CJD may be necessary to avoid missing genetic cases. Specialist investigations, including EEG, CSF 14-3-3, and MRI brain scan, may be positive in genetic forms of human prion disease, but in many cases these investigations may not be diagnostic.

Iatrogenic cases of CJD are recognized on the basis of identifying a relevant risk factor in a patient with a suggestive neurological presentation. A history of exposure to human pituitary hormones, human dura mater graft, or corneal graft should be sought in all suspected cases of CJD as the clinical phenotype in iatrogenic CJD may be similar to sCJD. Iatrogenic transmission through contaminated neurosurgical instruments has not been recognized for decades, but any potential exposure through this mechanism requires specific epidemiological investigation. Specialist investigations are less likely to be helpful in diagnosis of iatrogenic CJD, but should be carried out in all cases of suspected CJD.

In vCJD, the suspicion of the diagnosis is raised by the occurrence of a progressive neuropsychiatric syndrome in an individual in a younger age range than expected for sCJD. The EEG is usually normal or exhibits nonspecific slowing in the great majority of cases, although periodic complexes at $1\,s^{-1}$ have been found in two cases in the terminal stages of the illness. The 14-3-3 CSF immunoassay is positive in approximately half the cases and is of limited diagnostic value. The MRI brain scan is the most useful investigation, showing high signal in the posterior thalamus, the pulvinar sign, in more than 90% of cases. These appearances are virtually pathognomonic for vCJD in the appropriate clinical context. Tonsil biopsy, showing positivity for disease-associated prion protein, is also a highly reliable diagnostic aid, but this investigation is invasive and has risks, including hemorrhage. A definite diagnosis requires the identification of the specific neuropathological features of vCJD, including widespread deposition of florid plaques of abnormal prion protein in a number of cortical regions, usually at postmortem. Diagnostic criteria for vCJD have been formulated and validated and provide a reliable framework for case classification. As with sCJD, most authorities report definite and probable cases of vCJD because of the diagnostic uncertainty in cases classified as possible vCJD.

CJD and Public Health

Following the identification of BSE in 1986, the possible public health implications were considered by a number of agencies in the UK and Europe, and the consensus was that BSE was unlikely to pose a significant risk to the human population owing to the fact that BSE was likely caused by transmission of sheep scrapie to cattle and there was no evidence that this condition was transmissible to humans. However the origin of BSE was, and remains, uncertain and it was known that prions can change their characteristics, including species specificity, after transmission across a species barrier. A range of measures were introduced in the UK in order to control the BSE epidemic, but the critical measure aimed at minimizing human exposure to BSE in the food chain was the specified bovine offals (SBO) ban, which was introduced in 1989. The tissue distribution of infectivity in BSE was not known and a range of bovine tissues from all cattle were excluded from the human food chain, although this measure was not fully enforced. Subsequently the tissues designated as SBOs were extended, bovine-sourced MRM was banned in 1995 and in 1996, following the identification of vCJD, and the Over Thirty Months Rule was introduced, which excluded cattle aged more than 30 months from the human food chain. Other countries in the European Union introduced a ban of specified risk materials (SRMs) in 2000 and a compulsory testing of all animals for human consumption over the age of 30 months in 2001, following the development of rapid tests for the presence of BSE, which could be utilized in abattoirs.

The impact of these measures on reducing the risk of vCJD is difficult to estimate because of the long mean incubation period in vCJD and the fact that human exposure to BSE was significant in the UK before these measures were introduced and probably before BSE was first identified. It has been estimated from a mathematical model that between 1 and 3 million infected cattle may have entered the human food chain before the introduction of the SBO ban in 1989 and infected cattle are also likely to have been consumed in other European countries before the introduction of measures to minimize human exposure to BSE, although in much lower numbers than in the UK.

An important question is why the epidemic of vCJD has been relatively limited in the UK and other European countries despite the extent of human exposure to BSE. The most parsimonious explanation is that there is a significant barrier to the transmission of bovine prions to humans, and the so-called species-barriers to transmission are well recognized in experimental prion disease. The possibility that there may be cofactors necessary for transmission in the form of concurrent

conditions, for example, inflammatory bowel disease, easing the spread of infected prions across the gut wall, or occupational exposure to BSE have been largely excluded by detailed analysis of the vCJD patient characteristics. Genetic factors other than variation in *PRNP* are potential determinants of susceptibility to BSE, but no such genetic factors have been identified. A crucial issue for public health is the prevalence of infection in the general population, and studies of routine appendix and tonsil specimens in the UK suggest that there may be a population of individuals who are infected with BSE, with an estimated prevalence of 237 per million. The mismatch between the observed incidence of vCJD and the estimated number of individuals who are infected may be explained by subclinical infection in which disease does not develop in lifespan.

The identification of transfusion transmission of vCJD has raised concerns regarding the possibility of the spread of infection in the population through transmission of vCJD from person to person, a concern that has been heightened by the estimated prevalence of infection in the general population. Measures to reduce the risk of secondary transmission of vCJD have included the sourcing of plasma for plasma product manufacture from outside the UK and the deferral of transfusion recipients as blood donors. As with the SBO ban and other measures to reduce the risk of BSE, most of the policies to minimize the risk of secondary transmission of vCJD were introduced well before the demonstration of an actual risk, underlining the importance of precautionary action in relation to potential public health consequences of prion diseases.

Future Prospects

The epidemics of BSE and vCJD are in decline and there will be an increasing pressure to review the current measures in place to control BSE, not least because of the costs involved in the active testing program and the SRM ban. This has to be balanced against the risks to the human population from BSE, and the decisions about relaxing the protective measures are essentially political rather than scientific. However, the identification of atypical forms of BSE and scrapie in recent years indicates that there are continuing uncertainties about the extent and nature of animal prion diseases.

There are also uncertainties about the future of vCJD. A possible case of vCJD in an individual with an MV codon 129 genotype has been described, raising the possibility of further outbreaks of vCJD in individuals with MV and perhaps VV genotypes. Mathematical predictions of the likely numbers of cases in these genotypes suggest that these will be no greater than the primary outbreak in the MM population, but these analyses make a range of assumptions that cannot be verified. The identification of abnormal prion protein in the spleen of an individual with hemophilia A has raised the possibility of a further outbreak of vCJD cases in the population treated with plasma products. There may be further cases of transfusion transmission of vCJD, and individuals who donated blood and later developed vCJD have been identified in Ireland, France, Saudi Arabia, as well as the UK. There are concerns about the potential for secondary transmission of vCJD through contaminated surgical instruments and perhaps vertically from mother to child.

Prion diseases have had a major impact on the general population, policy makers, and industry, and the difficulties in managing diseases with long incubation periods, a fatal outcome, and no means of identifying those that are infected suggest the need for continuing caution.

See also: Analytical Methods: Transmissible Spongiform Encephalopathy Diagnosis. Prions and Agents of TSEs: Bovine Spongiform Encephalopathy in Cattle

Further Reading

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PROTOZOA

Contents

Cryptosporidium spp.

BR Dixon, Health Canada, Ottawa, ON, Canada

Glossary

Genotype A genetically distinct group of organisms within a species.
Immunocompetent Having the ability to develop an effective immune response following exposure to an antigen.
Immunocompromised Having impaired immune response due to a disease or condition, or as a result of medical treatment.
Oocyst The infectious environmental stage of *Cryptosporidium* and other apicomplexan parasites, which is produced following the sexual stage of the life cycle.
Zoonotic An infectious disease transmissible from animals to humans.

Background

Cryptosporidium spp. is a protozoan parasite of the phylum Apicomplexa. It is found worldwide in a large number of different hosts, including humans. There are currently more than 20 valid species of *Cryptosporidium*, and more than 40 distinct genotypes. Some species, such as *Cryptosporidium hominis*, have very narrow host ranges, whereas others, such as *Cryptosporidium parvum*, have a wide range of hosts. A growing number of *Cryptosporidium* sp. have been reported to infect humans (Table 1), and several genotypes have also been identified. However, approximately 90% of reported human infections involve *C. hominis*, which is found primarily in humans, and *C. parvum*, which is an important zoonotic species. *Cryptosporidium hominis* accounts for more human cases than *C. parvum* in North America, Australia, Asia, and Sub-Saharan Africa, whereas *C. parvum* is more common in Europe and the UK. Generally speaking, *C. parvum* is more prevalent in rural or agricultural regions, a likely result of zoonotic transmission.

Cryptosporidium was regarded as a benign commensal until the early 1970s when it was recognized as an important cause of diarrhea in animals. It was only recognized as a pathogen in humans in 1976. In recent years, human infection with *Cryptosporidium* has emerged as a global public health problem and this parasite is now considered to be a common cause of gastroenteritis in immunocompetent individuals, and of severe illness in immunocompromised individuals. Prevalence rates based on oocyst excretion vary from approximately 1–3% in industrialized countries, up to 10% or higher in developing

Table 1 *Cryptosporidium* species reported in humans

Cryptosporidium sp.	*Major hosts*
Cryptosporidium hominis	Humans
Cryptosporidium parvum	Humans, cattle, pigs, sheep, and mice
Cryptosporidium meleagridis	Turkeys
Cryptosporidium cuniculus	Rabbits
Cryptosporidium canis	Dogs
Cryptosporidium felis	Cats
Cryptosporidium suis	Pigs
Cryptosporidium muris	Mice
Cryptosporidium baileyi	Chickens
Cryptosporidium andersoni	Cattle
Cryptosporidium ubiquitum	Ruminants
Cryptosporidium fayeri	Kangaroos
Cryptosporidium viatorum	Humans

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countries. The higher prevalence in developing countries is likely due to a lack of clean water and poor sanitation, crowded housing conditions, and closer contact with domestic animals. Those people at greatest risk of infection include young children, people in contact with young animals or children, international travelers, recreational water users, and consumers of poor quality drinking water. *Cryptosporidium* oocysts are found in the stools of 10–20% of patients with acquired immunodeficiency syndrome (AIDS)-associated diarrhea, and chronic intestinal cryptosporidiosis is currently listed as an AIDS-defining disease.

Cryptosporidium are intracellular parasites, which generally inhabit epithelial cells of the small intestine but have been detected throughout the gastrointestinal tract, and even in the respiratory tract. These parasites undergo asexual multiplication, followed by sexual stages leading to fertilization and formation of oocysts, which are then shed with the feces of the host and transmit the parasite through direct contact with feces, or through contaminated water or food. An important distinction to be made between *Cryptosporidium* spp. and bacterial pathogens is that the former does not grow outside the host, so no multiplication will take place, for example, in water or on contaminated fruits regardless of the environmental conditions. Mature oocysts of *C. parvum* are round, thick-walled structures approximately 4–5 μm in diameter and contain four sporozoites. They are environmentally resistant and can survive for many weeks under cool and moist conditions. The oocysts of most other species are morphologically indistinguishable from *C. parvum*, with some exceptions such as *Cryptosporidium muris* and *Cryptosporidium andersoni* which are somewhat larger.

Transmission of Cryptosporidiosis

The transmission of cryptosporidiosis is facilitated by the ability of oocysts to survive for weeks to months in the environment. Routes of transmission include waterborne, person-to-person (i.e., the fecal–oral route), zoonotic, and foodborne. Although there is considerable overlap amongst these routes of transmission, water is numerically the most important mode of transmission. Numerous waterborne outbreaks of cryptosporidiosis have occurred worldwide as a result of oocyst contamination of drinking water sources. For example, the largest waterborne outbreak of any kind in the US occurred in the spring of 1993 when an estimated 403 000 people became ill with cryptosporidiosis in Milwaukee, Wisconsin. Recent surveys worldwide have indicated that low levels of *Cryptosporidium* oocysts are relatively common in raw and treated water. Fecal material from domestic animals has the potential to contaminate surface water through run-off, and may lead to infections in other animals and in humans. This source of oocysts is, in fact, generally thought to be responsible for the majority of waterborne outbreaks. The mishandling of sewage may, however, represent another important source. In addition to drinking water, numerous outbreaks worldwide have been associated with recreational water, including swimming pools, splash pads, and aquatic parks.

Direct person-to-person transmission may occur following the ingestion of oocysts in fecal matter, and is associated with poor hygiene (e.g., children in daycare, institutionalized patients), sexual practices, and caring for an infected person. Autoinfection may also occur when the so-called thin-walled oocysts are released into the gut lumen. These oocysts are responsible for chronic and life-threatening disease in immunocompromised individuals.

In the case of zoonotic species of *Cryptosporidium*, such as *C. parvum*, calves, rodents, puppies, kittens, and many other animals serve as important reservoir hosts in zoonotic transmission. Although many such infected animals are asymptomatic, *Cryptosporidium* can cause clinical disease and death in young animals. Young farm animals are very commonly infected with *C. parvum*. For example, a number of studies have reported a 100% cumulative prevalence in herds of young cattle. Given the extremely large number of oocysts excreted by an animal in a single day, veterinary personnel, cattle farmers, and other animal handlers, including petting zoo visitors, are at an increased risk of infection. There are also serious implications with respect to contamination of surface water through agricultural runoff.

Foodborne transmission of cryptosporidiosis is thought to be much less common than waterborne or person-to-person transmission. For example, approximately 8% of domestically acquired cases of cryptosporidiosis in the US are foodborne. Nevertheless, a number of foodborne outbreaks have also been reported worldwide. Contamination of food or equipment during harvest, packaging, transport, or food preparation may occur directly from the hands of food handlers who are infected, or have family members that are infected, and is associated with poor personal hygiene, namely insufficient hand washing. The irrigation of crops with contaminated water or the use of contaminated water to mix pesticides or wash produce are other possible sources. Contamination of produce by livestock, either through direct access or through the application of manure to crop lands, has also been proposed.

Symptoms, Diagnosis, and Treatment

Fewer than 10 *Cryptosporidium* oocysts may cause infection in healthy adults, although infectivity and virulence may be dependent on the species and genotype. The incubation time has been estimated to be between 2 and 10 days, with 7 days being the most frequently reported period. Cryptosporidiosis is an enteric disease which is self-limiting in immunocompetent individuals. The disease is characterized by watery diarrhea and a variety of other symptoms including cramping, abdominal pain, weight loss, nausea, vomiting, fever, and headache. More than 90% of acute infections present with watery diarrhea, 50% present with nausea, vomiting, and abdominal pain, and 36% present with fever. Symptoms generally last 1–2 weeks in immunocompetent patients; however, oocyst excretion persists for 1–4 weeks after symptoms are resolved. Asymptomatic infections were originally thought to be rare but more recent evidence suggests that they are not uncommon. Cryptosporidiosis is an AIDS-defining illness, and symptoms in some immunocompromised patients become chronic, debilitating, and potentially life threatening.

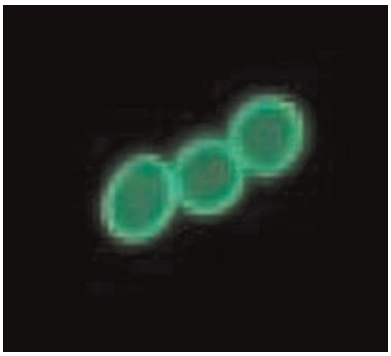

Figure 1 Oocysts of *Cryptosporidium parvum* stained with fluorescein isothiocyanate (FITC)-labeled monoclonal antibodies.

Specifically, patients may experience profuse diarrhea and considerable weight loss.

Detection of *Cryptosporidium* oocysts in stools by microscopy is the principal method of diagnosing cryptosporidiosis. Oocyst shedding may be intermittent, requiring that multiple stools be examined by microscopy. Oocyst concentration methods such as sucrose flotation, or sedimentation in a solution of formalin-ethyl acetate or formalin-ether, can be used when there is low oocyst shedding. Though wet-mounts are often used in clinical laboratories, permanent stains such as acid-fast generally reveal the parasite. Fluorescent stains such as auramine–rhodamine are also commonly used. Fluorescence-labeled monoclonal antibodies specific to epitopes on the oocyst wall are commercially available and, when used with an epifluorescence microscope, generally provide higher sensitivity and specificity than conventional staining methods (Figure 1). Various enzyme immunoassays, including the enzyme-linked immunosorbent assay, are also available commercially but are not commonly used in diagnoses. A number of different polymerase chain reaction (PCR) assays to detect *Cryptosporidium* deoxyribonucleic acid (DNA) in fecal samples are currently available, and offer a very high level of sensitivity and specificity. PCR in conjunction with DNA sequencing and/or restriction fragment length polymorphism can also be used to identify *Cryptosporidium* species, genotypes, and subtypes. PCR is generally used in research settings, however, and is not widely used in diagnoses.

As infections are generally self-limiting in immunocompetent individuals, specific therapy is not always necessary in these patients. However, due to the severity of symptoms in immunocompromised patients, effective treatment is extremely desirable. Unfortunately, drug treatment has been largely unsuccessful against cryptosporidiosis. Therapy development has been hampered by the lack of good culturing systems and small animal models that mimic human disease. Although many drugs have been evaluated, none have been completely effective. Paromomycin is used in treating amoebiasis and has some activity against *Cryptosporidium*. Spiramycin and azithromycin have both produced favorable results in children on chemotherapy and in AIDS patients. Nitazoxanide is approved in the US for treating diarrhea caused by *Cryptosporidium* in immunocompetent patients. Short of drug therapy, however, fluid balance and nutritional status should be monitored, and treatment with antidiarrheal agents is often attempted. The advent of highly active antiretroviral therapy in human immunodeficiency virus (HIV)/AIDS has decreased the occurrence of life-threatening diarrhea in these patients.

Prevention of infection is very important, particularly for immunocompromised individuals. Probably the most important measure in preventing infection and transmission is frequent and effective hand washing, especially after using the toilet or changing diapers, before eating, when caring for an ill individual, or following contact with young animals. Food handlers should also take special precautions, including frequent hand washing, thorough washing of fresh produce, avoiding cross-contamination, and avoiding any handling of foods during, and immediately following, diarrheal episodes. Other important preventative measures include drinking only properly treated water, avoiding public swimming during, and for two weeks after, experiencing diarrhea, and heeding all public health recommendations regarding travel in developing countries.

Surveillance in Foods

Although foods are not as important in the transmission of cryptosporidiosis as water or person-to-person contact in terms of the numbers of cases or outbreaks reported, numerous surveys performed worldwide have reported the presence of *Cryptosporidium* on fresh produce. One study in Costa Rica reported the presence of *Cryptosporidium* oocysts on cilantro leaves and roots, and on lettuce obtained from open markets, with lower contamination rates found on radishes, tomatoes, cucumbers, and carrots. A second study in Costa Rica reported oocysts on lettuce, parsley, cilantro, and blackberries. A study on a variety of vegetables obtained from markets in Peru reported the presence of *C. parvum* oocysts on a variety of leafy greens and herbs. In Norway, oocyst contamination was found on imported lettuce and mung bean sprouts. In Poland, leeks, celery, and a variety of cabbages were found to be contaminated. Oocysts have also been detected in water spinach in Cambodia, and on lettuce and cabbage in Spain. Studies in Canada reported the presence of *C. parvum* on spinach, as well as on washed apples and in unpasteurized apple cider. A recent Canadian study found a relatively high prevalence of *C. parvum* on a variety of packaged leafy greens, both domestic and imported, purchased at retail.

A number of studies worldwide have reported on the presence and survival of *Cryptosporidium* oocysts in the gills and tissues of oysters and other molluscan shellfish, including clams, cockles, and mussels, and the potential for transmission to humans through consumption. The presence of oocysts in marine environments is thought to result from the routine dumping of raw sewage and/or agricultural run-off, and filter-feeding shellfish have been shown to readily take them up. Although there have been no reports of human cryptosporidiosis associated with the consumption of shellfish, this is a concern given that shellfish are often consumed raw.

There are numerous hurdles faced in making an association between the consumption of a suspected food and human illness due to a parasitic infection. These include the

relatively long incubation period of the parasite, the difficulty in diagnosing and reporting human cases, and the short shelf-life of some foods. Even when foods are available for testing, there are no standard methods available, and published methods often have low and variable recovery efficiencies. Furthermore, the concentration of oocysts on foods is generally low and, due to the porous and irregular surface of some fruits and vegetables, they are often difficult to remove from foods. As enrichment steps are not possible for protozoan parasites, the success of the recovery is largely dependent on optimization of elution and concentration procedures.

The elution and concentration of protozoan parasites from foods generally involves agitation of a food sample in a buffer with detergent, followed by centrifugation. Other methods for eluting and concentrating oocysts from foods include sieving, sedimentation, flocculation, formal ethyl acetate, filtration, and immunomagnetic separation. Once oocysts are eluted, a variety of microscopical methods (e.g., bright-field, permanent stains, phase-contrast, differential interference contrast, and fluorescence) can be used for their detection on foods. Molecular methods developed for clinical diagnoses have been useful in detecting the presence of DNA from *Cryptosporidium* on foods, as well as in genotyping and subtyping for the purpose of source-tracking.

Foodborne Outbreaks

A number of foodborne outbreaks of cryptosporidiosis associated with the consumption of fresh produce have been reported in the US. One such outbreak occurred in Washington state in 1997 following a catered dinner, and some epidemiological association was made with the consumption of foods containing uncooked green onions. In another outbreak in 1998, a large number of cases were associated with eating at a university cafeteria in Washington, DC. Although no specific food item was implicated in this outbreak, an ill food handler with laboratory-confirmed cryptosporidiosis prepared the raw produce used in meals served during the expected exposure period. This outbreak illustrates the need for good hygienic practices by food handlers, and a restriction from working during periods of diarrheal illness. More recently, an outbreak at a summer camp in North Carolina, USA in 2009 was associated with sandwich-bar ingredients, including ham and lettuce, and possibly tomatoes and onions.

Fresh produce has also been implicated in a number of recent cryptosporidiosis outbreaks reported in Northern Europe. An outbreak in Sweden following a wedding reception resulted in gastroenteritis in both guests and restaurant employees. Fresh parsley was determined to be the most likely source of these illnesses. Another outbreak was associated with the consumption of peeled whole carrots, grated carrots, or red peppers at a company cafeteria in Denmark. Again, the vegetables in this case were thought to have been contaminated by an infected food handler. A large outbreak in Finland was thought to have been associated with the consumption of contaminated lettuce mixture.

There have also been at least four cryptosporidiosis outbreaks associated with drinking unpasteurized apple cider, all in the US. The first such outbreak occurred in 1993 in Maine and involved students and staff attending a school agricultural fair at which fresh-pressed apple cider was consumed. *Cryptosporidium* oocysts were detected in leftover cider and on swabs from the cider press. Apples used in the cider were likely contaminated after falling to the ground near the edge of a pasture on a farm on which an infected calf was identified. In 1996, an outbreak in New York state was associated with drinking commercially produced, unpasteurized apple cider. The apples used in the cider may have been contaminated following washing with fecally contaminated well water. A third outbreak associated with apple cider in 2003 was associated with the consumption of ozonated apple cider in Ohio. In 2004, a fourth outbreak of cryptosporidiosis associated with unpasteurized apple cider was reported in New York. It was not clear, however, how many of the illnesses in this outbreak could be attributed to *Cryptosporidium* and how many to the bacterium, *Escherichia coli* O111, both of which were detected in the cider and in clinical and environmental samples. It is important to note that *Cryptosporidium* oocysts are acid tolerant and are able to survive in apple cider for up to 4 weeks.

Unpasteurized milk has also been associated with outbreaks of cryptosporidiosis in Australia and the UK, as well as in a group of Canadians traveling in Mexico. In the outbreak in the UK in 1995, cases were linked to drinking school milk which was thought to have been inadequately pasteurized. An outbreak associated with a processed food occurred following a social event in Minnesota, US in 1995. This outbreak was associated with the consumption of chicken salad that may have been contaminated by a food worker, who also operated a daycare facility.

Control Measures

Cryptosporidium oocysts are relatively temperature resistant and, as a result, oocysts on fresh produce or in water may remain infectious for considerable periods of time even under extreme environmental temperatures. For example, oocysts can survive for 2 weeks at temperatures as high as 30 °C, and for short periods of time in water which has been frozen at temperatures down to −20 °C. *Cryptosporidium* oocysts will only be rapidly inactivated following exposure to temperatures above approximately 50–60 °C or below −20 °C.

Dessication is another important factor limiting the survival time of protozoan parasites in the environment. Studies have reported very low viability rates of *C. parvum* oocysts after only 2 h of air drying on glass slides at room temperature, or after 4 h of air drying at room temperature on stainless steel surfaces. Although there is little supporting data, dessication may be responsible for significant inactivation of oocysts on fresh produce in the field or during storage, and on surfaces and equipment. However, some fruits and vegetables, such as berries, have moist, irregular surfaces and will likely protect any contaminating oocysts from drying out.

Control measures to reduce the likelihood of contamination of produce at the preharvest stage with *Cryptosporidium* include the use of good quality water for irrigation, mixing of pesticides, or washing and processing, restricting access of livestock and other animals to crop lands and surface waters, monitoring the health of farm workers and encouraging good hygiene, and

using only composted manure as fertilizer. Postharvest control measures include the use of good quality water for washing and processing produce, monitoring and enforcing good personal hygiene in food handlers, prevention of cross-contamination, and the incorporation of Hazard Analysis Critical Control Point plans. *Cryptosporidium* is generally very resistant to most chemical disinfectants. For example, oocysts are much more resistant than bacteria to chlorine, which largely accounts for the importance of the waterborne route of transmission of this pathogen. However, a variety of physical disinfection procedures may be used postharvest. These include high hydrostatic pressure, which has been found to be effective in inactivating *Cryptosporidium* oocysts in fruit juices, and irradiation, which is effective in killing *Cryptosporidium* oocysts on fresh produce. *Cryptosporidium* oocysts in milk, juice, and water are also readily destroyed through pasteurization treatment. At the consumer level, thorough washing of fresh produce is recommended but will not likely be fully effective in removing all contaminating oocysts. Although oocysts are somewhat resistant to freezing, they can be inactivated at −20 °C for more than 24 h or at −15 °C for approximately a week. Alternatively, oocysts will be readily destroyed in foods that are subsequently cooked or baked.

See also: Disciplines Associated with Food Safety: Parasitology. Food Safety Assurance Systems: Hazard Analysis and Critical Control Point System (HACCP): Principles and Practice. Food Technologies: Food Irradiation; High Pressure Processing; Pasteurization. Protozoa: *Cyclospora cayetanensis*; *Cystoisospora belli* (Syn. *Isospora belli*); *Entamoeba histolytica*; *Giardia lamblia*; *Sarcocystis*; *Toxoplasma gondii*. Safety of Food and Beverages: Fruits and Vegetables

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Wikipedia.

Cyclospora cayetanensis

VA Cama, Centers for Disease Control and Prevention, Atlanta, GA, USA
YR Ortega, University of Georgia, Griffin, GA, USA

Glossary

Anthroponotic A disease that has humans as its source of transmission, and human to human transmission is typical (related to anthroponoses).
Oocyst Cyst that contains zygotes or other immature stages of protozoan parasites.
Sporocyst Cyst that contains reproductive or infectious stages of a protozoan parasite. In the case of *Cyclospora*, two of these structures are observed after the parasite has matured to become infectious; each sporozoite contains two infectious stages called sporozoites.
Sporulation Process by which the immature (freshly excreted *Cyclospora*) further develops into an infectious parasite. A sporulated cyst of *Cyclospora* is characterized by the presence of two sporocysts, each containing two sporozoites.
Unsporulated or nonsporulated cysts Cysts of *Cyclospora* that have not differentiated into the infectious stage. The oocysts of *Cyclospora* are excreted unsporulated by humans.

Background

Historical Information

Cyclospora cayetanensis is an important foodborne gastrointestinal pathogen. It is endemic in several regions of the world, primarily in the developing countries. *Cyclospora* is not endemic in the developed world but has been associated with significant foodborne outbreaks, and in travelers returning from *C. cayetanensis* endemic areas.

Date of Discovery

Cyclospora cayetanensis was fully recognized as a parasite in 1993. It gained significant recognition because of its association with several foodborne outbreaks in the US and other developed countries. The name *C. cayetanensis* dates back to 1992; however, a few other studies previously described organisms that were similar to *Cyclospora*. Perhaps the first report was the 1979 description of a parasite with morphometric and microscopic characteristics of immature (nonsporulated) oocysts. Although the authors considered the observed organism as a possible coccidian, the presence of two sporocysts per sporulated oocysts led them to conclude that it was probably a new species of *Isospora*.

In the following years, other investigators reported similar organisms that autofluoresced when observed under ultraviolet (UV) light. These observations opened the hypothesis that these organisms could be blue-green algae or cyanobacteria, and the term cyanobacterium-like body (CLB) was proposed. The acronym CLB, however, was also used to describe the same organism as a coccidian-like body. Another name also used before *C. cayetanensis* was 'big *Cryptosporidium*.'

Person and Specific Context

Studies from Ortega *et al.* demonstrated that organisms matching the description of CLBs were also found in the fecal specimens from Peruvian patients. In those studies, Ortega demonstrated the coccidian nature of the parasite through sporulation of immature organisms (previously described as CLBs) into a mature oocysts that had two defined sporocysts, and then by excystation showing the presence of two sporozoites per sporocyst. These characteristics allowed the proper identification of this pathogen as a coccidian parasite from the genus *Cyclospora.* Ortega later proposed the species name *C. cayetanensis*. These findings were first presented at the Annual Meeting of the American Society of Tropical Medicine and Hygiene in November 1992 and later published.

Characteristics

Nomenclature

The sporulation of oocysts, followed by excystation led to the classification of the new parasite as *C. cayetanensis.* The genus name corresponds to sporulated coccidian oocysts, having two sporocysts, each with two sporozoites. The species name '*cayetanensis*' was in honor of Universidad Peruana Cayetano Heredia, the Peruvian University where the preliminary studies were conducted.

Taxonomy and Classification

Domain	Eukaryota
Superphylum	Alveolata
Phylum	Apicomplexa
Class	Coccidia
Order	Eucoccidiorida
Suborder	Eimeriorina
Family	Eimeriidae
Genus	*Cyclospora*
Species	*cayetanensis*

Description of the Organisms

Morphology

The oocysts of *C. cayetanensis* are spherical, 8.6 μm in diameter (range 7.7–9.9 μm) and are not sporulated upon excretion. After sporulation, each oocyst contains two ovoid dizoic (containing two sporozoites) sporocysts that measure 4.0 by 6.3 μm (range 3.3–4.4 by 5.5–7.1 μm).

Hosts

Evidence so far indicates that *C. cayetanensis* only infects humans, and no animal model exists. Unsuccessful attempts to infect multiple animal species included nine strains of mice (adult and neonatal immunocompetent and immune-deficient inbred and outbred strains), rats, sand-rats, chickens, ducks, rabbits, jirds, hamsters, ferrets, pigs, dogs, owl monkeys, and rhesus and cynomolgus monkeys.

There are anecdotal reports of the microscopic detection of *C. cayetanensis* in the feces of ducks, chickens, and dogs, and by detection of deoxyribonucleic acid (DNA) in samples from monkeys, however, none of these reports provided evidence of tissue infections.

Nonhuman primates can be naturally infected with other species of *Cyclospora*. Studies in Kenya found *Cyclospora papionis* in baboons, vervet monkeys were infected with *Cyclospora cercopitheci*, and colobus monkeys with *Cyclospora colobi*. This study used sequence analysis of the small subunit ribosomal ribonucleic acid (rRNA), and the findings suggested that those species of *Cyclospora* had marked host specificity.

Survival in Food and/or Environment

Infections with *C. cayetanensis* have been linked to the consumption of some fruits and vegetables eaten raw. Although it is not yet elucidated how the parasite survives in foods or the environment until it becomes infectious, it appears that specific characteristics or topology of the surface of certain fruits and vegetables favor the life cycle of *Cyclospora*. In laboratory experiments, *Cyclospora* oocysts became sporulated after 2 weeks of incubation at room temperature in liquid suspension. However, it is not clear either how soon sporulation occurs, or how long sporulated oocysts do remain infectious in nature.

Laboratory methods are still being generated toward understanding the parasite biology outside the host, mainly in food products. Parasite recovery methodologies are based on elution from the different food matrices. The recovery rates of *Cyclospora* from seeded lettuce were determined at 13–15%. This method was used in a longitudinal survey of produce purchased in a *Cyclospora*-endemic area and collected more than 200 different samples from 15 different markets at three different seasons of the year. Most of the samples were leafy greens, whereas fruits were primarily strawberries. Among all the samples, three vegetables usually eaten raw had *Cyclospora*: huacatay (black mint, *Tagetes minuta*), yerba buena (*Micromeria douglasi*), and lettuce (*Lactuca sativa*). This study highlighted the presence of *Cyclospora* in vegetables likely to be consumed raw, and the challenges associated with persistence of the parasite in produce frequently eaten raw.

Life Cycle

Cyclospora cayetanensis (*Cyclospora*) is considered a monoxenus parasite, infecting only humans, therefore the term anthroponotic. The developmental stages of *Cyclospora* were described from jejunal biopsies of infected humans. Cyclosporiasis starts after a susceptible person ingests sporulated oocysts, i.e., the infectious stage. After ingestion, oocysts are ruptured in the upper gastrointestinal tract and two sporocysts are liberated. Shortly thereafter, two sporozoites are released from each sporocyst, which proceed to infect epithelial cells of the intestine and form type I meronts. In this stage there is asexual reproduction leading to the generation of 8–12 merozoites. The released merozoites invade other enterocytes to form type II meronts, characterized by the presence of 4 merozoites. Merozoites can invade other cells to form additional meronts, or to differentiate into micro- and macrogametocytes, the sexual stages. Oocysts are formed as a result of sexual reproduction when the macrogametocyte is fertilized by the microgametocyte. These oocysts are not sporulated (noninfectious) and are released into the environment, becoming infectious only after sporulation, which is estimated to occur in approximately 2 weeks (**Figure 1**).

Incubation Time

The oocysts of *Cyclospora* are excreted unsporulated and require time outside the host to fully sporulate and become infectious. Laboratory data using oocysts from infected patients has estimated that it will take 1–2 weeks (reported range 1–23 days) for the oocysts to become fully sporulated, thereby infectious. Once a person ingests the infectious oocysts, the infection of epithelial cells will start shortly after excystation. The gastrointestinal manifestations including diarrhea, fatigue, and abdominal cramps are likely to be observed between 1 and 2 weeks postinfection.

Duration of Infection

The length of infection and severity of clinical manifestations will vary between people living in endemic and nonendemic areas. In endemic settings, the infections usually occur in children, mainly those older than 2 years of age, and infrequently in children older than 10. Most infections may resolve spontaneously, and more than 50% may have an asymptomatic course. As children get older, the episodes tend to become shorter, with a decrease in the severity of associated

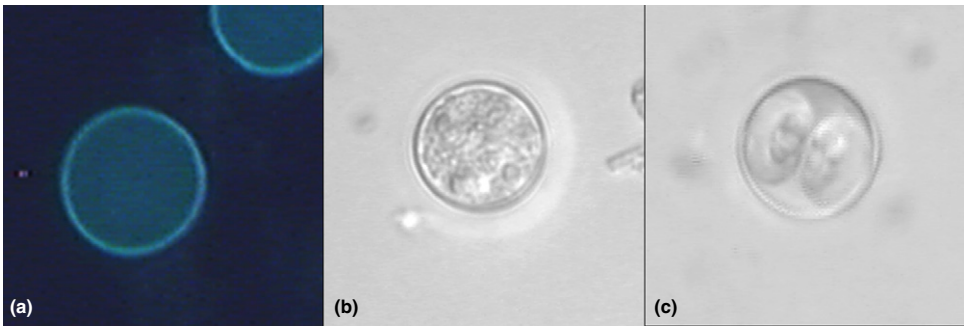

Figure 1 Microscopic observation of oocysts of *Cyclospora cayetanensis*. (a) Autofluorescence under UV light, (b) nonsporulated oocysts observed under differential interference contrast (DIC) microscopy, and (c) sporulated oocysts, containing two defined sporocysts, DIC microscopy.

symptoms. This suggests that immunity may play a role in the clearance of infections. This is not the case in people from nonendemic areas, where confirmed infections usually require antibiotic therapy. Infections usually last 1–2 weeks or longer with intermittent diarrhea if untreated. Longer episodes have anecdotally been reported in immunocompromised patients.

Symptoms

Symptoms vary between unexposed (naïve) and exposed people. Among people not living in the endemic areas, symptoms occur in the vast majority of infected people. The clinical manifestations include: diarrhea, malaise, lack of energy and appetite, mild fever, nausea, flatulence, and abdominal cramps.

Among the residents of endemic areas, clinical infections are primarily detected in children 4–10 years of age. Approximately 40% of infected children may have diarrhea or other gastrointestinal discomforts, including malaise, bloating, and anorexia; however, infections can also be asymptomatic. In these endemic settings, *C. cayetanensis* is infrequently detected in adults.

Jejunal biopsies from infected people have shown altered mucosal architecture with shortening and widening of the intestinal villi due to diffuse edema and infiltration by a mixed inflammatory cell infiltrate. There was reactive hyperemia with vascular dilatation and congestion of villous capillaries. Parasitophorous vacuoles containing sexual and asexual forms, meronts of type I (containing 8–12 merozoites) and II ($n=4$ merozoites) were found at the luminal end of the epithelial cells. These findings demonstrated the complete developmental cycle of the parasite in the jejunum, which may be associated with the host clinical manifestations due to *C. cayetanensis*.

Carrier Duration

It is not known. It is suspected that once the infection has cleared, that person is no longer infectious.

Infectious Dose

It is not known but suspected to be low. *Cryptosporidium*, another coccidia that also causes gastrointestinal illness, requires a minimum of 100–200 oocysts to infect people as demonstrated in human volunteer studies, whereas other investigators propose that only one oocyst is needed to cause illness. The lack of information on *Cyclospora* is in part due to the absence of an animal model to study human cyclosporiasis. Human volunteer studies were unsuccessful in reproducing infections in controlled trials. Laboratory studies have attempted to infect immunocompetent and immunodeficient animals; however, these attempts were unsuccessful and none of the species evaluated developed patent infections or associated clinical symptoms.

Sequelae/Chronic Effects and Complications

Guillain–Barré syndrome and Reiter's syndrome (ocular inflammation, sterile urethritis, and inflammatory oligoarthritis) have been reported in very few cases after infections with *Cyclospora*.

Risks of Misdiagnosis

Cryptosporidium oocysts could be misdiagnosed as *Cyclospora* when stained using the modified gram stain. When using this technique oocysts must be measured as *Cyclospora* oocysts, which measure 10 μm, twice the size of *Cryptosporidium*. Misidentification of *Cryptosporidium* as *Cyclospora* may result in the unnecessary administration of trimethoprim/sulfamethoxazole (TMP/SMX) or ciprofloxacin. Conversely, if *Cyclospora* would be misdiagnosed as *Cryptosporidium*, the error will lead to different therapeutic approaches and patients not receiving TMP/SMX as they should be.

Epidemiology

Statistical Data on Prevalence and/or Incidence of the Disease

Foodborne outbreaks in the US have increased in absolute numbers over the past 30 years. Data analysis from the Foodborne Outbreak Surveillance System revealed 190 outbreaks between 1973 and 1997. During this period, *Cyclospora* and *E. coli* O157:H7 were newly recognized as causes of foodborne illness. In a recent study, it is estimated that more than 11 000 *Cyclospora* illnesses occur in the US.

The transmission of *Cyclospora* through the waterborne route has also been reported. The first report came from

Nepal, where contaminated chlorinated water was identified as the source of *Cyclospora* infections between expatriates in Nepal. A retrospective epidemiological investigation of an outbreak within a hospital in Illinois identified drinking water as the source of infection. A prospective epidemiological study in Haiti identified an artesian well with *Cyclospora* oocysts before the study; however, none of the wells was positive thereafter. Despite reporting important temporal variations in the frequency of *Cyclospora* infections, from 12% in February to 1.1% in April 2001, no epidemiological associations could be established between sources of drinking water and cyclosporiasis. Molecular investigations have reported the detection of parasite DNA in surface waters from California. Overall, these findings indicate that *Cyclospora* may be present in water and that the potential waterborne transmission needs to be further studied.

Currently, *Cyclospora* is primarily considered a foodborne parasite, although before 1996, cyclosporiasis was originally considered to be a diarrheal disease primarily affecting returning travelers. This concept changed in the spring of 1996, when a large foodborne outbreak of cyclosporiasis occurred in North America, affecting 1465 people from 20 states, the District of Columbia, and two provinces. Epidemiological investigations confirmed the vast majority of cases were associated with 55 events that served raspberries. The investigations later demonstrated a significant association between cyclosporiasis and consumption of raspberries imported from Guatemala. Cluster investigations in Florida confirmed that the only food common to this outbreak was raspberries from Guatemala.

Detailed epidemiological descriptions of additional specific events included a wedding reception in Massachusetts and a luncheon in Charleston, SC, USA, where 38 of the 64 attendees met the case definition of cyclosporiasis.

In the spring of 1997, another large outbreak of cyclosporiasis affected the US and Canada. Epidemiological and trace-back investigations identified 41 infection clusters that comprised 762 cases and 250 sporadic cases of cyclosporiasis. Similar to the 1996 outbreak, there were significant associations between *Cyclospora* infections and the consumption of Guatemalan raspberries. As a consequence of this second outbreak, exportation of Guatemalan raspberries was voluntarily suspended in May 1997.

Other fresh produce have also been implicated in the transmission of *Cyclospora*. Basil was directly implicated in a 1999 outbreak in Missouri, where 62 cases were identified. All of these people had previously eaten either pasta chicken salad at one event or tomato basil salad at another event. European countries also have documented cases of foodborne cyclosporiasis. An outbreak in Germany identified that infections were associated with the consumption of fresh salad spiced with fresh herbs, whereas the lettuce was from southern Europe. Snow peas were implicated in an outbreak where 50 potential cases were linked to the consumption of snow peas from Guatemala.

Cyclospora infections had been previously reported in travelers returning from developing countries. Although infections have been reported from multiple countries around the world, the true prevalence of this parasite in any population is frequently unknown. In 1997, *Cyclospora* was reported in 5 of 795 travelers returning from nonindustrialized nations. A study in Nepal reported, that among expatriates living in endemic areas, the annual attack rate for *Cyclospora* was 32% during their first 2 years of residence.

Geographic Distribution

Endemic cyclosporiasis has been reported in several areas of the world, mainly in the developing countries. Although there are socioeconomical and geographical similarities with other related pathogens including *Cryptosporidium*, *Giardia*, and bacterial and viral diseases, there are also marked differences. In general, *Cyclospora* has been reported as affecting children in areas where access to clean water or sanitation is marginal or suboptimal.

Cyclospora infections have been reported in pediatric and adult patients with diarrhea in Tanzania and the sub-Saharan region. In Lagos, Nigeria, the overall prevalence among inpatients with gastrointestinal disease was 0.9%. In Yunnan, China, 4% of preschool children with diarrhea had *Cyclospora*. A prospective study in Peru found that children between the ages of 3–6 years of age were more frequently affected, and infections were rare after 10 years of age. However, cyclosporiasis in upper middle class Peruvians closely resembled the epidemiological and clinical features observed in the industrialized countries. The two study sites in Peru were only a few miles apart; however, the sanitary and socioeconomic infrastructure was markedly different, suggestive of the role of sanitation in the epidemiology of *Cyclospora*.

Age and Locations

Endemic cyclosporiasis has been spottily reported around the world, and more frequently in young people. The first reports suggested that *Cyclospora* infections were more commonly found in children especially those under 5 years of age. In a 2-year study of 5836 Peruvian children 2–4 years of age living in endemic settings, infections were detected in 63 (1.1%) participants. *Cyclospora* infections were reported in children with diarrhea (2/315) in Brazil and 6.1% of people living in impoverished areas in Venezuela. An epidemiological study among Guatemalan children reported significant differences in age susceptibility and gastrointestinal symptomatology. *Cyclospora* was detected in 117 (2.1%) of 5520 specimens, mainly in children 5 years of age. When compared to cryptosporidiosis, *Cyclospora* infections were more strongly associated with diarrhea than *Cryptosporidium* infections. Seven of 132 otherwise normal Venezuelan children ages 1–12 had *Cyclospora* infections, and the highest frequency was observed in children 2–5 years of age. The presence of *Cyclospora* and its association with diarrheal disease was studied in Nepal (Oct 1999–Aug 2002) and Lao PDR (Feb 2002–Jun 2003). Infections were detected in 128/1397 Nepalese participants, with higher rates among children younger than 10 years. Interestingly, only 1/686 specimens collected from Lao PDR was positive for *Cyclospora*. A survey conducted in 285 adolescents from a middle school in West Java, Indonesia found only one case of *Cyclospora*. These reports show that in nonindustrialized nations young children are more frequently

infected, and that the prevalence of cyclosporiasis is not homogenous between different sites or countries.

Nourishment and HIV Status

A study in Egypt found that 5.6% of malnourished children had cyclosporiasis, versus 2.8% of controls. A survey among malnourished children, people infected with the human immunodeficiency virus (HIV), and farm workers from Guatemala identified *Cyclospora* in 1.5% of participants; however, none was identified in raspberry farm workers.

Cyclospora is not considered as HIV opportunistic agent, with similar incidence rates among immunocompromised and immunocompetent people. Nonetheless, a study from Egypt reported 6% *Cyclospora* infections among patients receiving chemotherapy. Additionally, *Cyclospora* infections were reported in 7 of 71 Venezuelan HIV-infected patients.

Seasonality

Findings from a 3-year longitudinal study (1995–98) in children living in an impoverished area of Peru, showed an incidence rate of 0.20 cases per child year, which was constant among children 1–9 years of age. Infections were more frequent during the warmer months, December to May, showing a defined seasonal pattern. Data from a large three-tier survey in Nepal detected *Cyclospora* oocysts in 632/2123 fecal specimens from three health care facilities. Samples from water and leafy greens were also examined, and oocysts of *Cyclospora* were detected in four occasions. A longitudinal study in several areas of Nepal from April 1995 to November 2000 also identified marked seasonality, with higher infection rates occurring during the summer and rainy seasons of the year.

Reservoir of Pathogen

No reservoirs are known. There are anecdotic reports of *Cyclospora* oocysts in the feces of ducks, dogs, chickens, and monkeys. Two dogs from Sao Paulo, Brazil were reported positive for *Cyclospora*, although a follow-up study of 140 dogs from the same city did not find any infected dogs. These findings, in addition to the unsuccessful attempts to establish experimental infections in multiple laboratory animal models, suggest that only humans are susceptible to infections.

Causes of the Outbreaks/Incidences

The factors more frequently associated with the outbreaks of *Cyclospora* are the consumption of raw vegetables or fruits, primarily raspberries and basil. These were either whole or prepared, such as raspberry filling in desserts, and pesto. Other potential sources of infection are herbs consumed raw, for example, black mint, yerba buena, lettuce, and cilantro. Environmentally associated risks have not been reported.

Analytical Methods

Isolation or Recovery from Fresh Produce Matrices

The protocols for the recovery of oocysts of *Cyclospora* are based on the elution of the parasites by physical removal, and there are protocols for water, and fruits and vegetables. From water, the recovery of parasites is achieved through filtration and concentration as described in the United States Environmental Protection Agency (EPA) Method 1623. After the sample is fully processed, the oocysts of *Cyclospora* will be found in the eluate, where they can be observed either by epifluorescence microscopy or detected by the polymerase chain reaction (PCR)-based molecular assays.

The recovery of oocysts from food matrices presents more challenges due to the apparent adhesion of *Cyclospora* to certain vegetables and the inability to culture this parasite The oocysts can be recovered from vegetables through washes using either water, phosphate buffered saline, and elution buffer (laureth-12, Tris, ethylenediaminetetraacetic acid (EDTA), sodium phosphate, and antifoam A) as described in Method 1623 from the EPA, however, no final standard protocol has been agreed upon. The efficiencies of the recovery of *Cyclospora* will vary not only by the method used but also by the vegetable matrix tested. Shields reported that the use of 0.1% Alconox, a common laboratory soap (33–43% sodium carbonate, 10–20% sodium alkylbenzene sulfonate, 5–15% sodium tripolyphosphate, 5–15% tetrasodium pyrophosphate, 1–10% sodium carbonate, and 1–5% sodium alcohol sulfate) yielded higher recovery rates (70–80%) when compared to washes and elution with deionized water. The Bacteriological Analytical Manual from the Food Drug Administration (FDA) also has a method to isolate *Cyclospora* from fresh produce. Briefly, samples of 10–25 g are placed in filter bags with 100 ml of distilled water, and are mixed by rocking for 30 min at room temperature. The washed material is transferred to conical tubes and centrifuged at 2000*g* for 20 min. After the supernantant is discarded, the remaining pellet is resuspended in 2.5 ml of 20% celite in sodium chloride EDTA tris–bovine serum albumin (NET–BSA) buffer (0.1 M Tris, pH 8.0, 0.15 M NaCl, 0.001 M EDTA, 1% weight/volume bovine serum albumin), followed by mixing for 15 min. Then, 1 ml of 10% polyvinyl polypyrrolidone is added to the resuspended pellet, mixed again, and then filtered through a 0.45–0.2 μm membrane. The resulting sample can be split for microscopic examination (approximately 10%) and the remainder can be used for filtration on Flint Technology Associates (FTA) membranes for PCR detection. Among vegetables and fruits, special consideration needs to be placed on berry preparations, as they need to be processed as soon as possible. Other leafy vegetables such as lettuce can be stored and examined after overnight storage. Regardless of the elution method used, the concentrated washes are examined either by microscopy, or DNA extraction followed by PCR amplification.

Identification

The identification of *Cyclospora* can be done by microscopic observation of oocysts in fecal samples of infected people, water samples, or from the eluates from contaminated produce. The oocysts can be observed using phase contrast, bright field, or differential interference contrast (DIC) microscopy. Autofluorescence, a diagnostic feature of *Cyclospora*, can be observed using epifluorescent microscopy, using excitation

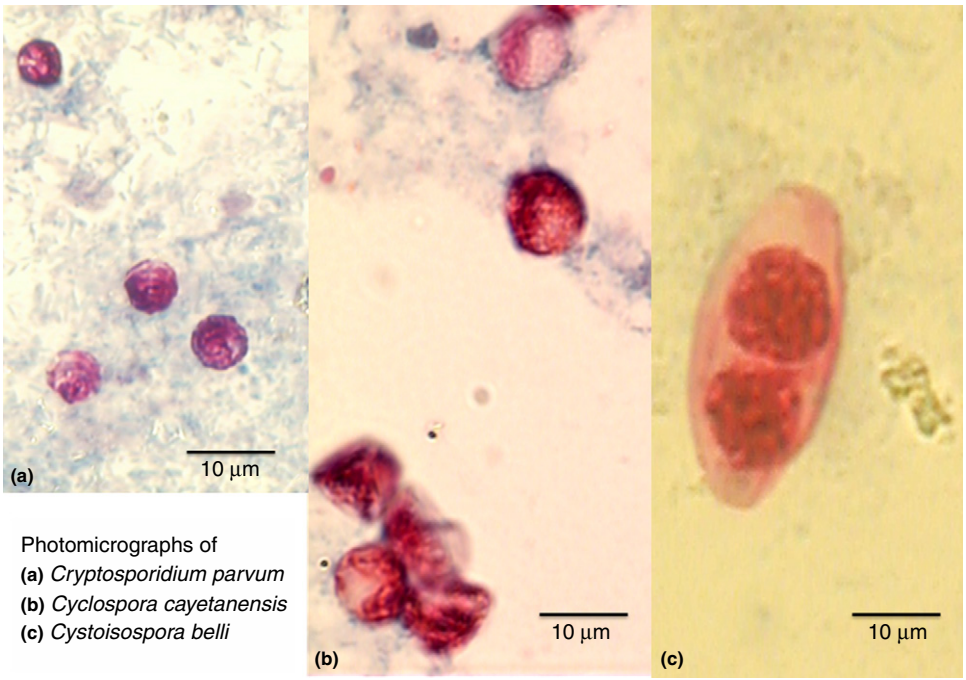

Figure 2 Acid fast staining of coccidian parasites with foodborne potential. (a) *Cryptosporidium parvum*, (b) *Cyclospora cayetanensis* – note the characteristic uneven staining, and (c) *Cystoisospora belli.*

filters of 360/40 nm, long pass dichroic mirrors of 400 nm, and emission filters of 420 nm (Figure 1a). Kinyoun, Ziehl Nielsen, or carbolfucsin-modified acid fast stains can be used to visualize the oocysts; keeping in mind the characteristic uneven staining of *Cyclospora* oocysts (Figure 2b). Safranin stains are also available and it was previously reported that most oocysts will uptake this stain showing a bright pink color.

Microscopic methods are not always the best for examining the environmental or produce samples. Oocysts could either be in small numbers, their morphology might have been affected during the handling and processing of the samples, or many other food or environmental structures may look alike and even autofluoresce. In these cases, PCR-based methods can be very useful for the identification of *Cyclospora*.

A PCR assay amplifying the 18S rRNA gene was developed for the detection of *Cyclospora* in clinical specimens. This PCR method is very sensitive, however not specific when used to test environmental or food samples. The digestion of the amplified products with the restriction enzyme *Mnl* I allowed the discrimination between *Cyclospora* and *Eimeria* from animal species. Other molecular PCR-based assays include oligonucleotide-ligation assays as well a real-time PCR amplification of the ITS1 locus. The detection limits of the PCR-based assays could be optimized by improvements in the yields of DNA extraction as well as the avoidance of PCR inhibitors. An alternative for sample collection and PCR amplification was the use of FTA filter membranes, where the eluates or test solutions are filtered through the FTA, which is then used directly for PCR amplification.

Control and Preventive Measures

Primary Sources (Prevention of Contamination in Food), or Underlying Factors (i.e., Prevention of Contamination in Environment, Water Treatment)

The transmission of *Cyclospora* is associated with the consumption of contaminated foods. In areas of known endemnicity, infections can be reduced by avoiding the consumption of raw fresh produce and drinking purified or boiled water, as *Cyclospora* is highly resistant to chlorination. Thus, chlorination alone should not be considered as a safe water treatment. Preventative measures at the farm level, should be based on the establishment of good agricultural practices, with an emphasis on the use of clean potable water for spraying the crops.

The current methods to evaluate the efficacy of any control and preventative measures against contamination with *Cyclospora* are based either on data from oocyst sporulation/excystation, or results from surrogate coccidian parasites. These limitations are the result of the lack of infectivity assays, either animal or culture models.

Any disinfection or sanitation method has to also be compatible with the organoleptic characteristics of the food products. Thus far chemical methods have not proven very effective. Short-time exposure to commercial bleach (5% sodium hypochlorite) has been used to purify and clean viable *Cyclospora* oocyts for laboratory experiments, therefore, commercial bleach does not appear to inactivate the parasite. Exposure to chlorine dioxide, at a concentration of 4.1 mgl^{-1} for 20 min was not effective in reducing the sporulation of *Cyclospora*, although it was highly effective at inactivating

Cryptosporidium parvum, another important coccidian parasite of humans and mammals.

Physical methods rely on timed temperature exposures. Freezing at −15 °C for 24 h or −20 °C for 48 h did not interfere with the sporulation of *Cyclospora*. Exposure to temperatures close to refrigeration or ambient temperature such as 4 and 23 °C for periods of at least 7 days had no effect on sporulation. Exposure to warmer temperatures of 37 °C for up to 4 days, or 50 °C for 1 h resulted in the reduction of the sporulation rates, however, minimal parasite sporulation was still detected.

Extreme temperatures were more effective against the viability of *Cyclospora*. Exposures to temperatures of −70 °C for at least 60 min, 70 °C for at least 15 min, or parasite exposure to 100 °C were effective in preventing sporulation. Microwave-induced heating also can inactivate *Cyclospora* oocysts, occurring when the internal temperature of the product reaches ≥80 °C.

The lack of an animal model or *in vitro* system to determine the viability of the parasites has also led to the use of other coccidians like *Eimeria* and *Toxoplasma* as surrogate parasites. The evaluation of γ-ray irradiation showed that exposures of 0.5 kGy were effective against *Toxoplasma* oocysts, whereas 1 kGy was needed to inactivate the oocysts of *Eimeria acervulina*. High hydrostatic pressure and exposure to UV light have also been tested. The rationale for the hydrostatic pressure was that it would release the labile sporozoites from the environmentally resistant oocyst and sporocyst walls, whereas the exposure to UV light intended to interfere with biological processes within the parasites. Pressures of 550 MPa, applied at 40 °C for 2 min, or UV exposure at 261 mW cm^{-2}, led to an effective reduction of the infectious potential of both *Toxoplasma* and *Eimeria*. However, data from *in vivo* assays using animal infectivity studies demonstrated that these methods did not completely inactivate *Eimeria*, and only reduced its infectious potential. The use of a lower pressure of 340 MPa, applied for only 60 s was shown to be equally effective.

Measures at Different Levels of the Food Chain

At the farm level, water quality is of extreme importance. Although ozone and UV radiation have proven effective to kill *Cryptosporidium* oocysts, it is suspected that *Cyclospora* is resistant to these inactivation strategies. This in part is due to the autofluorescence of *Cyclospora* oocysts, suggesting that the

Table 1 Representative outbreaks of *Cyclospora cayetanensis*

Year (Reference)	*Location*	*Cases*[a] (*n*)	*Implicated food product*
1996 (Caceres *et al.*, 1998)	Charleston, SC, USA	38	Fresh raspberries and potato salad
1996 (Katz *et al.*, 1999)	Broward and Palm Beach, FL, USA	60	Fresh raspberries
1996 (Herwaldt and Ackers, 1997)	USA and Canada	1465	Raspberries
1996 (Sewell and Farber, 2001)	ON and QC, Canada	160	Fresh raspberries and possibly blackberries
1997(Centers for Disease Control and Prevention, 1997)	Cruise ship, FL, USA	224	Unknown
1997 (Herwaldt and Beach, 1999)	USA/Canada	1012	Raspberries
1997 (Centers for Disease Control and Prevention, 1997)	Virginia, District of Columbia and Baltimore, MD, USA	308	Basil pesto
1997 (Herwaldt, 2000)	FL, USA	12	Mesclun salad
1997 (Sewell and Farber, 2001)	ON, Canada	31	Fresh raspberries and possibly blackberries
1998 (Herwaldt, 2000)	GA, USA	17	Fruit salad?
1998 (Centers for Disease Control and Prevention, 1998)	Toronto, ON, Canada	192	Berry garnish, raspberries
1999 (Herwaldt, 2000)	ON, Canada	104	Berry dessert
1999 (Herwaldt, 2000)	FL, USA	94	Fruits, berry
1999 (Lopez *et al.*, 2001)	MO, USA	62	Chicken pasta, tomato basil, and vegetable pasta salads
2000 (Ho *et al.*, 2002)	Philadelphia, PA, USA	54	Raspberry filling, wedding cake
2000 (Doller *et al.*, 2002)	Baden-Wuerttemberg, Germany	34	Salad, leafy vegetables and herbs
2001 (Hoang *et al.*, 2005)	Vancouver, BC, Canada	17	Thai basil imported from the US
2004 (Centers for Disease Control and Prevention, 2004)	PA, USA	96	Snow peas
2004 (Torres-Slimming *et al.*, 200627)	Lima, Peru	27	No food identified
2005 (Blans *et al.*, 2005)	Bogor, Indonesia	14/29	Not identified
2005 (Milord *et al.*, 2012)	QC, Canada	142	Basil
2009 (Insulander *et al.*, 2010)	Stockholm, Sweden	12	Sugar snap peas
2009[a]	Victoria, BC, Canada	160[b]	No food identified
2011[c]	Atlanta, GA, USA	>100	Not determined

[a] http://www.cdc.gov/nceh/vsp/surv/outbreak/2009/april21amsterdam.htm (accessed May 2012).

[b] Estimated number of cases, based on meeting definition.

[c] http://www.wsbtv.com/news/news/food-illness-makes-100-sick-catering-georgia-aquar/nDxzG/ (accessed May 2012).

Note: ?, Suspected but not confirmed.

UV light may be reflected from the oocysts and only lower doses may actually penetrate the oocysts.

The simplest measures are based on the detection of markers of contamination with human fecal material. which should not be present in the agricultural soils or water, and emphasizing that good sanitation practices within the farms are crucial to prevent the contamination of crops. From the farm perspective, the facilities should consider adequate bathrooms, with potable water and soap. Among farming practices, workers should avoid the use of the same tools for soil operations and harvesting, primarily to minimize potential cross-contamination by farm tools.

Regulatory or Education Measures

Farm workers should be aware that good hygiene will prevent fecal contaminants from being introduced into the food products. There are no specific regulations specifically addressing *Cyclospora* in foods. However, in the USA, the Global Food Safety Initiative (GFSI) and the Food Safety Modernization Act (FSMA) are two recent efforts aimed to assure the overall quality and safety of food products. GFSI is a private initiative that is focused on providing technical assistance and support to less developed businesses in the development of food management systems. They use a step-wise approach that begins with a self-assessment of practices by the producer, which leads into passing to a higher level of technical requirements and assessment. The overall goal is that the producers will eventually reach accreditation against recognized international standards.

The FSMA came into law in the USA as a response of the public health burden presented by foodborne diseases. A significant difference is that this law is focused on preventing contamination of the food products. The law provides the USFDA the authority to regulate practices starting at the farm level, all the way to postharvest processing. This law will cover products that could be contaminated with *Cyclospora*.

Additional measures to prevent contamination of food products come from the guide on good agricultural practices and manufacturing practices for fruits and vegetables These documents provide recommendations to growers, packers, transporters, and distributors that are aimed to minimize the risk of foodborne diseases. It includes guidance on the basic principles for the proper use of water and organic fertilizers, employee hygiene, sanitation of the fields and processing facilities, as well as transportation (**Table 1**).

Monitoring

No routine monitoring procedures are in place.

See also: Disciplines Associated with Food Safety: Parasitology. Protozoa: *Cryptosporidium* spp.; *Toxoplasma gondii*. Public Health Measures: Surveillance of Foodborne Diseases

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Alconox Material Safety Data Sheet.

http://www.cdc.gov/foodborneburden/2011-foodborne-estimates.html
CDC: For tracking the estimates of foodborne illness in the USA.

http://dpd.cdc.gov/dpdx/HTML/Cyclosporiasis.htm
CDC: Up-to-date information and assistance for diagnosis of *Cyclospora* infections.

http://www.fda.gov/Food/FoodSafety/FSMA/ucm239907.htm
FDA: Key information on the Food Safety Modernization act.

http://www.mygfsi.com/
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Entamoeba histolytica

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Glossary

Amebiasis An infection with *Entamoeba histolytica* with or without clinical manifestations. Its use is usually restricted to this pathogen although some references to other parasitic amebae have used this term.

Dysentery An inflammatory infection of the intestine, particularly the colon, with severe diarrhea containing mucus and/or blood. *Shigella* dysentery usually has more pus in the feces whereas amebic dysentery contains more blood. Historically, dysentery was called flux or bloody flux.

Historical Evidence

The history of *Entamoeba histolytica* has been reviewed. Feder Losch (1875) found amebae in fecal samples in Saint Petersburg, but it was Fritz Schaudinn (1903) who first established the differentiation between *E. histolytica* and *Escherichia. coli* and decided on the species name *E. histolytica* as the parasite was seen to cause tissue lysis. Emile Brumpt (1925) established that *E. histolytica* contained two morphologically indistinguishable species, *E. dysenteriae* in symptomatic infection, and *Entamoeba dispar* in asymptomatic carriers but description of the former came after the description of *E. histolytica* and was therefore not accepted. During the 1960s, axenic culture medium for *E. histolytica* was developed which allowed *in vivo* and *in vitro* studies and in the 1970s isoenzyme electrophoresis was used to separate *E. histolytica* virulent and avirulent strains. William Petri *et al.* (1987) demonstrated that the 170 kDa protein with greater antigenicity was the Gal/GalNac-specific lectin. Diamond and Clark (1993) described again Brumpt's original 1925 hypothesis, concluding that there was enough evidence to support the existence of two morphologically indistinguishable species, a pathogenic and a nonpathogenic one, corresponding to *E. histolytica* and *E. dispar,* respectively. The World Health Organization (WHO) accepted this hypothesis in 1997. Other nonpathogenic *Entamoeba* species are known to occur in humans, including *Entamoeba hartmanii*, *E. coli*, *Entamoeba gingivalis*, *Entamoeba chattoni*, and *Entamoeba moshkovskii*. *Entamoeba moshkovskii* has been considered a potential pathogen in Australia but the evidence is limited. *Entamoeba histolytica*-like organisms from monkeys are genetically distinct from human strains.

Definitions

Entamoeba histolytica and *E. dispar* are anaerobic parasitic protozoa which have trophozoites with a single nucleus. They form cysts (10–16 μm diameter) with a single nucleus when immature, which differentiate into four nuclei when mature. The cysts contain glycogen in a vacuole and usually have chromatoid bodies. The nucleus is vesicular, spherical, and its membrane is lined with small chromatin granules and a small central spherical karysome. Because *E. histolytica* and *E. dispar* cysts are morphologically indistinguishable using light microscopy they are reported as *E. histolytica/dispar*. Trophozoites with ingested red blood cells in fresh stool or other specimens, and trophozoites in tissue biopsies are both strongly correlated with amebic dysentery caused by *E. histolytica*. *Entamoeba histolytica* and *E. dispar* cysts are differentiated using polymerase chain reaction (PCR) protocols that differentiate them.

Cultural Characteristics

Culture of *E. histolytica* has proved useful in the investigation of virulence factors but is unhelpful as a diagnostic procedure. Media used in maintaining trophozoite viability include Roswell Memorial Park Institute (RPMI-1640), Dulbecco's Modified Eagle Medium, phosphate-buffered saline for ameba, and Hank's balanced salt solution.

Entamoeba dispar can outgrow *E. histolytica* in axenic cultures.

Typing

Typing pathogenic amebae has been useful in establishing the species *E. histolytica* and *E. dispar,* but subtyping has had less utility than, for example, *Cryptosporidium*. The differentiation of pathogenic and nonpathogenic amebae in the 1970s was conducted using isoelectric enzyme focusing of the enzymes glucose phosphate isomerase, phosphoglucomutase, and L-malate: NADP + oxidoreductase (oxalacetate-decarboxylating) (ME) (5;10;11). PCR analysis of the

chitinase genes has been used to examine the differences in the isolates of *E. histolytica*, and a microarray system has potential for typing *E. histolytica*. A tandem repeat PCR method was used to differentiate *E. histolytica* and *E. dispar*.

Virulence Factors

Entamoeba histolytica causes amebic colitis through disruption of the mucus layer, followed by binding to and destruction of the epithelial cells. It is unclear why only some infections with *E. histolytica* result in the disease. A study of human colon invasion found that *E. histolytica* infection removed the protective mucus coat during the first 2 h of incubation, detached the enterocytes, and then penetrated into the lamina propria by following the crypts of Lieberkuhn. Cell lysis and inflammation result in the secretion of proinflammatory molecules such as interleukin 1 beta, interferon gamma, interleukin 6, interleukin 8, and tumor necrosis factor (TNF) within 4 h of incubation. TNF-alpha protein is expressed in people infected with *E. histolytica* infection. The virulence of pathogenic *E. histolytica* has been attributed to the capacity of the parasite to destroy tissues through the expression and/or secretion of various molecules, but animal models of the disease suggest the host's inflammatory response is primarily responsible for tissue damage. Host–parasite interactions mainly involve anchored glycoconjugates localized in the surface of the parasitic cell. The damage observed in invasive amebiasis is thought to be linked to interactions between polymorphonuclear leukocytes (neutrophils and lymphocytes) and *E. histolytica* trophozoites. *Entamoeba histolytica* has an effect on the intestinal epithelial cells, causing DNA degradation, mitochondrial dysfunction, and apoptosis. Several essential biochemical processes are situated in mitochondria and in *E. histolytica*, its anaerobic and parasitic lifestyle has led to the evolution of tiny mitochondria known as mitosomes, which are one of the simplest known mitochondrial compartments of all eukaryotes. In liver abscesses there is destruction of liver cells as a result of a strong inflammatory response. The monocyte locomotion inhibitory factor, a heat stable oligopeptide found in the supernatant fluid of *E. histolytica* axenic cultures, may contribute to the delayed inflammation observed in amebic hepatic abscess. Three virulence factors (Gal-lectin, cysteine proteinases, and amebapores) were thought to be the main proteins involved in pathogenesis, but other molecules such as lipophosphopeptidoglycan, peroxiredoxins, arginase, and lysine and glutamic acid-rich proteins are also implicated. *Entamoeba histolytica* possesses an array of protein kinases.

Virulent and avirulent strains from the same culture collection have been examined to investigate the determinants of virulence and differential gene expression has been examined using outputs from the *E. histolytica* genome. In chronic amebiasis, the host immune response is not able to eliminate the parasites. The parasite stimulates active immunosuppression of the host.

Entamoeba histolytica exhibits an abundant population of small RNAs with an unusual 5-polyphosphate structure that may play a role in gene silencing. *Entamoeba histolytica* possesses a higher level of NADP-dependent alcohol dehydrogenase activity than *E. dispar*, but it is difficult to directly link these raised levels to virulence. Iron chelation interrupts the completion of the fermentative pathway of *E. histolytica* by removing the metal cofactor indispensable for the structural and functional stability of alcohol dehydrogenase 2, thus affecting trophozoite survival. Virulent *E. histolytica* has a greater ability to reduce O_2 and H_2O_2 than avirulent isolates. Hydrogen peroxide induces an apoptosis-like programed cell death. The susceptibility of people to *E. histolytica* seems to be linked to leptin (an adipocytokine) signaling in enterocytes.

Gal/GalNAc Lectin

URE3-BP is important in the regulation of two amebic virulence genes, the Gal/GalNAc lectin and ferredoxin. N-glycoconjugates with terminal alpha (1–3) linked mannose residues participate in the adhesion and subsequent cytotoxicity of *E. histolytica* to cultured hamster hepatocytes. These include the Gal/GalNAc lectin.

Cysteine Proteinases

Entamoeba histolytica contains at least 50 cysteine proteases; however, only three *Entamoeba histolytica* cysteine proteinases (EhCPs) (EhCP1, EhCP2, and EhCP5) are responsible for approximately 90% of the cysteine protease activity in this parasite. EhCPs contribute to the disruption of the epithelial cell during invasion of the colon. The degree of virulence of *E. histolytica* isolates, as determined in hamster liver, has been linked to different levels of CP5 mRNA in trophozoites freshly isolated from hepatic amebic lesions and suggest cysteine protease activity is involved in pathogenesis and the digestion of erythrocytes. *Entamoeba histolytica* infection and protease activity can cause selective damage to enteric neurons.

Amebapores

Amebapores are proteins that form oligomeric pores in target cell membranes that are present in the cytosol of the parasite within cytoplasmic granules. When released they can rapidly puncture the host cells. The expression of amebapores is more important in liver disease than colonic infection.

Iron and Infection

Entamoeba histolytica can grow using free iron, lactoferrin, transferrin, ferritin, hemoglobin, or hem as the sole iron supply and the ferritin is internalized through clathrin vesicles. It has been found that the amebae internalized holotransferrin (holoTf), which transports iron into all cells, through clathrin-coated pits, suggest that holoTf endocytosis could be important for the parasite during colonization and invasion of the intestinal mucosa and liver.

Genetics and Evolution

Some eukaryotic parasites have evidence that horizontal gene transfer (HGT) has played a role in their evolution. A study screening the completed genomes of *E. histolytica* and *Trichomonas vaginalis* found 68 and 153 recent cases of HGT respectively, with the gene enzymes involved in metabolism. These genes were from prokaryotic donors that share similar environmental niches (e.g., gut and vagina). *Entamoeba histolytica* isolates from across Africa are genetically heterogeneous.

Animal Models

Hamsters are a model for the development of amebic liver abscesses. Experimental infection of rhesus monkey's (*Macaca mulatta*) results is a disease that is similar to that seen in humans. Amebiasis in the murine model can be prevented by vaccination with the Gal/GalNAc lectin through a T cell-dependent mechanism. Trophozoites of *E. histolytica* HM-1:IMSS can become less virulent with long-term maintenance in axenic cultures, but cholesterol appears to prevent this.

Detection Methods

Diagnosis has traditionally required observation of trophozoites of *E. histolytica* containing erythrocytes in stool samples, aspirates from intestinal and other organs, biopsy material, or in mucus from rectal ulcers. Enzyme-linked immunosorbent assay tests are available for the detection of *Entamoeba* antigen and specific *E. histolytica* lectin antigen in fecal samples. Although the demonstration of active amebae or cysts is the best way to make definitive diagnosis, serology is the method of choice for the diagnosis of amebic liver disease. For microscopic examination, the Eosin-Y staining technique was found to be the easiest to perform, and was as accurate as the commonly used Wheatley trichrome technique for the detection of trophozoites in feces. Samples need to be examined within 6 h of stool collection to obtain good microscopic results.

PCR has been used in the developing countries for parasite detection. Primer pairs have been designed to amplify species-specific products for the differentiation of the species *E. histolytica* and *E. dispar* by PCR, and real-time PCR can be useful in the diagnosis of amebic abscesses. Preparation methods can improve PCR sensitivity.

A commercially available fecal antigen detection test specifically identifies *E. histolytica*. A novel one-step, closed tube, loop-mediated isothermal amplification assay for detecting *E. histolytica* has been reported. A multiplex tandem PCR assay for the detection and identification of four common pathogenic protozoan parasites, *Cryptosporidium* spp., *Dientamoeba fragilis*, *E. histolytica*, and *Giardia intestinalis*, from human clinical samples has been established. Freezing the fecal samples has been shown to give better PCR detection.

Health Effects

Entamoeba histolytica invades the colonic mucosa producing ulcerative lesions and profuse bloody diarrhea (amebic dysentery). Acute amebic colitis has a gradual onset, with a 1–2 week history of mild-to-moderate abdominal pain and tenderness, tenesmus (feeling the need to defecate). There are frequent watery diarrheic stools (e.g., five or more episodes per day) usually with abundant mucus and blood, and contain motile amebic trophozoites. During the chronic stages of the infection these symptoms disappear, and only cysts are found in the feces. Most patients experience abdominal pain, whereas some have intermittent diarrhea alternating with constipation. Fever is less unusual, whereas other associated symptoms include weight loss and anorexia.

Feces are almost always positive for occult blood if frank blood is not present. The dysenteric symptoms usually resolve within a few days following appropriate treatment. Severe cases of amebic colitis are characterized by bloody dysenteric stools, diffuse abdominal pain, high fever, and severe dehydration, and patients usually appear quite ill. Other presentations of acute intestinal amebiasis include extensive fulminant necrotizing colitis, toxic megacolon, and perianal ulceration.

Systemic infection can lead to abscess formation, particularly in the liver and lungs. Clinical complications, such as fulminating necrotic colitis and intestinal perforations, are the main cause of death in cases of invasive intestinal amebiasis.

Epidemiology

Entamoeba histolytica occurs worldwide, with natural infections in humans and other primates. Infection is most common in developing countries, where sanitation, water supply, and hygiene are rudimentary. Infection due to *E. histolytica* in endemic areas has been difficult to estimate and dysentery or bloody diarrhea can be misdiagnosed as amebiasis where laboratory diagnosis is unavailable. Human susceptibility to *E. histolytica* appears to be the norm. Although asymptomatic carriage of *E. dispar* can be common, carriage of *E. histolytica* is not uncommon. As a result the mortality rate of infected people is difficult to estimate. However, the WHO suggest that there are over 100 000 amebiasis-related deaths worldwide every year and mortality is probably linked to nutritional status.

In developing countries mixed parasitic infections can be common and can influence postinfection chemokine and cytokine responses. The frequency of invasive intestinal amebiasis is thought to range from 2.2% to 16% of patients with acute amebic dysentery and case fatality from 0.5% in uncomplicated cases to 40% in patients with amebic dysentery complicated by peritonitis (**Figure 1**).

Outbreaks

In the early twentieth century, outbreaks were commonly attributed to food handlers, but an outbreak in three hotels in Chicago in 1933, and a stockyard fire outbreak in 1934 emphasized the importance of waterborne transmission, particularly where there is direct contamination of drinking water with wastewater. A waterborne outbreak on a Royal Air Force base in 1950 was also linked to gross fecal contamination of a drinking water borehole source. Outbreaks of *E. histolytica* can

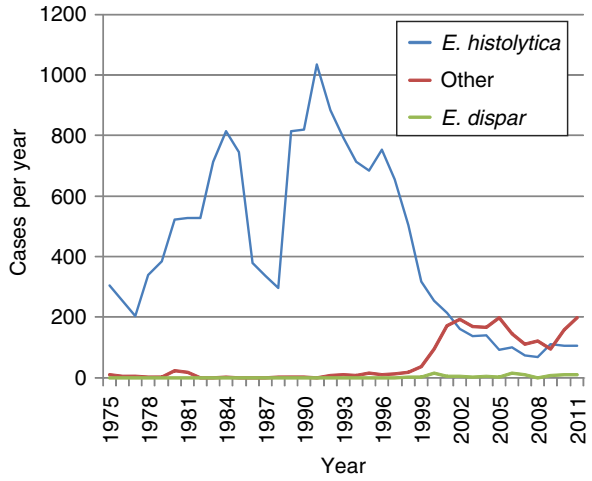

Figure 1 Reported cases of *E. histolytica* and *E. dispar* in England and Wales.

occur in institutions with poor hygiene such as mental homes. A community outbreak in Hecun Town, Jiangshan City, China was thought to be linked to poor personal hygiene. Outbreaks linked to sexual transmission have been reported. Outbreaks of gastroenteritis can involve *E. histolytica*. A review identified nine waterborne outbreaks involving *E. histolytica* and inadequate treatment or breaks in distribution can be important as can the contamination of groundwater with sewage. A waterborne outbreak of gastroenteritis in Taiwan affecting 730 students was due to the contamination of underground well water by sewage. Both *Shigella sonnei* and *E. histolytica* were isolated from the patients. As with other enteric pathogens, family outbreaks can occur. In developing countries outbreaks may be linked to particular farms, even where an obvious transmission route may not be identifiable. Poor hygiene or contaminated water supply were the likely reasons for the outbreaks. Travel is a significant risk factor for sporadic amebiasis, and outbreaks linked to travel have been reported. An outbreak in people returning from Thailand identified consuming ice to be a risk factor. An unusual outbreak of amebiasis associated with a chiropractic clinic and the source of infection was contaminated equipment used for colonic irrigation. The direct contamination of the colon, where the amebae invade, was thought to be important in this outbreak. A review of waterborne outbreaks of protozoan parasites undertaken by Karianis *et al*. in 2007 found nine outbreaks associated with water.

Entamoeba histolytica can contaminate fresh produce, and in developing countries, the unsanitary conditions in markets make transmission likely, with no hand washing facilities and contaminated water sources. Indeed, there is thought to be a close relationship between personal hygiene, sanitary food conditions, and amebic infection. In developed countries, outbreaks are very unlikely unless a water supply becomes grossly contaminated, although infection in travelers returning from the Indian subcontinent is not uncommon. Asymptomatic carriage of the organism appears to be relatively common in certain populations and this has implications for foodborne transmission from food handlers.

An epizootic outbreak of amebiasis affected two mantled guerezas (*Colobus guereza*) and one Hanuman langur (*Semnopithecus entellus*) in an open range recreation park. An outbreak in a colony of spider monkeys has also been reported.

Case–Control Studies

Sexually active men who have sex with men (MSM) were more likely to be seropositive for antibodies to *E. histolytica* than either low-risk MSM or a control group. This is true for developing as well as developed countries.

A case–control study conducted between 2006 and 2009 examined factors associated with the presence of anti*E. histolytica* antibody titers of ≥ 128 by indirect hemagglutination assay, among people seeking voluntary counseling and testing (VCT) for human immunodeficiency virus (HIV) infection. It was shown that 57 out of 4802 persons (1.2%) were seropositive for *E. histolytica* infection compared to 228 seronegative controls. The MSM, fecal–oral contamination, lower educational achievement, and older age were associated with increased risk for amebiasis among persons seeking VCT for HIV infection.

Routes of Transmission

Transmission of *E. histolytica* generally occurs by fecal excretion of cysts followed by oral ingestion of contaminated food or water. However, fecal–oral transmission may occur within households and long-term care institutions, and sexual transmission can occur heterosexually through oral–anal sex and among MSM. Cysts of *Entamoeba* survive well in moist conditions and may be transmitted via contaminated food or water, or direct contact. Waterborne spread appears to occur where there is gross contamination of drinking water with sewage.

Reservoirs and Environmental Occurrence

Amebic trophozoites are only present in the human body and do not play a role in transmission, except for the occasional case associated with organ transplant. As *E. histolytica* is not found in agricultural animals and people do not usually come into close contact with monkeys and apes, the source of infection is exclusively other infected humans. The cysts can survive outside the body for only a few days in feces but can be present in night soil, sewage, and water, and for short periods on foods, on the hands of infected food handlers, or through flies contaminating food. The cysts remain alive outside the body at temperatures just above freezing for periods of up to 3 months. At moderate temperatures they will survive between 9 and 30 days in water. They cannot withstand desiccation or high temperatures. Cysts are killed within 10 min by desiccation on the surface of hands, but survive for longer (45 min) if trapped in fecal material under fingernails.

Infections in Animals

Entamoeba histolytica can be detected in both old world and new world monkeys, including rhesus monkey, silver leafed

monkey, baboon, spider monkey, cynomolgus monkey (*Macaca fascicularis*), macaque, mantled guereza (*C. guereza*), Hanuman langur (*S. entellus*), colobus monkey, Abyssinian colobus monkey, De Brazza's guenon, white-faced saki, and Geoffroy's spider monkey. Some simian isolates can be differentiated from human strains but are still regarded as *E. histolytica*, and others better fit into *Entamoeba nuttalli* or the nonpathogenic *E. chattoni*. The *E. histolytica* like strains are able to cause liver abscesses in monkeys. *Entamoeba histolytica* has also been isolated from gorilla and chimpanzee. Dogs, cats, rodents, and other mammals can be infected but are not thought to contribute to transmission.

Water Treatment

Entamoeba histolytica cysts are larger than *Cryptosporidium* oocysts and are removed from water by slow sand filtration and conventional flocculation, sedimentation, and rapid sand filtration. *Entamoeba histolytica* cysts are relatively resistant to chlorine ($>3\ \mathrm{mg\,l^{-1}}$ for 30 min needed for killing). They are readily removed from water by conventional water treatment process of coagulation, sedimentation, and filtration. Brief boiling or pasteurization is sufficient to eliminate/kill *E. histolytica* cysts, which are sensitive to 60 °C for 1 min. Solar disinfection has been used to kill *E. histolytica*, with temperatures above 56 °C being effective. Cysts of *E. histolytica* can survive for 9 and 30 days in water, but cannot withstand desiccation or high temperatures. Cysts die within 10 min by desiccation on the surface of hands. Although *E. histolytica* is distributed worldwide, infection is not endemic in Europe or generally in the industrialized world and most reported cases are in people returning from abroad or regions of the world with poor sanitary infrastructure. The risk of infection from drinking water is low as *E. histolytica* cysts are unlikely to be present in large numbers in source waters of these regions and multiple barrier treatment including disinfection will provide effective control.

Foodborne Transmission

Although the evidence for foodborne transmission of *E. histolytica* is slim, there is circumstantial evidence that sporadic transmission occurs. Food handlers probably play an important role in sporadic food-related disease in developing countries where carriage is common. The detection of parasite ova and cysts on raw vegetable is much reduced by washing. Hand washing is thought to be protective for people catching *E. histolytica* and other parasites. Disinfectants are more effective at decontaminating foods contaminated with *E. histolytica* compared to *Cryptosporidium parvum*.

Preventive and Control Measures

Entamoeba histolytica can be transmitted by the poor personal hygiene of food handlers, contamination of ready-to-eat foods such as fruits or vegetables, from drinking water, and by insect vectors such as flies.

As with other protozoa, cooking food is an effective control, although contamination from the hands of infected food handlers can still be important. Because restaurants in developed countries commonly employ recent immigrants from developed countries, reducing transmission should focus on pathogen screening of new employees and good kitchen hygiene training. In the developing countries *E. histolytica* cysts can be transmitted through foods such as salads, fruits, or vegetables that have been contaminated from irrigation or wash water, or through handling at market (where toilets and hand washing may not be available), or in food preparation. Water contaminated by human waste is the primary vehicle for infection and treatment should include filtration as well as chlorination. In developing countries, improvements to reduce amebiasis and other fecal–oral pathogens need to focus on waste disposal, improvements in water infrastructure, treatment and household storage, and hygiene education and support in order to reduce the exposure of people to human feces.

Entamoeba

Entamoeba histolytica is an anaerobic parasitic protozoan that affects primates. *Entamoeba histolytica* is estimated to infect approximately 50 million people worldwide although there can be many asymptomatic cases. Symptoms can include fulminating dysentery, bloody diarrhea, weight loss, fatigue, and abdominal pain. The ameba can penetrate the intestinal wall and reach the blood stream and internal body organs, such as the liver, spleen, lungs, and even the heart and brain. There is an approximately 1–3% mortality rate in those with overt symptoms. A closely related species, *E. dispar*, is considered to be nonpathogenic but this species are hard to distinguish morphologically. The cysts are transmitted through consumption of contaminated water or food, such as salads, fruits, or vegetables that have been washed in water containing the cysts; handling objects that have been in contact with contaminated soil or animal feces, and by anal sex. More cases occur in the rainy season than in the dry season. One cyst may be enough to initiate an infection with an incubation period of 2–4 weeks. Although water is the primary vehicle for infection, raw fruits and vegetables may also be contaminated, and normal chlorination treatment for potable water is not effective in destroying the cyst. The most dramatic incident in the USA was the Chicago World's Fair outbreak in 1933 caused by contaminated drinking water. There were 1000 cases and 58 deaths; defective plumbing allowed sewage to contaminate the drinking water. More recently, food workers are suspected of causing a few sporadic infections, but there has been no single large outbreak in industrialized countries

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PROTOZOA

Giardia lamblia

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Glossary

Cyst The environmentally stable form of the life cycle for protists with complex life cycles. This form has reduced metabolic activity and does not replicate.

Diplomonad (Literally two bodies) a phylogenetic class of organisms that includes the *Giardia* species as well as other genera of free-living and parasitic species. They are characterized by a body with dyad symmetry and two symmetrically placed nuclei.

Encystation This is a developmental process in which the trophozoite stage is transformed into the cyst stage.

Excystation This is a developmental process in which the cyst stage is transformed into the trophozoite stage.

Ova and parasite A microscopic examination of feces for the detection of eggs and parasites; it is used in the diagnosis of multiple helminthic and protozoan intestinal infections.

String test A diagnostic test for giardiasis (or for several other infections) in which a capsule at the end of a string is swallowed, followed by removal after 4 h to overnight and microscopic examination of the end of the string.

Trophozoite The vegetative form of the life cycle for protists with complex life cycles. This form replicates within the host and causes the disease symptoms.

Ventral disk The ventral surface of the trophozoite which attaches by mechanical means to the intestinal wall of the host, or to other surfaces.

Background

The first description of *Giardia lamblia* (synonym *Giardia duodenalis*, *Giardia intestinalis*) was reported by Leeuwenhoek who gave a classic description in 1681 of what is probably *Giardia*, as he observed his own diarrheal stool under the microscope. His description was translated by Dobell and reads as follows:

> "I have ordinarily of a morning a well-formed stool; but now and then hitherto I have had a looseness…when I went to stool some 2, 3, or 4 times a day…My excrement being so thin, I was at divers times persuaded to examine it…All the particles aforesaid lay in a clear transparent medium, wherein I have sometimes also seen animalcules a-moving very prettily; some of 'em a big bigger, others a bit less, than a blood-globule, but all of one and the same make. Their bodies were somewhat longer than broad, and their belly which was flatlike, furnisht with sundry little paws, wherewith they made such a stir in the clear medium and among the globules, that you might e'en fancy you saw a pissabed (woodlouse) running up against a wall; and albeit they made a quick motion with their paws, yet for all that they made but slow progress."

Later, Lambl independently discovered *Giardia* in 1859 and described it in greater detail, and the organism was subsequently named after him.

Characteristics

Giardia species are flagellated protists that are classified within the diplomonads (which literally means two bodies) (Figure 1). Most of the diplomonads have two nuclei, each forming one of the two bodies. The other diplomonads include *Spironucleus* spp. and *Hexamita* spp. These other diplomonads are not pathogenic for humans, but include fish pathogens and free-living organisms. *Giardia* and other diplomonads were proposed as early diverging eukaryotic organisms on the basis of rudimentary metabolism and cell biology that was lacking some of the canonical eukaryotic features. These observations suggested that these 'archaea' had emerged from the eukaryotic lineage before the acquisition of mitochondria and other organelles. This hypothesis was supported by small subunit rRNA sequences that suggested an ancient divergence from the eukaryotic line. However, subsequent data have suggested that most or all of the absent eukaryotic features have been lost secondarily. *Giardia* may still be a relatively early diverging organism, but probably not more than some of the other protists (protozoans).

Giardia trophozoites lack several of the organelles that are found in most eukaryotes. Mitochondria appear to have been replaced by a remnant called a mitosome. Standard Golgi have not been identified, although Golgi can be identified in encysting organisms. Peroxisomes have not been identified. Nucleoli were thought to be absent, but recent reports have described nucleoli. Trophozoites have minimalist metabolisms that match their minimalist genomes. As predicted by the lack of mitochondria, the metabolism of the trophozoites is strictly anaerobic, utilizing anaerobic glycolysis when using sugar for energy production. Both purines and pyrimidines are obtained from the host by the use of salvage pathways. Similarly,

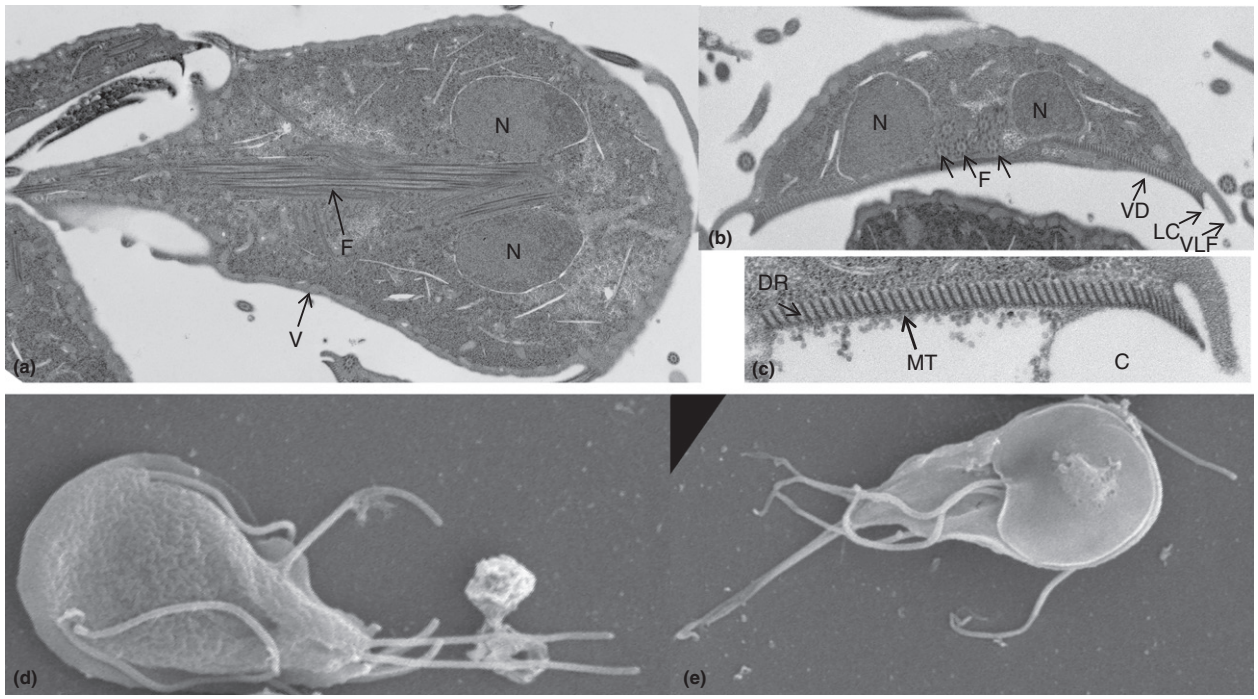

Figure 1 Electron micrographs of *Giardia trophozoites*.

there is no endogenous fatty acid synthesis, so lipids are obtained from the host, as are most amino acids.

Giardia lamblia was not generally accepted as a human pathogen until the 1960s when it was clearly associated with a number of outbreaks of diarrheal disease, and subsequently became recognized as the most common cause of waterborne outbreaks of diarrhea. In addition, a number of epidemiologic studies have shown a link between diarrhea and the presence of *Giardia* trophozoites or cysts in fecal specimens. These epidemiologic observations have also been supported by descriptions of trophozoites in the small intestinal mucosa accompanied by abnormalities of the small intestinal mucosa in patients with diarrhea.

Giardia species were initially named according to the host species from which they were obtained, leading to a large number of *Giardia* species names. Subsequently, a detailed microscopic description of *Giardia* by Filice in 1952 described only three morphologic types that were distinguishable by light microscopy, *Giardia agilis* from amphibians, *Giardia muris* from rodents, and *Giardia duodenalis* from a variety of mammals and birds. However, with the advent of electron microscopy, several species within the *G. lamblia* (*G. duodenalis*) morphologic type were distinguished by ultrastructural differences, including *Giardia microti* (voles and muskrats), *Giardia ardeae* (herons), and *Giardia psittaci* (psittacine birds). Because of these changes in species designation, all the organisms remaining within *G. lamblia* are mammalian parasites (Table 1).

Even within this narrower species definition of *G. lamblia*, there are seven distinct genotypes or assemblages with different levels of host specificity (see Table 1). None of these genotypes can be distinguished morphologically, but all can be easily distinguished by molecular sequencing. Genotypes A (Nash Groups 1 and 2) and B (Nash Group 3) are the only types known to infect humans. Genotypes A and B have also been identified in a variety of wild animals, so there are probably occasions when zoonotic transmission occurs. One of the biggest controversies revolves around whether transmission between dogs and humans occurs, and if so, whether it occurs commonly. Some studies, including our own recent results from a shantytown near Lima Peru, have found that dogs rarely carry Genotypes A or B. However, other studies

Table 1 *Giardia* species and genotypes

Species	*Genotype (of* G. lamblia*)*	*Host*
Giardia lamblia (*G. duodenalis*, *G. intestinalis*)		
	A	Humans and other mammals
	B	Humans and other mammals
	C	Dogs
	D	Dogs
	E	Livestock
	F	Cats
	G	Rats
Giardia agilis		Amphibians
Giardia muris		Rodents
Giardia microti		Rodents
Giardia psittaci		Psittacine birds
Giardia ardeae		Herons

have found the opposite. In addition to Genotypes A and B, five *G. lamblia* genotypes have subsequently been described from dogs (C and D), livestock (E), cats (F), and rats (G), and have never been identified in humans. Genotypes A through G differ substantially when compared by molecular sequencing. So far, the sequences of Genotypes A (WB isolate) and B (GS isolate) have been reported. The sequences of these two organisms differ by nearly 20%, so it is likely that they will be designated as separate species in the future. Ironically, we have nearly returned to the pre-1952 convention of naming *Giardia* species after the host of origin, although with substantially greater understanding of the molecular basis for the distinction.

Life Cycle

The *Giardia* species have a two-stage life cycle which includes the environmentally resistant cyst that initially establishes infection and the vegetative trophozoite which replicates in the small intestine and causes the disease manifestations. The cyst wall has an outer filamentous layer composed of *N*-acetylgalactosamine and three cyst wall proteins (CWPs 1–3). Inside the outer layer are two cell membranes. The cyst has a markedly decreased metabolic activity, which along with its protective outer layer allows it to survive for more than a month in the environment, especially at cooler temperatures. Infection is initiated by the ingestion of as few as 10–100 cysts and is followed by excystation when the cyst passes through the acidic environment of the stomach followed by entry into the duodenum, resulting in the production of two trophozoites from each cyst. However, despite the role of a low pH in inducing excystation, it should be noted that excystation has also been induced by bicarbonate at a neutral pH, and that achlorhydria does not prevent human infections. Therefore, it is clear that the acidic pH is not required for excystation. Pancreatic and endogenous proteases probably play an important role in excystation.

The trophozoite colonizes the proximal small intestine where it obtains its nutrients from the host and causes the disease manifestations. The trophozoites are pear shaped and are approximately 12–15 μm in length and 5–9 μm in width (**Figure 1**). The dorsal surface is convex, whereas the ventral surface attaches to the intestinal epithelium by means of its concave ventral (sucking) disk. The attachment appears to be purely mechanical in nature, and trophozoites adhere just as vigorously to a glass surface while being cultivated *in vitro*. The four pairs of flagella play roles in attachment and motility. A median body is also part of the cytoskeleton, and its morphology can be used to distinguish some of the *Giardia* species, but its function remains unknown.

Trophozoites replicate by binary fission and *Giardia* has always been assumed to be asexual. However, recent population genetic data in addition to the presence of meiosis-specific genes in the sequenced genome, have raised the possibility that there may be a cryptic sexual cycle as well. *Giardia* species are the only human pathogens that have two nuclei. The nuclei are symmetrically placed anteriorly in the trophozoite and are similar in appearance. They replicate at nearly the same time (in contrast to ciliates such as *Paramecium* that have a micronucleus and macronucleus). The nuclei are both transcriptionally active and each has a complete copy of the genome. In fact, trophozoites are tetraploid or approximately tetraploid, so that each nucleus is diploid.

Encystation occurs in the small intestine as a result of exposure to bile salts and cholesterol starvation. Trophozoites complete nuclear replication and karyokinesis, so a single cyst has four nuclei and is poised to divide into two trophozoites when it excysts after infecting the next host.

Pathogenesis

The mechanism by which *Giardia* causes intestinal disease is not well understood. Invasion of the intestinal epithelium has never been convincingly demonstrated, and extra-intestinal infection does not occur. It has been suggested that the malabsorption occurs as a result of trophozoite adherence followed by mechanical blockage of absorption. However, contradicting this hypothesis is the observation that the intestinal wall is highly efficient for absorption of nutrients, so it is unlikely enough that the small intestinal surface is covered by trophozoites to cause malabsorption. Therefore, most of the recent studies have focused on a potential immunopathologic etiology of malabsorption. The intestinal epithelial microvilli are diffusely shortened by activated T lymphocytes which are activated as part of the response to the trophozoites. Lymphocyte activation and subsequent apoptosis of the epithelial cells results in disruption of the epithelial tight junctions, increasing permeability and causing the diarrhea.

However, there is also evidence that the host benefits from an effective immune response. There is evidence for acquired resistance to disease from epidemiologic studies. For example, a large waterborne outbreak of giardiasis at a Colorado ski resort resulted in a much higher frequency of symptoms in visiting skiers than in local residents despite having the same water source. This suggested that the local residents had developed partial protection from symptomatic disease as a result of prior exposure. In addition, there is evidence supporting a beneficial role for the humoral immune response from multiple studies in which the severity and duration of giardiasis were increased greater in patients with common variable immunodeficiency. Despite the evidence in animals for a potentially beneficial role of the cell-mediated immune response, there is little evidence in humans that giardiasis is increased in frequency or severity in patients with HIV infection or other illnesses characterized by deficiency of cell-mediated immunity. It may well be noted that the immune response is a double-edged sword in which there may be situations where the immune response decreases symptoms and others where it contributes to the symptoms of infection.

The organism itself does not have any known toxins or virulence factors. The genome does encode a family of approximately 300 cysteine-rich proteins, called variant-specific proteins (VSPs). One of the 300 VSPs coats the trophozoite surface at a time. The organism is then able to switch expression from one VSP to another during the course of an intestinal infection so that one coat is replaced by another VSP that is resistant to antibodies stimulated by the original VSP. It is likely that antigenic variation of the VSPs contributes to the prolonged

nature of the infection, but whether the VSPs contribute to the symptoms of infection is not known. In addition to their potential role in evading the immune response of the host, there is also circumstantial evidence that they may allow the trophozoite to adapt to different intestinal environments. Different hosts have different intestinal proteases and different VSPs can have different protease susceptibilities. This observation has led to the interesting hypothesis that the role of antigenic variation is to allow the organism to adapt to different intestinal environments.

Clinical Manifestations

The hallmark presentation of giardiasis is chronic diarrhea with malabsorption and weight loss. However, that presentation occurs only for a minority of infected persons. Overall, the majority of infections are asymptomatic, but there are reports of outbreaks of giardiasis with symptomatic attack rates of 100% or near 100%. The most likely explanations for the differences in the rate of symptomatic disease are differences in virulence of organisms, or the presence or absence of prior exposure and partial immunity. The role of partial immunity is noted above and the evidence for differences in pathogenicity for different organisms comes from human volunteer studies in which no recipients of a Genotype A1 organism developed infection, whereas all five recipients of a Genotype B isolate developed infection and three were symptomatic. It is also possible that other differences such as inoculum size or differences in intestinal bacterial flora could affect the degree of symptoms, but these latter possibilities have not been studied.

Infection is initiated by the ingestion of as few as 10–100 cysts and when symptoms develop, they typically occur in approximately 1 week after exposure, but the incubation period can range from 1 or 2 days to 2 weeks. Symptomatic patients nearly always have diarrhea, consisting of loose stools that are greasy in consistency and described as malodorous (Figure 2). Other gastrointestinal symptoms include nausea and vomiting, abdominal bloating, and flatulence. Because *Giardia* infects only the small intestine and is a noninvasive pathogen, blood and white cells are not seen in stools. Fever is unusual but a low-grade fever is sometimes present at the beginning of illness. Systemic symptoms are unusual with the exception of fatigue or malaise, which are found in approximately two-thirds of symptomatic patients.

The duration of symptoms is nearly always greater than 1 week, which distinguishes giardiasis from most forms of viral or bacterial gastroenteritis. The average duration ranges from 2 to several weeks in different series and is sometimes measured in months in the absence of treatment. Malabsorption with weight loss is a key component of the presentation for these patients with prolonged illness. The history of weight loss is helpful in distinguishing giardiasis from irritable bowel syndrome, which is not associated with weight loss. Diarrhea with weight loss can be seen with celiac disease or tropical sprue as well as less common illness such as Whipple's disease, so these illnesses should be considered in patients with a compatible history. It is also noteworthy that approximately one quarter of patients with symptomatic will have symptoms of irritable bowel syndrome after recovery from the acute episode. These patients should be worked up for persistence of giardiasis, but in the absence of documented infection, they do not benefit from anti-Giardia therapy.

When giardiasis is suspected, the diagnosis can usually be established by microscopic examination of fecal material for the presence of cysts or trophozoites (stool for ova and parasite). The cyst is detected much more commonly than the trophozoite, but identification of the trophozoite correlates more closely with symptomatic infection. The yield from a single ova and parasite examination is inadequate, so three successive stool samples are examined when suspicion is high. Alternatively, there are enzyme immunoassays (EIAs) or fluorescent antibody-mediated tests that have about the same sensitivity when used with a single specimen as ova and parasite examination done with three stool specimens. The

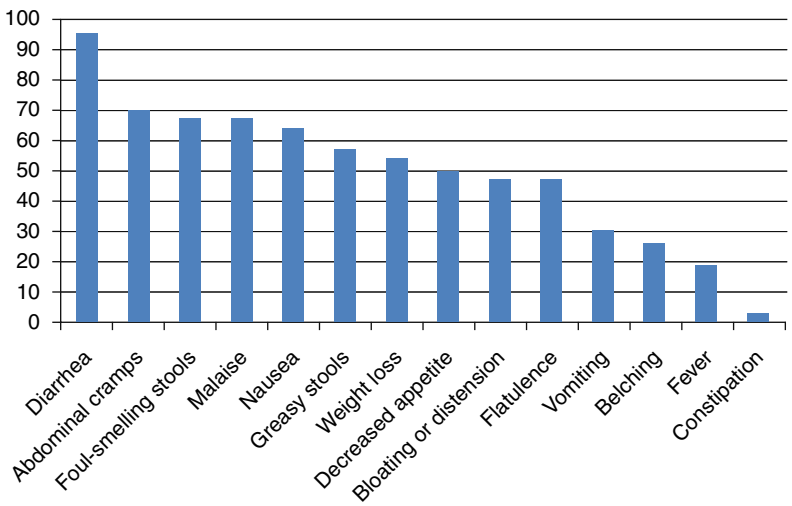

Figure 2 The frequency of symptoms found in patients with symptomatic giardiasis. The numbers on the left indicate the percentage of patients with the designated sign or symptom; the numbers represent a weighted average from a series of clinical studies of symptomatic patients. Signs and symptoms of giardiasis are along the x-axis.

fluorescent antibody-mediated test, which detects Giardia cysts, may have slightly higher sensitivity and specificity than the EIA (and also detects *Cryptosporidium* oocysts at the same time), but the EIA is more commonly used because it is less labor-intensive. Obviously, if detection of other intestinal parasites is desired, then the ova and parasite examination is preferable. Polymerase chain reaction (PCR) tests have been described in the literature, but are not yet clinically available. There are no serologic tests that are useful for the diagnosis of giardiasis.

There are occasional patients with malabsorption and weight loss due to *Giardia* who have consistently negative diagnostic studies. In these patients, *Giardia* trophozoites can be detected in small intestinal contents by endoscopy or by using the string test. The string test is performed by having the patient swallow a capsule at the end of a string. The capsule advances to the small intestine where it stays 4 h to overnight. The string is removed followed by microscopic examination of the end of the string for trophozoites. The advantage of the string test over endoscopy is that it is simpler, but the disadvantage is its inability to identify other illnesses such as celiac disease, which can be in the differential diagnosis.

Epidemiology

Giardia is the most commonly identified intestinal protozoan throughout the world, in both developed and developing areas. However, the epidemiology differs markedly between developed areas which generally have good water supplies and developing areas with inadequately purified water. The typical pattern in developing regions is a high rate of endemic transmission, which may include surprisingly low levels of symptomatic disease. For example, 95% of children living in a shantytown near Lima, Peru, were infected by the age of 2 years and after treatment 98% were reinfected within 6 months. Other studies of children in highly endemic areas have also demonstrated nearly universal infection in early childhood and reinfection shortly after treatment. These children are rarely symptomatic, although growth stunting may be associated with these apparently asymptomatic infections.

In contrast, giardiasis in developed regions is less common and frequently occurs in outbreaks of symptomatic disease. *Giardia lamblia* is one of the most commonly documented causes of waterborne outbreaks of illness in the US and in other developed regions. The most recently reported large outbreak was from Bergen, Norway, where more than 3000 residents of this small city developed symptomatic infection. Although drinking water was incriminated as the source of infection, the source of the original contamination was never determined. Waterborne outbreaks are facilitated by the fact that infected individuals may excrete up to 10^9 cysts per day in the feces in comparison with an infective dose of 10–100 cysts. The cysts can survive for more than a month in temperatures that are near 4 °C. At higher temperatures, the duration of cyst viability decreases substantially, which may explain that within the US, the incidence of giardiasis is higher in the northern states. The annual frequency of reported cases in the US varies from approximately 4 to 20 cases per 100 000 population among the various states with an average of 7 cases per 100 000 for the entire country. However, it is likely that these numbers obtained by a passive case reporting system greatly underestimate the case rate. A report taking this likelihood into account estimated the rate in the US to be between 50 and 1000 cases per 100 000 population annually. In that report, the annual rate of hospitalization for severe giardiasis was estimated at 2 per 100 000, a rate that is similar to that for shigellosis.

Municipal water supplies in developed regions have very low rates of contamination with *Giardia*, although occasional studies have raised the possibility that ingestion of tap water is a risk for acquisition of giardiasis. The risk is increased when drinking water is obtained from shallow wells and from surface water that is not adequately purified (such as chlorination without filtration). The source of contamination of the shallow well and surface water remains highly controversial in that zoonotic sources are frequently blamed but seldom documented. The controversy centers on how often genotypes of *Giardia* that infect humans (A and B) are found in animals. Genotypes A and B have been found in beavers, and beavers have occasionally been incriminated as sources of contamination that led to human infections. However, data incriminating other animals as sources of contamination are lacking. As noted above, some studies have found Genotypes A and B in dogs, whereas others have not, so it is unclear if dogs are a potential source. Genotypes A and B have not been identified in livestock animals, so they cannot be considered as a source of contamination leading to human infection.

Water used for recreational purposes is probably more common than drinking water as a source of human infection in the US. The implicated sources range from swimming pools to wilderness water supplies. Mountain backpacking is a well-recognized risk factor for acquisition of giardiasis. Beavers are commonly blamed as the source of contamination of the wilderness water, but this remains controversial. Within the US, the incidence of giardiasis peaks in the late summer, which probably reflects the high use of recreational water facilities in the summer. The frequency of giardiasis is also substantially higher in children less than 10 years of age than for adults, which may reflect greater use of recreational water facilities. Alternatively, the higher incidence in children could be the result of a lack of preexisting immunity, or alternative sources of infection, such as day care centers.

Although less frequent than waterborne outbreaks, a number of foodborne outbreaks of giardiasis have been reported in the literature (**Table 2**). When considering the potential risk of foodborne giardiasis, the key question is how and when the contamination occurs. Theoretically, the contamination can occur at any time before harvest until after preparation of the food. Cysts passed by the mammalian host are fully infectious as during the time of passage in the feces, so unlike some other parasitic infection, there is not a delay between the time of contamination and the beginning of the risk to those ingesting the food. It is also important to note that because the cyst is not a replicative form, they will not replicate in food items. Cysts are also not heat stable, so cooking will inactivate the cysts. When outbreaks occur, they are typically associated with food handlers who are themselves infected and contaminate the food during the preparation

Table 2 Foodborne outbreaks of giardiasis

Year of report	*Foods implicated*	*Source of food contamination*	*Number of people ill*	*Attack rate (%)*
1993	Raw sliced vegetables	Asymptomatic food handler	27	14% of those who ate the raw vegetables
1992	Ice	Asymptomatic carrier in household of food handler	27	75
1990	Fruit salad	Food handler who had a 2-year-old child with asymptomatic giardiasis	10	40 (82 for those who ate the fruit salad)
1989	Sandwiches	Food handler with an asymptomatic child	88	35% of nursing home residents
1989	Taco ingredients	Unknown	22	19
1988	Noodle salad	Symptomatic food handler with two asymptomatic *Giardia*-infected children	13	81
1981	Home-canned salmon	Asymptomatic carrier in household of food handler	29	48

process. For example, an outbreak of giardiasis in Minnesota resulted in infection of half of 60 employees at a school, but none of the students had documented giardiasis. The source of the outbreak was traced to home-canned salmon opened by a woman caring for her grandson who was subsequently found to have asymptomatic giardiasis. This outbreak points out the potential risk of transmission from asymptomatic carriers of *Giardia*. Other foodborne outbreaks have also been associated with asymptomatic food handlers who had significant contact with infected children (Table 2).

Sexual transmission has been reported on several occasions in men who have sex with other men. It is also worth noting that transmission of *Entamoeba histolytica* has been identified in women having sex with other women, and because the transmission patterns of *Giardia* and *E. histolytica* are similar, it is likely that sexual transmission in these settings results from ororectal or orogenital contact.

Children in day care centers are at an increased risk for acquisition of giardiasis. These infected children may be symptomatic or asymptomatic, and sometimes symptomatic infection results in family members of children who became infected in day care centers.

There are several factors that make the detection of environmental contamination with *Giardia* challenging. The cyst is the environmentally stable form of the life cycle, and as so, it is the transmissible stage. However, the cyst does not replicate in the environment, so culturing them is not an option. Attempts to identify *Giardia* cysts in the environment generally focus on detection in water. Methods that are currently used by the US Environmental Protection Agency (EPA) to detect *Giardia* cysts include filtration followed by cyst concentration. The cysts are then separated by an immunomagnetic method, which allows the examination of large volumes of water and separates the cysts from other artifacts that might be confused with *Giardia* cysts. The cysts are then evaluated microscopically using fluorescent staining and differential interference contrast (DIC). However, because cysts can remain morphologically normal even after they are no longer viable, additional testing is required to determine whether the cysts are viable. Viability is ultimately defined as the ability to establish infection in a host after ingestion. The best surrogate test is to place the cyst under conditions that promote excystation and determine their ability to develop into trophozoites. However, this approach is laborious and not widely applicable, so the usual determination of cyst viability is by the exclusion of vital dyes, such as fluorescein diacetate or propidium iodide. To further complicate the analysis, the cysts from multiple *Giardia* species all appear morphologically identical, but only Genotypes A and B of *G. lamblia* have the potential to cause human disease. Thus, it is difficult to know whether the identified cysts pose a risk of human infection or not. In addition, PCR-mediated approaches to identifying the cysts after immunomagnetic separation are coming into use. PCR-based methods have the potential of identifying cysts at the species and genotype level, which will be useful in determining whether the cysts pose a risk to humans.

Control and Prevention Measures

Symptomatic giardiasis should be treated. Asymptomatic giardiasis should generally be treated in areas with low prevalence or where there is significant hazard to others from the infected person (e.g., food handling). A number of effective agents are available for treatment (Table 3). The agent of first choice in most cases is metronidazole or one of the other nitroimidazoles, especially tinidazole. The key advantage of tinidazole is the high degree of efficacy of single dose treatment, whereas metronidazole must be given for 5–10 days to allow a similar degree of efficacy. Severe toxicity is rare with the nitroimidazoles, but nausea and vomiting occur frequently, as does a metallic taste. These drugs have a disulfiram-like effect, so the concurrent use of alcohol is contradicted. These drugs are mutagenic for bacteria, but are now very widely used with no evidence for carcinogenesis in humans, so the concern of cancer causation has generally been laid to rest. Likewise, early concerns about use in pregnancy have been ameliorated by the lack of demonstrated teratogenesis despite extensive use during the second and third trimesters for other purposes. However, there are inadequate data to allow the same level of comfort in using the nitroimidazoles during the first trimester. In fact, the only agent commonly

Table 3 Treatment of giardiasis

Drug	*Full dose (adults or children weighing more than 40 kg)*	*Pediatric dose (or adults weighing less than 40 kg)*	*Duration (days)*	*Comments*
Metronidazole	250 mg tid	5 mg kg^{-1} tid	5–7	Most commonly used treatment in the US
Tinidazole	2 g	50 mg kg^{-1}	Single dose	The only available single dose treatment
Albendazole	400 mg qd	Same as adult	5	Better tolerated than metronidazole, but may be less effective Contraindicated in pregnancy
Nitazoxanide	500 mg bid	100 mg bid (ages 1–3 years), 200 mg bid (ages 4–11 years)	3	
Quinacrine	100 mg tid	2 mg kg^{-1} tid	5	Not commercially available in the US, but can be obtained from a compounding pharmacy
Furazolidone	100 mg qid	2 mg kg^{-1} qi	7–10	Somewhat less effective than other agents, but commonly used in children because of the available liquid form
Paromomycin	8–10 mg kg^{-1} tid	8–10 mg kg^{-1} tid	5–10	Less effective, but commonly used in pregnancy because it is not absorbed

recommended during early pregnancy is the nonabsorbed aminoglycoside, paromomycin. However, paromomycin is less effective than nitroimidazoles, so the choice of treatment during early pregnancy should take these factors into consideration.

Albendazole is a tubulin inhibitor with broad spectrum antihelminthic activity and has been shown to be highly effective for treating giardiasis, although perhaps slightly less effective than the nitroimidazoles. The related drug, mebendazole, has also been studied, but is much less effective. Nitazoxanide has been developed more recently and is reasonably effective for giardiasis as well as other protozoan and bacterial pathogens, but is less effective than the nitroimidazoles. For many years, quinacrine was the treatment of choice, but in the years since metronidazole effectively replaced its use in the US, it is no longer generally available. It is still useful in combination with metronidazole for treatment of refractory giardiasis, and if necessary can be obtained from a compounding pharmacy. Furazolidone has commonly been recommended for children in the past because it is better tolerated than quinacrine, but is seldom used today.

Human giardiasis can be prevented to the extent that ingestion of cysts can be prevented. There are no available vaccines for preventing human infection and, although there is a vaccine used in animals, its efficacy is not well documented. Therefore, successful prevention results from interrupting the cycle of transmission from human (or perhaps infected animal) to the environment and back to another human. For these reasons, the single most important factor in effective control of giardiasis is the availability of pure water. Giardiasis became a nationally reportable infection in 2002 and is reported by state health departments to the CDC through the National Electronic Disease Surveillance System. This approach should facilitate efforts to maintain water purity by earlier and more comprehensive detection of outbreaks when they occur.

Municipal water supplies typically utilize a process that includes coagulation/flocculation, clarification, and filtration through sand or diatomaceous earth. These steps are highly effective at removing *Giardia* cysts. The final step of the purification process uses chlorination, which plays an important role in inactivation of bacterial and viral pathogens. However, *Giardia* cysts are relatively resistant to chlorination, so this step is less important for *Giardia*.

When people travel to developing areas or backpack within the US, additional water purification steps are necessary. Portable or household filters are available with pore sizes of less than 1 μm; these filters are highly effective in removing *Giardia* cysts. Because *Giardia* cysts are rapidly inactivated by heat, adequate purification can be provided boiling for 1 min or by treatment at 55 °C for 5 min. Halogenation with iodine or chlorine is also effective for inactivating cysts, but requires a longer duration of exposure than for bacterial pathogens.

Foodborne transmission occurs most frequently as a result of handling food by infected preparers. These food preparers may be symptomatic or asymptomatic, so exclusion of people with diarrhea from handling food can be helpful in preventing giardiasis and other foodborne illnesses, but certainly will not prevent all risks of contamination. Therefore, consistent attention to hand washing and the use of gloves or other mechanical barriers to direct hand to food contact are important. It is also possible for fresh produce to be directly contaminated by cysts, so washing these items in purified water may reduce the risk by washing away some of the cysts, but will not eliminate the cysts. It is important to note that washing with inadequately purified water can actually introduce contamination where none existed before.

Research Priorities

There are at least two major research questions that would inform attempts to prevent human giardiasis. The first is a better understanding of the sources of environmental contamination. If zoonotic transmission occurs frequently, then significant effort should be given to preventing water or direct human exposure to fecal material of these infected animals. However, if most human cases result from other infected humans, then the major effort should go into interrupting transmission that might occur from human contamination of water or food, or human infections that result from day care contact or sexual transmission. Another major priority is the development of improved methods of detecting significant environmental contamination. It is a challenge to identify small numbers of cysts, especially in nonaqueous sources, and if cysts are found, to determine whether they pose a risk of human infection.

See also: Disciplines Associated with Food Safety: Parasitology. Veterinary Drugs Residues: Antibacterials

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Cystoisospora belli (Syn. *Isospora belli*)

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Glossary

Anthroponosis An infectious disease in which its etiological agent is carried by humans and is transferred to other humans and animals.
Foodborne disease Any disease resulting from the consumption of contaminated food, pathogenic bacteria, fungus, viruses, or parasites that contaminate food, as well as chemical or natural toxins such as poisonous mushrooms.
Organism life cycle A period involving all different generations of a species succeeding each other through means of reproduction, whether through asexual reproduction or sexual reproduction; a period from one generation of organisms to the same identical point in the next.
Surveillance In public health and epidemiology, the discipline of continuously gathering, analyzing, and interpreting data about diseases, and disseminating conclusions of the analyses to relevant organizations, to intervene in their patterns in order to control and prevent them.
Taxonomy The science of classification, in microbiology the arrangement of microorganisms into a classification.

Introduction

Cystoisosporiasis (formerly isosporiasis) is a human intestinal disease caused by the parasite *Cystoisospora belli* (syn. *Isospora belli*). The coccidian parasite, *C. belli*, infects the epithelial cells of the small intestine, and is one the least common intestinal coccidia that can infect humans (e.g., *Toxoplasma*, *Cryptosporidium*, *Microsporidium*, and *Cyclospora*).

The Pathogen

Cystoisosporiasis is found worldwide, especially in tropical and subtropical areas. Infection often occurs in immunocompromised individuals, notably in patients with human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS), and outbreaks have been reported in institutionalized groups in the USA. The first documented case was in 1915. Until 2005 the etiological agent belonged to the genus *Isospora* (Table 2). In 2005 it was included in the genus *Cystoisospora*. In Table 1 other species belonging to this genus are shown. These genuses belong to different families. *Isospora* belongs to family Eimeriidae (Table 2) and *Cystoisospora* to Sarcocystidae. Both families belong to the suborder Eimeriorina (order Eucoccidiorida of the phylum Apicomplexa).

Table 1 Species included in the genus *Cystoisospora*, characterized and included in the NCBI Taxonomy Browser (2012)

Family Sarcocystidae
Cystoisospora belli
Cystoisospora cf. *ohioensis*
Cystoisospora felis
Cystoisospora ohioensis
Cystoisospora rivolta
Cystoisospora suis
Cystoisospora timoni
Cystoisospora sp. 1-MM
Cystoisospora sp. 2-MM

Source: NCBI Taxonomy Browser (2012) (http://www.ncbi.nlm.nih.gov/taxonomy).

Table 2 Species included in the genus *Isospora*, characterized and included in the NCBI Taxonomy Browser (2012)

Isospora gryphoni
Isospora hypoleucae
Isospora insularius
Isospora lesouefi
Isospora orlovi
Isospora peromysis
Isospora robini
Isospora sp. Harbin/01/08
Isospora sp. iSAT1
Isospora sp. iSAT2
Isospora sp. iSAT3
Isospora sp. iSAT4
Isospora sp. iSAT5
Isospora sp. iSAT6
Isospora sp. SG-2010a

Source: NCBI Taxonomy Browser (2012) (http://www.ncbi.nlm.nih.gov/taxonomy).

Humans are the only known hosts for *C. belli*, which has no known animal reservoir, however other species are present in animals such as *Cystoisospora suis* (pigs), *Cystoisospora rivolta* and *Cystoisospora felis* (cats), and *Cystoisospora canis* and *Cystoisospora ohioensis* (dogs) (Table 1). Owing to this animal-species specificity it has been suggested this infection cannot be zoonotic.

Cystoisospora belli infection usually causes a mild and protracted illness unless the patient is immunocompromised. Clinical presentation may mimic those of inflammatory bowel disease and irritable bowel syndrome.

Transmission

Exposure to contaminated food or water predisposes to this infection. Because an external stage in the environment is required for the oocysts to mature (Figure 1), direct person-to-person transmission is unlikely. Accordingly, cystoisosporiasis is more common in areas with poor sanitation. The disease is also more common in patients with AIDS.

At time of excretion from an infected patient, the immature oocyst contains usually one sporoblast (rarely two) (Figure 1, step A). In further maturation after excretion, the sporoblast divides in two (the oocyst now contains two sporoblasts); the sporoblasts secrete a cyst wall, thus becoming sporocysts; and the sporocysts divide twice to produce four sporozoites each (Figure 1, step B). Infection occurs by ingestion of sporocysts-containing oocysts: the sporocysts excyst in the small intestine and release their sporozoites, which invade the epithelial cells and initiate schizogony (Figure 1, step C). On rupture of the schizonts, the merozoites are released, invade new epithelial cells, and continue the cycle of asexual multiplication (Figure 1, step D). Trophozoites develop into schizonts which contain multiple merozoites. After a minimum of 1 week, the sexual stage begins with the development of male and female gametocytes (Figure 1, step E). Fertilization results in the development of oocysts that are excreted in the stool (Figure 1, step A).

Foodborne *C. belli* Infection

Cystoisospora belli can be transmitted through food and water. It is an uncommon infectious agent compared to other viruses, bacteria, and protozoa that cause waterborne and foodborne illness. The immature form of the parasite known as oocytes are ingested with water and food and then need to mature in the human gut before it becomes infective. After completing its life cycle in the human body, newly formed oocytes are then eliminated in the stool. Contaminated vegetables and raw food serves as a source for this parasite infection.

The parasite is transmitted via the feco–oral route. Oocytes that are passed out in the stool of infected humans or animals

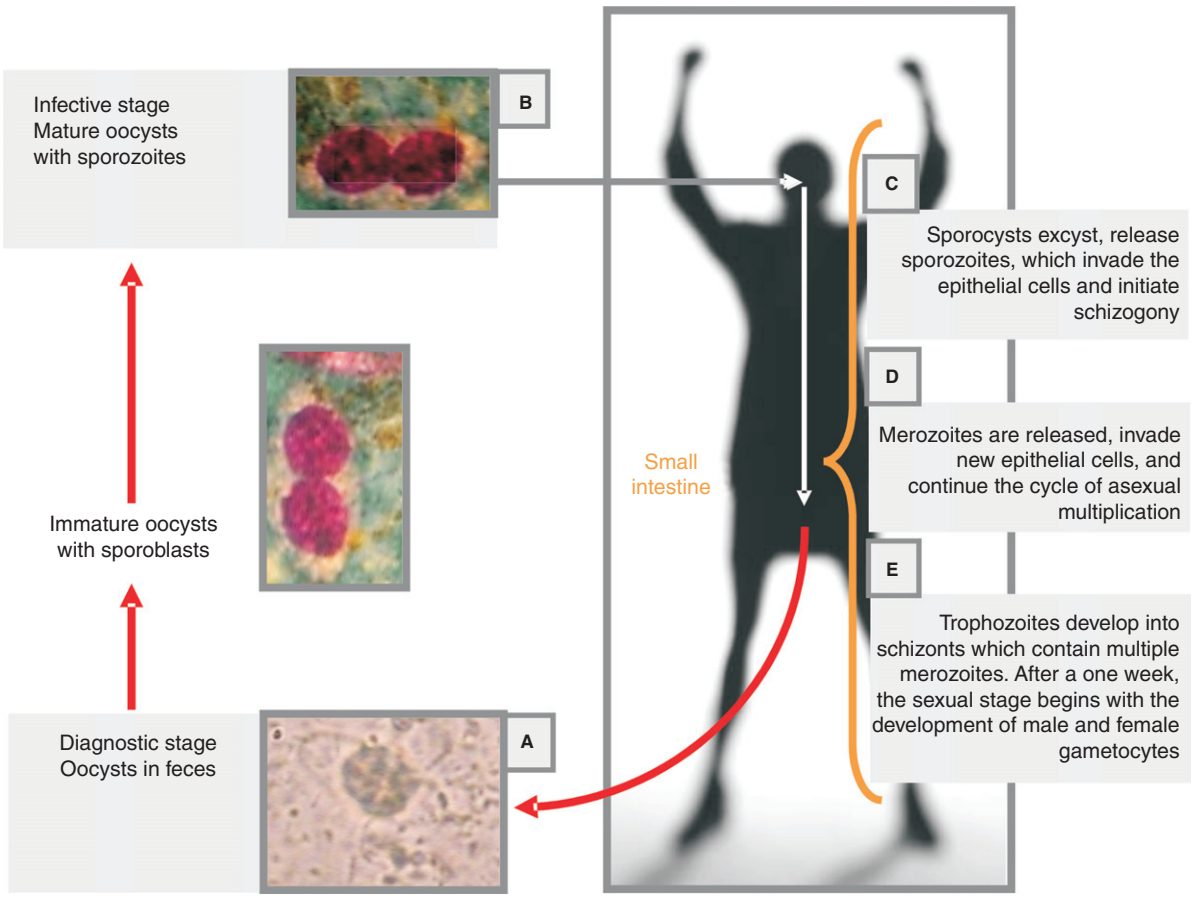

Figure 1 Transmission and life cycle of *Cystoisospora belli* in human beings.

may contaminate water that is then ingested. Outbreaks may occur when a common water supply and food is contaminated. However, these infections are uncommon in most developed nations. People with weakened immune systems, patients who are immunocompromised and those living in areas with poor sanitation are at the greatest risk of infection.

Particularly during past century, sporadic outbreaks were reported in mental institutions and in daycare centers in the US.

Epidemiology

Cystoisosporiasis has a worldwide distribution, although it is more common in tropical and subtropical climates. The exact incidence of cystoisosporiasis in humans is unknown. *Cystoisospora belli* has been reported as the cause of outbreaks of diarrheal illness in daycare centers and mental institutions and has been implicated in traveler's diarrhea in endemic areas, although is not clear how to define an specific endemic area for this parasite. Some data support the endemicity concept. A study of cystoisosporiasis among persons with AIDS in Los Angeles County, California, USA, found that this foodborne parasite was reported in 127 (1.0%) of 16 351 persons with AIDS during the study period. Prevalence of infection was highest among foreign-born patients (3.2%), especially those from El Salvador (7.4%) and Mexico (5.4%), and in all persons of Hispanic ethnicity (2.9%). It has been estimated that among patients with AIDS who are from South America, 10% with chronic diarrhea have cystoisosporiasis. In patients with AIDS who are from Haiti and Africa, 7–20% with chronic diarrhea have cystoisosporiasis.

This parasite has rarely been reported as a cause of travelers' diarrhea in immunocompetent patients. However, in a immunosupressed traveler, particularly with persistent diarrhea, this disease should be considered in the differential diagnosis.

The infection often occurs in immunodepressed individuals although some reports in immunocompetent and asymptomatic individuals have been published. Outbreaks have been reported in institutionalized groups in the USA. In spite of this, there are few studies designed to study the population prevalence of this parasite as well as the burden of this foodborne disease. Even more, this parasite and disease is not usually considered in the record or surveillance in most countries.

Clinical Presentation

The infection causes acute, nonbloody diarrhea with crampy abdominal pain, which can last for weeks and result in malabsorption and weight loss. In immunodepressed patients, and in infants and children, the diarrhea can be severe. Eosinophilia may be present (differently from other protozoan infections). Although not common, extraintestinal disease in HIV/AIDS patients has been described (compromising mediastinal and mesenteric lymph nodes, liver, spleen, and lamina propria of the intestinal mucosa).

Although isosporiasis is generally a self-limited infection, patients who are treated tend to improve in 2–3 days, whereas those who do not remain sick considerably longer. Immunocompetent hosts generally respond very rapidly to antiparasitic therapy. Immunocompromised hosts also respond well, though less rapidly; however, they have a high relapse rate once therapy is stopped and thus typically require indefinite prophylaxis after therapy.

Diagnosis

The diagnosis of cystoisosporiasis is based on a combination of clinical, epidemiological, and diagnostic tests. Cystoisosporiasis is an AIDS-defining illness, so an appropriate workup for HIV infection should be performed, if necessary, particularly CD4 T-cell counts.

Microscopic demonstration of the large, typically shaped oocysts (**Figure 1**), is the basis for diagnosis. Because the oocysts may be passed in small amounts and intermittently, repeated stool examinations and concentration procedures are recommended (three or more).

If stool examinations are negative, examination of duodenal specimens by biopsy or string test (Enterotest®) may be needed.

The oocysts can be visualized on wet mounts by microscopy with bright-field (**Figure 1**), differential interference contrast, and epifluorescence. They can also be stained by modified acid-fast stain (**Figure 1**).

Prevention and Control

Cystoisosporiasis is a foodborne and waterborne illness. Consequently, the practice of hygienic measures may help in preventing transmission. It is important to avoid possible exposure to contaminated food and water as far as possible. Appropriate isolation measures should be used because shedding of oocysts may last for weeks.

See also: Bacteria: *Aeromonas*. Food Safety Assurance Systems: Microbiological Testing, Sampling Plans, and Microbiological Criteria. Foodborne Diseases: Overview of Biological Hazards and Foodborne Diseases. Institutions Involved in Food Safety: Trust in Animals and Food Safety (TAFS) Forum; World Health Organization (WHO). Public Health Measures: Surveillance of Foodborne Diseases

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DPDx Laboratory Identification of Parasites of Public Health Concern.

http://www.fao.org/
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http://www.who.int/
WHO.

Sarcocystis

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Glossary

Coccidia A subclass of spore forming, single-celled, microscopic, obligate intracellular parasite.
Heteroxenous Requiring more than one host to complete a life cycle.
Oocyst Hardy, thick-walled spore resistant to environmental conditions and able to survive for prolonged periods of time outside the host.
Protozoa A diverse group of unicellular eukaryotic organisms.
Sporocysts Thin-walled cysts containing sporozoites. Two sporocysts are contained within a *Sarcocystis* oocyst.

Background

Miescher, for the first time, in 1843, reported *Sarcocystis* cysts in striated muscle of a house mouse. These cysts had the appearance of white threads and were thereafter referenced as Miescher's tubules. These tubules, when mature, contain a large number of cellular forms known as spores or Rainery's corpuscules. Similar observations were reported in muscle from a pig in 1865, which Kuhn called *Synchytrium miescherianum*. Finally in 1882, Lankester introduced the name *Sarcocystis* and renamed the organism as *Sarcocystis miescheriana*. The taxonomic position of *Sarcocystis* was uncertain, and in 1967 electron microscopy demonstrated that the sarcocysts had organelles similar to those in the Apicomplexa. In 1972, Fayer cultivated the spores in mammalian cells, thereby obtaining the sexual stages. Later, other investigators demonstrated that *Sarcocystis* was a heteroxenous parasite because sexual and asexual stages were produced in two different hosts. In 1976, *Sarcocystis hominis* was described by Dubey and in 1977 Heydorn described *Sarcocystis suishominis*. Future transmission studies using *Sarcocystis fusiformis* to infect various animals (potential definitive hosts) provided more information on the biology, role of intermediate and definitive hosts, and the naming of three new species, *Sarcocystis bovicanis, Sarcocystis bovifelis*, and *Sarcocystis bovihominis*.

In general, a host can be infected with pathogenic and nonpathogenic species of *Sarcocystis*. Among the four species found in cattle (*S. bovifelis, S. bovihominis, Sarcocystis cruzi*, and *Sarcocystis hirsuta*), *S. cruzi* is pathogenic to cattle. Of the *Sarcocystis* in sheep (*Sarcocystis arieticanis, Sarcocystis tenella, Sarcocystis gigantean*, and *Sarcocystis medusiformis*), *S. arieticanis* and *S. tenella* are pathogenic species to sheep, of the four species reported infecting pigs (*S. medusiformis, S. meischeriana, Sarcocystis porcifelis* and *S. suihominis*), only the *S. porcifelis* is confirmed to be pathogenic to pigs. There are five pathogenic species infecting horses, including *Sarcocystis asinus, Sarcocystis bertrami, Sarcocystis equicanis, Sarcocystis fayeri*, and *Sarcocystis neurona*. Condemnation of meats for human consumption occurs if *S. cruzi* is identified in beef meats and *S. hirsuta and S. meischeriana* infections can result in condemnation as it affects the meat quality (visible cysts easily identifiable).

The taxonomy of genus *Sarcocystis* is still not clear and its relationship with other closely related genera including *Besnoitia, Caryospora, Cystoisospora, Frenkelia, Isospora, Hammondia, Hyaloklossia, Lankesterella, Neospora*, and *Toxoplasma* is being examined by experts worldwide. Phylogenetic studies have revealed *Isospora* to be the closest genus to *Sarcocystis*.

Characteristics

Sarcocystis belongs to the phylum Apicomplexa, class Sporozoasida, subclass coccidiasina, order Eucoccidiorida, suborder Eimeriorina, family Sarcocystidae, and subfamily Sarcocystinae. Much confusion over the biology and variability of the tissue stages in the intermediary and definite hosts has made it difficult to describe and validate different *Sarcocystis* species. In 1986, Levine listed all the recognized names of 122 species, of which definitive and intermediary hosts are known to only 56 species. Intestinal sarcocystosis in humans is believed to be caused exclusively by *S. hominis* and *S. suishominis*.

The life cycle of *Sarcocystis* is similar to that of most of the other Apicomplexa. Two hosts are required by *Sarcocystis* to complete its life cycle: an intermediate and a definitive host (Figure 1). Sporulated oocysts (Figure 2(a)) containing two sporocysts each with four sporozoites are excreted in the feces of the definitive host.

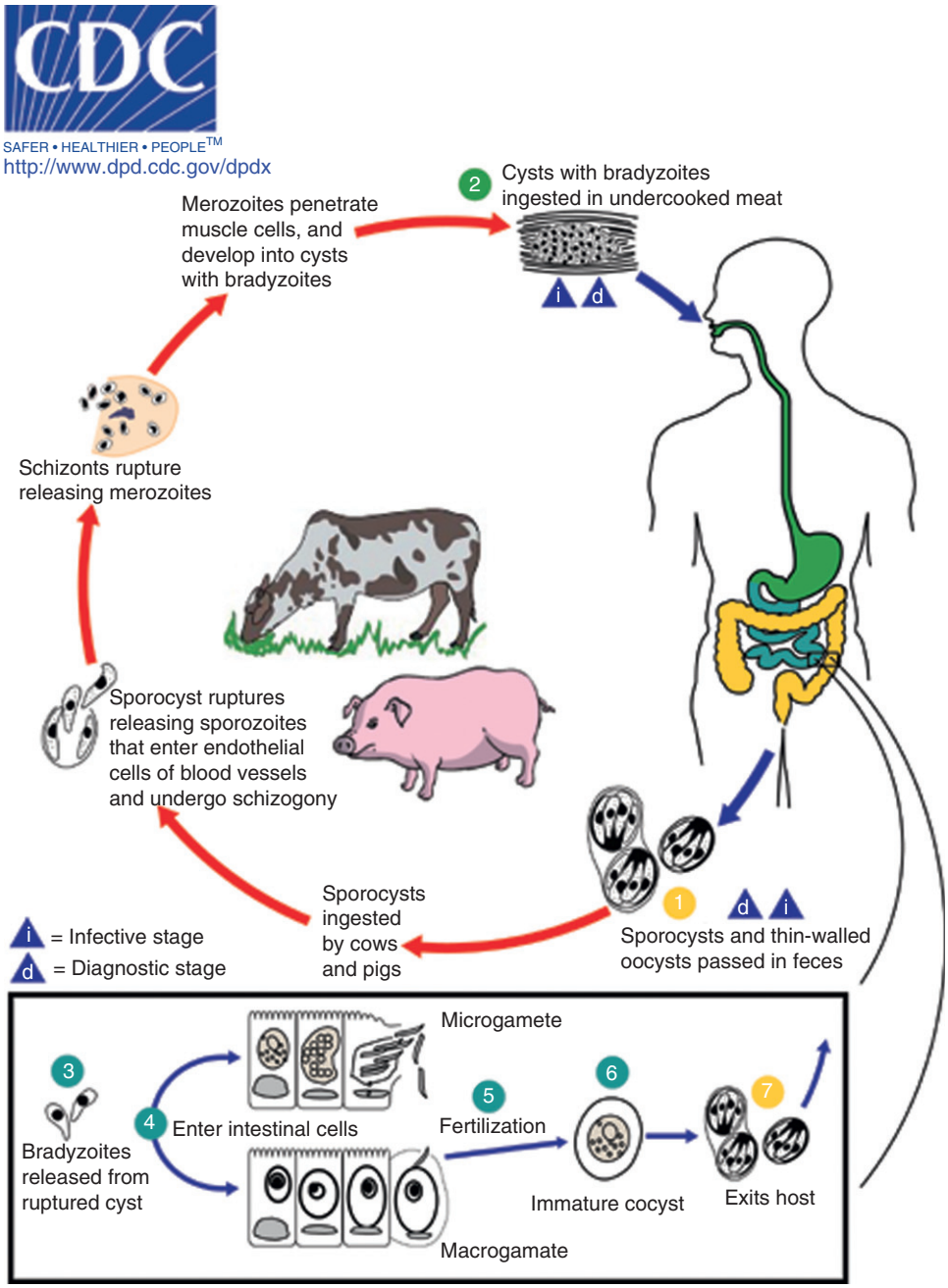

Figure 1 Life cycle of *Sarcocystis* parasite. http://www.dpd.cdc.gov/dpdx.

In the intermediate host (pigs, cattle, sheep, and other animals), the sporozoites are released in the intestinal lumen, migrate through the gut epithelium, and eventually invade small arteries throughout the body where the first generation of asexual stages is produced (merogony). Two subsequent generations of merozoites are reproduced asexually downstream in the direction of the blood flow to arterioles, capillaries, venules, and veins throughout the body. The fourth generation of merozoites (metrocytes) is formed in the muscles and initiate sarcocyst formation. As the sarcocyst matures, the metrocytes (small, rounded, and noninfectious) will begin to form the crescent-shaped bradyzoites. This process can take up to 2 months and the sarcocysts in the muscle can persist for several months. The definitive host acquires infection when the sarcocysts are ingested via contaminated undercooked meat.

The sarcocysts can be found in any striated muscle of the body and to a lesser extent, also in smooth muscle. Sarcocysts have also been reported in neural tissue such as the spinal cord, brain, and Purkinje fibers of the heart.

When the definitive host ingests meat containing mature sarcocysts (full of bradyzoites), the sarcocyst wall is ruptured or digested and the bradyzoites are released, which in turn infect the cells from the intestinal lamina propria. Each bradyzoite will then differentiate to form the macro- and

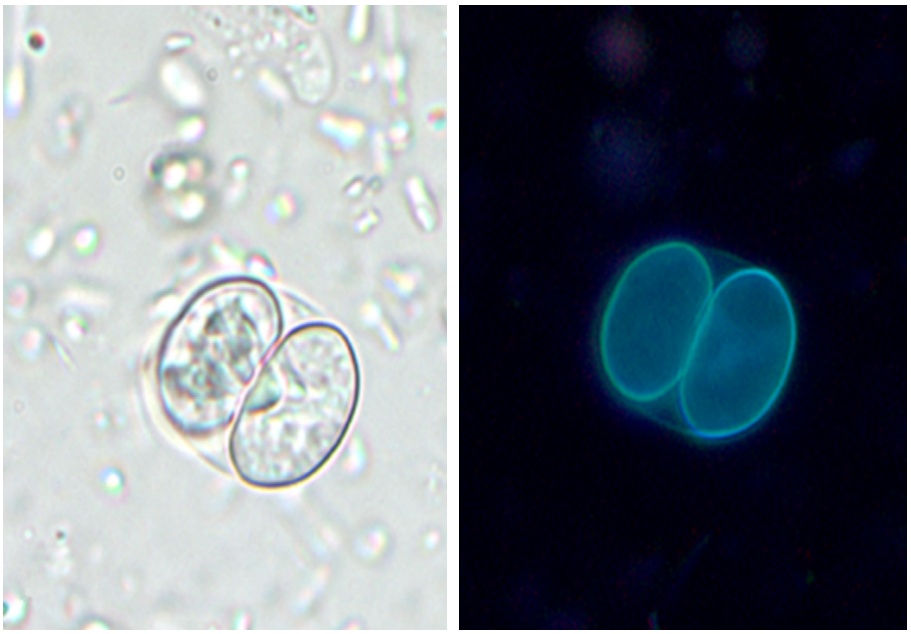

Figure 2 Sporulated *Sarcocystis* sp. oocysts: (a) bright field microscopy, (b) autofluorescence. http://www.dpd.cdc.gov/dpdx/HTML/ImageLibrary/Sarcocystosis_il.htm.

microgametocytes. After fertilization, sporogony occurs, producing a mature oocyst, each containing two sporocysts. The oocyst and sporocysts are then excreted in the feces; however, it is uncommon to find intact oocysts in the feces, but rather individual sporocysts.

Oocysts of *S. suishominis* measure 12.3–14.6 μm × 18.5–20 μm and the sporocysts contain four sporozoites and a residual body. Sporocysts of *S. hominis* and *S. suishominis* measure 9.3 × 14.7 μm and 10.5 × 13.5 μm, respectively.

Intermediate host specificity for *Sarcocystis* spp. is thought to be high in some species but not in others. In the case of *S. hominis*, cattle and water buffalo can acquire the infection, whereas *S. suis* can infect pigs. Humans can serve as intermediate hosts for several *Sarcocystis* spp. Humans and other nonhuman primates can serve as definitive hosts of *S. hominis* and *S. suishominis*. Dogs and coyotes serve as definitive host for *S. cruzi*. The use of molecular methods may help clarify and provide a more accurate speciation of sarcocysts in infected individuals. The presence of virulence factors in *Sarcocystis* has not been described and needs to be studied. This parasite can be found worldwide, particularly where livestock are bred.

Clinical Manifestation

In general, patients with intestinal sarcocystosis (anthroponotic) present with chills, mild fever, diarrhea, vomiting, and respiratory problems, whereas the zoonotic sarcocystosis disease leads to the formation of muscle cysts with symptoms of muscle weakness and transitory edema. In humans, infection with *Sarcocystis* is of short duration and often asymptomatic.

Studies with human volunteers have demonstrated that the first signs of infection are observed 3–6 h after ingestion of contaminated meats. Human volunteers in Germany had nausea, stomach ache, and diarrhea. These symptoms lasted for approximately 36 h. In China, volunteers ingested meats containing a large number of sarcocysts. They developed abdominal pain, distension, watery diarrhea, and eosinophilia between weeks 1–4. When humans act as intermediate hosts, most have no symptoms. Fewer have been diagnosed by the presence of intramuscular cysts. In other cases, patients can present with vasculitis and/or myositis. In China, a man developed these cysts in the chest intermittently for 16 months. Masses appeared with overlaying erythema and then subsided spontaneously after 2 weeks. In another report, an individual from India developed lumps and pain in his limbs.

Diagnosis

Sarcocystis oocysts can be identified in the feces using bright field microscopy, phase contrast microscopy, or differential interference contrast microscopy. The two sporocysts can be observed (Figure 2(a)); however, the oocyst wall is barely visible. Sporocysts can be easily observed using an epifluorescence microscope where sporocysts appear as two bright oval structures (Figure 2(b)). The thin oocyst wall usually breaks, releasing the sporocysts and diagnosis is based on identification of the sporocyst. Diagnosis can be facilitated by flotation methods using cesium chloride, zinc sulfate, or sucrose rather than sedimentation methods such as the formalin-ethyl acetate.

In humans, sarcocysts are usually found in skeletal and cardiac muscle and less frequently in the larynx, pharynx, and upper esophagus. Sarcocysts can be identified in hematoxilin–eosin stained tissue sections. When histological

Table 1 Identification of *Sarcocystis* species in various hosts and using various diagnostic methods

Sarcocystis *spp.*	*Country of origin*	*Detection method*	*Host (prevalence)*	*Reference*
S. buffalonis, S. levinei, S. dubeyi	Iran	Macroscopy, histology	Water buffalo (83% of 100)	Oryan *et al.* (2010)[a]
S. neurona	USA	Seroprevalence	Domestic cats (7% of 441)	Hsu *et al.* (2010)[b]
S. cruzi	Iran	Macroscopy	Cattle (89% of 100)	Nourani *et al.* (2010)[c]
S. neurona	USA	ITS1-PCR	Opossums (100% of 10)	Rejmanek *et al.* (2010)[d]
S. falcatula	USA	Immunohisto-chemistry, PCR	Bald eagles, golden eagles (100% of 4)	Wünschmann *et al.* (2010)[e]
Not determined	Japan	Ultrastructure	Raccoon dogs, red foxes, martens, badgers (100% of 18)	Kubo *et al.* (2009)[f]
S. camelicanis	Egypt	Electronmicroscopy	Camels (64% of 180)	Abdel-Ghaffar *et al.* (2009)[g]
S. hirsuta, S. cruzi, S. hominis	Vietnam	18 S PCR	Cattle, water buffalo	Jehle *et al.* (2009)[h]
S. falcatula	Brazil	Pathophysiology	Psittacine birds (100% of 32)	Godoy *et al.* (2009)[i]
S. ovicanis	Egypt	Macroscopy, ELISA	Sheep (95% of 120)	Abdel-Baki *et al.* (2009)[j]
S. murinus	Taiwan	Light microscopy	Farm rodents (93.7% of 98)	Tung *et al.* (2009)[k]
S. spp.	USA	18 S rRNA PCR	Hawks (66.8% of 238)	Yabsley *et al.* (2009)[l]
S. grueneri, S. rangi, S. hardangeri, S. rangiferi, S. tarandi, S. tarandivulpes	Norway	18 S rRNA PCR	Reindeer (100% of 18)	Dahlgren & Gjerde. (2007)[m]

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sections are stained using the periodic acid-Schiff reaction, the cyst wall can be observed; however, it can vary in morphology, overall size, and presence or absence of septa depending on the age of the cyst and type of infected tissue.

In recent years several immunologic and molecular biologic methods have been developed and are used in the detection and differentiation of the pathogenic and nonpathogenic *Sarcocystis* species. Immunologic methods have been found to be highly conserved and cross-reactive throughout the genus; therefore, these methods cannot be used for genetic differentiation at species level. However, nucleic acid-based molecular tools have been found to be more powerful and can differentiate *Sarcocystis* at an interspecies level. For this purpose, the small subunit (SSU) rRNA gene has been most extensively characterized. In general, SSU rRNA-based molecular methods have been effectively employed in species identification in animal hosts (Table 1). Nevertheless, these molecular tools have not been used to detect *Sarcocystis* species in humans.

Treatment

Currently there is no recommended prophylaxis or therapeutic treatment available for treating intestinal or tissue sarcocystosis disease in humans. Because infection is usually asymptomatic,

self-limiting, and of short duration, controlled studies to determine the efficacy of various drugs to control this infection have not been done. Cotrimoxazole or furazolidone are frequently used to treat coccidial infection, but have not been fully evaluated for treatment of human sarcocystosis. Whether immunosuppressive drugs may help control the vasculitis and myositis while at the same time allowing parasite proliferation has not been determined.

Epidemiology

The prevalence and incidence of the sarcocystosis disease caused by pathogenic *Sarcocystis* species are widespread, but vary among various infected hosts. In humans, prevalence of intestinal sarcocystosis disease is low and generally not associated with illness, except in human volunteer studies where large numbers of sarcocysts (>100) were ingested. The number of cases of *Sarcocystis* infection is probably underreported. Most cases have been reported by physicians, public health workers, and research scientists.

In humans, infection has been reported in individuals ranging in age from a 26-day-old infant to a 75-year-old man. Most cases of muscular sarcocystosis infection have been reported in tropical and subtropical regions, particularly Asia and Southeast Asia. Intestinal sarcocystosis in humans has been reported more frequently in humans in Europe. In a study in children from Germany, 10.4% and 7.3% were positive for intestinal sarcocystosis. In another study in Tibet, more than 42% of beef samples collected from a marketplace had sarcocysts and when fecal samples of locals were examined, 21.8% were positive to *S. hominis*.

A very high percentage (80% or more) of *Sarcocystis* infection has been reported in several domestic and companion animals including cattle, sheep, camels, pigs, goats, water buffalo, and yaks. Potential and unknown sources of sarcocystosis include camels, llamas, water buffalo, yaks, and pigs (other than domestic *Sus scrofa*). Meat from reptiles, birds, and wild mammals may also be a source of this infection in humans. Dogs and cats could also excrete sporocysts infectious to humans.

Sarcocystis can be acquired by ingestion of raw or undercooked meats containing sarcocysts. The infectious dose for *Sarcocystis* is unknown; however, in Chinese volunteers fed with less than 2000 sarcocysts developed symptoms within 1 week. In another study in Germany, 11 medical students and seven members of staff ate raw pork meat containing various concentrations of sarcocysts. The symptoms on day 2 were the same for all the participants, but the severity of the illness seemed to be related to the amount of contaminated meat ingested. Those that ingested a low number of sarcocysts were asymptomatic but clinical presentation varied according to the individual.

Prevention and Control

Intestinal sarcocystosis can be prevented by thoroughly cooking or freezing meat. Infected pork and beef products were noninfectious when cooked at high temperature (more than 60 °C for at least 20 min) or frozen for 48 h at -4 °C and 24 h at -20 °C before feeding to dogs. Under controlled experimental conditions, anticoccidial drugs such as amprolium and salinomycin have been effective in precluding acute illness and death in infected calves and lambs. Therefore, in order to prevent *Sarcocystis* infection in humans, animals for human consumption should not be exposed to contaminated water and feed containing *Sarcocystis*. If this is not feasible, meat of these animals should be thoroughly frozen for at least 2 days or fully cooked to kill pathogenic infectious parasites. Municipal water treatment procedures are generally effective at stopping *Sarcocystis* from contaminating tap water. However, if water control measures of municipal and well water are breeched, additional kill steps should be taken to prevent contamination. Boiling water can inactivate the oocysts that may be present in drinking water. These precautionary measures should control and prevent the *Sarcocystis* infection.

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See also: Foodborne Diseases: Prevalence of Foodborne Diseases in South East and Central Asia. Safety of Food and Beverages: Meat and Meat Products

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Relevant Website

http://www.dpd.cdc.gov/dpdx
Centers for Disease Control and Prevention (CDC).

PROTOZOA

Toxoplasma gondii

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Glossary

Bradyzoite The slowly dividing forms of *Toxoplasma gondii* that persist in cells within tissues of the body, including muscle; these forms are enclosed in a cyst wall and are resistant to degradation by digestive enzymes. This is the stage in meat that transmits the infection when meat is eaten undercooked or raw (Greek, *brady* = slow; *zoite* = small life).

Oocyst The stage of *T. gondii* in the feces of cats that is responsible for transmission of the parasite through contaminated soil, water, or food. This is an environmentally resistant stage that is surrounded by a resistant wall (Greek, *oo* = egg, and *cyst* = cyst).

Paratenic host These are hosts that serve to increase the transmission of a parasite to its final host. In the paratenic host, the parasite may undergo multiplication or development, but they are not required for transmission. (The feline final host can be infected either via the ingestion of infective oocysts directly or by the ingestion of hosts containing bradyzoites, i.e., paratenic hosts; the paratenic host is not required for the completion of the life cycle of this species.) Infections in paratenic hosts can be passed from host to host, such as mouse, to rat, to ultimately the final host, the cat.

Sabin-Feldman dye test (SFDT) A test based on the presence of certain antibodies that prevent methylene blue dye from entering the cytoplasm of *Toxoplasma* organisms; it is used for serologic diagnosis of toxoplasmosis.

Tachyzoite The stage of *T. gondii* that divides rapidly in many cell types during acute infections, before rupturing the cells and infecting additional cells (Greek, *tachy* = fast; *zoite* = small life).

General Information

Toxoplasma gondii is an obligate intracellular parasite of virtually any nucleated cell of warm-blooded vertebrates. The definitive host of *T. gondii* is the cat and related wild felids; these are the only hosts that shed stages into the environment to contaminate pastures, food items, and water. People get infected through the ingestion of oocysts. However, because the parasite can be in the tissues of warm-blooded vertebrates, including pigs, sheep, goats, chickens, etc., people can also be infected through the ingestion of undercooked or raw meat. Tragically, whatever the route of infection, the establishment of a primary infection in a pregnant woman can have marked effects on the developing fetus that may become infected *in utero*. Rare forms of transmission to people have also included tissue transplantation and blood transfusions. The ingestion of raw goat milk and human milk has been linked circumstantially to the transmission of infection.

In summary, infections in humans occur primarily in four different ways (Figure 1):

1. Accidental ingestion of the oocysts that have developed to the infective stage a day or so after being passed in the feces of cats (touching hands to mouth after gardening, cleaning a cat's litter box, or ingesting something contaminated with sporulated oocysts).
2. Ingestion of infective stages in animal tissue through the consumption of raw or undercooked meat, or raw milk, from infected animals.
3. Transmission from mother to fetus.
4. Transmission through organ transplants or blood transfusions.

History

In 1908, Nicolle and Manceaux in studies on leishmaniasis at the Pasteur Institute in Tunisia, found an organism in the tissues of a hamster-like rodent, the gundi, *Ctenodactylus gundi*. They named the new organism *Toxoplasma gondii*, based on the morphology (*toxo* = arc or bow, *plasma* = life or form, Greek) and the host in which it was found. At about the same time, Splendore discovered the same parasite in a laboratory rabbit in Brazil, and Darling observed the first human case in Panama. In 1923, the first case of congenital toxoplasmosis was recognized in the retina of a blind, hydrocephalic 3-month-old child in Prague. The role of this parasite as a human pathogen became more widely known in the 1930s and 1940s after it was reported as the cause of encephalitis in a child who died after birth, and descriptions of fatal cases in adults. The 'dye test' developed during this time by Sabin and Feldman for the detection of infection supplied much of our

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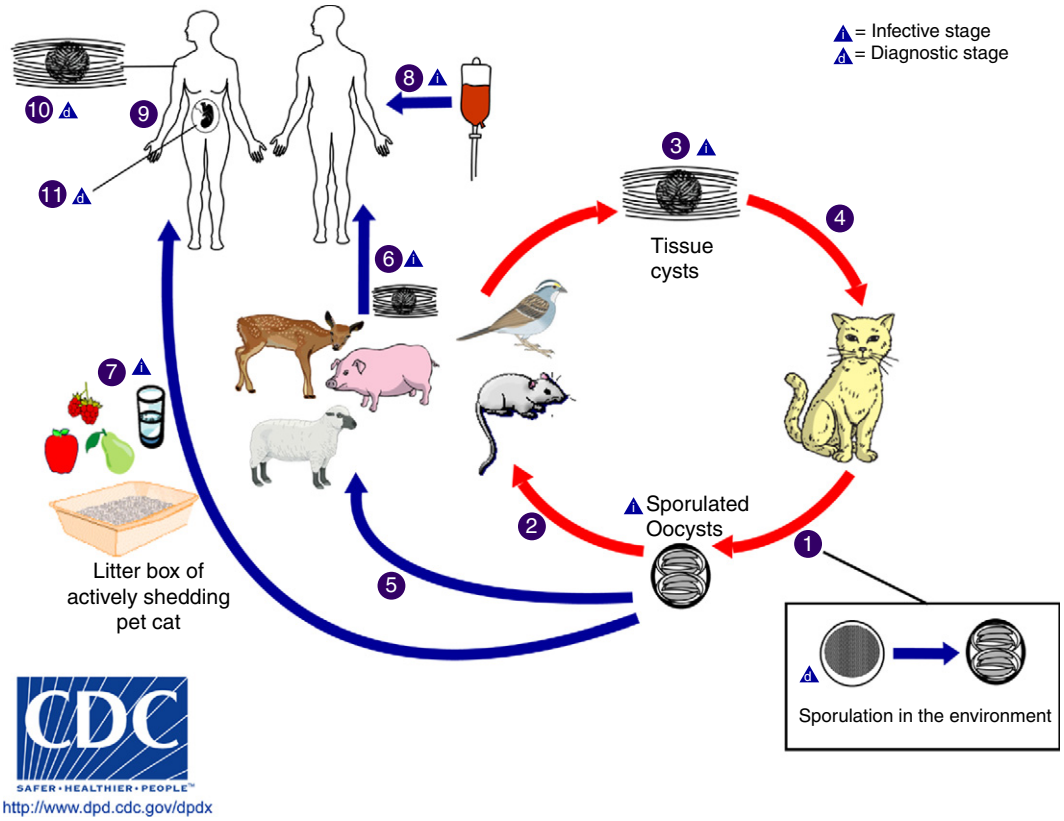

Figure 1 Life cycle and epidemiology of *Toxoplasma gondii*. (1) The only known definitive hosts are members of family Felidae (domestic cats and their relatives). Unsporulated oocysts are shed in the cat's feces. (2) Although oocysts are usually shed only for 1–2 weeks, large numbers may be shed. Oocysts take 1–5 days to sporulate in the environment and become infective. Intermediate hosts in nature (including birds and rodents) become infected after ingesting soil, water or plant material contaminated with oocysts. (3) Oocysts transform into tachyzoites shortly after ingestion. These tachyzoites localize in neural and muscle tissue and develop into tissue cyst bradyzoites. (4) Cats become infected after consuming intermediate hosts harboring tissue cysts. (5) Cats may also become infected directly by ingestion of sporulated oocysts. Animals bred for human consumption and wild game may also become infected with tissue cysts after ingestion of sporulated oocysts in the environment. Humans can become infected by any of several routes: (6) eating undercooked meat of animals harboring tissue cysts; (7) consuming food or water contaminated with cat feces or by contaminated environmental samples (such as fecal-contaminated soil or changing the litter box of a pet cat); (8) blood transfusion or organ transplantation; or (9) transplacentally from mother to fetus. (10) In the human host, the parasites form tissue cysts, most commonly in skeletal muscle, myocardium, brain, and eyes; these cysts may remain throughout the life of the host. Diagnosis is usually achieved by serology, although tissue cysts may be observed in stained biopsy specimens. (11) Diagnosis of congenital infections can be achieved by detecting *T. gondii* DNA in amniotic fluid using molecular methods such as PCR.

knowledge on the prevalence of *T. gondii* in people through the monitoring of serum for antibodies to *T. gondii*.

It was in 1960 that Leon Jacobs and others suggested that transmission of infection might occur through the ingestion of undercooked meat, and supported this idea by demonstrating the resistance of *T. gondii* from tissue cysts to proteolytic enzymes. Studies then identified various food-animals as being infected with this parasite. Then, a small outbreak of disease was described in a group of medical students at Cornell University in New York, NY, USA, who had eaten undercooked hamburger. The ingestion of meat and vertical transmission during pregnancy were the only means of transmission that were known for the next 10 years.

In 1969–1970, it was shown that a stage passed in the feces of cats was capable of transmitting infection with this agent. Dr JP Dubey who was involved with this work published a recent review of the history of these events. The cat was first implicated as a potential source of infection when it was shown that transmission involved the shedding of a stage called 'oocysts' in the feces of cats. Later work revealed that cats excrete oocysts into the environment following either the ingestion of oocysts or by the ingestion of bradyzoites in the tissues of prey animals such as mice and birds.

Life Cycle

Relative to food safety, three stages in the life cycle of *T. gondii* are of most importance: the oocyst, the bradyzoite, and the tachyzoite. The oocyst is the environmentally resistant stage shed in the feces of cats. The oocyst is not infectious when passed, but requires a day or longer in a soil or water environment where with warmth, moisture, and sufficient aeration, the contents of the oocyst sporulate to produce the

infectious sporulated oocyst. The sporulated oocysts contain sporozoites that will infect a host on the ingestion of an oocyst. The bradyzoite is the 'dormant' stage that is found in the tissues of warm-blooded vertebrates, and is the stage obtained by the consumption of contaminated meat. Ingestion of either sporulated oocysts or bradyzoites by a human, results in an in infection with the rapidly dividing tachyzoite stage. After a series of multiplications as tachyzoites in various tissues, the organisms will undergo a change to the bradyzoite form that will slowly divide within cells and form the cysts that can contain thousands of bradyzoites. It is the tachyzoite form that causes acute disease in people. This is the stage that rapidly multiplies in many cells of the host, crosses the placenta to infect prenatally, and is responsible for disease in acute infections, including people who are infected via transplantation and blood transfusion, and in cases of new infections and recrudescences in the immunocompromised.

These three stages vary in their resistance to environmental extremes. The oocysts in the environment are quite resistant to environmental and chemical destruction. Oocysts will survive dilute bleach or chlorine, but are readily destroyed by desiccation or high temperatures. The oocysts can persist in moist environments for months to years. The bradyzoites are resistant to pepsin digestion (compared with tachyzoites), which provides their ability to infect a host following ingestion and passage through the stomach. The tachyzoites have the function of undergoing rapid multiplication within cells for the purpose of increasing numbers and infecting more cells before being controlled by the host's immune system, thus they are the stage that is most sensitive to environmental conditions outside the body of a warm-blooded vertebrate.

Prevalence in People

A recent review of infections in people shows that, internationally, various countries have prevalence rates that can vary anywhere from less than 10% to more that 60% of the population (Figure 2). It can be assumed that the majority of asymptomatic infections are acquired from the ingestion of oocysts in water, food or soil, or tissue cysts in meat, because prenatal infections are often associated with sequelae that manifest as disease. In Turkey, seropositivity by the Sabin-Feldman dye test (SFDT) to toxoplasmosis was 42.5% in healthy blood donors.

Risk of congenital toxoplasmosis varies greatly with the area where the studies have been performed. In Brazil, some 9 (95% confidence interval 6–13) of 10 000 newborn infants were infected congenitally with *T. gondii*, and among 200 newborns who had been exposed to *T. gondii in utero*, the maternal to fetus transmission rate was found to be 18.5%. In the Netherlands, the rate of congenital infection was found to be approximately 20 newborns per 10 000 births. Other incidence studies have shown rates of congenital toxoplasmosis of 0.7/10 000 in Sweden, 0.8/10 000 in Massachusetts, USA,

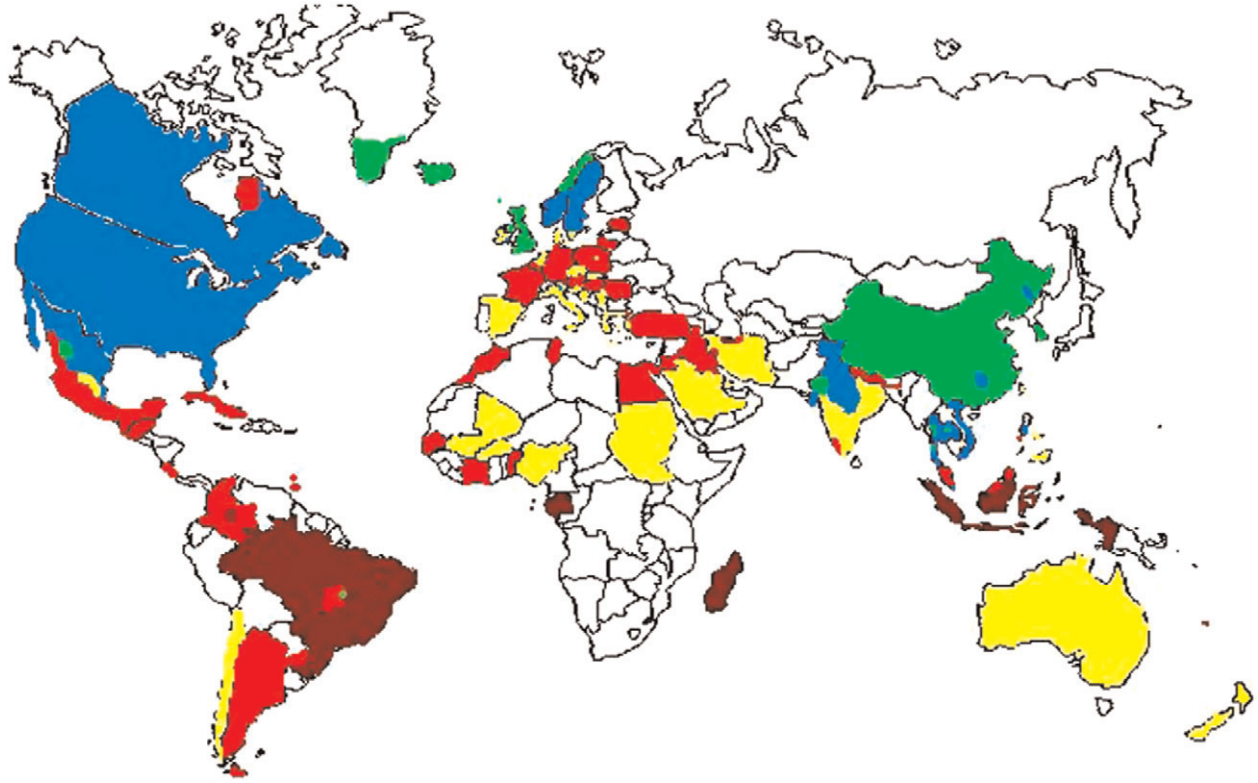

Figure 2 Global status of *Toxoplasma gondii* seroprevalence. Dark red equals prevalence above 60%, light red equals 40–60%, yellow 20–40%, blue 10–20%, and green equals prevalence <10%. White equals absence of data.

0.8/10 000 in London, England, 1.2/10 000 in Switzerland, 3/10 000 in Spain, 4/10 000 in Denmark, 11/10 000 in Poland, and 19 to 32/10 000 in Paris, France.

Foodborne Transmission

Dr Dubey (2010) recently published the second edition of his book *Toxoplasmosis of Animals and Humans* where he summarizes the literature and presents in-depth tables on the serology of food animals for the prevalence of infections from around the world. He has articles on sheep, goats, pigs, cattle, water buffalo, horses, camels, chickens, Australian marsupials, marine mammals, deer, and bears; other chapters cover humans, cats, dogs, wild canids, rodents, and various wild animals, including antelope and bats. For the sections that follow, this information is a targeted summary of Dr Dubey's in-depth articles that are fully referenced.

As described below, the animals that are important potential sources of infection through meat are sheep and lambs, goats, and camels. Pigs and chickens raised in commercial large-scale facilities are virtually *T. gondii*-free, but these animals are likely sources of oocysts if the animals are raised outdoors. Work in Europe has shown that the management of commercial hogs under organic conditions can produce *T. gondii*-free meat if careful attention is paid to rodent control and preventing the access of cats. Cattle and horses do not appear to be significant as food-animal sources of toxoplasmosis. People are also likely to be infected directly from soil or from the ingestion of unwashed fruits and vegetables contaminated with soil. Recently, it has also been shown that waterborne transmission may be more common than once believed.

Transmission via Meat

The prevalence of *T. gondii* in different hosts will vary depending on how they are raised; animals on pastures have easy access to oocysts as opposed to animals in confinement facilities with adequate biosecurity in place. Thus, sheep, goats, horses, and camels that tend to be pastured for the most part wherever they live in the world are likely to have infections from oocysts. Although, however, in much of the developed world, pigs and chickens are often raised indoors on large farms where they have only a slight chance of getting toxoplasmosis. However, if these same hosts are raised outside, as they are by smallholders in the developed world and throughout much of the developing world, they are very likely to acquire infections with *T. gondii*. It appears that cattle are not important in the epidemiology of toxoplasmosis even though they are raised on pasture.

1. Cattle: Although cattle have been diagnosed as serologically positive for *T. gondii*, there have been very few successful isolations of the organism from beef. Dr Dubey reports that although he isolated *T. gondii* from one seropositive cow, the muscle tissue was not infectious to cats (12 cats fed 6 kg of beef) nor to 150 infected mice. A homogenate of the intestinal mucosa was found to contain *T. gondii* that produced an infection in mice. In a survey of beef in the US, 2094 meat samples from commercial grocery stores were fed in pools (100 g from each of six beef samples) to 350 cats. None of the cats shed oocysts. Attempts to isolate *T. gondii* in Uruguay by feeding 20 g beef samples to cats failed to isolate any organisms. Polymerase chain reaction (PCR) performed on meat in Switzerland did detect *T. gondii* DNA in meat of 3% of adult cows, 6% of 50 heifers, 2% of 100 bulls, and 1% of 100 calves.
   Dr Dubey states in his text that beef does not appear to be important in the epidemiology of foodborne toxoplasmosis, but it cannot be ruled out. There are two reports from more than 30 years ago that link infection to the consumption of raw beef, in one case in five medical students mentioned above who bought meat from a butcher who claimed that pork contamination was highly unlikely, and three people who developed toxoplasmosis after eating raw beef in a Syrian restaurant in Pennsylvania.
2. Sheep: Sheep are known to be infected with *T. gondii* because it induces abortions in pregnant ewes, and organisms can be found in the placenta and fetuses. Sheep have high serologic titers to *T. gondii* throughout the world. *T. gondii* has been isolated from the tissues of sheep in Brazil, the US, France, England, Denmark, Egypt, and Iran. In Switzerland, *T. gondii* DNA was detected by PCR from 6% of 150 sheep.
3. Goats: Goats appear similar to sheep in having persistent infections with *T. gondii* and in having abortions associated with infections obtained during pregnancy. As with sheep, *T. gondii* has been isolated from tissues of goats. In Italy, attempts to isolate *T. gondii* from 10 seropositive goats failed. However, overall it is believed that goats harbor the organisms in a fashion similar to sheep.
4. Camels: Camels have been examined serologically in Egypt (two studies, 25.3 and 17.4%), Iran (4.2%), Saudi Arabia (two studies, 16 and 0%), and Sudan (67%). *Toxoplasma gondii* infections have been identified in camels in Egypt by feeding meat from the tissues of 38 asymptomatic camels to 4 cats. The oocysts recovered were verified to be *T. gondii* by bioassay in mice. The only report of disease in a camel is from Iowa, USA, where the animal became ill approximately a month after becoming anorectic and aborting a near-term fetus. This camel developed fluid in the thoracic cavity that contained numerous tachyzoites of *T. gondii*. The camel was treated with trimethoprim sulfadiazine but still succumbed. A necropsy was not performed.
5. Horses: Horses have been shown to be serologically positive for *T. gondii* around the world. A low seroprevalence (less or equal to 1%) of anti-*Toxoplasma* antibodies has been observed in horses in some European countries. Higher antibody prevalences have been observed in Turkey, and it has been suggested that the major risk for horses becoming infected is farming as opposed to animals held for racing.
   There have been no isolates of organisms made from horses. Two horses inoculated intravenously with tachyzoites of the RH strain of *T. gondii* in Brazil developed transient fever 4–8 days postinoculation, but had no

organisms detectable in the tissues at 3 months post-inoculation. There is a report of transplacental transmission of *T. gondii* in a pregnant horse fed oocysts. Thus, overall, it would appear that horses pose little threat of transmission of *T. gondii* through the consumption of their meat.

6. Pigs: Serologically, the prevalence in pigs around the world is quite high. This is due to pigs coming into contact with oocysts from cats as they root in soil, and probably through the ingestion of rodents. In pigs in poorly managed farms, the prevalence of infection will be higher than in well-managed farms. The recent trend to move pigs back outdoors will likely increase the rate of infection in pigs. In a study in the Netherlands, 0 of 621 conventionally raised indoor pigs, 8 of 660 organic pigs, and 30 of 635 free-range pigs were found to be serologically positive for *T. gondii*. Seventy-two percent of the organic farms did raise *T. gondii*-free pigs. However, a number of the organic farms, and the farms that had free-range pigs, kept cats for rodent control, and this probably provided a source of oocysts for the pigs.
   It has been possible to recover *T. gondii* from pigs on numerous occasions. Organisms have been recovered from pigs in Argentina, Brazil, Uruguay, Austria, Portugal, the Czech Republic, Yugoslavia, and the US. In a sampling of 2094 commercial meat samples from grocery stores in 28 metropolitan areas in the US that was fed in pools to cats as a bioassay, *T. gondii* was isolated from seven of the samples, i.e., from 0.3%. In a sampling of 2800 pig hearts in Canada, there were no *T. gondii* isolated, although 8.6% of these pigs were serologically positive for *T. gondii*.
7. Chickens: Chickens that are raised in backyard flocks have an excellent chance of being serologically positive for *T. gondii* and having organisms in their tissues. In samplings of a number of free-range chickens from around the world, *T. gondii* was isolated from up to 16% of the birds. When chickens were kept indoors, the numbers were markedly less, and a number of samplings had no organisms being isolated. There was no positive breast meat in the 2094 meat samples from grocery stores in the US where the samples were pooled and fed to cats as part of the bioassay. Overall, commercial chicken meat in the US and elsewhere where the chickens are raised in confinement appears basically free of *T. gondii*, whereas outdoor chickens have a very high chance of containing organisms in their tissues. The prevalence of *T. gondii* in chicken eggs is extremely low, and the consumption of raw eggs is not considered an important risk for toxoplasmosis.
8. Wild animals: *Toxoplasma gondii* organisms have been found in muscles of naturally infected deer, bear, moose, and pronghorn antelope, and can encyst in elk. Thus, wild animal meat can serve as a source of infection for hunters and their families, especially when care is not taken while eviscerating and handling, or when their meat is served undercooked. More importantly, viscera and meat scraps left at the site of the kill could serve to infect cats and further spread *T. gondii* in the environment.
9. Marine mammals: The finding of *T. gondii* in marine mammals deserves mention. The marine environment is contaminated with *T. gondii* oocysts, and fish-eating marine mammals are found infected with *T. gondii*. The prevalence of *T. gondii* infection in susceptible marine mammals can be quite high, with prevalences of 36% in sea otters, 42% in sea lions, 16% in ringed seals, 50% in bearded seals, 11.1% in spotted seals, 98% in Atlantic bottlenose dolphins, and 6% of 53 walruses using an IFAT.
10. Australian marsupials: Australian marsupials are highly susceptible to infection with *T. gondii* and many have died in zoos around the world from acute disease due to oocyst ingestion. Kangaroos have died following attempted vaccination with the attenuated vaccine that is used to prevent the disease in sheep in Europe. Organisms have been isolated from harvested kangaroos in Tasmania. Also, kangaroos, wallabies, bandicoots, and wombats have been found to be serologically positive for the infection, suggesting chronic infections with bradyzoites.

Transmission via Milk

Milk was implicated as a source of infection of *T. gondii* in several reports. *Toxoplasma gondii* was detected in the milk of goats that were experimentally inoculated during lactation. *Toxoplasma gondii* tachyzoites were found in the milk of ewes and cows, and an infection has been described in a breast-fed child whose mother had recently acquired the infection. Also, camels experimentally infected with *T. gondii* oocysts can excrete the tachyzoites in their milk, and *T. gondii* cysts were also detected in the brains of milk inoculated mice and suckling camel calves. The presence in milk, and the ability of some tachyzoites to survive for up to 2 hours in pepsin digest solutions may be of public health significance for nomads and others who choose to consume raw milk. However, other agents in raw milk can also be harmful, so there are many good reasons for pasteurizing or boiling milk before it is consumed.

Transmission via Fruits and Vegetables

Cats, both domestic and wild, are the sole source of environmental contamination. Many farms own cats for rodent control and many people have cats as pets. From six pig farms in the USA, cat feces, feed, and water samples have been found to contain the oocysts of *T. gondii*. Calculations made for the state of California, USA, has suggested that the annual burden of oocysts produce by cats in that state is 94 to 4671 oocysts per square meter.

The high prevalence of infection, based on serology, in groups of vegetarians, reaching almost 50%, provides evidence that oocysts represent an important source of infection in humans. Infection may occur by working in soil containing oocysts, as when gardening, or eating unwashed fruits or vegetables (shown to be a risk factor in a study with pregnant women). Also, in the US, a large serological survey of people by the Centers for Disease Control and Prevention in Atlanta, GA, for *T. gondii* and the soil transmitted nematode *Toxocara canis*, found the odds ratio equal to approximately 2.0 (meaning that one was twice as likely to be infected with the

second agent if they already had the other), which is an indication that *Toxoplasma* may be acquired in the same manner as *T. canis* which is considered unlikely to be transmitted in meat.

Waterborne Transmission

The first verified waterborne outbreak occurred in 1979 amongst US Army soldiers during training exercises in Panama, who drank unfiltered water from streams contaminated with oocysts, which were likely from jungle cats. In 1995, a large outbreak occurred associated with a single water reservoir in Victoria, British Columbia, Canada. There were some 100 people known to be affected, of which 19 had retinitis and 51 had lymphadenopathy. Based on routine serological screening done on pregnant women in the area served by the reservoir, and controls from other regions, it was estimated that some 2900 to 7700 people had been infected during the outbreak. In another large outbreak in Brazil, 155 people are believed to have been infected drinking unfiltered water from an underground storage tank. The water was contaminated by oocysts washing into the tank, and the water was shown to contain oocysts by both PCR and bioassay. Other places where waterborne toxoplasmosis has been suggested or implicated include Columbia, India, Poland, Turkey, Cuba, Granada, Taiwan, and China. There is some indication that endemic toxoplasmosis, as opposed to outbreaks, may be due to the drinking of unfiltered municipal water or well water. There is also some suggestion that recreational waters may also be a source as they are for *Giardia* and *Cryptosporidium*. It may be that the large number of cases of toxoplasmosis in developed countries that cannot be explained by foodborne or soil transmitted infections may be due to waterborne oocysts.

Food-Associated Risks for People

There have been reports from around the world of people being infected by many different foods of animal origin, including: dried seal meat, seal liver, raw caribou meat, rare kangaroo meat, undercooked lamb satay, raw mutton, raw spleen and liver of domestic pigs and wild boars, and others. These studies indicate that transmission of the parasite to humans is influenced not only by the potential contamination of various food sources, but also by the individual behavior of consumers in different ethnic groups and geographical regions. General eating habits have a significant impact because, among the major food animals, viable *T. gondii* has been found in pork, mutton, chicken, and horse meat and the proportion of human beings with *T. gondii* infection from these meats is higher in populations that eat undercooked meat (European and Asian) than in populations that thoroughly cook meat before consumption (Oriental and African). Of course, the background prevalence in the animals being eaten will also play a role; if people eat rare pork in an area with a high prevalence of *T. gondii* in the pigs, then the chance of acquiring an infection will be greater than in another country where commercial pork is virtually *Toxoplasma* free. Also, it is possible for animals to be seropositive and not have cysts in their tissues. Thus, beef and buffalo meat rarely contains viable organisms, although these animals may have a very high level of seroprevalence in some areas. However, if animals such as pigs, sheep, and goats are seropositive, they are very likely to contain infectious tissue cysts. Of course, people who are strict vegans and infected with *T. gondii* have acquired their infections from oocysts, whether in soil due to poor hand hygiene, ingesting oocyst contaminated fruits and vegetables, or drinking contaminated water. Overall, gustatory habits and hygiene are more important than other socio-cultural factors for preventing foodborne toxoplasmosis.

Travelling from an area of low risk to a region of high risk is another factor that can increase one's chances of acquiring a primary infection with *T. gondii*. Usually people tend to change their habits when entering different environments, and thus, they may become temporarily at risk from sources of infection that are not important epidemiologically in their home situation. Likewise, changing eating habits and developing preferences for new food types may impact the potential sources of foodborne infections with *T. gondii*, for example, meat from camels and kangaroos, which are often served undercooked in restaurants in areas where they are found, is a potential new source of infection for non-indigenous visitors.

Detection in Animals, Meat and Environmental Samples, and Determination of Clonal Types

The *T. gondii* infective stages are present in low numbers in contaminated water and foods, so rapid and sensitive methods are necessary for their detection. Tissue culture and animal models available for *Toxoplasma* are time-consuming, expensive, and labor-intensive. Therefore, PCR amplification has become the preferred method. Most PCR assays used for *T. gondii* identification use primers targeting the *B1* gene. It is a 35-fold-repetitive gene that is highly specific and conserved among strains of *T. gondii*. Ultimately, it is hoped that the determination of clonal types may elucidate the ability to assess the potential virulence of a specific isolate and will lead to means of tracing infections back to the specific source.

Detection in Meat and Determination of Viability

There are several means by which *Toxoplasma* can be detected in meat. Some of these assays monitor the presence of antibodies in sera, body fluids, or tissue juices, some examine tissue for the presence of organismal DNA, and some assess the presence of infection by inoculation of tissue into other hosts. Some of these assays identify animals as having an infection (serology and PCR), whereas other assays assess the presence of viable organisms in the tissue (mouse inoculation and the feeding of tissue to cats); often detection by PCR is considered as indicative of an infection with viable organisms.

The most commonly used methods to assess prevalence have utilized the sera of animals to determine the presence of antibodies. In animals, the detection of chronic infections often relies on serological testing with the fairly species-independent modified agglutination test (MAT). The other assay

that is commonly used to determine the serologic status of infection has been the enzyme-linked immunosorbent assay (ELISA), where the typical antigen that is used is the crude soluble extract of tachyzoites. This assay has also been adapted to work with meat juice. The ELISA-type tests, as opposed to the MAT, require the availability of secondary antibodies that recognize individual species such as cow, sheep, pig, etc.

The development of the PCR has made it possible to detect DNA in the tissues of animals. This can be done before or after pepsin digestion of the meat. The material is then subjected to PCR with different primers. Unlike the MAT or ELISA, this methodology detects the presence of DNA from actual organisms. The typical primers that are used are the B1 primer, for which there are 35 copies in the genome, and a 529 base-pair fragment, for which there are 300 copies in the genome, the latter being 10 to 100 times more sensitive than the B1 primer. Although it is likely that the recovered DNA is from living organisms, it is possible that they are nonviable and that simply the DNA remains.

Viability is typically detected through the inoculation of mice with ground tissues or material concentrated by pepsin digestion of tissue, or feeding the suspect tissues to cats that are never known to have been exposed to *T. gondii*. Mice are inoculated either intraperitoneally or subcutaneously. After the mice are infected, they need to be monitored closely. If the mice die during the first 2 weeks of infection, tachyzoites can be found in peritoneal washes and in stained tissue press preparations. After 2 weeks, the organisms become scarcer, and over several weeks it may be somewhat difficult to tell if the mice are infected. If the mice live, organisms can be found in brain tissue following grinding and dispersion in saline or presses of small amounts of tissue between microscope slides. In some cases, the infection of mice can also be monitored using serology using either the MAT or an ELISA for mouse IgG. Cats can be used for bioassays by feeding them tissue from animals; cats can be fed up to 500 g of flesh, whereas a mouse inoculum is usually restricted to approximately 0.25 g. After infection, the feces of cats are monitored for the presence of oocysts by daily fecal examination for several weeks beginning several days after the cat is fed the meat.

Detection in Water or Food

The detection of *T. gondii* oocysts in water is difficult. The problem is that the numbers are small and, unlike the case for *Cryptosporidium* oocysts and *Giardia* cysts, there are no immunomagnetic beads to aid in the diagnosis. *T. gondii* was successfully isolated from a waterborne outbreak in Brazil from small reservoirs held on roofs. This was done by filtering the water through membrane filters and then feeding the filters to *T. gondii*-free pigs and chickens. The chickens and the pigs fed with the filters developed *T. gondii* infections. Chickens have been utilized to sample soil for the presence of oocysts by allowing them to feed from the ground in areas where the oocysts might be present; this has also allowed the sampling of different areas for the presence of different genotypes of the organism using oocyst-derived infections.

Molecular Characterization of Different *Toxoplasma gondii* Genotypes

In North America and Europe, the majority of the isolates of *T. gondii* obtained from humans and livestock appear to fall into three clonal lineages. The three *T. gondii* lineages or genetic types are called I, II, and III. In North America and Europe, type II is most commonly associated with human toxoplasmosis, both in congenital infections and in patients with AIDS. Several studies have indicated that the majority of isolates from agricultural animals are also type II, including pigs in the USA and sheep from Britain and France. Chickens in North America show a higher prevalence of type III strains than type II, consistent with an early survey that indicated both type II and type III strains being common in animals. The reasons for the apparent differences between animal (types II and III) and human (largely type II) infections are unclear. More recent surveys, using an expanded set of restriction fragment length polymorphism (RFLP) markers, have indicated that although the majority of isolates from sheep in North America are type II, a number of other distinct genotypes are also present. Also, i South America, there appears to be a greater diversity of genotypes than elsewhere. It has not yet been possible to directly identify specific types with specific signs or disease outcomes in humans or animals. Also, at this time, the molecular methods used in toxoplasmosis do not seem to generate the same type of molecular fingerprinting that allows trace-backs to occur when associated with discovering the source of outbreaks. However, it is likely that methods will soon become available for *T. gondii* that may help indicate from what specific source an individual has acquired his or her infection.

Risks from Food Animals

Foodborne toxoplasmosis in humans may result from exposure to different stages of *T. gondii*. Thus, besides infections obtained from oocysts, infection can also occur from the ingestion of tissue cysts or tachyzoites contained in the meat of food animals. It is likely that transmission of the parasite to humans is influenced not only by the potential contamination of various food sources, but also by the individual behavior of consumers in different ethnic groups and geographical regions.

Pigs

Infected pork is an important source of *T. gondii* infection for people in many countries. This parasite also causes mortality in pigs, especially neonatal pigs. Most pigs acquire *T. gondii* infection postnatally by ingestion of oocysts from a contaminated environment or ingestion of infected tissues of animals. Pigs infected experimentally by inoculation of tachyzoites or by the ingestion of oocysts or tissue cysts, undergo weight loss, anorexia, and fever, but generally recover after three weeks. *T. gondii* organisms can survive in living pigs for more than 1 year after experimental infection.

Sheep

Among livestock, toxoplasmosis causes its greatest losses in sheep. *Toxoplasma gondii* may cause embryonic death and

resorption, fetal death and mummification, abortion, stillbirth, and neonatal death in these animals. Antibodies to *T. gondii* have been found in sheep with high prevalence rates worldwide. The prevalence of antibodies in ewes was more than twice that in lambs, and seroprevalence was shown to increase with age, reaching 95% in 6-year-old ewes in some flocks. A few studies analyzed risk factors associated with *T. gondii* seropositivity in sheep, and found the important factors to be the presence of cats on a farm, the use of surface water for drinking. Farm size and altitude were also factors associated with infection rates, with prevalences being higher at lower altitudes and on larger farms.

Goats

Infection with *T. gondii* in goats not only results in significant reproductive disorders, such as abortion or neonatal mortalities, but also has implications for public health because consumption of infected goat meat can facilitate zoonotic transmission. *Toxoplasma gondii* is pathogenic for goats, and the parasite has been detected from soft tissues such as brain, cardiac muscle, liver, lungs, lymph nodes, and diaphragm. The microscopical lesions consist of multifocal necrotic areas in most tissues. Symptoms in goats include acute enteritis, necrosis of mesenteric lymph nodes, encephalomyelitis, interstitial pneumonia, and necrotic placental lesions with groups of numerous *T. gondii* tachyzoites and sometimes tissue cysts.

Poultry

Although *T. gondii* cysts may be found in edible tissues of poultry products, they are probably not important in the transmission of toxoplasmosis to human beings because these products are usually frozen for storage and are thoroughly cooked to avoid diseases that could be caused by contamination with other organisms. A recent paper reviewed worldwide reports of clinical toxoplasmosis in experimentally and naturally infected chickens and concluded that clinical toxoplasmosis is rare in chickens. However, free-range chickens are considered important indicators of soil contamination with the environmentally-resistant oocysts, and so may serve as a source of *T. gondii* infection in cats, and possibly in humans.

Beef (Cattle and Buffaloes)

Currently, it is believed that beef (and probably buffalo meat) play a very small role in the epidemiology of toxoplasmosis. It appears that this niche in cattle is filled by *Neospora caninum* with its dog–bovine cycle. *Neospora caninum* is not zoonotic.

Horses Used for Food

Currently, horses, like cattle, are considered of little or no importance in the epidemiology of toxoplasmosis, even in countries where horse meat is eaten.

Prevention and Control

Animal Production Practices

Keeping cats out of the animal barns, and housing animals indoors, can reduce *T. gondii* infection. It is also important to keep rodents out of barns as they are likely a source of infection in pigs (via tissue cysts). Producers should also avoid garbage feeding. As a result of changes in animal husbandry, the prevalence of viable *T. gondii* in animals is reduced, and hopefully, this will also reduce the prevalence of *T. gondii* in humans.

Food Processing and Preparation Practices

Vegetables should be washed thoroughly before eating because of possible contamination with soil, and people should wash their hands thoroughly with soap and water after handling potentially contaminated soils or meat. Before consumption, meat should be cooked until a temperature of 66 °C (151°F) has been reached. Heating at 60 °C or 100 °C for 10 min, freezing at either − 10 °C for 3 days or − 20 °C for 2 days, or irradiation at doses of 75 or 100 krad kills tissue cysts. The cysts of bradyzoites can be killed by freezing at − 12 °C, but at 4 °C to 6 °C, the cysts can survive for up to 2 months.

Cats

Cats should be fed only dry, canned, or cooked food, and never be fed uncooked meat, viscera, or bones. Efforts should be made to keep pet cats indoors to prevent hunting. Also, spaying and neutering is important to try and reduce as much as possible the burgeoning feral cat populations around the world. Seronegative pregnant or immunocompromised individuals should avoid cleaning the cat's litter box, especially before the consumption or preparation of food. Also, because younger animals are more likely to be seronegative and shed oocysts if infected, these households should not consider the adoption of a kitten, whereas an older cat in the household has probably already been infected with *T. gondii* and is unlikely to shed oocysts.

Vaccination

The only vaccines available for *T. gondii* are to prevent abortion in sheep and neonatal mortality in lambs. This vaccine, Toxovax® (MSD Animal Health), is available in Europe and New Zealand. The vaccine contains attenuated tachyzoites that does not persist in tissues of sheep. Ewes vaccinated with the S48-strain vaccine can retain immunity for at least 18 months. Attempts to date to produce a commercial vaccine to stop oocyst shedding by cats have not been successful. At this time, it is unlikely that a vaccine will be developed to prevent infections in humans, but the development of recombinant vaccines that would prevent fetal damage during pregnancy are being considered. Other objectives of vaccination would be to prevent the development of bradyzoites in pigs or other animals. These vaccines remain fairly theoretical at this point in time, but they would be embraced by the industry if developed.

Serologic Screening During Pregnancy

Serologic screening in women during pregnancy is an important way to reduce the transmission of *T. gondii* to the fetus. This tends to be cost effective in countries with a fairly high prevalence, but becomes cost ineffective in areas where there are low prevalence rates. When women are found to seroconvert during pregnancy, they can be treated to protect the developing infant.

Consumer Education

Of major importance to the consumer is that the meat of some animals represents a potential source of infection if their meat is consumed raw or poorly cooked. In many parts of the world, people prefer raw or undercooked pork and mutton, and these are important sources of infection if the meat comes from producers that have not been carefully controlled for the presence of cats within the area where the animals are reared. Unfortunately, there is not yet a means of certifying the meat *Toxoplasma*-free, but there is a chance that in certain parts of the world this could become a reality using proper production methods coupled with diagnostics that could identify infected pigs at slaughter. It should also be remembered that when pregnant women travel from a country of low prevalence to a country of high prevalence, they may be increasing their risk of infection at this critical time.

Conclusion

Toxoplasma gondii remains a common infection in humans that can be acquired from undercooked meat containing bradyzoites, or oocyst-contaminated foods and water. Commercially grown chickens and pigs can be reared virtually *T. gondii* free, and with care, hogs defined as organic can also be reared virtually *T. gondii* free. Mutton and goat meat remains a major potential source of infection if eaten raw or undercooked. Backyard chicken flocks and small holder farms with sheep, goats, pigs, and cats on the premises are very likely to produce meat containing viable *T. gondii*. Beef is no longer considered important in the epidemiology of this disease. An increased risk of becoming infected with *T. gondii* is associated with ingestion of raw uncooked vegetables, uncooked lamb, goat, or pork, and various other meats such as camel, deer, rabbit, etc. Recently, it has also been found that contaminated water may be a more common source than considered previously. The diagnostic tests to detect infections in humans and animals continue to improve, and it is expected that at some point it might be possible to determine a specific source for an individual infection. Women who are pregnant and seronegative for antibodies to *T. gondii* should be cautioned by medical practitioners to remain cautious about participating in behaviors that could jeopardize the developing fetus through the acquisition of and the infection with this agent.

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HELMINTH-CESTODE

Contents

Echinococcus granulosus and *Echinococcus mutilocularis*

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Glossary

Human alveolar echinococcosis An infiltrative lesion of the liver with metastasizes to other organs in the late stage caused by the larval (metacestode) stage of *Echinococcus multilocularis*. It is often fatal if not treated.

Human cystic echinococcosis A disease resulting in a space occupying lesion in any organ, but most commonly in the liver or lungs. The lesion is cystic in nature and caused by the larval (metacestode) stage of *Echinococcus granulosus*.

Hydatid disease An alternative name given to cystic echinococcosis. Occasionally it is also used for alveolar echinococcosis.

Metacestode The larval form of a tapeworm or cestode found in an intermediate host. The metacestode causes disease as a space-occupying lesion resulting from the growth of the metacestode. In humans the metacestode of Echinococcus spp. cause echinococcosis.

Characteristics of *Echinococcus* and Life Cycle

Echinococcus spp. are cestode parasites belonging to the family Taeniidae. All taeniid parasites have complex life cycles that include a carnivorous definitive host and a mammalian intermediate host in which the larval or metacestode develops. It is the larval stage that is pathogenic to humans and other mammals. The adult stage is a small tapeworm 2–7 mm in length that inhabits the small intestine of carnivores such as dogs or foxes. *Echinococcus granulosus* generally infects dogs (Figure 1) whereas for *Echinococcus multilocularis* the usual definitive host are foxes, but the parasite will also infect dogs (Figure 2). Molecular evidence suggests that *E. granulosus* is really a complex of several species with different intermediate host preferences and variable pathogenicity to man (Table 1, Figure 1). *Echinococcus granulosus* sheep strain (*E. granulosus* sensu stricto) is the most important human pathogen. *Echinococcus* adults characteristically produce taeniid eggs, approximately 40 μm in diameter. These eggs are morphological indistinguishable from eggs produced by tapeworms of the genus *Taenia*. The eggs are passed in the feces of the definitive host and are infective to the intermediate host. The larval stage in intermediate hosts develops as metacestode usually in the visceral organs (see Figure 1). Humans are aberrant intermediate hosts and become infected by ingestion of *Echinococcus* spp. eggs. When humans are infected with the *E. granulosus* complex, cystic echinococcosis (CE) develops, which is a space occupying fluid filled cyst. Alveolar echinococcosis (AE) develops when humans are infected with *E. multilocularis* larvae. This results in a more infiltrative lesion, initially in the liver, which can metastasize in the late stage and is usually fatal if untreated. Echinococcosis can be directly transmitted through contact with infected dogs or foxes or indirectly through a variety of ways including contaminated food or water.

Human Echinococcosis

Human echinococcosis occurs following ingestion of eggs and the development of the metacestode in one or more organs. The liver is the most common site for CE followed by the lungs. Other organs are occasionally affected (Figures 3 and 4). The disease has been recorded in all age groups with the peak age at the time of diagnosis is 30–50 years. Symptomatology depends on: (1) the organ involved, (2) the size and location of the cyst within the invaded organ(s), (3) pressure induced within organ(s), and (4) complications such as rupture and spread of larval tissue with formation of secondary cysts and possible sepsis. Cyst rupture may also lead to anaphylaxis.

The primary lesion for AE is the liver. The slowly growing infiltrative lesion may be present for several years before clinical signs become apparent. These include abdominal pain, jaundice, sometimes fever, and anaemia. This advances

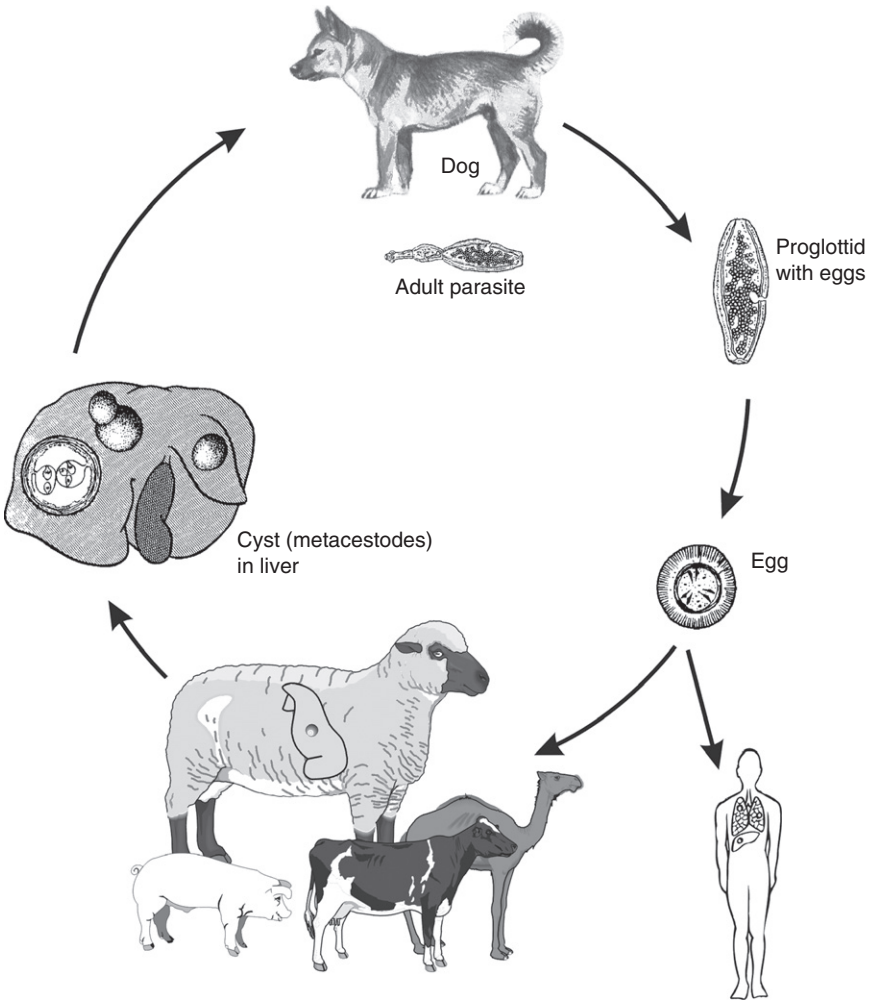

Figure 1 Life cycle of *E. granulosus* complex of genotypes frequently encountered in man. *E. granulosus* sensu stricto commonly utilizes sheep as the intermediate host. *E. ortleppi*, *E. intermedius*, and *E. canadensis* are often associated with cattle, pigs or camels, or wild ungulates respectively. © Institute of Parasitology, University of ZurichDogProglottidwith

to severe hepatic dysfunction, and is often associated with portal hypertension. The lesion will continue to grow and eventually metastasize to other organs and usually has a fatal outcome in the absence of treatment. In Europe, the peak age of diagnosis is approximately 50–55 years. In other endemic areas such as China this may be lower. However, the disease has been recorded in all age groups.

Diagnosis

Ultrasound examination (US), a widely used technique, can confirm the diagnosis of abdominal echinococcosis and indicate if lesions are active. Pulmonary echinococcosis cannot normally be detected by US. Classification systems based on the US appearance of the cyst have been developed for CE and AE, which may provide important information to the physician managing the case.

Nuclear magnetic resonance (Figure 3) or computer aided tomography (CAT-scan) (Figure 4) can be used to confirm the diagnosis, especially for lung and brain cysts, and to perform a pretherapeutic assessment of the lesions, for all locations. Unfortunately, in many remote endemic areas, such facilities are not available. In this case, serological back-up tests may be required to give additional information regarding the nature of the lesion detected by US.

A large number of serological assays have been developed for the diagnosis of human echinococcosis. These are generally more reliable for AE than for CE. Such tests can be used to give the clinician additional information when imaging techniques are inconclusive.

Treatment

There are numerous important reviews on the therapeutic management of CE. However, it still is a controversial subject. Consensus opinion was reached within the World Health Organization-Informal Working Group on Echinococcosis (WHO-IWGE) and the main conclusions published in 1996

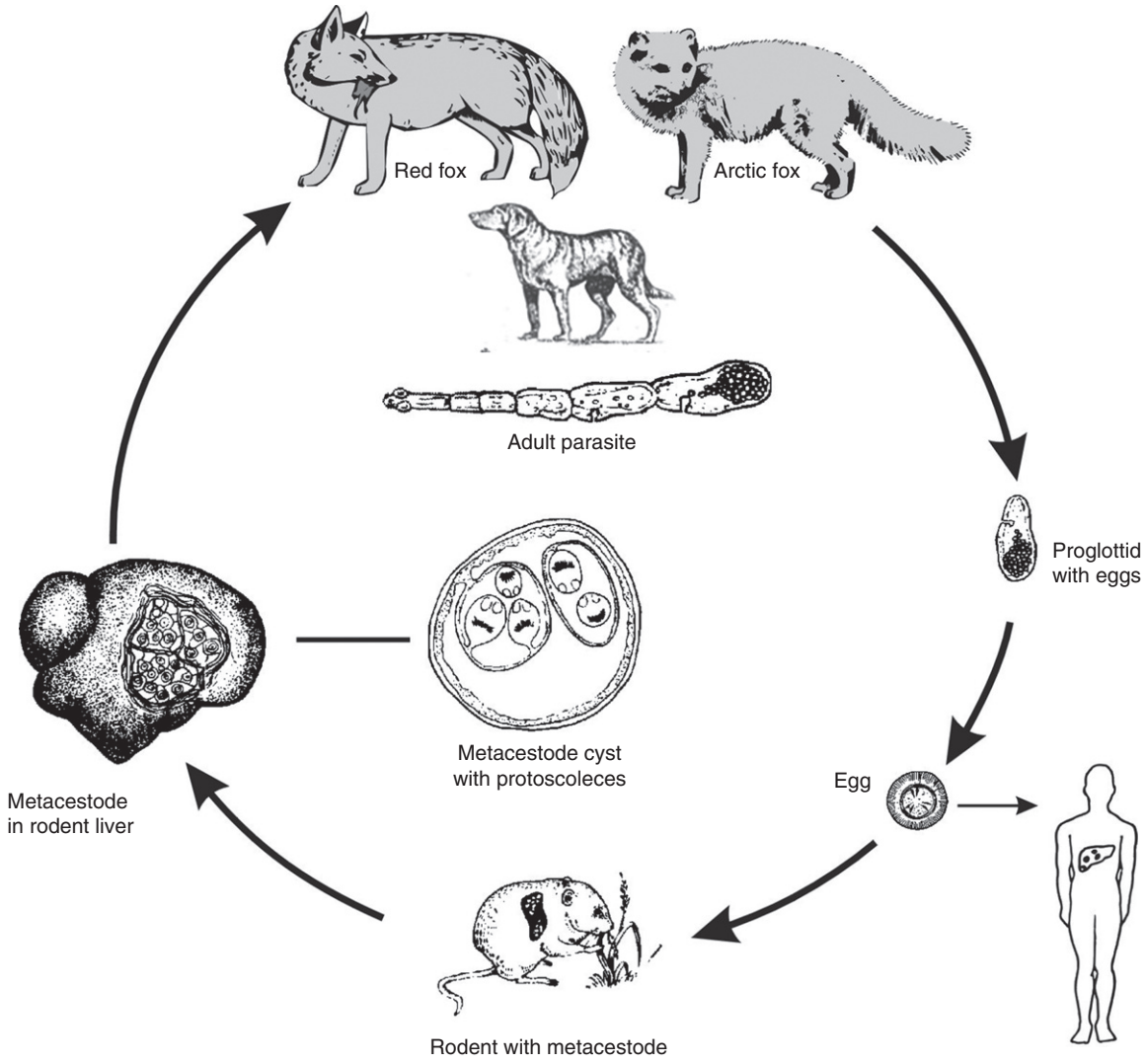

Figure 2 Life cycle of *E. multilocularis*. Man is an aberrant intermediate host and is infected directly or indirectly (for example via contaminated food) from fox or dog feces. © Institute für Parasitologie, Universität Zürich.

Table 1 Aetiology of cystic and alveolar echinococcosis in humans

Echinococcus *species*	*Name (abbreviation) and synonym of disease*	*Common intermediate hosts*	*Geographical distribution*
E. granulosus (sensu stricto)	Cystic echinococcosis (CE)	Sheep	Global
E. ortleppi[a]	Cystic echinococcosis	Cattle	Europe, South Africa, India, Russia, possibly South America
E. intermedius[a]	Cystic echinococcosis	Pigs, camels, possibly goats	Middle East, central Asia, China, India, Africa, eastern Europe, Argentina
E. canadensis[a]	Cystic echinococcosis	Wild ungulates such as deer	Northern Eurasia and North America
E. multilocularis (Eurasia, North America)	Alveolar echinococcosis (AE)	Small mammals	Northern Hemisphere

[a]Previously classified as *E. granulosus*. Further possible species exists – *E. equinus* and *E. felidis*. The former is not thought to be infectious to man whilst the latter's infectivity to man is unknown.

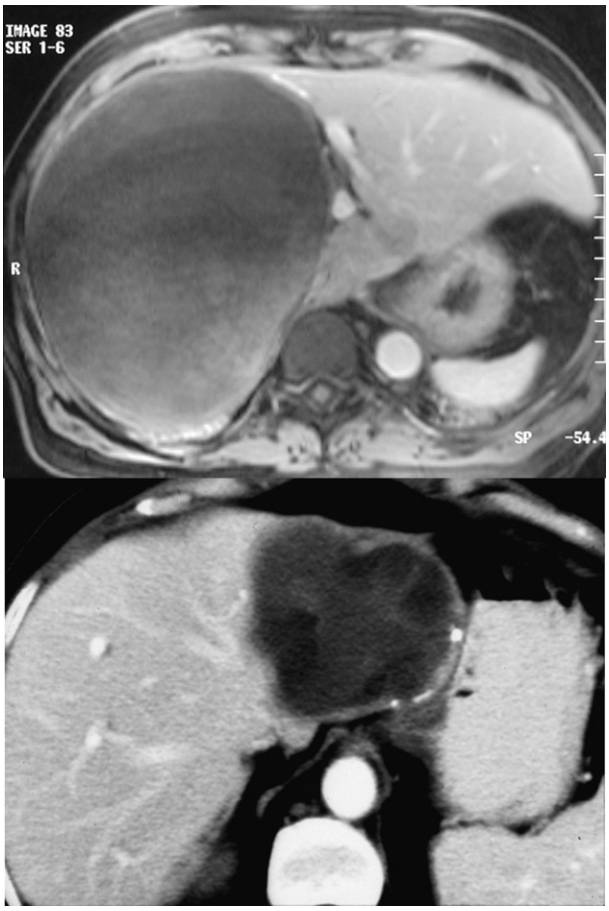

Figure 3 NMR images of cystic echinococcosis. Hydatid cysts can be clearly seen as space occupying lesions in the liver. Images with permission from Aliev MA, Baimakhanov BB, Samratov TU, and Toksanbaev DS (2004) Surgical treatment of cystic and alveolar echinococcosis of the liver. In: Torgerson PR and Shaikenov S (eds.) *Echinococcosis in Central Asia: Problems and Solutions*, pp. 214–218. Almaty, Kazakhstan: Publishing House Dauir.

with updated recommendations published in 2010. Surgery was the only option until the late 1970s. Complementary or alternative options are now available, which include nonsurgical interventional and chemotherapy with antiparasitic drugs. Treatment indication should be based on a multidisciplinary discussion and depends on cyst type, number and location, and presence or absence of cyst complication. A proper and long-term follow-up of the patients should assess the efficacy of the treatment, detect treatment complications, and timely disclose recurrences. Percutaneous puncture (PAIR) for inoperable cases is currently accepted as an alternative to surgery in selected cases. This includes puncture of the cyst, aspiration of the fluid content of the cyst, introduction of a protoscolicide, such as hypertonic saline or, preferably, alcohol, and reaspiration. It is carried out under ultrasonic guidance. Detailed practical guidelines have been published by the IWHO-IWGE.

With AE, patients should always be treated with benzimadazoles combined with surgical resection of the parasitic lesion. Based on the WHOPNM (P, parasitic mass in the liver; N, involvement of neighboring organs; M, metastasis)

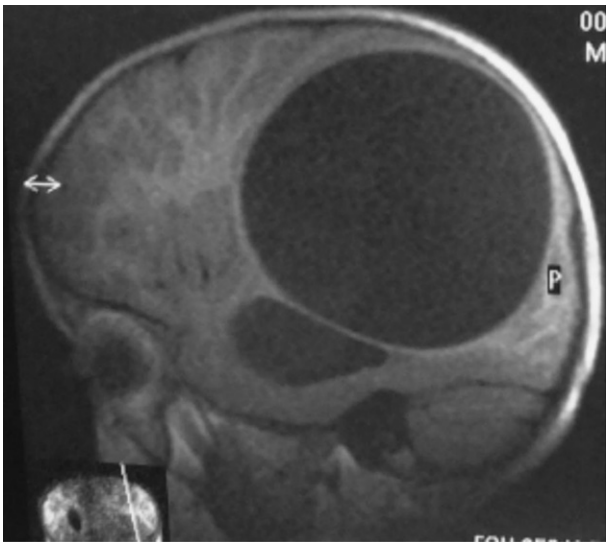

Figure 4 CAT scan of cerebral echinococcosis. Although CNS involvement of echinococcosis is rare, when it does occur it can be a devastating condition. Image supplied by the Government Pediatric Hospital, Bishkek, Kyrgyzstan.

classification of AE cases, an international group of experts has recently suggested various approaches of therapeutic procedures depending on the stage of the disease and on the available resources.

Epidemiology and Transmission to Man

The highest incidences of CE are seen where there is a close association with man and domestic livestock, often using working dogs. A common source of infection for dogs is offal from infected sheep. The resultant high infection levels in these dogs then pose a risk to humans. The cohabitation with dogs and feeding of uncooked viscera is a known risk factor for human CE.

The potential for domestic transmission of *E. granulosus* is highest in countries where the level of education may be poor, veterinary, and medical services are inadequate, and where home slaughter is commonly practiced. In such circumstances, prevalences in dogs can reach between 20% and 50% with perhaps an excess of 50% of the sheep population being infected. Occasionally other species, such as camels and pigs, may also be important intermediate hosts. Dogs themselves are more likely to become infected if they are young, allowed to roam, and fed on raw offal, offal in the community is not disposed of properly, the dogs do not receive anthelmintic treatment, or the dogs' owners are ignorant of the disease.

The distribution of CE is global (Figure 5), but different parts of the world have markedly different human incidences. In Europe, autothochonous CE is generally rare in central and northern Europe, although there are some foci in Eastern Europe, which are believed to be mainly through transmission of the pig strain. The most intensely endemic areas are Spain; where in some districts human incidence rates are 1.1–3.4 per 100 000 per year and Italy, particularly Sardinia where annual human incidence rates are 3.5 per 100 000. CE is also an

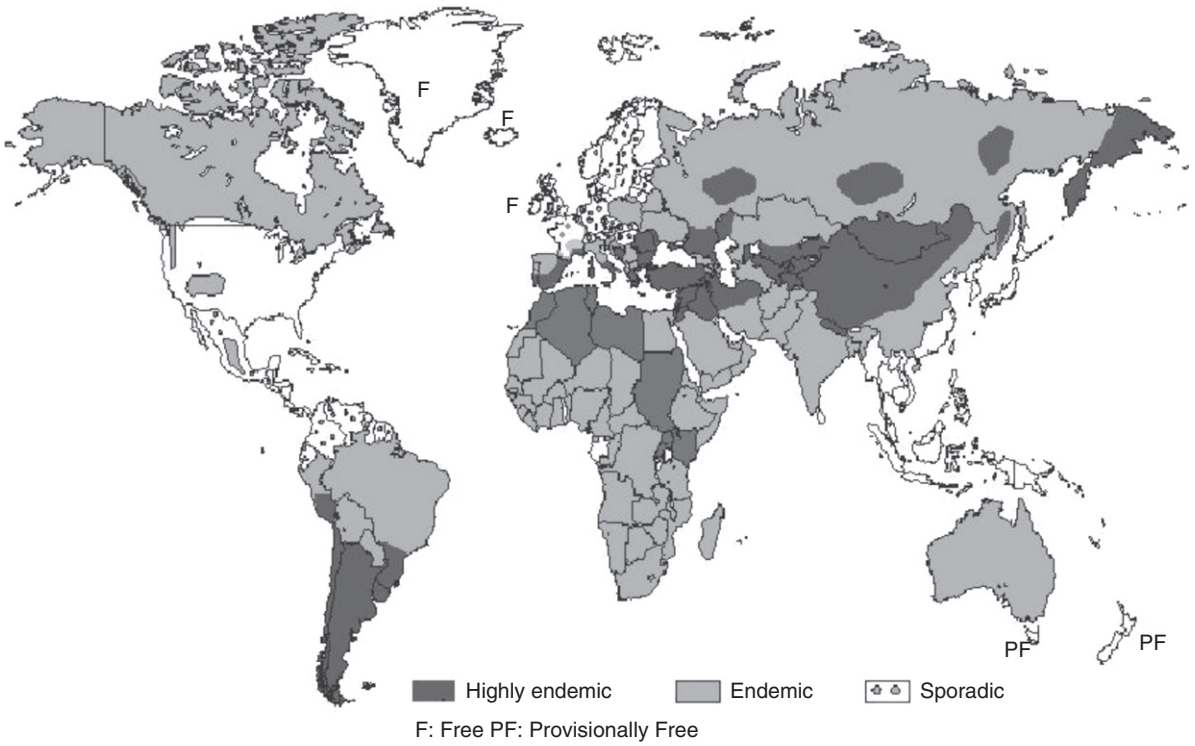

Figure 5 Approximate geographical distribution of the zoonotic strains of *E. granulosus*. Ireland is free of zoonotic *Echinococcus* spp., but is endemic for *E. equinus*. Reproduced from Eckert J, Conraths FJ, and Tackmann K (2000) Echinococcosis: an emerging or re-emerging zoonosis? *International Journal for Parasitology* 30: 1283–1294; Eckert J, Schantz PM, Gasser RB, *et al.* (2001) Geographic distribution and prevalence. In: *WHO/OIE Manual on Echinococcosis in Humans and Animals*, pp. 100–140. Geneva: WHO. © Institute für Parasitologie, Universität Zürich.

emerging problem in Greece, Bulgaria, and Romania where incidences of 3.3 per 100 000 per year have been recorded. There is also a small focus in Wales in the UK.

CE is a significant problem across much of the Middle East and North Africa. In Jordan the annual incidence is 2.9 per 100 000, in Tunisia 15 per 100 000, and in Turkey 0.67–6.6 per 100 000. In central Asia there has been a resurgence of CE following the collapse of the Soviet Union. This was due to poor funding of veterinary public health services, closure of centralized meat processing units and the privatization of large collectivized livestock enterprises. This enabled increased transmission of *E. granulosus* between dogs and livestock and from dogs to man. Consequently, surgical incidence rates are now commonly between 10 and 20 per 100 000 in much of central Asia. A similar pattern is also emerging in other former communist countries like Bulgaria.

Certain communities in Tibet have some of the highest incidences of CE. In some villages, US prevalences range between 5% and 10%. Similar disease burdens have also been recorded in transhumant pastoral communities in East Africa, such as the Turkana and Masai in Kenya and Tanzania, Toposa in Sudan, and the Dassanetch and Nyangatom in southern Ethiopia.

In Latin America, there are large endemic areas throughout the Andean regions of Peru, Argentina, Chile, Uruguay, southern Brazil, and sporadic cases being reported elsewhere such as Mexico.

In the US and Canada, CE tends to be sporadic and rare with cases occasionally being reported in certain groups of native Americans or particular ethnic groups.

In Australasia, CE was introduced with European colonisation and became a problem in large sheep rearing areas. Successful control programs have resulted in the elimination of the parasite from New Zealand and Tasmania. In continental Australia, the parasite has established a wild life cycle between macropod marsupials and dingoes. Therefore, prospects for elimination are now considered bleak. There are often descriptions of transmission within the domestic cycle, and human CE is frequently recorded.

Echinococcus multilocularis occurs in the northern hemisphere within a large belt stretching from the Arctic (80° N) southward to some regions approximately the 30° N latitude (**Figure 6**). The currently known endemic zone includes regions in Europe, Asia (extending eastward to Japan), and North America. More than 90% of human AE cases are in China where there is a heavily endemic region on the Tibetan plateau. In some communities 5% or more of the population may be infected. Elsewhere there are human cases reported from central and Eastern Europe, Turkey, much of Russia, central Asia, and Japan.

In western and central Europe, *E. multilocularis* is typically perpetuated in a wildlife cycle, involving red foxes (*Vulpes vulpes*) as definitive hosts and rodents (*Microtus arvalis, Arvicola terrestris, Myodes* [=*Clethrionomys*] *glareolus* and other species) as intermediate hosts. Prevalences of *E. multilocularis* in red foxes range from approximately 1% to more than 60% in various regions. In other regions such as China and central Asia, dogs are also infected as definitive hosts. This may increase the possibilities for transmission to man or contamination of food with parasite eggs.

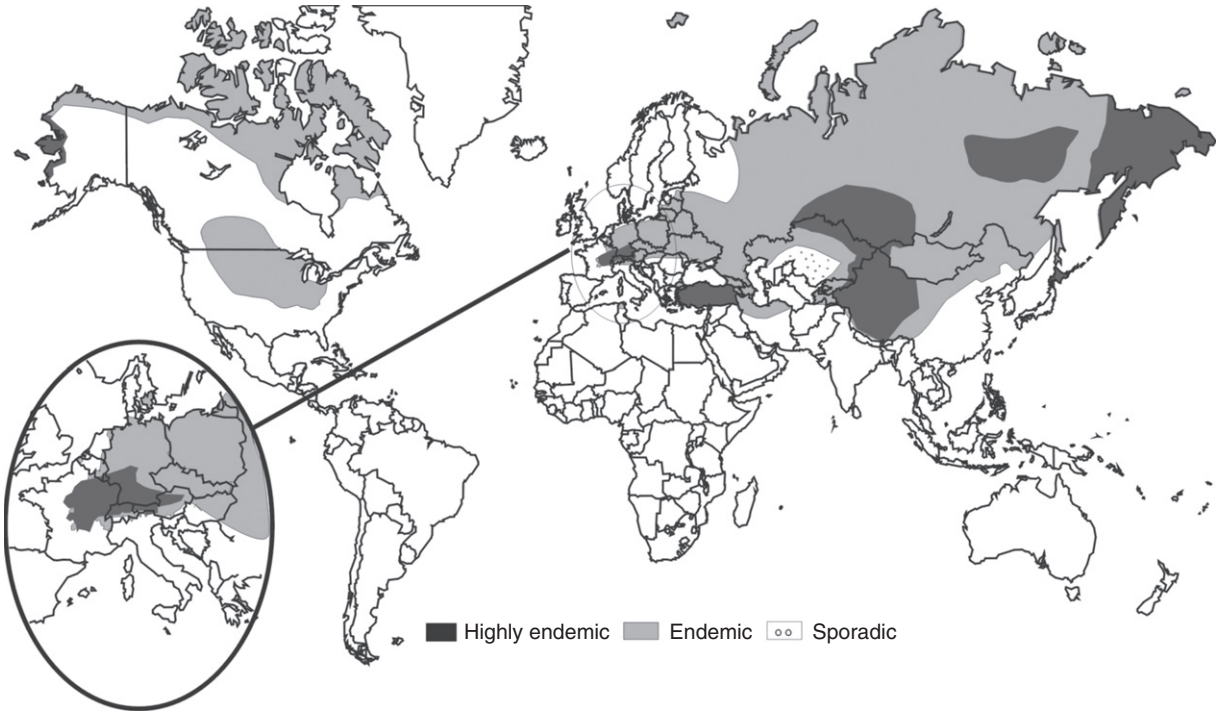

Figure 6 Global distribution of *E. multilocularis*. Image supplied by the Institute of Parasitology, Zurich.

Echinococcosis is not always associated with dog ownership or indeed contact with dogs or other carnivores. This may be because in some highly endemic areas dog ownership is universal and therefore cannot be used to discriminate between infected and noninfected subjects. Some groups such as Muslims regard dogs as unclean and therefore are unlikely to have close contact with them. Despite this CE infection amongst many of these people is evidence for indirect transmission of echinococcosis to man through contaminated food or water supplies. Epidemiological and experimental evidence has demonstrated that parasite eggs can be transmitted considerable distances by mechanical carriers such as insects. Thus, in highly endemic areas it is quite possible for individuals to contract CE even in the absence of dog contact.

Women are often reported to have a higher incidence of disease than men and this may be because they are more likely to tend to the household dogs and be involved with food preparation. In some populations men are found to have a higher incidence. This may reflect that in some societies men are more likely to be treated because they are more economically active, rather than an actual increased risk for men. Increasing age is also often reported as a risk factor with the peak age being 30–50 years. This is most likely due to continuous infection opportunities over time and the chronic and asymptomatic nature of abdominal CE.

Foodborne Transmission

Both cystic and alveolar echinococcosis can be transmitted to man through food. This is through contamination of food with parasite eggs and thus could occur where there is the possibility of contamination of food with dog or fox feces. Although not a consistent finding, there are studies that indicate an association of human infection with home grown vegetables, which presumably have been contaminated by dog feces. In addition, studies have shown an association with leaving food uncovered and thus available to flies. This is a potentially important means of human infection as flies have been shown to be capable of transmitting parasite eggs after they have fed on dog feces.

An unsafe water supply (such as from a stream) is also associated with infection with CE or AE in some studies and this may be due to water contamination with dog feces. This water can then contaminate food during food preparation.

With alveolar echinococcosis there is also a study linking the disease with eating wild fruit. This may be due to wild berries being contaminated with fox feces. However, a major issue that is difficult to resolve is source attribution for echinococcosis. This is because there is a long time lag, often of a number of years, between infection and the development of clinical signs, which makes it extremely difficult to identify the source of infection.

One recent study in Libya identified parasite eggs in vegetables and these could well include *Echinococcus* eggs. Tomato, cucumber, lettuce, and cress samples were examined for parasite eggs, and *Taenia*/*Echinococcus* eggs were found in 3%, 8%, 37%, and 33% of samples, respectively. This, therefore, provides strong evidence that inadequately washed and prepared salad vegetables are a potential source of infection.

An important point with regard to public health is animal offal that contains hydatid cysts (e.g., infected sheep liver). Such infected organs cannot directly infect humans even if consumed raw as the metacestode is not infectious to humans but must first infect a dog. Generally offal containing hydatid cysts would be condemned and removed from the food chain. This is for two reasons. First the organ would be regarded as

unwholesome and hence not suitable for human consumption. More importantly, the organ could be infectious to dogs and thus must be destroyed to prevent dogs becoming infected. A dog infected with *Echinococcus* is a substantial health hazard to its owner or indeed anyone coming in contact with this dog.

Economics and Societal Burden

CE presents a considerable societal and economic burden to a number of societies where *E. granulosus* is endemic. In terms of animal health the disease can lead to significant losses of production due to liver condemnations, lowered milk, meat and wool production, or decreased fertility in animals. This particularly impacts on societies with low socio-economic development, which have the highest prevalences in livestock.

A number of studies have estimated the economic costs of CE in various countries and it is often considerable. There is also a financial estimate of the global costs of CE, which suggests it could be as much as $3 billion annually if estimates for underreporting of the disease are accounted for.

A preliminary estimate of the global burden on echinococcosis has also been made, which indicates a loss of approximately one million DALYs. This is of a similar magnitude to the global burden of Trypanosomiosis or Schistosomiosis and considerably more than diseases such as Dengue or Leprosy. Similarly, the global burden of AE has been estimated to be approximately 600 000 DALYs with approximately 18 000 new cases annually.

Burden estimates present important methods in developing cost effective means of controlling this often neglected zoonosis. These estimates confirm that both CE and AE are major health problems in many parts of the world. However, what is less certain is the proportion of echinococcosis that is transmitted to man through the consumption of unsafe food that has been contaminated with *Echinococcus* eggs.

Prevention and Control

Control of CE has always involved a combination of routine anthelmintic treatment of dogs, control and reduction of stray dog populations, supervision of the slaughter of livestock and subsequent disposal of offal, and education of the public. A sheep vaccine has also recently been developed. This will prevent sheep becoming infected and hence break the cycle. Because this would then remove the source of infection for dogs, transmission to humans will be reduced or ceases. The various options for controlling CE are reviewed by Torgerson *et al*. in 2011. By implementing a carefully planned control strategy it is possible to eliminate *E. granulosus* and this has occurred in some Island nations such as New Zealand.

There is concern in Europe that fox populations are increasing and foxes are being found in greater numbers in the urban environment. This has led to an increase in the numbers of human AE cases in countries such as Switzerland. Control of AE is more problematic than CE as *E. multilocularis* has a wild life cycle. However, there has been some success by the distribution of praziquantel impregnated baits to foxes. In China, it is possible that dogs play an important role in the transmission of AE. Thus, periodic treatment of dogs with praziquantel may ameliorate transmission.

Conclusions

Echinococcosis can be a foodborne disease but the extent that it is foodborne is difficult to quantify. To prevent food becoming contaminated with *Echinococcus* eggs, dogs, and other carnivores must be totally excluded from any food preparation or storage areas. They should also be frequently treated with praziquantel. Dog feces should be properly disposed of and never used as a fertilizer for crops. Adequate cleaning and cooking of food should also minimize the risk of foodborne echinococcosis. Finally, food should not be accessible to flies as flies can carry *Echinococcus* eggs and thus act as a conduit of eggs from canine feces to food.

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Taenia saginata and *Taenia solium*

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Glossary

Cysticercus Tapeworm larva.
Cysticercosis Infection with tapeworm larvae.
Neurocysticercosis Cysticercosis located in the human brain.
Taeniasis Infection with the adult tapeworm.
Taeniid Adult tapeworm found in human intestine.

Introduction

Human taeniasis is caused by infection with the adult stage of the tapeworms *Taenia saginata* and *T. solium*, whereas human cysticercosis/neurocysticercosis results from infection with the larvae (cysticerci) of the latter species. Both of these parasites are zoonoses because the usual hosts for the cysticerci are cattle and swine respectively, from which humans become infected with the adult tapeworm. Food hygiene issues therefore arise if meat that is infected with viable cysticerci reaches the consumer or if food designed for human consumption is contaminated with eggs of *T. solium*. The worldwide prevalence of these parasites reflects inadequate socioeconomic conditions and inappropriate animal husbandry practices in the cattle and pig populations. A third, more recently identified taeniid, *T. asiatica* is also found in humans, cattle, and pigs but is restricted to Asia. As with the other two taeniids, humans are the definitive hosts, but in contrast both cattle and pigs can serve as intermediate hosts. Fortunately, humans do not act as intermediate hosts for *T. asiatica*. There is a continuing awareness in both the scientific community and the United Nations agencies of public health importance of human taeniasis and cysticercosis/neurocysticercosis, and of the economic importance of cysticercosis in cattle and pigs. The purpose of this article is to outline the history and biology of *T. saginata* and *T. solium*, their current medical/veterinary and economic importance, and to discuss in more detail the prospects for improving control by introducing new methods of treatment or animal management and by adopting a rational approach to the development of diagnostic assays and immunoprophylaxis. *Taenia asiatica* is considered for comparison, where appropriate.

History and Biology

Tapeworms were first documented as parasites of man *c.*1500 BC as the Ebers papyrus described worms that were probably species of *Taenia*, but it is likely that the existence of such large and obvious parasites was known well before that time. *Taenia asiatica* was identified in the 1980s. It is similar to *T. saginata* but is considered a unique species.

Parasites of the family Taeniidae have two mammalian hosts in their life cycle (Figure 1). The strobilated adult tapeworms inhabit the small intestine of the final host. The adult tapeworms of both *T. solium* and *T. saginata* are parasitic in man, causing human taeniasis. The larval cysticerci of these species were also known to man in ancient times. However, it was only in the eighteenth century the association of the larval cyst and adult tapeworm was suspected, and it was not until the following century that the life cycles of various taeniid species were fully demonstrated.

The morphological characteristics and life cycles of these parasites are well described in the literature. As with most taeniids *T. solium* bears a double row of hooks on its rostellum, whereas *T. saginata* does not have a rostellum or hooks. The gravid terminal segments of the adult tapeworms are either passed out in the feces or, in the case of *T. saginata*, can actively migrate out of the anus. The gravid segments of *T. saginata* and *T. solium* contain up to 80 000 and 40 000 eggs, respectively. The eggs released from these segments form the only free-living stage in the life cycle and are immediately infective for the intermediate host. Although the eggs of *T. saginata* can survive up to several months on pasture, *T. solium* eggs are generally not considered so resilient. After the eggs have been ingested by a suitable host, the six-hooked, hexacanth larvae or oncospheres hatch and activate in the small intestine before migrating to the development site, for example, the musculature or elsewhere, as is the case with *T. solium*, which can pass the blood–brain barrier via the lymphatics and blood stream. They then develop into the intermediate stage, termed cysticerci.

Cysticercosis is the term used to describe infection of the mammalian intermediate host by those taeniid cestodes, including both *T. solium* and *T. saginata*, whose larvae (metacestodes) are called cysticerci. They take the form of unilocular

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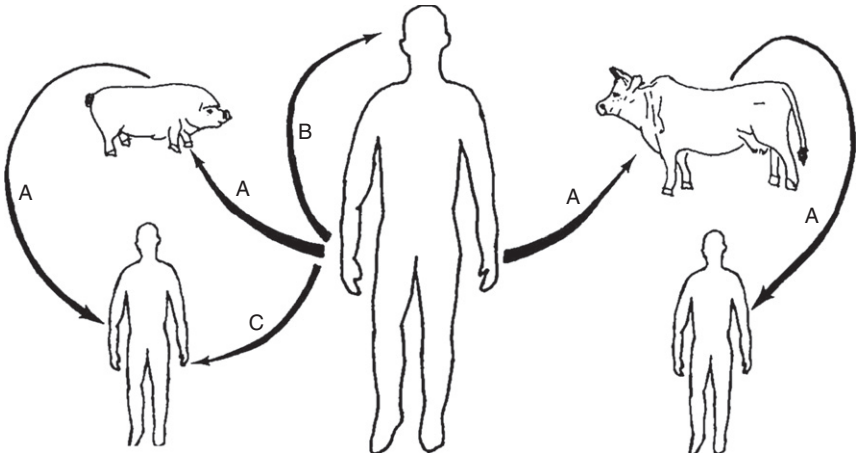

Figure 1 Diagram of the life cycles of *Taenia solium* (left) and *T. saginata* (right). The adult tapeworm of both species is located in the small intestine of the human host. When *T. solium* eggs are ingested by pigs or *T. saginata* eggs are ingested by cattle the eggs hatch and the larvae migrate to the tissues where they develop into cysticerci. Man becomes infected with either tapeworm when he consumes meat containing viable cysticerci (route A). However, *T. solium* cysticerci may develop in humans (routes B and C) including those already infected with the tapeworm.

cysts each containing a single scolex. The oncospheres of *T. saginata* and *T. solium* require at least 12 weeks but probably longer to develop to fully infective cysticerci, the characteristic bladder form may be identified within 2–4 weeks. The most common intermediate host for *T. solium* is the domestic pig. However, the cysticerci of this cestode can also infect a number of other mammalian hosts, including dogs and, unfortunately, man where generalized human cysticercosis can also occur with the cysticerci located in the musculature. In contrast, the cysticerci of *T. saginata* are more restricted in their host range and are usually only found in domestic cattle, both *Bos indicus* and *Bos taurus*. Mature cysticerci of *T. saginata* and *T. solium* are approximately 1-cm long and 0.75-cm wide but *T. solium* cysticerci can be larger.

Typical sites for the cysticerci of both species include the skeletal, cardiac, diaphragmatic, or lingual musculature but some cysts may develop in the liver of cattle and pigs and in other viscera. A strain of *T. saginata* has been reported in the Republic of Sudan, and Somalia, for which the predilection site is the liver, where unlike other geographical areas, large numbers of live cysts can be located. This is thought to be associated with a preference for the consumption of raw or very lightly cooked liver.

Unlike *T. saginata*, a characteristic of *T. solium* is that the parasite can cross the blood–brain barrier. Thus, in humans and pigs, the cysticerci of *T. solium* may be found in the brain or eye. It is this characteristic that is responsible for the major risk to human health caused by this parasite. Neurocysticercosis in man can cause severe symptoms including epilepsy and death.

Humans become infected with the tapeworm when they consume undercooked, raw, or rare meat containing viable cysticerci. If meat is well cooked until it is no longer pink then the cysticerci are killed and the transmission risk averted.

Epidemiology

The variations in the epidemiological patterns of taeniasis/cysticercosis are a reflection of the numbers and distribution of the human, cattle, and pig populations and also of the different management methods used with these domestic animals. Pigs are not commonly kept in Islamic countries. Their geographical distribution is thus more restricted than cattle but they are kept in large numbers in highly populous areas of the world such as Asia. In East Africa, because of the regulations governing the control of African swine fever, pigs tended to be maintained in tick-free commercial piggeries or if not should be kept tethered so that they cannot roam. Commercial piggeries are also found in west, central, and southern Africa but as with many countries in Latin America/Asia where intensive and semiintensive production systems are located, village-reared pigs or periurban pig production is becoming more common. Such pigs are often left to forage around human dwelling places, where, due to poor or nonexistent sanitary facilities they have access to human feces. This practice, combined with the omnivorous and coprophagous habits of pigs, accounts for the fact they are often found to have massive burdens of *T. solium* cysticerci. Cattle, being herbivorous, are usually taken to graze in areas away from direct contact with human dwellings, although they may still be in close contact with their herdsmen. Consequently, the intensity of infection in cattle is usually less than pigs.

Following a series of epidemiological studies, it was concluded that the difficulty in eradicating the large tapeworms such as *T. saginata* and to a lesser extent *T. solium* may lie in an interaction between their high biotic potential and the effective immunity acquired by their intermediate hosts. Thus, the balance between parasitic survival and attrition, on the one hand, and host immunity and fitness on the other hand, are the principal components in a complex system of interactions. For example, in areas of high prevalence of *T. saginata*, young cattle rapidly develop resistance to reinfection as a result of ingesting eggs from pasture and will retain that resistance because of the continual challenge. A result of this acquired resistance is that the numbers of cysticerci in the cattle when they are slaughtered are lower than would otherwise be expected from the high infection pressure. If the numbers of eggs on the pasture drops, perhaps as a result of the application of

control measures, young cattle may fail to acquire this resistance and remain susceptible to sporadic challenge. This is the situation that occurs in areas of low prevalence, where cattle involved in localized outbreaks may become heavily infected.

Naive cattle of all ages are susceptible to primary infection with *T. saginata*, however, they rapidly acquire a strong resistance to reinfection and will usually begin to destroy those cysts, which become established following the primary infection within 10–12 months. Thus, an explanation is needed for the fact that adult cattle of five or more years of age are commonly found to be carrying viable *T. saginata* cysticerci at slaughter in countries such as Kenya, where cattle often mature more slowly that the 18 months to slaughter as is common in say European countries. One possibility is that these cysticerci are those that have survived because their hosts were first infected very early in life, as it has been shown experimentally that cysticerci acquired during the neonatal period are able to survive in an otherwise resistant population. Although *T. solium* has a high biotic potential, it appears to be more susceptible to control by conventional means. This may be partly due to the relatively lower egg production of adult *T. solium* as compared with adult *T. saginata* and to the ease with which management practices for pigs can be modified.

Human and Animal Health Importance

Taeniasis causes various symptoms and signs, which probably depend very much on the psychological and physical characteristics of the host and are often not apparent but if they are they can include: abdominal pain, itchiness around the anus; appearance of active proglottids from the anus (especially in the case of *T. saginata* infection); and nausea. Some patients lose their appetite and thus lose weight, others experience increased appetite and gain weight, some patients tolerate the infection, whereas others do not. Other less common signs can include: diarrhea or constipation, dizziness, headache, vomiting, and weakness.

Taeniasis is known to cause pathomorphological changes in the jejunal mucosa and also functional disorders; reversible achlorhydria or lowered gastric secretion being found in 70% of patients. As a result of the reduced gastric acidity, bacterial or viral gastroenteritis is common, especially in those who are permanently exposed to various intestinal pathogens. Despite the relatively mild symptoms caused by the adult tapeworm, the infection is esthetically unacceptable. In addition, infection with adult *T. solium* is associated with the risk of an acute infection with the cysticerci. Infected persons require treatment, whereas the rest of the population requires protection from infection and the assurance that their beef and pork do not contain viable cysticerci of either parasite.

In humans, the symptoms of *T. solium* cysticercosis are also generally of minor importance, in the absence of nervous system involvement, being limited principally to muscular aches and pains. Neurocysticercosis is however a serious condition, often debilitating and sometimes fatal. The most frequent feature is epilepsy, in particular late onset epilepsy, but almost any focal neurological abnormality may occur. Locally acquired human neurocysticercosis continues to be a major cause of morbidity. It has been found to be the commonest identifiable cause of epilepsy in certain ethnic groups in South Africans, accounting for 32% of the epileptic patients in this population. However, the prevalence of human neurocysticercosis in most of Africa is not as well recorded as it is in other areas of the world such as Mexico, India, and China. An epidemiological survey carried out in northern Togo also pointed to a link between neurocysticercosis and epilepsy in that area with 38% of epileptic patients also found to have neurocysticercosis.

It has been commonly assumed that, cysticercosis per se is of little direct health importance in domestic animals, only causing occasional acute disease following rare massive infections or leading to esthetically unacceptable infections in pet dogs. However, there is some evidence that suggests that these parasites may not be totally innocuous. This is because, in common with many other parasites, such infections appear to be associated with general debilitation and some degree of suppression of their host's immune response. If this suppression results in the hosts being less able to control concurrent diseases or to respond adequately to vaccines, these parasites may be of greater general importance. Clearly, such considerations may also apply to human cysticercosis/neurocysticercosis patients.

Meat Inspection

Although most animals slaughtered under village conditions in some countries are not subjected to any form of meat inspection by qualified personnel, infected meat may be noted and rejected as unfit for consumption as was the case with carcasses of *T. solium* infected pigs 'measly pork' in Europe as early as the thirteenth century, well before the association with tapeworm infections was recognized.

The public health importance of larval taeniids in meat has led to the practice of condemnation or treatment – of infected organs or whole carcasses observed during meat inspection in centralized modern slaughter houses in an attempt to prevent completion of the parasitic life cycle. Research into the control of *T. saginata* taeniasis/cysticercosis was stimulated in Africa largely as a result of the desire by the beef producing nations to develop an export trade in beef to Europe and elsewhere. The presence of cysticerci in the meat would be a serious obstacle to meeting the import regulations of the recipient countries.

Studies were made by veterinary surgeons supervising meat inspection in abattoirs, who were primarily concerned with finding the most reliable routines for detecting the cysts in naturally infected carcasses by direct knife-and-eye inspection and also ways of handling such carcasses, usually by prolonged freezing or boiling, so as to eliminate the possibility of them transmitting the parasite to man. At the same time, the data obtained in abattoirs were used to assess the prevalence of cysticercosis in cattle and pigs in the catchment areas.

Meat inspection still relies exclusively on visual examination of the intact and cut surfaces of the carcass in the slaughter house by meat inspectors who follow officially laid down procedures. These vary from country to country and between the two parasitic infections. In the case of *T. saginata* the organs examined usually include the heart, diaphragm,

tongue, and cheek muscles, these being on the whole the less valuable parts of the carcass. Several of these are also the sites at which the cysts are concentrated in experimentally infected animals and may therefore be the optimal sites to examine in areas where the cattle have usually acquired the infection relatively recently. However, under production systems where cattle are matured relatively slowly, and where the infections are most commonly long-standing, the heart in particular is usually less heavily infected at slaughter. It was also shown some years ago that it is advantageous to include one or more cuts into the shoulder muscles of African cattle due presumably to the differing distribution of skeletal muscle between *B. taurus* and *B. indicus*.

Parasite burdens tend to be overdispersed, consequently light infections with *T. saginata* are relatively common. Only the more heavily infected carcasses can be reliably detected by these traditional methods of knife-and-eye inspection. Hence, it is recognized that existing procedures fail to detect many of the infected carcasses. Accordingly, meat inspection (even where it is routinely carried out) cannot be relied on to adequately control these parasites, although it may prevent the esthetically unacceptable heavily infected carcasses from appearing in the market place. Unfortunately, it is precisely these lightly infected carcasses, which usually escape detection during meat inspection, that are also most readily overlooked by consumers, so that the parasites in them are more likely to be eaten by humans and so infect those with a preference for raw or lightly cooked meat. Although heavily infected carcases tend to be condemned, more lightly infected carcases can in some circumstances either be chilled, frozen, or the meat processed, thus rendering it fit for human consumption. The value of such downgraded carcases is of course reduced and that combined with the cost of treatment results in an economic loss to the meat industry.

In the case of *T. solium* in pigs generalized infections are more common, and the cysticerci are larger so they are more easily detected at meat inspection. This may be another reason why this parasite has proved easier to control by conventional means.

Risk Factors Contributing to Transmission

Various practices and factors are of importance in maintaining a high prevalence level of *Taenia* infections in man and animals. From the life cycle of *Taenia* and the well-known transmission routes a series of possible risk factors can be anticipated. Many of these involve basic and food hygiene and can be summarized as follows:

- Risk factors for bovine cysticercosis (*T. saginata*/cysticercosis):
  - Application of human sewage slurry on pastures or highly permeable soils with possible contamination of water-bearing surface and drinking water.
  - Defecation of man in places frequented by cattle or in cattle feed production areas.
  - Tapeworm carrier on the farm.
  - Poor hygiene of stable personnel (e.g., handling of bovine feed or hand milking with egg-contaminated hands).
  - *Taenia* eggs in effluent water from sewage treatment plants.
- Risk factors for human taeniasis (*T. saginata* and *T. solium*):
  - Consumption of raw or undercooked meat, even when officially declared fit for consumption.
  - Consumption of uncontrolled meat.
- Risk factors for swine cysticercosis (*T. solium* cysticercosis):
  - Application of human slurry over pastures or human feces located within reach of free ranging pigs.
  - Tapeworm carrier on the farm, when swine have access to human feces.
  - Swine that are used as garbage collectors (including eating human feces).
- Risk factors for human cysticercosis/neurocysticercosis (*T. solium* cysticercosis):
  - Initially, man may have become infected with a *T. solium* tapeworm by consumption of raw or improperly cooked pork.
  - Poor personal hygiene (self-infection).
  - Consumption of food handled or prepared by infected persons.
  - Cohabitation and/or close contact with infected persons.

Public Health Measures

The consequences to man of infection with the cysticerci of *T. solium* together with the economic losses caused by the disease in man and animals have led to many national efforts to control or eradicate tapeworm diseases. In most developed countries, a combination of meat inspection and other factors, such as general improvements in sanitation and the availability of effective drug treatment for humans infected with tapeworms, have succeeded in almost or completely eradicating *T. solium*, but not *T. saginata*, in many countries. These measures have not so far been consistently applied in most of the less-developed countries. At present only a few such countries even have definite plans for controlling these parasites, although the large-scale use of praziquantel has proved useful in the control of *T. solium* taeniasis. It must therefore be expected that taeniasis/cysticercosis/neurocysticercosis will continue to be a problem for the foreseeable future.

Economic Impact and Assessment of Burden

Bovine and porcine cysticercosis both have an economic impact due to the losses incurred by infected carcasses, but because of the different public health/veterinary balance between the two parasites, the main driver for control of *T. saginata* is considered to be economic factors, whereas public health factors are the major concern with *T. solium*. The parasites can cause both severe illness and productivity losses in human and agricultural animal populations. Several recent studies indicate the extensive societal impacts on endemic areas. Estimates of burden provide essential, evidence-based, baseline data for conducting cost–benefit and cost–utility analyses. This information can be used to underpin calls for control and thus help secure political support, releasing the necessary financial and technical resources. To evaluate the

burden, the monetary and nonmonetary impacts of these zoonoses on human health, agriculture, and society must be considered across the board. Attempts to reduce the prevalence of *T. solium* and *T. saginata* in humans and their cysticerci in pigs and cattle may have a considerable impact on the economics of the meat production industries. This is particularly important where export industries are involved, because most importing countries have stringent regulations designed to prevent the importation of infected meat.

The costs can be broken down into those involved in treating human taeniasis and more importantly human cysticercosis; those incurred when pig and cattle carcasses are treated or condemned; and the costs involved in the inspection procedures themselves. Although there have been attempts to assess the economic burden caused by these parasites, in most countries there are no accurate data on any of these factors, especially in relation to *T. solium*. It was estimated that bovine cysticercosis was costing the economies of both Kenya and Botswana approximately UK £1 million each per annum. In the former country, this mainly arose from the loss of value that results in small abattoirs from boiling the meat to kill the cysts. In Botswana, a lower prevalence in fewer animals caused similar losses because of the reduced export potential. The losses will also vary with the prevalence.

Species Identification and Differentiation, Epidemiological and Other Diagnostic/Monitoring Tools

Taeniasis

It is particularly desirable to identify and treat *T. solium* infections in man because of the risk of either the host or his contacts developing *T. solium* cysticercosis. It is important, therefore, to be able to discriminate between *T. saginata* and *T. solium* tapeworm infection in humans. The morphological characteristics of the gravid proglottids such as their size and the number of uterine branches are usually used as the criteria for such identification. However, there can be considerable difficulty in distinguishing the two species because such features may overlap and accordingly many clinical reports refer only to '*Taenia* spp.'

Although the two species can be differentiated biochemically by differences in gene products, i.e., proteins or enzymes as the electrophoretic profiles of their proteins and their isoenzyme patterns differ. The samples required for use in these procedures are labile and must be either processed immediately or carefully frozen, preferably in liquid nitrogen. Fixatives such as alcohol or formalin cannot be used, so samples cannot easily be transported from one reference laboratory to another. Thus, this methodology proved of restricted value.

The application of species-specific deoxyribonucleic acid (DNA) probes to the identification of these tapeworm proglottides has immediate utility precisely, because DNA is very stable and samples can be stored in alcohol for transportation, before DNA extraction and analysis. In the clinical situation, it is important to have DNA probes, which will positively identify each individual sample. This is now feasible with the development of polymerase chain reaction (PCR) tools specific for *T. saginata*, *T. solium*, and *T. asiatica*, which allow the positive identification and differentiation of these parasites. Such tools can also make a major impact in epidemiological studies as specific reagents for the identification of discrete strains or geographical isolates of the parasite. Serological assays, based on polyclonal antibodies against adult tapeworm components, for the detection of taeniid worm antigen in the feces (coproantigen detection) are described in the literature, but a lack of species specificity limits their use.

Cysticercosis/neurocysticercosis

In contrast to adult tapeworm infections, where the proglottides or eggs may be found in the feces, the identification of hosts harboring cysticerci by direct parasitological examination poses problems. This is because the cysticerci are relatively small and widely disseminated in the tissues of the host. In humans *T. solium* cysticerci can sometimes be identified by biopsy of subcutaneous nodules, whereas in pigs it is often possible to observe them under the tongue, especially in relatively heavy infections. Cysticercosis is rarely diagnosed during life in cattle. The presence of dead calcified cysticerci can be detected by X-rays, but while this may be of some value in assisting the diagnosis of human cysticercosis, it is of little practical value in domestic animals. These reservations have stimulated a search for alternative methods of parasite detection.

Computerized tomography scanning and magnetic resonance imaging procedures provide a reliable diagnosis for neurocysticercosis in humans. These procedures are, however, neither cheap nor widely available in rural areas where *T. solium* persists. Nor is it practical to screen large numbers of people by these techniques.

An alternative diagnostic procedure is serodiagnosis, which allows the rapid screening of patients with neurological symptoms. Infected domestic animals might also be identified by antemortem serological screening and treated to kill the cysticerci, excluded from feedlot systems or subjected to close postmortem examination followed by treatment of the infected carcasses by freezing or cold storage.

All of these parasitological or clinical procedures require specially trained personnel and have the added disadvantage that each individual test is processed serially. Modern serology, through the widespread adoption of the enzyme-linked immunosorbent assay (ELISA) however, is rapid, cheap, may be automated and has the major logistic advantage that the samples are processed simultaneously with consequent decrease in turnover time.

Requirements for a serodiagnostic assay include levels of sensitivity, specificity and stability, and ease of performance, especially when used under tropical conditions. It is particularly important when diagnosing human neurocysticercosis that each infected patient is accurately identified. The criteria could perhaps be less stringent for herd animals, such as cattle, which can be treated on a group basis. Diagnostic assays for larval cestode infections should be directed toward the detection of those hosts harboring viable larvae. They should also provide an indication of the severity and progress of the infection, thus identifying those hosts that are suitable candidates for drug or surgical treatment. A highly

useful tool in the diagnosis of taeniid cysticercal infections is a serological test for the presence of viable parasites. This could be used to provide an immediate estimate of the prevalence of these infections in humans, cattle, or pigs and, as a rational indicator for clinical action for *T. solium* neurocysticercosis in man.

It has long been known that taeniids and other helminth parasites contain many cross reactive antigens, hence diagnostic assays based on the use of crude parasite extracts for antibody detection met with limited success. Problems included lack of specificity and sensitivity due to poor signal to background ratios. The problem is particularly serious in the diagnosis of helminth infections in domestic animals as they tend to be exposed to a large range of parasites. Also, although not generally the case with *T. solium* infection in pigs, *T. saginata* tends to be overdispersed in the cattle population with the majority of animals harboring only a small number of cysticerci so that they do not develop a strong serological response to infection. The reason why assays of an acceptable degree of sensitivity and specificity for the detection of *T. saginata* and *T. solium* infections were not developed was primarily a failure to identify sufficiently specific antigenic epitopes.

A detailed study at the molecular level of parasite antigens is a prerequisite for the logical design of serodiagnostic assays. This is particularly the case when dealing with complex metazoan parasites such as the taeniids. The application of modern analytical techniques such as molecular cloning, Western blotting and the direct or biosynthetic radiolabeling combined with sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) analysis allowed the identification of those parasite products, which are of diagnostic potential. The detection of specific antibodies in is one diagnostic tool for suspected cases of neurocysticercosis in man. Attempts to improve the performance of serological tests by partial purification of antigen extracts have been superseded by the development of peptide and recombinant protein based assays. A monoclonal antibody ELISA capture assay is now available, which detect surface and excreted products of cysticerci in the serum of *T. saginata* infected bovines and, due to a convenient cross reaction, the assay also detected similar excretions/secretions of *T. solium* cysticerci in the serum and cerebrospinal fluid of humans with confirmed *T. solium* cysticercosis. Once a specific serodiagnostic system has been identified, the sensitivity of modern ELISA-based technology, notwithstanding the possibility of further refinements, such as the development of rapid (pen-side) or lateral flow assays, offers the necessary sensitivity and versatility for field use. Effective serodiagnosis will undoubtedly lead to an increased number of pigs or cattle identified as infected with *T. solium* or *T. saginata* because the direct knife-and-eye methods for detecting cysticerci are known to be inaccurate.

As with taeniasis, PCR tools are also now developed that allow both the positive identification of cysticerci obtained either at biopsy or autopsy and the differentiation of these lesions from other possible infections such as sarcocystis. It is also possible to employ PCR techniques to detect parasite DNA in body fluid such as cerebrospinal fluid in the case of human neurocysticercosis.

Chemotherapeutic Control

Treatment of infection with the adult tapeworms of *T. saginata* or *T. solium* is relatively easy with the appropriate dose of suitable chemotherapeutic drugs to kill the parasite and/or the use of purgatives to stimulate expulsion of the worm. The drugs of choice are niclosamide and praziquantel, which were introduced in the late 1970s. The drug has been used in wide-scale treatment programs for the control of *T. solium* tapeworm infections in humans. Until the beginning of the 1980s there was no effective treatment for cysticercosis in man other than surgical intervention to excise cysticerci or relieve hydrocephalus in neurocysticercosis. Praziquantel is not only an effective treatment for adult taeniids but will also destroy viable cerebral cysts of *T. solium*. Adverse effects are fortunately few, and inflammatory reactions due to dying cysticerci are treated with corticosteroids. Other drugs such as albendazole are also efficacious.

Chemotherapy has mainly been used in the experimental treatment of cysticercosis in pigs and cattle. Albendazole, which appeared to offer promise as a dual purpose drug already licensed for use in many countries as a nematocide in cattle and pigs, is not fully effective against the cysticerci of *T. saginata*. Another benzimidazole derivative, fenbendazole, is effective against the cysticerci of *T. solium* in pigs and high doses of fenbendazole have been reported effective against immature and mature cysticerci of *T. saginata*. Praziquantel kills *T. saginata* cysticerci in cattle at dose rates of 100 mg kg^{-1} and above but there is some controversy as to its efficacy against immature cysticerci. Unfortunately, praziquantel is still an expensive drug, and it is not generally economic to use it in cattle. However, alternative sources or analogs may become available, such as the Chinese pyquiton and another Chinese drug Mienang No. 5 also reportedly kills cysticerci.

Even when all the cysts have been killed by a suitable drug, any calcified residues derived from those cysts that were already dead or dying at the time of treatment remain detectable in the carcass for months. Meat inspectors would have to be fully informed that drug treatment has taken place and appreciate that such residues, although possibly unacceptable esthetically, are quite innocuous as regards the transmission of the parasite to man. This is of particular importance while meat inspection is relied on as the main means of postmortem diagnosis and control. It would clearly be preferable in the long term to seek means of preventing infection in the first place.

Immunoprophylaxis

Because of the importance of acquired immunity in regulating the intensity of infection with cysticerci of *T. saginata*, a vaccine would assist during a control program by replacing this natural immunity until the parasite is eradicated and thus reduce the risk of cysticercosis 'storms.' This may apply even if effective chemotherapy was introduced as an additional control measure.

The development of vaccines against *T. saginata* cysticercosis in cattle and *T. solium* cysticercosis in pigs was made feasible because taeniid larval infections can be prevented by

antibody-mediated immune mechanisms. Resistance to secondary infection can be produced either by primary infection with the parasite or by immunization using homologous or heterologous parasite extracts. The oncosphere, or material secreted or shed by the oncosphere, is one potent source of immunogens. Extracts of cysticerci were used to vaccinate pigs against *T. solium* infection, as have extracts of *T. saginata* proglottids to immunize cattle against *T. saginata* infection. More recently, work was conducted on the molecular characterization of the antigens involved.

A considerable amount of evidence pointed to the importance of humoral immunity in resistance to the early intermediate stages of taeniid parasites. Protection by passive transfer of immune sera from animals infected with cysticerci indicated that antibodies play a role in immunity and that it is the invasive larvae or oncospheres, which are most vulnerable to such attack. Although the invasive oncosphere is considered the prime 'target' of a protective immune response, components of the cysticercal stage also constitute possible 'targets' and cysticercal extracts have been used to vaccinate animals. Studies on *T. solium* have suggested that resistance against the older cysticerci may have a cellular component in particular involving eosinophils.

There would be clear advantages in having a vaccine, which was directed against both the early invasive and later developmental stages of the parasites. If any invasive parasites survived the first line of immune defense they could then be killed at a later stage in development but while the parasites were still small and their death unlikely to result in lesions in the meat. In addition, investigations into possible antigenic variation between different strains of these parasites should be initiated to ascertain if they have the same antigenic and genomic characteristics and to ensure that vaccines are directed toward uniformly available antigens.

Progress has been made on the application of genetic engineering to the development of vaccines against taeniids. Although there are many practical difficulties to be overcome in the production and large-scale testing of such vaccines, extending the application of these techniques to parasites such as *T. solium* and *T. saginata* could do much to overcome the problems encountered when trying to produce vaccines against a parasite, where the main source of parasitic material is man or cattle. The vaccination of cattle and pigs in endemic areas remains a long-term aim for control of *T. saginata* and *T. solium* infection.

Conclusions

Conventional approaches that can be taken for the control of cysticercosis in man and domestic animals, and taeniasis in man include improved standards of human hygiene, public health education, and a more realistic approach to management practices and efficacy of meat inspection. It would be rational to adopt the attitude that when any carcasses in a group, herd, or village are infected then all the animals from that group are presumed to be infected and treated accordingly by prolonged cold storage, freezing, or processing the meat by boiling and canning. These approaches may well-eliminate *T. solium* but only reduce the prevalence of *T. saginata*. Additional control methods such as immunoprophylaxis and wide-scale application of chemotherapy either in cattle, pig, or the human population would augment conventional methods, speed up control programs and possibly eliminate *T. saginata* from the cattle population. However, mass chemotherapy to reduce the human *T. solium* tapeworm population must be treated with a degree of caution due to the potential risk that humans may also suffer from neurocysticercosis, either symptomatic or asymptomatic. Chemotherapy to kill the adult tapeworm may damage the cysticerci resulting in undesirable inflammatory reactions.

Improvements in the accuracy of detection of infected animals or humans by the application of highly specific and sensitive PCR, recombinant protein, peptide antibody detection assays, or monoclonal antibody-based assays, which detect the characteristic products of metabolism of viable cysts in the circulation would be very advantageous. Such assay can feasibly be presented either as conventions ELISA or enzyme-linked immunoelectrotransfer blot (EITB) assays or as rapid (pen side) lateral flow assays which are quick and simple to use in the clinical or farm situation. This would allow the identification of human patients, young animals, or groups of animals, which require treatment or act as an incentive for farmers with an identified problem to instigate control measures as early in the production system as possible. The detection of those animals that present a genuine hazard to human health and the exclusion of animals that only contain dead cysts could be a major factor in rendering such serological screening acceptable to owners, butchers, and public health authorities.

Although the possibility of effective chemotherapy against cysticercosis has become a reality, praziquantel is still too expensive for routine use in animals, even though this situation may well change. There is a continuing need to develop commercially viable larvicidal cestocides suitable for treating both humans and animals. Indeed, in view of the appearance of resistance to previously highly effective anthelmintics by other helminths it would be preferable not to place total reliance on any one drug. However, the instigation of a control program involving the use of chemotherapy would probably need to be undertaken by government because such a program could not be properly coordinated if it was to rely solely on the initiative and action of individuals. It will be desirable, especially in hyperendemic areas, that cysticidal drugs should mainly be used in young animals to reduce treatment costs and allow time for complete reabsorption of dead cysts before slaughter. Control program could now potentially include effective and cost-effective vaccines. Such recombinant vaccines should possibly be incorporated in with other vaccines used in the same young stock.

Effective transmission control would naturally reduce the prevalence of both porcine and bovine cysticercosis and thus helps to eliminate the continued meat hygiene problem caused by these parasites, and the production losses ensuing from attempt to control transmission by inspection of animals very late in the production system.

See also: Disciplines Associated with Food Safety: Parasitology. Safety of Food and Beverages: Meat and Meat Products

Further Reading

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HELMINTH–NEMATODE

Contents

Anisakid Nematodes

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Glossary

Accidental host Host that is not part of the normal life cycle of a parasite.
Anaphylaxis Exaggerated hypersensitivity reaction to antigen.
Anisakiasis Disease caused by infection with larval roundworms (or allergens from these worms) belonging to the family Anisakidae.
Definitive host Host in which an adult parasite lives and reproduces.
Intermediate host Host in which a parasite passes a larval stage and which is an obligatory part of the life cycle.
Sibling species Species that are morphologically similar, but reproductively isolated.
Urticaria Skin eruption characterized by transient, itchy wheals.

Background

Anisakiasis (anisakidosis) refers to infection with, or an allergic reaction to, larval stages of nematodes belonging to the family Anisakidae (and possibly also Raphidascaridae). These worms, commonly called anisakids, are found in the flesh, viscera, or body cavity of fishes or cephalopod molluscs. People contract anisakiasis by consuming infected fishes or cephalopods, or, more rarely, by exposure to allergens when handling fish products. The first case of anisakiasis was described by Van Thiel, Kuipers, and Roskam in the Netherlands in 1960, when they reported the presence of a larval anisakid in a patient suffering from acute abdominal pain. There has been a dramatic increase in the reported prevalence of anisakiasis throughout the world in the past two decades.

Characteristics

Taxonomy and Classification

Anisakid nematodes, broadly defined, are those members of the superfamily Ascaridoidea with an aquatic definitive host (fish, reptile, piscivorous bird, or mammal), whose transmission is dependent on water and usually involves aquatic invertebrate and fish intermediate hosts. More than 20 different genera of anisakids have been described. Although there is still some taxonomic uncertainty within the group, these genera are usually classified into one of the two families; Anisakidae or Raphidascarididae.

Cases of human infection have been reported with worms from a number of genera within these families. However, the two genera that are most often associated with anisakiasis are *Anisakis* and *Pseudoterranova*, both from the family Anisakidae. Historically, only two major zoonotic species were recognized; the 'herring worm' or 'whale worm' *Anisakis simplex* and the 'codworm' or 'seal worm' *Pseudoterranova decipiens*, both with an apparently cosmopolitan distribution. However, recent molecular genetic studies have found that both these morphospecies actually comprise a number of genetically differentiated sibling species, often with distinct host and geographic ranges.

Three different species have been described within the *A. simplex* complex; *A. simplex* (*sensu stricto*), *Anisakis pegreffi*, and *Anisakis simplex* C. In addition to these three sibling species, six other species of *Anisakis* have been confirmed using genetic

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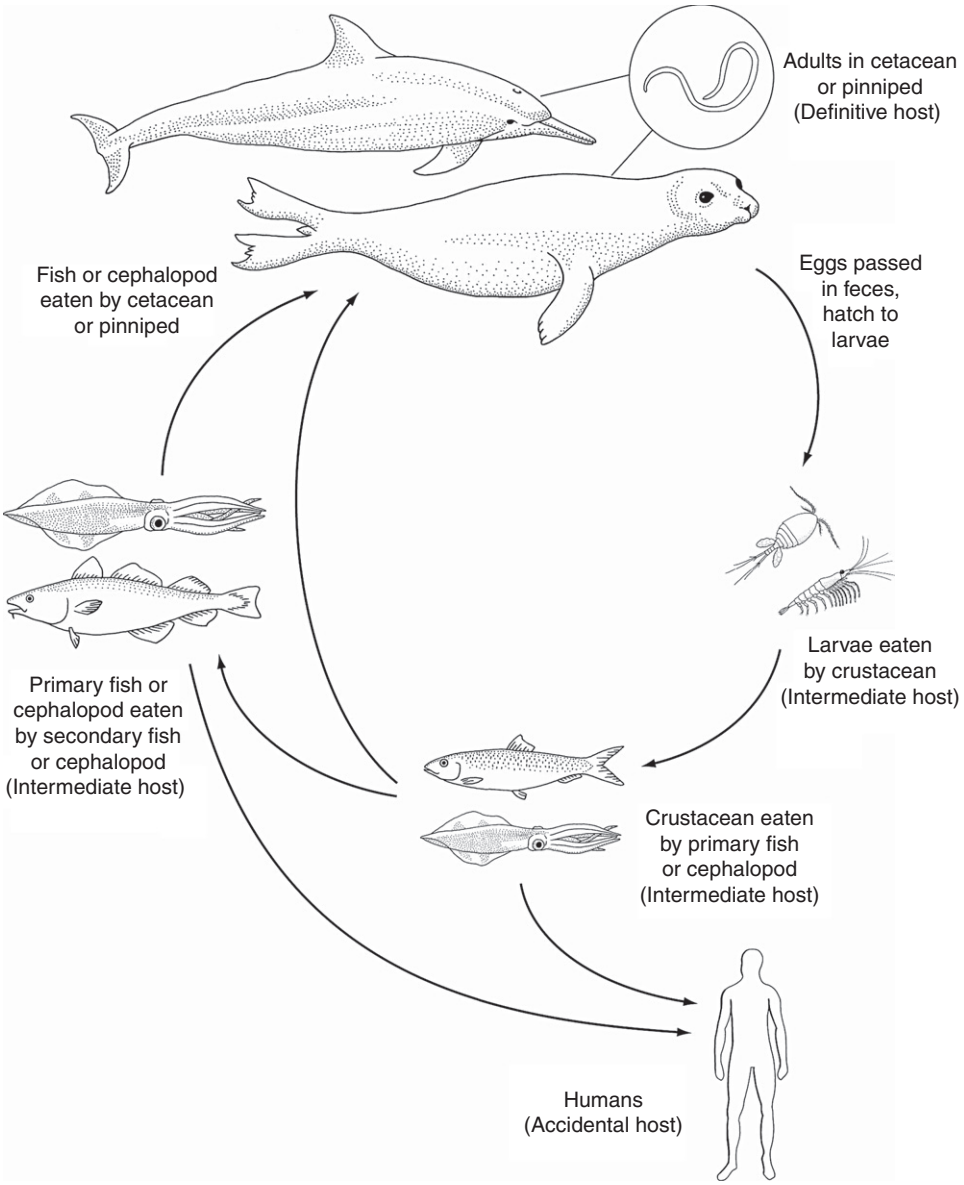

Figure 1 Life cycle of anisakid nematodes belonging to the genera *Anisakis* and *Pseudoterranova* (original drawing by Belinda Cale).

markers; *Anisakis typica*, *Anisakis physeteris*, *Anisakis brevispiculata*, *Anisakis zhiphidarum*, *Anisakis paggiae*, and *Anisakis nascetti*. Six different species have been described within the *P. decipiens* species complex; *P. decipiens* (*sensu stricto*), *Pseudoterranova krabbei*, *Pseudoterranova bulbosa*, *Pseudoterranova azarasi*, *P. decipiens* E, and *Pseudoterranova cattani*. In addition to these six sibling species, two other species of *Pseudoterranova* have been described on morphological criteria (*Pseudoterranova kogiae* and *Pseudoterranova ceticola*), although these species have not yet been confirmed genetically.

These species lists should not be regarded as definitive. Future systematic studies of the family using molecular genetic techniques are likely to uncover more species of anisakid nematodes and should be an urgent research priority. Molecular genetic analyses will also provide an essential tool for basic ecological studies of anisakids, providing us with a clearer understanding of geographic distribution, host range, and prevalence rates in definitive and intermediate hosts of different species within the family Anisakidae.

Life Cycle

Anisakids utilize aquatic mammals, piscivorous birds, aquatic reptiles, or fish as definitive hosts. Larval anisakids are found in aquatic invertebrates and fishes, although for most species, the precise details of the life cycle are uncertain and it is not clear whether the invertebrate and fish hosts are obligatory or whether the larval development occurs within them. For this reason, they have been referred to by different authors as both

Table 1 Forms of anisakiasis

Forms of anisakiasis	*Description*	*Pathology*
Noninvasive	Larvae do not penetrate mucosa	Usually asymptomatic
Invasive		
Oropharyngeal	Larvae penetrate the tissues of oropharyngeal cavity	Slight
Gastrointestinal	Larvae penetrate the gastric or intestinal mucosa	Slight–severe
Extra-alimentary	Larvae enter the body cavity	Severe
Gastroallergic	Allergic response to the larval antigens	Slight–severe

Source: Adapted from Lymbery and Cheah 2007.

intermediate and paratenic hosts; in this article, both invertebrates and fish are referred to as intermediate hosts, on the assumption that both of them are required for successful completion of the parasite's life cycle.

A generalized life cycle for species of *Anisakis* and *Pseudoterranova* is shown in Figure 1. Adults of *Anisakis* spp. are found in the alimentary tract (particularly the stomach) of cetaceans (dolphins, porpoises, and whales) and adults of *Pseudoterranova* spp. in the stomach of pinnipeds (seals, sea lions, and walrus), although the definitive host range of most species is still incompletely known. Eggs are shed in the feces and require an incubation period before hatching into free-living larvae. There is uncertainty over whether one or two molts occur within the egg and therefore whether the free-living larvae are in their second stage (L2) or third stage (L3). Free-living larvae are ingested by invertebrates, particularly crustaceans, such as copepods, amphipods, isopods, euphausiids, and decapods, where they grow within the hemocoel, perhaps undergoing one molt. Fishes and, at least for some species of *Anisakis*, cephalopod molluscs, such as squid and cuttlefish, become infected by eating crustaceans containing L3 larvae, which penetrate the intestine and invade the tissues, where they may continue to grow or become encapsulated. Fishes and cephalopods are referred to as primary hosts when they obtain their larval infection from crustaceans, and as secondary hosts when they become infected by eating other infected fishes or cephalopods. Definitive hosts are usually infected by eating fishes or cephalopods containing L3 larvae, which then develop to adults in the alimentary tract.

Infection of People

People may be accidental hosts in the life cycle of anisakids, becoming infected by eating intermediate hosts (usually fishes), which contain larvae. More than 90% of identified infections in people involve *A. simplex* (*sensu lato*), with most of the remainder involving *P. decipiens* (*sensu lato*), and other anisakid species, such as *A. physeteris* and *Contracaecum osculatum*, being found only rarely. Almost all reported cases of anisakiasis have involved L3 anisakid larvae, although L4 larvae have been identified in a small number of cases.

A very large number of fish species act as hosts for species of *Anisakis* and *Pseudoterranova*. Primary fish hosts are planktivores or predominantly planktivores, which acquire the parasite directly from crustacean invertebrate hosts. Secondary fish hosts are piscivores, which usually acquire the parasite from infected planktivorous fish. Prevalences and intensities of infection vary widely between fish hosts, both within and between anisakid species. These differences appear to be more related to geographic distribution, feeding habit, and growth rate of hosts than to behavioral or physiological host preferences of the parasites. Both prevalence and intensity of infection tend to increase with host age and size and are usually greater in secondary than in primary fish hosts.

As people usually become infected with anisakids by eating larvae contained within fish hosts, the distribution of larval nematodes within the tissues of fishes is epidemiologically important. After L3 larvae, contained within infected invertebrate or primary fish hosts, are ingested by a fish, they penetrate the intestinal wall. They may then remain within the body cavity, or migrate into the flesh or internal organs. Differences in relative abundance among these microhabitats may be affected by the species of parasite, the species and age of the infected fish, and the environmental conditions to which the fish are subjected after capture. Recent studies have suggested that the larvae of *A. simplex* (*sensu stricto*) are more likely to migrate into the flesh of fishes than the larvae of other species of *Anisakis*. Other studies have found that the larvae of *P. decipiens* (*sensu stricto*) are found more often in the flesh of young fishes than older fishes. In addition, there is evidence that larvae may migrate from the visceral organs to the flesh after death of their fish host, and that this migration may be enhanced by cold storage. The influence of all these factors is often difficult to disentangle; and at present, no general conclusions can be made concerning the risk factors for larvae being found in the flesh of fishes, where they are more likely to be eaten by people.

Clinical Manifestation

The disease associated with accidental infection or an allergic reaction to larval anisakid nematodes is usually called anisakiasis or anisakidosis. Clinically, human anisakiasis can take a number of forms, depending on the location and symptoms caused by the larvae (Table 1).

Noninvasive Anisakiasis

In noninvasive anisakiasis, often (but not exclusively) associated with infections with *Pseudoterranova* spp., larvae remain in the alimentary tract without penetrating the mucosal wall.

This often causes an asymptomatic infection, which may only be discovered when the worms are expelled by coughing, vomiting, or defecating hours, days, or even weeks after consuming a seafood meal. Occasionally, noninvasive infections give rise to a 'tingling throat syndrome,' which happens when worms migrate back up the esophagus into the oropharynx.

Invasive Anisakiasis

In invasive anisakiasis, larvae penetrate into or through the wall of the alimentary tract. Penetration of the buccal mucosa or pharyngeal mucosa occurs only rarely, and is usually associated with slight pain, feelings of discomfort, and difficulty in swallowing. Penetration of the gastric or intestinal mucosa is the most common form of invasive anisakiasis, with gastric anisakiasis more common than intestinal anisakiasis. Infections with *Anisakis* spp. are associated with both gastric and intestinal invasions, whereas infections by *Pseudoterranova* spp. are usually associated only with gastric invasions.

Symptoms of acute gastric anisakiasis appear 1–12 h after consumption of infected seafood, and include sudden stomach pain, nausea, and vomiting, with blood often found in gastric juices and stools. If acute cases are misdiagnosed, gastric anisakiasis may become a chronic disease, with clinical features very similar to peptic ulcer, gastric tumor, acute gastritis, and cholecystitis.

Intestinal anisakiasis usually manifests as an acute disease, occurring 5–7 days after seafood consumption. Clinical symptoms include nausea, vomiting, fever, bloody diarrhea, and severe lower abdominal pain, similar to acute abdominal syndromes, such as intestinal obstruction, appendicitis, or peritonitis.

Occasionally, anisakid larvae have been found to completely penetrate the wall of the alimentary tract and enter the body cavity. Larvae then usually lodge in the peritoneum or subcutaneous tissues, forming tumor-like, eosinophilic granulomas or abscesses. Associated symptoms include abdominal pain, vomiting, and bloody stools.

Gastroallergic Anisakiasis

Anisakiasis is often associated with a strong allergic response, mediated by immunoglobulin E and with clinical symptoms ranging from isolated swellings to urticaria and life-threatening anaphylactic shock. Allergic responses may occur with or without the gastrointestinal symptoms described above. The first signs of an allergic reaction usually occur within 2 h after eating infected seafood, although it may take up to 6 h to appear. Most cases of allergy reported to date have involved *A. simplex* (*sensu lato*). The allergens, which invoke a hypersensitivity reaction, appear to be highly resistant to heat and freezing, raising the prospect of an allergic response to parasitized seafood products, which have been prepared in a way that would normally kill nematode larvae. In addition, allergic symptoms have been found in fish processing workers, presumably from occupational exposure to contaminated fishes, and in people exposed to fishmeal or even to meat from chickens fed with a fishmeal-based diet. It is not yet clear whether the development of gastroallergic anisakiasis requires a prior, priming infection with live anisakid larvae or whether exposure to the anisakid allergens alone is sufficient to produce an allergic response.

Epidemiology

Prevalence and Geographic Distribution

Anisakiasis occurs throughout the world, with foci in North Asia and West Europe. More than 90% of the reported cases are from Japan, with the rest mostly from the Netherlands, France, and Spain. However, cases of anisakiasis have also been reported from many other areas of the world, including USA, Mexico, Canada, UK, Belgium, Egypt, Korea, Philippines, Chile, Australia, and New Zealand.

In the past 30 years, there has been a marked increase in the prevalence of anisakiasis throughout the world. This increase in reported cases of anisakiasis is probably due in large part to the use of new diagnostic techniques, particularly endoscopy. However, it is likely that the recent increased prevalence of anisakiasis is not solely due to the improved diagnostic methods, but also reflects a greater risk of contracting parasitic infections. As with other fish-borne parasitic diseases, the increasing global demand for seafood and a growing preference for raw or lightly cooked food, especially in many western countries, increase the risk of parasite exposure. It has also been suggested that the risk of exposure to anisakids has increased because greater regulatory controls over the exploitation of marine mammals has led to increasing population sizes of potential definitive hosts. However, evidence to support this view is mixed, and the relationship between definitive host and parasite population size is not straightforward for parasites, such as anisakid nematodes, which have a complex, multihost life cycle.

Risk Factors

The prevalence of anisakiasis and other fish-borne parasitic zoonoses is related to the traditions of consuming raw, lightly cooked, or marinated fish, such as Japanese sushi and sashimi, Dutch salted or smoked herring, Scandinavian gravlax (dry, cured salmon), Spanish boquerones en vinagre (pickled anchovies), Hawaiian lomi-lomi (raw salmon), Filipino kinilaw (chopped, marinated fish), and Latin American ceviche (raw fish seasoned with lemon juice). The risk of anisakid larvae in these dishes depends on the species of fish being used and may be enhanced if the fish are eaten whole (because worms are often found in the viscera rather than the flesh of the fish) or if the fish have been kept whole for some time after capture, rather than gutted immediately (because worms may migrate from the viscera to the flesh after death of the fish).

A major epidemiological issue which needs to be addressed is the reason why anisakiasis is most often associated with certain species of anisakid larvae. Almost all recorded cases involve infection with *A. simplex* (*sensu lato*) or *P. decipiens* (*sensu lato*). However, it is not clear to what extent this is a function of the geographic distribution of these parasitic species, their fish host range, their microhabitat distribution within fish, their propensity for postmortem migration, or

their invasive ability when ingested by humans. It is possible that all of these factors are involved, but determining their relative importance is essential because it will impact the advice that can be provided about the risk factors for anisakiasis.

Control

Preventive or control measures for anisakiasis focus on postharvest handling, storage, and cooking procedures for fish. However, consumption of live larvae in raw or undercooked fish is not the only way in which the parasite can cause disease and there is evidence that occupational exposure to fish products may be sufficient to trigger an allergic response to anisakid allergens. Furthermore, the allergens responsible for gastroallergic anisakiasis may be resistant to the freezing and cooking procedures recommended for killing anisakid larvae.

Postharvest Handling

In many countries with high prevalences of anisakid larvae in fish, fish are examined for infection at processing, with heavily infected fillets trimmed or discarded. The usual procedure is to examine fillets by candling on a light table. However, the candling procedure is very inefficient, often detecting as few as 33% of heavily infected fish (i.e., those with more than three worms per kg). Candling efficiency can be improved by slicing fillets longitudinally or by using different wavelengths of light and illumination sources to increase the contrast between the worm and flesh of the fish. Other approaches, such as laser candling, radiography, scanning laser acoustic microscope, pulse-echo technology, and electromagnetic detection have also been tested, but none of these are able to improve efficiency of detection to an extent that justifies increased processing costs or reduction in marketable fillet yield. Recent research has concentrated on molecular genetic approaches, with the development of primers for polymerase chain reaction amplification of anisakid gene sequences and the identification of restriction enzymes generating specific restriction profiles. Such techniques have demonstrated good specificity and sensitivity in experimental tests and have the advantage of being able to identify, if required, the species of anisakids present in fillets. They are likely to become the preferred methods for detecting anisakid larvae in fish flesh.

Freezing and Storage

Freezing kills nematode larvae, as long as the temperature is low enough and maintained for a sufficiently long period of time. Most health and food safety organizations recommend that the fish intended for raw or semiraw (marinated or partly cooked) consumption be frozen to at least $-20\ ^{\circ}C$ and stored at this temperature for 24 h or more. The available literature suggests that this is adequate to kill larval nematodes, although the US Food & Drug Administration provides more stringent recommendations, indicating that fish should be frozen to $-35\ ^{\circ}C$ or below for 15 h, or to $-20\ ^{\circ}C$ or below for a minimum of 7 days.

Preparation

Anisakid larvae are resistant to salting, smoke curing, and marinading. For home consumption, it is usually recommended that fish should be cooked by conventional means at 60 °C or higher, for at least 10 min. The effect of microwave cooking on anisakid larvae has not been well studied.

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Ascaris

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Glossary

Bolus of worms An aggregation of entangled worms that tends to occur in hosts with a high infection intensity.
Disability adjusted life years (DALYs) The sum of the years of potential life lost due to premature mortality and the years of productive life lost due to disability.
Infection intensity Defines the number of parasites in an infected host. *Ascaris* infection intensity is defined by the concentration of eggs per gram (epg) of stool.
Oviposition Deposition of eggs in which the embryo develops outside the maternal body.
Patent infection An infection in which the infectious agent is shed from the host.

Characteristics of the Genus

Within the family Ascarididae, the genus *Ascaris* describes intestinal roundworms with a three-lipped mouth. While 13–16 species are known from mammalian hosts, two species, *Ascaris lumbricoides* (Linnaeus, 1758) and *Ascaris suum* (Goeze, 1782), are of the greatest medical importance and, therefore, are the best studied. *Ascaris lumbricoides* and *A. suum* infect humans and pigs, respectively.

Since the two species are morphologically indistinguishable, there has been considerable debate concerning the taxonomic status of human and pig *Ascaris*. Modern classifications are based on molecular methods, which have shown that *A. lumbricoides* and *A. suum* differ in the mitochondrial genome (mtDNA) sequence. Therefore, *A. suum* and *A. lumbricoides* are closely related at a phylogenetic level but have been conclusively shown to be correctly separated as two species in their own right.

While human and pig *Ascaris* typically infect their conventional hosts, experimental crosstransmission studies indicate that the two species can infect both hosts. Furthermore, infected human hosts in *A. lumbricoides* nonendemic areas in for example the Netherlands, North America, and Denmark harbor *A. suum*, indicating that pigs are a potential reservoir for the human population. Contrastingly, in *A. lumbricoides*-endemic regions of Guatemala and China, crossinfection between host species is low or absent. Despite low levels of crossinfection in certain areas, ascariasis is a zoonotic disease and cases of transmission from pigs to humans have been documented in Europe.

Life Cycle

Hosts contract *Ascaris* infection via the fecal–oral route (Figure 1). Infection is by the ingestion of embryonated infective ova. Larvae hatch in the small intestine and migrate to the cecum and proximal colon, where they penetrate the mucosa. The parasites migrate to the liver via the portal blood and later advance to the lungs, where they penetrate the alveolar space and ascend the bronchial tree to the throat. At this point, the helminths are swallowed and return to the small intestine. Larvae mature and reach sexual maturity in the small intestine. Mature male and female adult worms measure 15–25 cm and 20–35 cm, respectively. *A. suum* in pigs tend to have an affinity for the first three quarters of the small intestine but particularly the latter portion of the first half.

Adult worms may reside in the intestines for approximately 1–2 years. Worm copulation takes place in the host's small intestine and female worms are estimated to produce approximately 200 000 ova daily but production is known to be density dependent as the number of eggs decreases with worm load. Ova are shed via host feces into the environment. The time from infection to the presence of ova in stools is approximately 67–76 days and 6–8 weeks in humans and pigs, respectively.

Egg Development and Viability

Ascaris eggs are ellipse-shaped to round, golden brown, and vary in size (*A. suum*: 50–70 μm in length and 40–60 μm in width; *A. l7umbricoides*: 45–75 μm in length and 35–50 μm in width). Both unfertilized and fertilized eggs may be found in an infected host's feces. Fertilized eggs tend to have adhesive properties, which is a result of a cortical layer composed of mucopolysaccharide.

The further development of eggs in feces is dependent on environmental variables such as oxygen, moisture content, temperature, and shade. Optimal conditions for development and to maintain viability are moist, warm shaded soil. Embryonation rates are variable depending on temperature, but full development to the infective stage can occur within 10–14 days at $30 \pm 2\,^{\circ}C$. *Ascaris* eggs consist of four layers, making

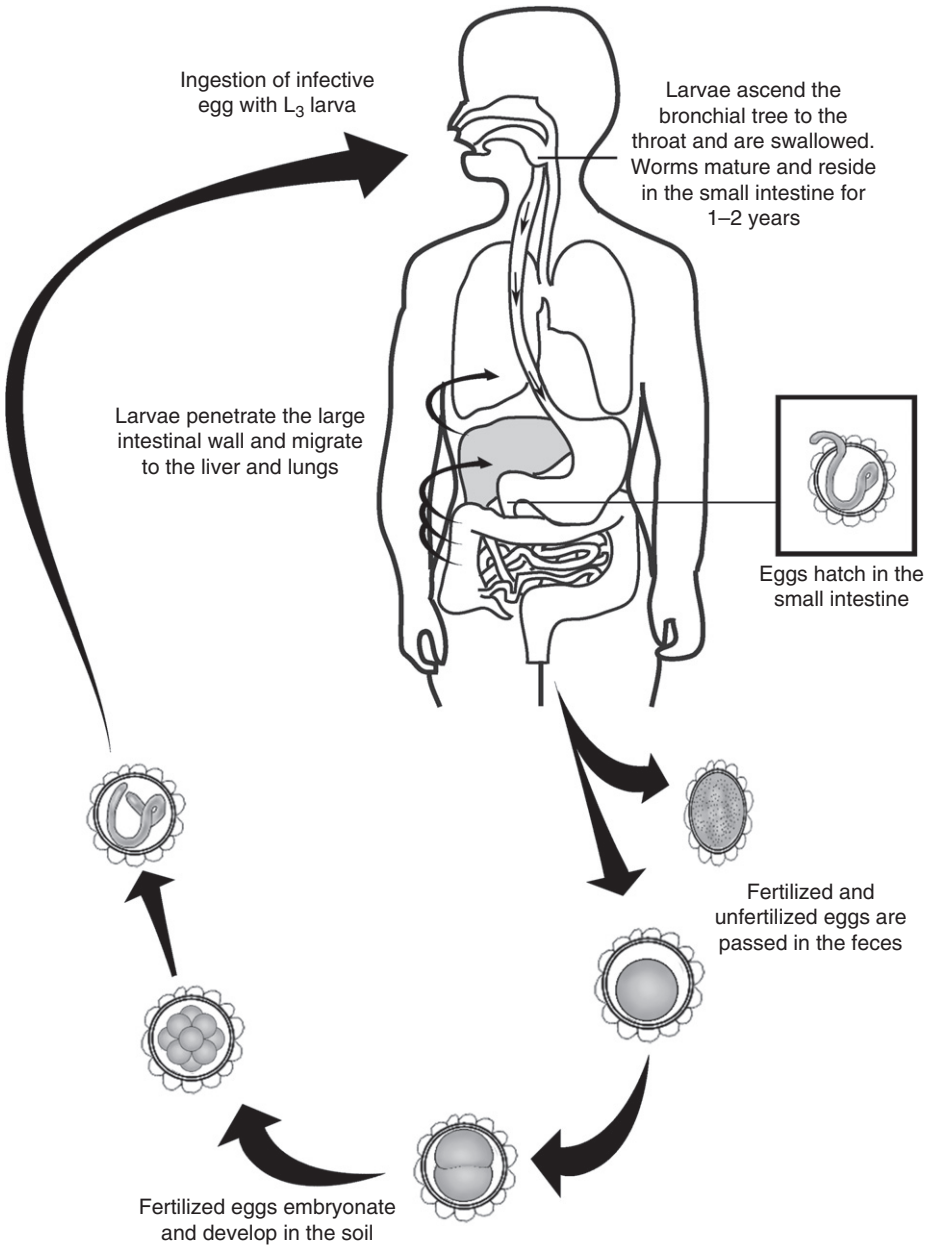

Figure 1 *Ascaris* life cycle.

them very resistant to adverse environmental conditions and while results vary, eggs can retain infectivity in the host environment for up to 15 years. The single-celled eggs that are shed in the feces develop into eggs containing third stage (L_3) larva, which have undergone two molts during development. The infective egg stage must be ingested to cause infection and eggs are triggered to hatch under intestinal conditions.

Infectivity

Epidemiological studies of *A. lumbricoides* infection in humans have shown that individuals tend to be reinfected with similar worm burdens as before treatment. The consistent observed heterogeneity in infection intensities between hosts is not fully understood but it has been calculated that a host genetic component accounts for 30–50% and 35–44% of variation in the worm load harbored in humans and pigs, respectively. Therefore, one cannot conclusively state an infective dose as it is largely dependent on the host individual. Despite this, the infectious dose is essentially low as each infective *Ascaris* egg is capable of developing into an adult parasite once ingested by its host.

High egg density inhibits embryonation and so stocks maintained for experimental studies should be diluted to ≤ 25 eggs μl^{-1} in order to maximize stock infectivity. Laboratory embryonated *A. suum* eggs can be maintained in 0.1 M sulfuric acid, for up to 5 years at room temperature. Many

experimental porcine studies are conducted since *A. suum* in the pig is a natural host parasite system and is therefore comparable to human infection. Coupled with this, rodent models have gained popularity since *Ascaris* undergoes a similar migratory route in both mice and pigs and thus humans. While adult worm infections cannot be studied in the mouse, this experimental host can provide information on the earlier stages of larval infection. Coupled with this, rodents are often infected in order to assess whether eggs are infective in studies focusing on egg embryonation and development. One can then isolate and enumerate the pulmonary larvae at approximately days 7 postinfection in mice. Therefore the experimental host provides a fast method for determining egg infectivity based on the infection intensities observed.

Health Consequences

The majority of hosts harbor few adult *Ascaris* worms and therefore tend to be asymptomatic. However, morbidity and mortality increase with burden and an estimated 60 000 people die annually from the infection. Migrating larvae are known to induce inflammatory reactions in the liver and lungs but the impact of *Ascaris* larval migration is less clearly understood than that of mature worms in the gut. The first symptoms evident in human infection are wheezing, coughing, and eosinophilia caused by pulmonary larval migration. The resultant respiratory distress is known as Loeffler's syndrome, but it is not evident until day 10–14 postegg ingestion. Characteristic symptoms of adult worm infections and chronic ascariasis include abdominal distension and pain, nausea, and diarrhea. In heavy infections, entangled worms can lead to mechanical intestinal obstruction, which is most commonly detected in children.

Ascaris infection is associated with appetite loss and impaired weight gain and therefore impacts on host nutrition. Intestinal malabsorption has been documented in *Ascaris*-infected children, which was attributed to infection-induced alterations in intestinal villi lengths. Studies have indicated that infection leads to reduced protein and fat absorption as well as vitamin A and C deficiency.

As discussed later (see Diagnosis of Infection), diagnostic techniques are dependent on detection and enumeration of worm eggs passed in host feces. Oviposition by the adult female occurs 2–3 months after infection is acquired, at which point hosts will have experienced acute symptoms (e.g., the Loefflers syndrome).

Epidemiology

It is currently estimated that 807 million people worldwide are infected with *A. lumbricoides*. Infection is most commonly documented in endemic developing countries but sporadic cases are reported in developed countries as a result of travel, consumption of contaminated imported crops, or zoonotic transmission from pigs. Prevalence in endemic countries varies and is generally low in countries with arid climates and high in those with consistent wet weather and warmth as embryonation and thus transmission are maintained throughout the year in latter conditions. Furthermore, the intestinal roundworm coexists with overpopulation, poor sanitation, and a high level of fecal contamination as well as inadequate sewage disposal, which plays an integral role in increasing transmission. The geographical distributions of *A. lumbricoides* and other helminth infections (*Trichuris trichiura* and hookworms) as well as malaria, tuberculosis, and HIV tend to overlap. Conflicting results of the effect of helminth infection on the 'big three' diseases indicate the need for further research as the full impact of large scale deworming programs is currently unknown.

The spectrum of disease associated with *Ascaris* infection is known as ascariasis. The majority of infected hosts are asymptomatic as morbidity tends to be associated with the worm burden harbored. Both human and porcine hosts experience acute lung inflammation and difficulty in breathing as a result of larval migration through the pulmonary tissue. Characteristic symptoms of chronic human ascariasis include abdominal distension and pain, nausea, and diarrhea. Entangled adult worms have also been documented as leading to mechanical intestinal obstruction in 0.005–2 per 1000 infections per year, which is the most common complication in human infections, with an associated mean case fatality rate of 5.7%. Combined with physical self-limiting symptoms, chronic infection can also lead to malaise, stunted growth, and malnutrition. Morbidity associated with *A. lumbricoides* infection is assessed as disability-adjusted life years (DALYs), which has been calculated at 10.5 million.

Foodborne Transmission

The infective agents of the helminths are the eggs, not the worms. Therefore, *Ascaris* infection is not acquired directly from consumption of infected porcine hosts. However, in *Ascaris*-endemic areas, human feces, raw sewage, and untreated wastewater are used as fertilizer to grow crops for human consumption. Therefore, many human infections can be attributed to the ingestion of polluted crops and contact with polluted feces or polluted wastewater. Furthermore, sludge is widely used as a fertilizer in agricultural fields; therefore it also poses the risk of dissemination of infection in *Ascaris*-endemic areas. Similarly, a high prevalence and intensity of *Ascaris* infection is observed in areas where human excreta are used as fertilizer for crops. The spread of these so-called 'nightsoils' maintains transmission and reinfection in endemic areas. Furthermore, transportation/exportation of food to developed countries can also increase the threat of contaminants in the importing countries.

Diagnosis of Infection

Detection in Feces

Diagnosis of ascariasis is generally dependent on the detection of *Ascaris* ova in the host's feces. The coprological diagnostic assays available are quantitative and, therefore, infection intensities can be determined based on a classification system provided by the WHO (Table 1). Originally devised to assess

Table 1 Classes of infection intensity according to *Ascaris* eggs detected per gram feces (WHO, 1998)

Eggs per gram feces	*Infection intensity*
1–4999	Light
5000–49 999	Moderate
≥50 000	Heavy

Schistosoma prevalence in Japan, the Kato–Katz method is the most commonly used diagnostic tool for intestinal helminth infections and involves examination of a thick smear of a approximately 50 mg stool sample. The long-term popularity of this WHO-recommended method is attributed to multiple factors such as its simplicity, relatively low cost, and suitability for rapid use under field conditions.

A further widely used diagnostic assay, the formol–ether-concentration method allows for concurrent diagnosis of intestinal protozoa as well as helminth infections. Samples are preserved in sodium acetate–acetic acid formalin (SAF) or dilute formalin, which is advantageous for large epidemiological surveys in which analysis is undertaken at later time points. The efficiency of detection is also increased in this method by the addition of ether, which removes fats and oils from the stool samples. Centrifugation results in a layered sample, from which the ether and formalin can be discarded, so that the remaining pellet containing the parasite ova can be examined.

A recently developed coprological method known as FLOTAC is based on the centrifugal flotation of a fecal sample suspension during which helminth eggs gather in the apical portion of the flotation column. This portion can be subsequently translated for viewing under a microscope. While this new method was originally designed for veterinary use, it has shown promising results for the detection of human parasites.

Sensitivity of Detection

A selection of studies has comprehensively assessed the relative diagnostic performance of the Kato–Katz and/or formol–ether-concentration methods along with the FLOTAC technique in order to determine the most reliable assay. The Kato–Katz method has a theoretical analytic sensitivity of 24 eggs per gram (epg) of stool, which can be increased by examining multiple thick smears prepared from the same stool sample or multiple stool samples. For example, one study reported an increase in sensitivity from 56.3% to 70.3% when one and three Kato–Katz smears were examined for *A. lumbricoides*. The FLOTAC technique requires a 24-fold higher amount of stool than a single Kato–Katz and consequently increases the likelihood of detecting ova. Therefore, the FLOTAC detects a higher number of *A. lumbricoides* infections in comparison to the Kato–Katz and especially formol–ether-concentration methods. The lower sensitivity of the Kato–Katz method tends to be a larger diagnostic issue for hookworm and *T. trichiura* since these helminths produce fewer ova than *A. lumbricoides*. However, the risk of misinterpretation increases when using the Kato–Katz method in assessing *A. lumbricoides* infections at low intensities. Despite lower sensitivities of Kato–Katz, this diagnostic method yields more accurate infection intensity results than FLOTAC. Therefore, the choice of diagnostic method should be based on the purpose of the study.

Alternative Methods

The routine diagnosis for ascariasis is primarily based on morphological identification of eggs or adult worms passed in the host's feces. However, there are alternative methods available based on detecting parasites through imaging techniques, *Ascaris*-specific nucleic acid markers, or antigens in host tissue or serum.

Imaging Diagnostic Techniques

The larval stage of *Ascaris* infection may also be diagnosed in the host's sputum by microscopy. Combined with this, imaging techniques such as chest radiography and high resolution CT show symptoms of pulmonary migration, including patchy alveolar infiltrate and alveolar hemorrhage. The use of radiography can also identify aggregations of adult *A. lumbricoides* worms as the parasites tend to form a bolus in the intestines of hosts, particularly children who present with abdominal pain. A further complication of *A. lumbricoides* infection, hepatobiliary ascariasis in which worms enter the common bile duct can be detected using imaging techniques. Ultrasonography can identify more than 85% of cases of biliary ascariasis. While scanning and imaging techniques are a tool useful for the detection of *Ascaris* infection, the availability of such diagnostic tools are limited in highly endemic areas and are not suitable for mass application.

Nucleic Acid Based Methods

The internal transcribed spacer (ITS) is the most common marker used to discriminate nematode species as it has highly variable nuclear loci. The first ITS (ITS-1) has been shown as a useful marker in identifying *Ascaris* in porcine liver and can therefore provide a useful tool for diagnosing infection at the early larval stages. However, much debate has arisen as to whether this approach can reliably differentiate between *A. suum* and *A. lumbricoides* infection. Determining host origin would be advantageous over existing microscopy diagnosis techniques. Since pig and human *Ascaris* are so closely related, significant similarity of the ITS region is observed between the two species. Even though some studies argue that the sequence difference is reproducible, others suggest that the use of this molecular marker in defining *Ascaris* sp. genotypes requires reconsideration. Therefore, while PCR methods would increase specificity and sensitivity in diagnosing *Ascaris* infection, such practices are yet to be adopted as standard protocol especially in endemic countries where there is a lack of available technologies.

Serological Analyses

Immunological methods can provide direct evidence that an infection is present. Researchers have undertaken enzyme-linked immunosorbent assays (ELISAs) in which *A. suum* antigens have been detected and therefore prove useful in herd examinations. The high level of sensitivity provided by antigen detection would overcome the problem of false negatives in cases of low *A. lumbricoides* intensities. However, such

techniques are unable to distinguish between current and past infections and therefore require further definition of assay performance prior to mass application.

Analytical Aspects

Detection and Treatment of *Ascaris* Eggs in Wastewater and Sludge

Ascaris is the most common helminth egg in wastewater and sludge and the WHO has cited helminth ova as one of the main targeted pathogens in its recent water reuse guidelines. While *Ascaris* eggs found in wastewater and sludge may not be infective, they may embryonate under suitable conditions once deposited through irrigation and fertilization of crops.

Assessment of *Ascaris* prevalence in communities with differing sanitation systems has shown that untreated wastewater is associated with increased helminth infection, particularly *A. lumbricoides* infection. The current WHO guidelines recommend treating wastewater so that ≤ 1 nematode egg l^{-1} remains prior to irrigation. This guideline is generally accepted as sufficient but some studies have suggested that a guideline of 0.1 egg l^{-1} is more appropriate when children are likely to be exposed. Increased *Ascaris* infection is associated with consumption of food items fertilized with nightsoils rather than with the act of spreading the human excreta. Treatment of human excreta with ovicides held in nightsoil tanks substantially reduces infection levels. Similarly to wastewater, the current guideline limit for sludge is ≤ 1 helminth ova g^{-1}. During wastewater treatment, the suspended solids are removed, thus removing all helminth ova. Therefore, while *Ascaris* eggs are removed from wastewater, they are later inactivated in sludge. Inactivation of *Ascaris* ova in sludge proves difficult due to their resistance to many chemicals and physical conditions.

In order to ensure that the prevalence of helminth ova is reduced to ≤ 1 helminth ova l^{-1} of wastewater or per gram of sludge, treatment techniques may need to be combined to increase efficiency. The removal of helminth ova in the suspended solids portion may be undertaken by sedimentation, filtration, or coagulation–flocculation. There are various treatment methods for inactivating helminth ova in sludge but in general the eggs are destroyed by raising the temperature, lowering moisture ($<5\%$), or adding certain disinfectants. Alkaline poststabilization is advantageous due to its low cost, yet has disadvantages such as the process' inability to destroy organic compounds that permit microbial regrowth. While *Ascaris* ova tend to show resistance to a range of chemicals, peracetic acid, for example enters the eggs and damages the nuclei, thus rendering them inactive. There are few studies on the effect of anaerobic digestion on the viability of helminth ova and the process is costly. However, a further step to this process involving thermal drying has been shown to inactivate the eggs. Studies differ in their outcome on the effectiveness of composting on inactivating helminth ova, yet it appears that this process in unable to produce sludge with <1 helminth ova per 4 g sludge. Similarly with the process of dehydration, there are contradictory results on its effectiveness in inactivating helminth ova. There is little information on the subject of sludge treatment to destroy helminth ova and therefore further research is required to assess the most efficient methods as well as the most cost-effective so that they can be implemented in endemic developing countries, where the prevalence of *Ascaris* of up to 85% in sludge has been recorded.

Since *Ascaris* ova are resistant to many treatments and have a high prevalence in raw sludge in endemic countries, these specimens are often used as indicator organisms in monitoring the efficiency of treatment. In order to assess the effectiveness of treatments, methods of detecting *Ascaris* eggs are undertaken. The US Environmental Protection Agency (EPA) describes a commonly used standard method to quantify the level of *Ascaris* ova contamination in wastewater, sludge, and compost. Samples are blended in water and surfactant and large particulates are removed by screening. The sediment is allowed to settle and the supernatant is subsequently decanted. Following density-gradient centrifugation using magnesium sulfate, a layer is formed, which will contain any helminth ova present. A further screening step is undertaken and followed by incubation for up to 4 weeks at 26 °C so that *Ascaris* eggs are fully embryonated, if viable, once microscopic examination takes place.

Standard methods in detecting *Ascaris* eggs in wastewater and sludge currently depend on labor-intensive and time-consuming identification by means of microscopy. Furthermore, such methods cannot identify the species of *Ascaris* or host of origin. The adoption of PCR can increase the specificity and sensitivity of detection as well as decreasing the time required. Current approaches require an incubation period of approximately 30 days in order to allow eggs to embryonate so that they can be identified. However, molecular methods can be undertaken within a day if only determination on total *Ascaris* eggs is required or 10 days if viability assessment is needed. A quantitative PCR (qPCR) approach has been developed in which one is able to detect the quantity and viability of the eggs in a given sample. Combined with this, since the nucleic target chosen (ITS-1 rDNA) differs between *A. lumbricoides* and *A. suum*, the eggs can be identified to species level (see Section Nucleic Acid Based Methods). Since ITS-1 rDNA increases as the eggs develop from the single-celled stage to a 600 cell infective larvae, this approach allows one to differentiate the stages of egg development, which is not always possible using microscopy.

Methods of Detection in Foods

While a standard method of detecting *Ascaris* eggs in food does not appear to be practiced, many protocols exist. In general, researchers assessing the quantity of *Ascaris* eggs on vegetables and fruit tend to firstly weigh the food item which then undergoes washing and sonication steps in a dilute detergent (e.g., 1% sodium dodecyl sulfate and 0.1% Tween 80). Centrifugation or sedimentation steps concentrate the parasite ova, which can then be examined by microscopy.

Importance to the Food and Water Industries

The use of untreated nightsoil, wastewater, and sludge all lead to higher levels of *Ascaris* infection by means of contaminating food. While these modes of transmission maintain infection,

it is difficult to assess the proportion of worms acquired solely through ingestion of contaminated foodstuffs especially in highly endemic areas. Imported crops have been implicated in cases of infection in nonendemic areas in the past but it is frequently difficult to associate outbreaks with a particular food item (e.g., Robertson and Gjerde, 2001). As discussed earlier (see Section Health Consequences), the first symptoms evident in human infection are not evident until day 10–14 postegg ingestion. Further to this, diagnosis does not generally occur until oviposition by the adult female 2–3 months after infection was acquired. As a result, retrospective inquiries are required in order to identify the source of infection. It is, therefore, likely that foodborne transmission, particularly in nonendemic regions, is underdetected.

Since *A. suum* infects humans, porcine waste is also an important vehicle for transmission. Studies in which *Ascaris* haplotypes have been identified have also shown that there is an association between the proximity of inhabitation to pig farms and human infection with *A. suum*. The increased interest in organic farming may exacerbate the potential of cross species infection especially since organic farms have a higher incidence of infection due to a lack of provision of anthelminthics.

Importance to the Consumer

Human infection is contracted after the ingestion of embryonated infective *A. lumbricoides* or *A. suum* eggs. Consumption of contaminated fruit and vegetables is a primary route of infection. Person-to-person transmission can also occur during food preparation if proper sanitation is not adhered to. While many studies undertaken vary in design and methodology, there is evidence that there is an effect of handwashing upon both prevalence and intensity of *Ascaris* infection and so personal hygiene plays a key role in transmission. As well as thorough washing or peeling of vegetables, cooking will decrease the potential spread of infection. Both *A. lumbricoides* and *A. suum* eggs have similar susceptibility to high temperatures. While results from studies vary, the majority of eggs are killed within 45–60 min at 50 °C and within 15 min at 55 °C. Contrastingly, *Ascaris* eggs are more resistant to low temperatures as unembryonated eggs can resume development after 40 days at −26 °C. Embryonated eggs however are less tolerant of colder temperatures; their viability is compromised within 20 days at −11 °C.

Since eggs are sticky (see Section Egg Development and Viability), they tend to adhere to many surfaces in the home, including food utensils. Therefore, sufficient washing of utensils are required prior to food preparation and consumption in order to reduce infection.

It is difficult to delineate the routes of transmission responsible for individual infections especially in highly endemic areas due to the number of possible routes of transmission and the length of time required for establishment of patent infection. Outbreaks in nonendemic countries can be avoided through appropriate hygiene and food preparation practices. It is unlikely that foodborne transmission in endemic countries will be eradicated, yet it can be minimized by the implementation of improved sanitation facilities, raw wastewater treatment combined with education and practice of improved consumer hygiene and food preparation practices.

Strategies for Control of *Ascaris*

The adequate treatment of wastewater and sludge, described above (see Detection and Treatment of *Ascaris* Eggs in Wastewater and Sludge), can successfully interrupt transmission of the parasite by reducing contamination of food items. Combined with this, the spread of helminth infection can be prevented through implementation of three major strategies: anthelmintic treatment, improvements in sanitation, and health education.

Anthelmintic Treatment

Treatment of *Ascaris* infection is an important means to prevent the transmission of the parasite as well as reduce morbidity. There are a range of drugs available for treatment of patent infection of *Ascaris* infection, which are also effective against other helminth infections. The most commonly administered anthelmintic drugs to treat *A. lumbricoides* are albendazole, levamisole, and mebendazole. Treatment leads to immobilization and expulsions of worms from the gut via the feces. The WHO aim to undertake anthelmintic drug treatment programs in order to reduce morbidity rather than eradicate helminths as control of these parasites is dependent on sanitation and interruption of transmission also. Since morbidity associated with helminth infection is the greatest in school-aged children, the WHO recommends regular administration of anthelminthics to at least 75% of this high risk group. Regular treatment is necessary due to high reinfection rates in endemic regions. For example, in one study conducted *A. lumbricoides* reached 55% of pretreatment rates within 11 months.

Improvements in Sanitation and Health Education

Improvements in sanitation interrupt transmission of infection by reducing the infiltration of eggs into the environment, which leads to the contamination of foodstuffs. Therefore, the long-term provision of improved sanitary facilities can gradually reduce worm loads and consequent morbidity. Health education programs also aim to reduce parasite burdens as they encourage the construction of improved sanitary facilities. Furthermore, health education focuses on behaviors related to food preparation as well as family hygiene (see Importance to the Consumer).

The multiple strategies implemented to control ascariasis each serve to interrupt different potential sources of infection. Anthelmintic treatment, health education, and improvements in sanitation prevent the dispersion of parasite eggs into the environment while wastewater and sludge treatment reduces the risk of food contamination. Furthermore, health education in the area of food preparation and hygiene prevents infection when crops are contaminated. The range of control strategies required in order to reduce the spread and intensity of *Ascaris* infection highlights the complexity of eradicating the ubiquitous parasitic nematode.

See also: Helminth-Nematode: *Trichuris trichiura*

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Capillaria hepatica and *Capillaria philippinensis*

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Glossary

Borborygmi Gurgling or rumbling stomach.
Embryonization The development of the helminth zygote into a larva within the egg.
Enzyme-linked immunosorbent assay An assay that relies on an enzymatic conversion reaction and is used to detect the presence of specific substances.
Intermediate host An animal wherein the asexual or larval stage of a parasite may reside during the parasite's life cycle.
Lagomorphs Members of taxonomic order that includes rabbits, hares, and pikas.
Larviparous Pertaining to laying live larvae.
Open defecation Evacuation of feces outside a latrine, often in open fields/natural environment.
Oviparous Pertaining to laying ova or eggs.
Relapse Recurrence of symptoms after an asymptomatic period, usually after treatment.
Zoonotic infection Infections that are transmitted between vertebrate animals and man.

Introduction

The genus *Capillaria* falls under the phylum Nematoda, order Trichurida, and family Trichinellidae. There are approximately 300 known species under the genus *Capillaria*, most of which infect animal species. Four species, *Capillaria aerophila*, *Capillaria hepatica*, *Capillaria philippinensis*, and *Capillaria plica*, have been reported to infect humans. Some species require intermediate hosts in their life cycle (i.e., *C. aerophila*, *C. philippinensis*), whereas some are directly transmitted (egg-to-egg cycle) and do not require an intermediate host (i.e., *C. hepatica*).

Capillaria hepatica

Rodents and lagomorphs are the natural hosts of *C. hepatica* (syn. *Calodium hepaticum*). Infection with *C. hepatica* involves the host's liver, resulting in fibrosis and parenchymal damage, and eventually death in some cases. Zoonotic infections in humans have been reported, although such cases are rare.

Parasite Biology

Hosts, including humans, are infected through the ingestion of embryonated eggs (i.e., mature, infective eggs) from contaminated food or water. The eggs hatch in the cecum, and the larvae penetrate the mucosal membrane, enter into the portal system, and migrate into the liver parenchyma. In about approximately 18 to 20 days postinfection, the larvae mature and female worms deposit fertilized unembryonated eggs in the surrounding parenchyma. Embryonation of helminth eggs occurs when the eggs reach the environment. The process takes approximately 5–7 weeks, depending on the temperature, humidity, and level of oxygen. In most cases, eggs are not passed out in the stool. Instead, eggs are released into and eventually exposed to the environment only after the death and disintegration of the host. In some cases, unembryonated eggs (i.e., immature, non-infective eggs) inside the host are ingested by and pass through the digestive system of carnivorous animals, and are eventually excreted (unchanged) through the feces into the environment where they embryonate. Embryonated eggs can stay infective for 25 months at room temperature.

Pathogenesis

The presence of larvae and adult worms, as well as the deposition of eggs in the liver parenchyma result in inflammatory reaction, granuloma formation, and focal necrosis. The reaction of hepatic tissue may result in clinical manifestations similar to that of acute hepatitis. The classic triad of persistent fever, hepatomegaly, and eosinophilia may be observed. Other findings may include abdominal pain, organomegaly involving the spleen or the kidneys, anemia, and weight loss. Death may occur in cases of severe liver damage and hepatic failure.

Epidemiology

The first reported case of hepatic capillariasis was on a British soldier who died in India in 1923. Postmortem examination revealed "an apparent suppurative condition of the liver with areas of spongy consistence." Large masses of eggs similar to

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Hepaticola hepatica (*Capillaria hepatica*) eggs were observed on microscopic examination of liver samples. From 1924 to 1996, there were 37 reported cases of hepatic capillariasis from countries such as the former Czechoslovakia, USA, Mexico, Brazil, South Africa, Japan, and Korea. Three more cases were reported in Brazil in 1999, and one in India in 2007.

Prevention and Control

Preventive measures include thorough washing or cooking of vegetables and boiling of water in areas where the infection is endemic. Proper disposal of animal carcasses is also important to avoid contamination of food and water, as well as transmission to other animal hosts.

Capillaria philippinensis

History

In 1963, a 29-year-old male patient from Bacarra, Ilocos Norte, Philippines, was admitted at the Philippine General Hospital. The patient was reported to have diarrhea of 3 weeks duration and recurrent ascites. The patient appeared to be emaciated and cachectic, and died a week after admission. Autopsy findings revealed a large number of tiny worms recovered from the intestines. At that time, the species of the parasite was unknown, but it was later identified as a member of the genus *Capillaria*. Soon after, the new species was called *Capillaria philippinensis*. This patient was the first reported case of capillariasis philippinensis, although a number of people from Bacarra were also reported to have died following chronic gastroenteritis around the same year.

In 1965, an outbreak of chronic gastroenteritis occurred in Pudoc West, Ilocos Sur, Philippines, involving cases from all age groups. The disease resulted in death in a number of patients, most of whom were middle-aged men. An investigation in 1966 reported that cases presented with profuse diarrhea, abdominal pain, borborygmi, and wasting. Stool examination showed 'unusual' *Trichuris trichiura* ova, whereas autopsy of a fatal case revealed findings of intestinal worms similar to that found in the initial case from Bacarra. Soon after, the disease spread to the neighboring towns that resulted in more than 1000 cases and 77 deaths. It is believed that the spread of the disease during the epidemic may have been a result of washing fecally-contaminated bed sheets in lagoons in the Tagudin area of Ilocos Sur, exposing the freshwater fish to infective *Capillaria* eggs. During the outbreak, people in the area maintained their practice of eating raw or poorly-cooked fresh water fish which may have contributed to increased transmission of the disease.

Parasite Biology and Life Cycle

Capillaria philippinensis adult worm is characteristically filamentous at its anterior end, with a thicker and shorter posterior end. Females may be both oviparous and larviparous, and their uteri may contain thick- or thin-shelled ova, and larvae. *Capillaria philippinensis* may be regarded as a bridge between the genera *Trichuris* (oviparous) and *Trichinella* (larviparous). *Capillaria philippinensis* eggs are peanut- or barrel-shaped, with striated shells, flattened ends, and prominent bipolar mucoid plugs.

The complete lifecycle of *C. philippinensis* involves the passage of unembryonated eggs from the feces of infected definitive hosts. The eggs embryonate in the soil or water and are ingested by an intermediate fish host. The egg hatches in the intestines of the fish and develops into an infective larva. The larva enters a definitive host through ingestion of raw or poorly-cooked fish and develops in the small intestines into an adult worm. Larviparous females produce larvae resulting in autoinfection, whereas oviparous females produce eggs that are then released into the environment through the feces.

Pathogenesis and Clinical Manifestation

Although the incubation period is unknown, early symptoms observed in the cases from the Philippines usually manifested 3 weeks after consumption of raw or poorly-cooked fish. Persons with *C. philippinensis* infection usually develop symptoms as the helminth population increases, and present with abdominal pains and borborygmi. Patients initially experience intermittent diarrhea, which progresses to passing out 8–10 voluminous stools per day. After a few weeks, there is weight loss, malaise, anorexia, vomiting, and bipedal edema. Physical findings include pallor, muscle wasting, low blood pressure, distant heart sounds, gallop rhythm, pulsus alterans, abdominal distention and tenderness, edema, and hyporeflexia.

The parasites are responsible for micro-ulcers in the intestinal epithelium and the compressive degeneration and mechanical compression of epithelial cells. The ulcerative and degenerative lesions in the intestinal mucosa may account for malabsorption of fluid, fat, protein, and electrolytes. Laboratory findings may include low serum potassium, sodium, calcium, albumin, and total protein levels. Anemia with hypochromic and microcytic red blood cell morphology may be seen. Eosinophil ratios may be elevated. Histologically, the intestinal villi may appear flattened and denuded with dilated mucosal glands. The lamina propria is infiltrated with plasma cells, lymphocytes, macrophages, and neutrophils. Endoscopic finding may reveal nonspecific segmental erythematous inflammation with superficial erosions with exudation in the small intestines. Upper gastrointestinal series with barium may reveal mucosal thickening and segmentation in the small intestines suggestive of malabsorption syndrome.

Severe electrolyte loss, dehydration, and heart failure may occur in untreated cases, and may result in fatality. Necroscopy findings may show serous fluid in the abdominal, pleural, and pericardial cavities. Histological sections of the small intestines may reveal large numbers of worms in the glands, crypts of Lieberkuhn, and within the lamina propria. Villi may be denuded or completely destroyed, and glands may be dilated and filled with eosinophilic debris. Plasma cells, lymphocytes, macrophages, neutrophils, and eosinophils may be seen as infiltrations in the lamina propria.

Diagnosis and Treatment

Suspicion of capillariasis may be made based on symptoms of chronic diarrhea, borborygmi, abdominal pain, and weight loss in a patient with a history of travel to an endemic area and

a history of eating raw or poorly-cooked fresh- or brackish-water fish. A definitive diagnosis may be made based on finding characteristic eggs in the feces by direct smear or wet mount, or by stool concentration techniques. Various larval stages of the parasites as well as adult worms may be seen in the feces. The parasites can also be recovered from the small intestines by duodenal aspiration.

The use of sandwich enzyme-linked immunosorbent assay (ELISA) in the detection of coproantigen prepared from stool samples of infected patients has been demonstrated to have high specificity for the diagnosis of capillariasis. This technique did not show cross reaction with coproantigen from patients with *Fasciola gigantica* and *Schistosoma mansoni*. In another study done in Thailand, cross reaction of capillariasis patient antibodies with *Trichinella spiralis* antigen in immunoblot assay suggested the prospective use of *T. spiralis* antigen for the immunodiagnosis of capillariasis. ELISA using *T. spiralis* antigen was shown to have a sensitivity of 100% (43 positive cases) and a specificity of 100% (57 negative cases) in the diagnosis of capillariasis. Examination of fresh water fish may show *C. philippinensis* larvae although this is not routinely done.

The current drug of choice for the treatment of capillariasis is mebendazole given at 200 mg twice a day for 20 days, for both adult and pediatric patients. Albendazole is the alternative drug, given at 400 mg as a single dose or in two divided doses for a period of 10 days for both adult and pediatric patients. Cases of relapse, or the recurrence of capillariasis after a period of improvement, have been documented, and have been attributed to the inability of the anthelminthics to affect parasite larvae. Extended treatments allow maturation of larvae into drug-susceptible adults. Mebendazole 200 mg twice a day may be given for an additional 30 days in cases with relapse. Alternatively, albendazole 400 mg day^{-1} may be given for an additional 20 days. Supportive treatment with replacement of fluid and electrolytes, as well as high protein diet must also be considered. A repeat stool examination may be done after treatment to monitor the infection status of the patient after treatment.

Epidemiology

After reports of capillariasis in the Northern Philippines in 1966, sporadic cases were then seen in neighboring countries. The first case of capillariasis reported in Thailand was in an 18-month-old girl in 1973, whereas the first epidemic was reported in 1981. In Taiwan, a review of local hospital data throughout Taiwan since the report of its first case in 1983 revealed a total of 30 capillariasis cases (from 1983 to 2003), 21 of whom were from two major Taiwanese aboriginal tribes. Cases of capillariasis were later reported in Iran, Japan, Indonesia, United Arab Emirates, South Korea, India, Egypt, and Lao People's Democratic Republic.

In the Philippines, nearly 2000 cases have been reported from the Northern Luzon provinces from 1967 to 1990. Cases have also been documented in Zambales, in Central Luzon, and Southern Leyte in the Visayas region. In Monkayo, Compostela Valley Province in Southern Philippines, an outbreak described as a 'mystery disease' in 1998 resulted in the death of villagers due to misdiagnosis. Intestinal capillariasis was diagnosed in 17% of the cases presenting with chronic diarrhea. Poor sanitation and defecation in fields and rivers were also reported. A more recently described endemic area in the Philippines includes Zamboanga del Norte, where 4.9% of those examined were confirmed to have capillariasis. Residents in the area have reported consumption of raw freshwater fish.

Disease Control and Prevention

Early diagnosis and treatment can greatly contribute to a decrease in mortality caused by capillariasis. Health service providers, especially in endemic areas, must be made aware of the common signs and symptoms of capillariasis, and should be familiar with the treatment regimens for the disease. Microscopists must also be trained in identifying *C. philippinensis* ova in stool to increase sensitivity of diagnosis. This can also contribute to more accurate disease surveillance in the community. Improvements in environmental sanitation may help reduce disease transmission. Discouraging indiscriminate or open defecation and consumption of raw or poorly cooked fish through health education may also contribute to disease prevention and control.

See also: Characteristics of Foodborne Hazard and Diseases: International Classification of Diseases. Disciplines Associated with Food Safety: Epidemiology. Food Safety Assurance Systems: Good Practices in Fisheries and Aquaculture. Foodborne Diseases: Prevalence of Foodborne Diseases in South East and Central Asia; Prevalence of Foodborne Diseases in Western Pacific Region. History of Food Safety and Related Sciences: History of Foodborne Disease in Asia – Examples from China, India, and Japan. Public Health Measures: Challenges of Developing Countries in Management of Food Safety

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Center for Disease Prevention and Control (CDC): Capillariasis.

Gnathostoma spinigerum

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Glossary

Copro-DNA technique A method used to detect the DNA of parasitic agents, especially intestinal parasites in the fecal samples of human or animal hosts.
Immunoblotting A sero-test for examining specific antibody; the results normally show a specific diagnostic band for an infection.
Intermediate host An animal in which a parasite can develop into a further developmental stage, but not an adult stage.
Migratory swelling A specific technical term for the symptom caused by a gnathostome worm migrating under the superficial layers of the skin.
Paratenic host An animal in which a parasite larva can live with suspended development.
Xanthochromic Pale blood-colored cerebrospinal fluid.

Background

Larvae of the nematode *Gnathostoma spinigerum* cause migratory swelling syndrome in humans. Superficial infections are painful and irritating, but the impact of deep infection is more devastating. If the infected organ is the brain, the person affected may be paralyzed or die. Infection is usually acquired by eating raw or insufficiently cooked fish, the second intermediate host; however, infection is also possible by direct penetration of actively moving larvae. Gnathostomiasis spinigerum is distributed mainly in Asia, and has recently appeared in countries where the disease was previously nonendemic, among travelers returning from endemic areas in Asia.

Characteristics and Transmission

The gnathostome nematode has a body and head-bulb covered with rows of spines. The number of rows, and the number and shapes of the spines are diagnostic characteristics of *Gnathostoma* species. There are 13 species in the genus *Gnathostoma*; among them, five species have been reported in humans (*Gnathostoma binucleatum, Gnathostoma doloresi, Gnathostoma hispidum, Gnathostoma nipponicum,* and *Gnathostoma spinigerum*), and one possible infection with another species, *Gnathostoma malaysiae*. *G. spinigerum* is the main causative agent of infection in humans in Asia, and its close relative, *G. binucleatum*, is mainly distributed in Latin America. *G. spinigerum* AL3 (Figure 1) can be distinguished from other species by the shape and number of hooklets in the head-bulb and the number of nuclei of the intestinal epithelial cell (Table 1).

Gnathostoma spinigerum has a complicated life cycle, involving many species of animals as first, second, paratenic, and final hosts. Adult males and females live in a tumor in the stomach wall of their feline and canine definitive hosts. Eggs are released by gravid females, and pass out of the host with the feces, entering natural water resources. Second-stage larvae hatch out and swim freely in the water, then develop into early third-stage larvae after being eaten by copepods (*Cyclops* spp.), the first intermediate host. Infected copepods are eaten by fish, the second intermediate host, and advanced third-stage larvae (AL3) develop and encyst in the liver and muscles of their fish host. AL3 can survive in many species of fish, amphibians,

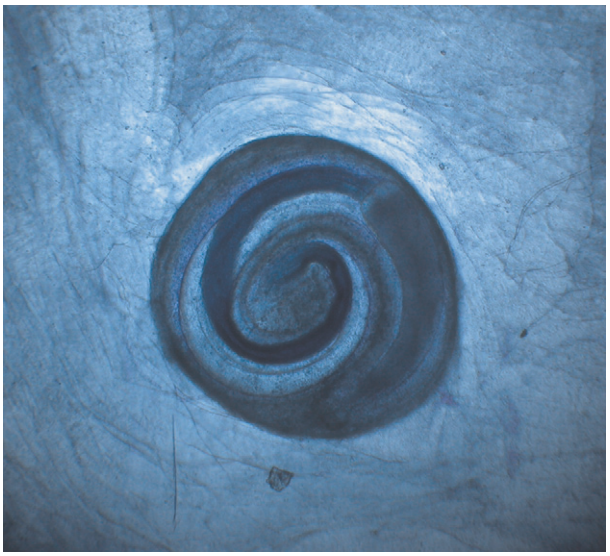

Figure 1 Advanced third-stage *G. spinigerum* larva encysted in frog's muscle.

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Table 1 Specific characteristics of advanced third-stage *G. spinigerum* larvae

- Body approximately 4.0 mm in length
- Head-bulb armed with four rows, and in each row there are 43, 44, 45, and 49 hooklets
- Head-bulb hooklets are oblongated
- Intestinal epithelial cell has 3–7 nuclei (those that morphologically closely resemble the species *G. binucleatum* has 2–5 nuclei)[a]

[a]Akahane *et al.* (1994) A morphological observation of the advanced third-stage larvae of Mexican *Gnathostom. Japanese Journal of Parasitology* 43(1): 18–22.

Table 2 Transmission routes of advanced third-stage *G. spinigerum* larvae (AL3) to humans (Daengsvang, 1980)[a]

- Ingestion of encysted or excysted AL3 in meat of fish and other animals – second intermediate hosts and paratenic hosts
- Ingestion of early third-stage larvae in copepods (first intermediate host) – *Cyclops* spp.
- Direct penetration of AL3 into human skin during preparing food (infected meat)
- Migration of AL3 via placenta (transplacenta) from infected pregnant mother to embryo in uterus

[a]Daengsvang S (1980) *A Monograph on the Genus Gnathostoma and Gnathostomiasis in Thailand*, pp. 1–87. Tokyo: SEAMIC/IMFJ.

reptiles, and birds without further development among these prey–predator cycles. This characteristic makes the gnathostome a unique nematode, as transmission can occur in several ways (Table 2). The most common route of infection is the ingestion of raw fish containing AL3. However, other intermediate and paratenic hosts also play important roles in parasite transmission. Freshwater fish are a common food source, and are prepared differently in several countries. Several local dishes are reported and commonly prepared, such as the fish dishes *som fak*, *pla raa*, *pla jom*, *koi pla*, and small amphibians, in Thailand; *ceviche* and *callos* in Latin America; *sashimi* and *sushi* in Japan; *naniura* (fish) in Indonesia; roast eel in Vietnam; raw bream in Zambia; and raw catfish in Tanzania. Rural inhabitants and the urban residents of some Asian countries eat the meat, viscera, and blood of paratenic hosts, such as snakes and aquatic snails. Visitors who encounter/enjoy eating local and exotic foods find these dishes interesting. Gnathostome infection may occur by skin penetration when handling infected fish with bare hands, while preparing food at home, and preparing home and industrial products using fish materials. While rare, the consumption of infected copepods with early-stage *Gnathostoma* larvae from water resources may cause accidental infection. Another possibility, the local application of reptile or amphibian flesh to a wound, may cause the penetration of larvae from raw meat.

Clinical Manifestations

Gnathostoma larvae are likely to migrate randomly throughout the body of paratenic hosts, including humans. The disease has been divided into two main categories, external or cutaneous gnathostomiasis, and internal or visceral gnathostomiasis, depending on the site of larval migration and the organs affected. The clinical features can be cutaneous, ocular, neurologic, or a combination of all, and presentation varies from 24–48 h to 1–2 years after ingestion of infected-stage larvae. Symptoms can vary widely, and may include fever and malaise, depending on the migration of the worm. The most helpful manifestation in establishing a diagnosis is localized, intermittent, migratory swelling in the superficial layers of the skin, depending on the depth of the skin affected; for example, the hands, arms, shoulders, face, feet, or trunk (especially the abdomen), or even sometimes in more fragile areas, such as the eye, the brain, or the spinal cord. Cutaneous swelling varies in size, with signs of inflammation, redness, indurated erythematous plaques, and pain with itching, which might be the cause of migrating panniculitis, eruptions, and pseudofurunculosis. In addition, the swelling is hard and nonpitting, and lasts for various lengths of time before it subsides. Reappearance of the swelling in another spot can occur after a symptom-free interval of 1 week to several months. Some symptoms of pleura-pulmonary involvement are frequent coughing with or without exudates or hemoptysis, chest pain, dyspnea, and pneumothorax due to effects on the respiratory system. Patients may suffer from epigastric pain, nausea, or vomiting, which are presumably due to penetration of the gastric wall by the ingested larvae; granuloma may form in the peritoneal cavity. Hematuria may occur, with or without pain, or irritation of the kidneys. Serious symptoms, such as paralysis, seizures, or coma, may occur, depending on the location of the worm in the central nervous system (CNS). Ocular involvement also occurs, probably by migration of the worm via the optic nerves, and causes pain, uveitis, iritis, intraocular pressure and hemorrhage, retinal scarring, detachment, deteriorated visual acuity, and blindness. Clinical findings on physical examination depend on the area of the body into which the worms migrate, and vary among causative *Gnathostoma* species. In *G. spinigerum* and *G. binucleatum* infection, the disease persists with relapse for over several years in a relative deeper part of the skin of the peripheral part of the human body. In *G. hispidum*, *G. nipponicum*, and *G. doloresi* infections, where migration is close to the body surface of the trunk with skin lesions, the disease may spontaneously disappear within 3 months, even without treatment.

Pathogenesis

The primary cause of infection is the consumption of third-stage *G. spinigerum* larvae from infected intermediate hosts.

Larvae can penetrate the stomach wall and migrate randomly to many parts of the human host, to continue causing lesions, such as destruction of the liver parenchyma, hemorrhage, granulation, and cicatrization. Infection results in initial nonspecific symptoms such as fever, arthralgias, myalgias, malaise, anorexia, nausea, vomiting, and abdominal pain, followed by skin lesions, which probably result from hepatic disorder. The migrating worm causes mechanical and chemical damage to the tissues, including the production and action of several kinds of secretions with inflammatory and allergic reactions by the host. The pathologic picture of the swelling and track-like necrosis comprises marked inflammation in both acute and chronic stages, necrotic changes, fibrogranulomatous formation, intense cellular infiltration with neutrophils, small mononuclear cells, plasma cells, and massive numbers of eosinophils. Leukocytosis with marked hypereosinophilia occurs in the peripheral blood, but does not correlate with clinical severity, and may also be associated with numerous other diseases. The parasite can also migrate into the ocular system and CNS, resulting in decreased visual acuity, blindness, and neurological symptoms, such as eosinophilic meningitis or meningoencephalitis and subarachnoid, as well as intracerebral and intraventricular hemorrhage. In cerebral gnathostomiasis, the cerebrospinal fluid (CSF) is usually bloody or xanthochromic, and a pleocytosis with increased white blood cells and prominent eosinophils.

Diagnosis and Analytical Methods

Patient diagnosis can first be performed using clinical symptoms, and confirmed by indirect antibody detection using serological methods, especially the immunoblotting technique. Stool examinations of the definitive canine/feline hosts for the presence of gnathostome eggs can confirm the disease transmission area and species of gnathostome nematode. Inspection of food materials and foodstuffs from intermediate and paratenic hosts must be conducted for food-safety precautions. Several species of fresh- and brackish-water fish, amphibians, crustaceans, reptiles, avians, and mammals have been reported as hosts harboring gnathostome worms. Several techniques have been used to detect parasites.

The current diagnosis of gnathostomiasis utilizes one technique or a combination of techniques, such as the detection of worms from a skin lesion or clinical manifestations and sero-testing the suspected case for specific antibody to *Gnathostoma*. Sero-detection is now routine practice, and the current sero-tests include the skin test, immunofluorescent test, ELISA, and immunoblot. Different antigen preparations from *G. spinigerum* have been used in these tests, such as crude extracts or sectioned samples, excretory–secretory products from infective larvae, and adult worms. DNA recombinant antigens have been developed and are being analyzed for their specificity to gnathostomiasis sera. Until now, native antigens have been used in sero-tests. Immunoblot using 24 kDa antigen from *G. spinigerum* infective larvae to serum IgG has shown 100% sensitivity (all worm-proven *G. spinigerum* cases) and an excellent specificity is determined by no reaction with 28 different diseases (not yet published). *G. hispidum* and *G. binucleatum* have been used as sources of antigens for ELISA and immunoblot. Immunoblot was used in the detection of IgG rather than IgG subclasses. For indirect ELISA, it is recommended that IgG1 and IgG2 subclasses yield higher sensitivity and specificity than total IgG for screening gnathostomiasis sera. However, the excellent sensitivity and specificity of these tests are based on the number samples of homologous and heterologous diseases used as reference.

The compression and digestion methods are commonly used for detecting and collecting gnathostome larvae in intermediate/paratenic host tissues. The compression technique is simple but tedious; *Gnathostoma* larvae are observed in host tissue samples pressed between two glass plates under a stereo-microscope. The tissue sample should be thin enough to observe larvae in encysted or unencysted forms. Gnathostome larvae are easily seen as red or pink spots in fish flesh or livers; sometimes, they appear pale yellow, which may cause detection difficulties, particularly for inexperienced laboratory staff.

The digestion method uses artificial gastric juice (1% HCl–1% pepsin) solution and activates the digestion at 37 °C in a shaking water bath or by stirring with a glass rod. Other protocols include the use of 0.6% pepsin in 0.08% HCl and incubation at 37 °C for 2–3 h, or 1.5% pepsin in water, adjusted to pH 2.0 with 1 M HCl, digestion at 37 °C for 4 h, or concentrated pineapple juice at 37 °C for 4 h. Pepsin digestion may be replaced with acid–trypsin digestion, but it is less efficient than pepsin. However, trypsin is cheaper than pepsin. This method is useful for digesting chopped tissues and tissue connected with pieces of host bone.

Control/Prevention Measures

It is recommended that meat be cooked using sufficient heat and time to kill the parasite before consumption. The physical and chemical methods used to preserve meat products, with the appropriate concentrations and times for killing gnathostome larvae, are summarized in Table 3. A protocol on methods for cooking meat and killing gnathostome larvae should be developed and distributed on media that are accessible to the public and the food industry. Offal of raw meat of intermediate/paratenic hosts must be segregated and not fed to the definitive hosts – dogs and cats – to prevent completion of the organism's life cycle.

Travelers entering endemic areas should be informed about gnathostomiasis, and how to prevent infection, by avoiding eating raw or improperly cooked freshwater fish, frogs, snakes, or even birds and chicken. Drinking water directly from natural water sources – especially ponds or lakes – should be avoided to prevent accidental intake of copepods infected with gnathostomes.

Information on gnathostomiasis and its prevention should be provided to the general public.

Research Needs

There is a need for further investigation of the epidemiology of gnathostomiases and *Gnathostoma* in many countries

Table 3 Estimated survival times for gnathostome larvae exposed to chemicals and other conditions

Condition	*Exposure time*	*Effect*
Temperature[abc]		
−4 °C	22 days	Encysted larvae survive
−9 °C	9 days	Encysted larvae survive
−20 °C	3–5 days	Encysted larvae survive
65 °C	5 h	Encysted larvae survive
Water at >70 °C	5 min	Killed encysted larvae
Boiling water	>5 min	Killed larva at 1 cm depth in muscle of fish
Boiling water and steaming	Few minutes	Killed encysted larva at 2 cm depth in fish
Salinity[ac]		
Soy	12 h	Killed encysted larva
Fish sauce (23% NaCl)	18 h	Encysted larvae survive
Salt (30%)	20 h	Encysted larvae survive
Physiological saline (0.9%)	12 days	Encysted larvae survive
Acid[abcd]		
Vinegar	6 h	Killed encysted larva
Vinegar (4% acetic acid)	5.5 h	Killed larva at 1 cm deep in muscle of fish
Vinegar (4% acetic acid)	8 days	Encysted larvae survive
Lime juice	5 days	Encysted larvae survive
Lime juice	5 days	Larvae survive
Lime juice containing 7.74% citric acid	7 days	Killed encysted larvae at 2–4 mm deep in the flesh of fish and frog
Others[c]		
Syrup (20% sugar)	6 days	Encysted larvae survive
28% ethyl alcohol	8 days	Encysted larvae survive
35% ethyl alcohol	9 days	Encysted larvae survive
Desiccation at room temperature	3 h	Encysted larvae survive
Minced meat salad	>8 days	Encysted larvae survive
De-chlorinated tap water	6 days	Encysted larvae survive
Radiation[e]		
Cobalt-60 gamma at 8 kGy	Not mentioned	Stop infectivity

[a]Daengsvang S (1949) Human gnathostomiasis in Siam with reference to the method of prevention. *Journal of Parasitology* 62: 88.
[b]Egashira M (1953) Intracutaneous reaction in human gnathostomiasis (Japan). *Japanese Journal of Clinical and Experimental Medicine* 28: 220–223.
[c]Setasuban P, Punsri W, Muennoo C (1981) Effects of temperature, chemicals and some native Thai food on its viability of the infective stage of *G. spinigerum*. *Journal of Parasitology and Tropical Medicine Association of Thailand* 4: 77.
[d]Tansurat P (1955) Gnathostomiasis (Thai text) *Journal of the Medical Association of Thailand* 38: 25–32.
[e]Setasuban P, Hiranyachattada P, Prachasitthisak Y, Pubumpen S, Rojekettikhun W, Dekumyoy P (1995) Effects of cobalt-60 gamma radiation on *G. spinigerum* larvae. *INIS Atomindix* 26: 1.

where information is lacking. *Gnathostoma* worms may exist in the same geographical region and similar environmental settings. Though gnathostomiasis may not occur in these countries, and the inhabitants do not consume raw fish, there is still a risk for travelers who consume raw fish.

A commercial kit needs to be developed for the diagnosis of gnathostomiasis and the improvement of larva-detection methods in intermediate/paratenic host meat. There is also a need for a copro-DNA technique to detect gnathostome eggs in fecal samples of definitive hosts, for epidemiological surveys to identify new endemic areas.

At present, the available treatment of gnathostomiasis is a long-term regimen (10–21 days) of 400 mg albendazole. In some reports, albendazole was administered in combination with praziquantel or ivermectin. A better treatment with either shorter-term regimen of a new drug, or a new combination of anthelmintic, is needed. More in-depth research on the genetic/biology of worms and factors inducing clinical involvement, using multidisciplinary advanced technologies, would create new findings to enrich our knowledge of *Gnathostoma* and gnathostomiasis. A research network for *Gnathostoma* and gnathostomiasis has been developed between Mexico and Thailand, and other international collaborators are welcome.

See also: Disciplines Associated with Food Safety: Parasitology. Food Technologies: Food Irradiation. Foodborne Diseases: Foodborne Diseases in Travelers; Prevalence of Foodborne Diseases in South East and Central Asia

Further Reading

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Relevant Website

www.dpd.cdc.gov/dpdx/HTML/gnathostomiasis.htm
Centers for Disease Control and Prevention.

Haplorchis

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Glossary

Cercaria The free-living larval stage produced by the intra-snail cycle of asexual multiplication in the life cycle of the parasite; the cercariae escape and swim from the snail, seeking the next host.

Intermediate host Fishborne trematodes (e.g., *Haplorchis*) have two intermediate hosts in their life cycle: an aquatic snail in which asexual multiplication of the larval trematode occurs, and fish the second intermediate host in which the metacercaria encysts.

Metacercaria The encysted resting or maturing stage of a trematode parasite in the tissues of an intermediate host. This is the trematode stage that is infective to the definitive host (in which the trematode parasite develops to the adult, sexually reproducing stage). For fishborne trematodes, the fish harbors the metacercaria.

Praziquantel An anthelmintic drug used for treatment of a wide spectrum of helminth infections, including almost all species of trematodes and many species of cestodes.

Background

Foodborne intestinal trematodes (flukes) are currently estimated by the World Health Organization (WHO) to infect 40–50 million people worldwide, and probably half of them acquired the parasites from fish. Belonging to the Heterophyidae family of helminth parasites, species of *Haplorchis* predominate among fishborne zoonotic parasites. *Haplorchis* is a common parasite of fish-eating birds and mammals in Africa, Eurasia, and Australia. Three species of *Haplorchis*, *Haplorchis pumilio*, *Haplorchis taichui*, and *Haplorchis yokogawai* are especially common in humans in these regions. Looss first described *H. pumilio* from pelicans and kites in Egypt in 1899, and it was recovered from humans in Taiwan by Nishigori in 1924. Also in 1924 in Taiwan, Chen recovered *H. taichui* from humans, and in 1932 Katsuta reported human infections with *H. yokgawai*. Since then, one or more of these species are frequently reported from China, Taiwan, Vietnam, the Philippines, Laos, Thailand, Malaysia, Indonesia, and Egypt. The genus *Haplorchis* has a very wide intermediate and definitive host range. As discussed below, the wide host range and human fondness for raw or lightly prepared fish foods are main factors for the wide geographic distribution of the haplorchids. For example, the number of species of fish (second intermediate host) reported to be susceptible to the infection with metacercariae (infective stage for mammals and birds) is more than 70. The number of fish-eating birds (including migratory species) and mammal species that are susceptible is also large.

Although *Haplorchis* has been recognized as potentially zoonotic for nearly a century, it is only during the past 20 years that recognition of it as a very common fishborne zoonosis has emerged, especially in the countries of South East Asia. In terms of infection intensity, it has been frequently shown to be the dominant intestinal fluke. This belated recognition is undoubtedly due to diagnostic confusion in routine fecal exams for parasite eggs (the primary detection procedure). It is very difficult to discriminate between the eggs of heterophyids ('minute trematodes') and those of other trematodes, especially those of the liver flukes. This has resulted in under reporting of intestinal flukes such as *Haplorchis* (until recently poorly known), and the over reporting of *Clonorchis sinensis* and *Opisthorchis* spp., which have long been recognized as important fishborne liver flukes. However, the increasing inclusion of worm expulsion methods in epidemiological studies, which yield adult stages for morphological and molecular analysis, is allowing more definitive species identification, and the resulting data are altering the human parasite diversity profiles in many regions and underscoring the poor specificity of fecal exams for small trematode eggs.

Characteristics

Taxonomy and Morphology

Adult *Haplorchis* trematodes are parasites of fish-eating birds and mammals, including humans and their domestic animals such as dogs, cats, and pigs which are important epidemiologically as reservoir hosts.

The *Haplorchis* genus is a member of the subfamily Haplochorinae, which is distinguished from other members of the Heterophyidae family by certain morphological features of the ventrogenital complex (Figure 1, adults). The Haplorchinae are characterized by the presence of a genital pore, gonotyle, and ventral sucker, which are sunk below the ventral surface in a single, common invagination, termed the ventro-genital sac; there is only a single opening on the ventral surface. Within the subfamily, *Haplorchis* is differentiated from other genera by

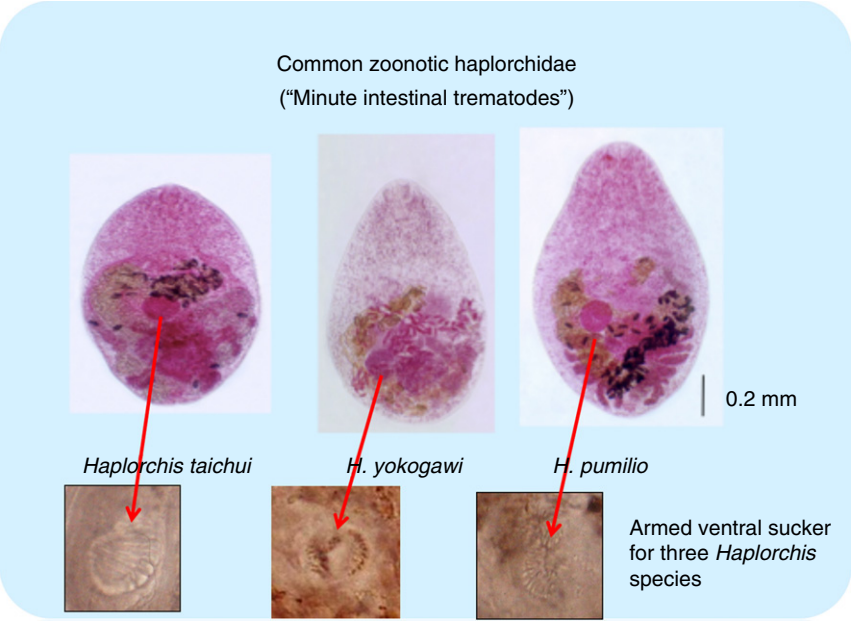

Figure 1 The difference in the number of spines around the ventral sucker between three species of *Haplorchis*, namely *H. taichui*, *H. yokogawi*, and *H. pumillio*, is shown. Ventral sucker of *H. taichui* armed with 12–16 spines, ventral sucker of *H. yokogawi* with apex comprising a large ventral lobe armed with numerous tiny spines and a larger variable but characteristic pair of sclerites ventro dextrally, and the ventral sucker of *H. pumilio* with 32–40 spines (Photos provided by Dr NT Lan Anh, National Institute of Veterinary Research, and Dr Do Dung, National Institute of Malariology, Parasitology and Entomology).

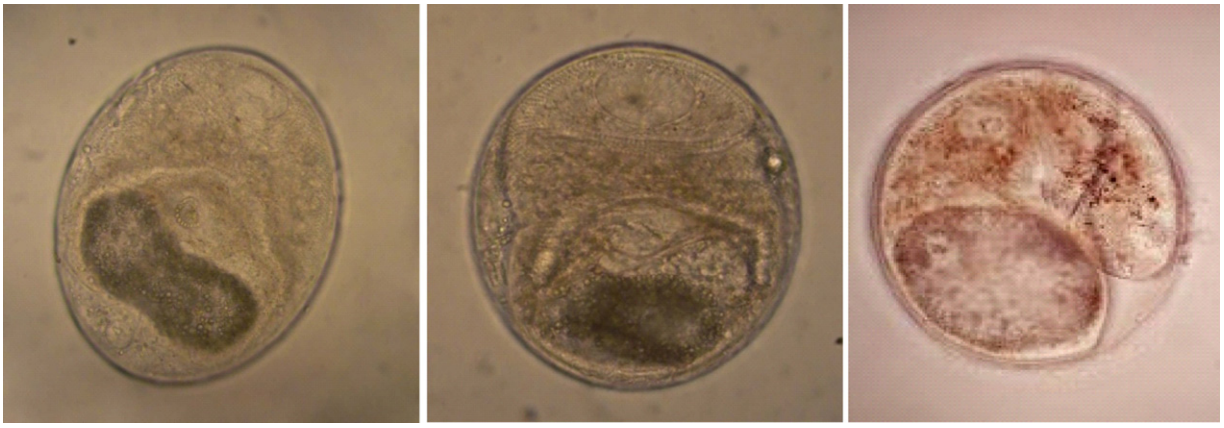

Figure 2 Metacercaria isolated from fish muscle seen through stereomicroscope (Photos of *H. taichui* and *H. pumillio* by Mr Jakob Skov and *H. yokogawi* by Dr Woon-Mok Sohn).

having a single testis and no gonotyle. The infective larval stage (metacercariae) for these hosts occurs in fish belonging especially to the Cyprinidae, Siluridae, and Cobitidae. Because the haplorchids are quite small, typically 30×18 μm in size, their species diagnosis can be difficult, especially in the metacercarial stage (Figure 2).

However, for the three most commonly encountered species in human infections, *H. pumilio*, *H. taichui*, and *H. yokogawai*, the number, size, and arrangement of spines on the ventral sucker are diagnostic (Figure 1). Recently, molecular methods have been developed which have promise for distinguishing the adult, metacercaria, and cercaria stages of *Haplorchis* spp.

Life Cycle

The life cycle of all *Haplorchis* species is similar and is characteristic of members of the family Heterophyidae (e.g., *Heterophyes* and *Metagonimus*). A schematic life cycle of Haplorchis is shown in Figure 3.

The adult worms residing in the small intestine of the definitive host shed eggs (typically less than 30 μm in length)

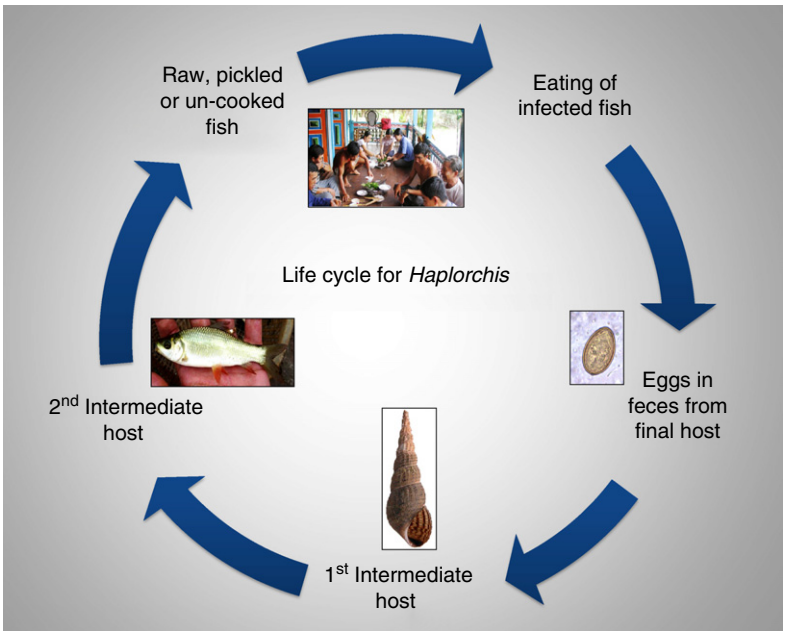

Figure 3 Schematic illustration of the life cycle for *Haplorchis* (Photos by Henry Madsen, Jesper Clausen, Darwin Murrell, FIBOZOPA).

which are voided with the feces. If deposited into water bodies they may be ingested by certain snails (for *Haplorchis*, snails belonging to the genera *Melanoides* and *Melania* are important vectors). In the snail the egg hatches releasing a miracidium, which invades the snail organs and metamorphoses to a sporocyst, in which, through asexual multiplication, daughter redial stages develop. In these, a second cycle of asexual multiplication occurs, producing large numbers of pleurolophocerus cercariae, which escape and swim from the snail. Attracted by fish, the cercariae penetrate under the fish's scales, or into the gills, muscle, and skin. Within a few days the cercariae develop into an encysted metacercarial stage which is infective to the definitive host. The life cycle is completed when the infected fish, or fish parts, is eaten by a bird or mammal, in which the metacercariae develop to the egg-producing adult stage within 4–5 days.

Virulence Factors

The pathology caused by intestinal infection has received little attention. However, it seems certain that because the adult worms insert themselves intimately with the intestinal villi the amount of damage to that layer is proportional to number of worms present. It is also not known if the adult worms secrete enzymes or other chemicals to facilitate their adherence and invasion of intestinal tissues; secretory products are not uncommon among other helminth parasites, such as *Trichinella*. These questions are only now beginning to be investigated.

Resistance Factors Facilitating Survival in the Intestine

There is little information on the longevity of adult *Haplorchis* in humans. In experimental infections in mice, a reduction of worm populations can be seen after 1 week of infection, however, infections in chickens have been reported to persist for up to 48 days. The earlier expulsion of worms from mice may be immune mediated because there is evidence that this 'self-cure' is density dependent. Long-term studies in suitable experimental models are needed to characterize the population dynamics of adult *Haplorchis* in infections in which metacercarial exposure is similar to the natural exposure doses and frequency experienced by humans.

Clinical Manifestations

Disease caused by minute flukes of the Heterophyidae family is called heterophyidiasis. Infections are less associated with mortality than other groups of parasites; however, cases with heavy infections of some well-studied heterophyid species (e.g., *Heterophyes* and *Metagonimus*) are often associated with diarrhea, mucus-rich feces, catarrhal inflammation, abdominal pain, dyspepsia, anorexia, nausea, and vomiting, the most prominent symptoms being malabsorption and diarrhea.

Because halporchid infections have only recently been recognized, it is not possible yet to assess their overall health impact. However, a recent report on *H. taichui* in Thailand indicates that mucosal ulceration, mucosal and submucosal hemorrhages, fusion and shortening of villi, chronic inflammation, and fibrosis of the submucosa can occur. A recent investigation on intestinal pathology caused by experimental infections of the rat with *Centrocestus armatus*, a close heterophyid relative of *Haplorchis*, is also suggestive. In the intestine the stroma of villi around the fluke became edematous and infiltrated by inflammatory cells such as lymphocytes, plasma cells, and eosinophils. The crypt became mildly hyperplastic and villi were moderately atrophied at 4 days postinfection. The intestinal lesion produced was confined to the areas around the

fluke, and the pathologic findings were not significantly different between infections with 1000 and 5000 flukes.

Epidemiology and Preharvest Control of Fish Infections

The geographical distribution of *Haplorchis* spp. is not well described in the literature, but it is likely very common in South East Asia, South Asia, the Middle East, and Africa. They are particularly endemic in the Philippines, Malaysia, Indonesia, Thailand, Lao People's Democratic Republic, Vietnam, People's Republic of China, and Egypt. Fish sources of infection include both wild caught and farmed fish.

The eating of raw, undercooked, or pickled fish is a key risk factor for humans to acquire infection. In South Asia and South East Asia especially, there are long traditions of eating raw, pickled, or undercooked fish. A good example is the tradition of eating different varieties of sour fermented fish in Thailand, Laos, and Cambodia. The raw fish is fermented giving it its sour taste and is considered a delicacy, especially in rural areas of North Eastern and Central Thailand, where it is called *Pla Som* It is known as *Pla Hok* in Cambodia and *Pa Som* in Laos. Other traditional ways of preparing fish are mixing it with raw crab meat spiced with soy sauce (ke-jang) in the Republic of Korea, and the eating of raw grass carp dishes in China and Vietnam. During food preparation, cross-contamination arising from using the same knife and chopping board for raw fish and other food products in kitchens and in restaurants may also be an important risk. Fish for these traditional raw, pickled, and uncooked dishes are normally obtained from fresh water reservoirs, lakes, rivers, or aquaculture ponds. The source of fish is also a risk factor for humans. Studies have shown that wild caught fish have a higher prevalence and intensity of *Haplorchis* metacercariae than fish reared in aquaculture ponds. Infection rates in pond fish appear to be influenced by the stocking density-at high densities metacercarial intensity can be nearly as high as in fish that are wild caught. As discussed in the Section on Clinical Manifestations, infections with *Haplorchis* are increasingly being reported. One important factor for this apparent emergence may be due to improvements in diagnosis (see Detection/Diagnosis Methods). Another possible explanation is that humans in endemic areas are increasing their consumption of fish.

Owing to an increased production of aquacultured fish more fish are available for consumption. The main production of aquaculture is being done in the Asian region, a region with many countries well known as endemic areas for fishborne zoonotic trematodes (FZTs). This increases the risk of exposure for consumers. However, the environments inside the aquaculture ponds, pens, or cages are often more controlled and manageable compared to natural environments. This increased level of control gives a unique opportunity to prevent FZTs from entering the cultured environment. Integrated control programs in fish nurseries and fish grow-out ponds have, therefore, been designed to lower the prevalence and intensity of metacercaria in fish being raised. The management practices of aquaculture can, therefore, be an important factor in controlling outbreaks and the spread of the parasite. Based on the risk-assessment studies that have been carried out in aquaculture systems, the following interventions should be included in a control program:

1. Because domestic animals such as pigs, cats, chickens, ducks, and dogs can act as alternative reservoir hosts, these domestic animals play a major role in the epidemiology of FZTs in aquaculture systems. Therefore, prevention of FZT infections in domestic animals must be included in aquaculture-management practices. This can be done by regular deworming of the animals and restricting their access to fish.
2. Humans residing and working on fish farms should also have regular parasite exams and be treated if infected.
3. Pond management must also include efforts to reduce the habitat conditions that promote snail invasion and multiplication (e.g., removing snails and mud from drained ponds when preparations are underway to prepare for new fish stocking). Control of run-off water to ponds and diversion of latrine drains (both from farm animals and from human facilities) are important interventions.
4. Education of fish farmers, fishermen, and the general public on the nature and consequences of eating raw or improperly prepared fish is also necessary.

Detection/Diagnosis Methods

The minute intestinal flukes can be detected in the three stages of their lifecycle: at the egg stage, the metacercaria stage, and the adult stage.

The metacercaria stage located in fish flesh or under the scales of the fish is the most important stage from a food-safety diagnosis perspective. To isolate the metacercaria from infected fish, two techniques are commonly used: the tissue compression technique and the digestion technique. Both techniques have their own advantages and it is up to the researcher or the analyst to choose the right method for the given task.

The compression technique is done by compressing fish flesh between two glass slides and examining the slides under a microscope. The advantage of this is that the analyst will know exactly where on the fish the sample is taken. The method is inexpensive and does not require chemical reagents.

The digestion technique is performed by subjecting either a whole fish or sections of a fish to pepsin enzymatic to liberate metacercariae. The advantages are that a large number of samples can be dealt with at the same time, and it is more efficient in recovery of any metacercariae present in the fish; the compression method is more likely to miss metacercariae in lightly infected fish. The metacercaria liberated from the flesh of the fish can then be easily isolated, identified microscopically, and utilized for either experimental infections or fixed for molecular or staining work.

To diagnose the potential infections of heterophyid intestinal flukes in humans (including *Haplorchis)*, a fecal sample from a host (human or animal) is processed and examined using simple techniques identifying the eggs. The most widely used methods are the Kato-Katz smear and formalin–ethyl–acetate techniques, because of their high sensitivity and because they allow for quantification of infection intensity.

Identification of adult flukes is done morphologically as the adults are easily recognizable compared to eggs and metaceraria stages. The major features and diagnostic characters of *H. taichui*, *H. yokogawi*, and *H. pumillio* metacercaria are shown in Figure 2. Some PCR-based methods have been developed in recent years to detect DNA from eggs and metacercaria in stool samples or from metacercaria which offer high sensitivity and specificity. However, molecular-based diagnostics are still expensive and it is considered unlikely at this current stage that they will become a standard tool for routine diagnosis.

Control and Prevention Measures

Postharvest control and prevention of the minute intestinal flukes are similar to the control measure for the liver flukes. There are reports that heating fish (e.g., sweet fish) for 10–15 min at 70 °C would be sufficient to inactivate metacercariae; however, the extent of research and available data are insufficient to determine the exact time–temperature treatment required. A review by WHO in 1995 of available data on processing conditions identified some parameters for heating, pickling, salting, freezing, and irradiating fish for the inactivation of parasites. Similar treatment conditions are also expressed in regulatory requirements of the US and the EU. For example, according to the US FDA, freezing and storing at –4 °F (− 20 °C) or below for 7 days (total time), or freezing at − 31 °F (− 35 °C) or below until solid and storing at –31 °F (− 35 °C) or below for 15 h, or freezing at –31 °F (− 35 °C) or below until solid and storing at –4 °F (− 20 °C) or below for 24 h is sufficient to kill parasites. FDA's Food Code recommends these freezing conditions to retailers who provide fish intended for raw consumption. Further, the heating of fish flesh to a temperature sufficient to kill bacteria is considered adequate for parasites.

Although brining and pickling of fish may reduce the risk, they do necessarily minimize it to an acceptable level. For example, some zoonotic trematode metacercariae require up to 8 days in 30% brine to be killed. Smoking to a temperature of 65 °C is considered to be effective for parasitic nematodes in fish, but there has been little research done on this process for intestinal trematodes.

Research Needs

Research is needed to better understand the importance of *Haplorchis* spp. and its public health impact on humans. Also needed are effective and sustainable aquaculture management practices to prevent infection in farmed fish. The rapid expansion of aquaculture in many areas of the world and the increasing demand for greater food safety and quality will only increase the need to control zoonotic parasites in fish. Although rapid and sensitive inspection procedures for use in fish processing would be helpful, much of the fish produced in less-developed countries do not pass a processing plant before being purchased by the consumer. However, postharvest inspection for exported and commercially high-value fish is feasible. Much more research would be needed, however, to develop cost-effective treatment technologies to render the product safe. Climate changes may be associated with an increase in snail habitats due to changed pattern of water flow. Snails are a key risk factor in the life cycle of fishborne zoonotic parasites and as a result of more snails, more people will potentially be at risk of getting infected with *Haplorchis* spp. Although the health and economic impact of many foodborne zoonoes in general has been at least partially estimated, this is not the case for recently emerged parasites such as *Haplorchis*. Impact evaluations of intestinal flukes on the health and economies of the countries with high endemicity are needed if these zoonoses are to be assigned their appropriate national priority for prevention and control resources. An integrated hazard analysis and critical control points (HACCP)-based approach including measures to prevent and control pollution with animal and human parasite eggs, the animal and snail intermediate hosts, and better aquaculture management practices is needed for a sustainable control of fishborne zoonotic parasites. The economic investment that will be required for poor and middle-income countries to develop sustainable control programs is difficult to project, but will probably be substantial, especially for fish export production systems. However, it is likely that the cost of not addressing the emerging risk of these flukes will be higher than the cost of taking action now.

See also: Helminth-Nematode: *Trichinella spiralis* and Other *Trichinella* Species. Helminth-Trematode: *Clonorchis sinensis*; *Heterophyes heterophyes*; *Metagonimus yokogawai*; *Opisthorchis viverrini* and *Opisthorchis felineus*

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Trichinella spiralis and Other *Trichinella* Species

HR Gamble, National Research Council, Washington, DC, USA

Glossary

Encapsulated Contained within a single muscle cell.
Muscle larvae First stage larvae of the genus *Trichinella* that are found encysted, or free, in host muscle tissue.
Newborn larvae First stage larvae of the genus *Trichinella* that are released by adult worms in the host intestine and migrate to muscle tissue.
Preharvest All processes of animal production that precede slaughtering.
Sylvatic That part of the transmission cycle of a disease or parasite that circulates in wildlife; opposite of domestic.

Background

The nematode parasite *Trichinella*, which people most commonly associate with pork, has historically been a serious cause for concern as a foodborne pathogen. It is distributed worldwide and has been found in virtually all carnivorous and omnivorous animals as well as less commonly in certain herbivores. *Trichinella spiralis* was first observed in 1835 by Paget, a British medical student, in the muscles of a man during postmortem dissection. The newly discovered worm was further described and named *Trichina spiralis* by Owen (1835). The name change to *Trichinella spiralis* was made in 1896 by Railliet because the genus *Trichina* had previously been attributed to a group of small flies.

A landmark in the history of clinical trichinellosis was Zenker's demonstration in 1860 that encapsulated larvae in the arm muscle caused the illness and death of a young woman. His subsequent collaboration with Leukart and Virchow confirmed the life history of the parasite by describing the various life cycle stages. Zenker's further investigations, using prior knowledge of the observation of *Trichinella* in pork, led him to identify pork as the source of human infection.

With the recognition of trichinellosis as an important foodborne disease, reports of its occurrence increased dramatically, with thousands of cases reported each year in Europe. As a result, mandatory testing programs for pork began as early as 1863 in Prussia and Germany and quickly spread to other countries in Europe. Although testing was not completely effective, it did remove the most heavily infected animals and greatly reduced the rate of human exposure. Some countries that did not adopt slaughter testing for *Trichinella*, most notably the US, relied on cooking and further processing methods to avoid human exposure. Over time, pig infections in most developed countries have been dramatically reduced due to improved management systems, including biosecure housing and the banning of waste feeding. However, pigs raised outdoors, under poor sanitary conditions and those exposed to wildlife, remain at risk. Further, due to the cosmopolitan distribution of *Trichinella* spp., human infection can result from eating meat from a wide variety of wild and domesticated animals.

Characteristics

Life Cycle

Trichinella spp. completes its entire life cycle in a single host (Figure 1). The infective stage is the first stage (L1) larva which is found in striated muscle cells of a wide range of hosts. On ingestion of *Trichinella*-infected muscle tissue by a new host, larvae are released by the host digestive processes. Larvae then enter the small intestine and burrow into the lamina propria of the villi in the jejunum and ileum where they undergo four molts to reach the adult stage. Adult male and female worms mate and produce newborn larvae. Adult female worms in the small intestine continue to produce larvae in most hosts for several weeks before they are expelled. Each female worm bears approximately 1500 newborn larvae, but this varies based on *Trichinella* species and host.

Newborn larvae leave the intestine and migrate, via the circulatory system, to the striated muscle tissue. During migration, the larvae are known to enter many tissues, including those of the myocardium, brain, and other sites, but here they either are destroyed or reenter the bloodstream. Generally, only larvae that reach striated muscles are able to continue development. They penetrate the sarcolemma of the fibers, where they mature. They become coiled within

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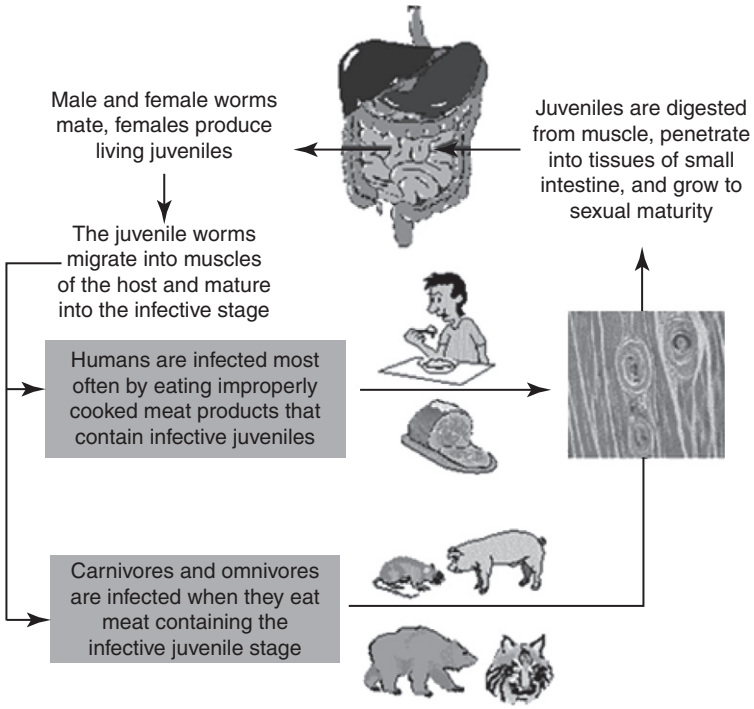

Figure 1 The life cycle of *Trichinella spiralis*.

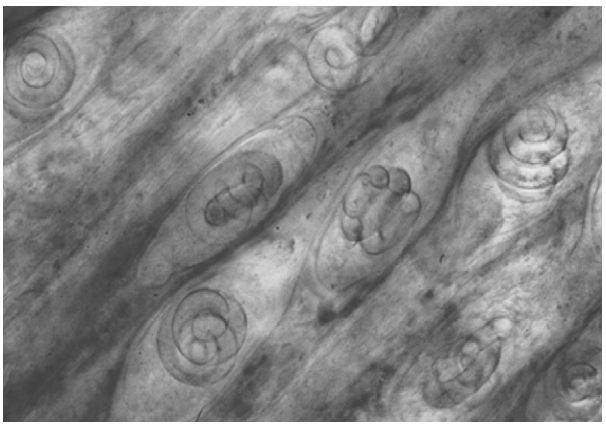

Figure 2 *Trichinella spiralis* larvae encapsulated in muscle tissue.

the fibers and, in the case of some of the species, are encapsulated as a result of the host's cellular response (Figure 2). This host–parasite complex, called the nurse cell, is capable of supporting the infective larvae for months or even years. An increased vascular supply to the nurse cell provides nutrients and oxygen vital to the parasite's survival. The encapsulated cyst eventually becomes calcified and as a result the larva dies.

The time required for complete development takes from 17 to 21 days (time from exposure to appearance and encystment of larvae in musculature). Once adult worms are expelled and migrating larvae reach and encyst in muscle tissue, no further increase in the number of worms can occur. An animal that is infected with *Trichinella* is at least partially refractory to a subsequent infection due to a strong and persistent immunity.

Morphology

The size of *Trichinella* larvae and adult stages varies by species and genotype. Muscle larvae (mature L1 stage) range in size from approximately 0.70–1.10 mm in length to 0.25–40 mm in width. Adult worms range in size from 0.60–1.60 mm in length to 0.25–0.35 mm in width for males and 1.25–3.35 mm in length and 0.30–0.40 mm in width for females. Newborn larvae (immature L1 stage) are approximately 110 μm in length and 7 μm in width. A distinguishing feature of the morphology of *Trichinella* spp. is the discoid shaped stichosome, which occupies the anterior third of the body. The secreted contents of these cells are important for serological detection of infection and also contain antigens which confer protective resistance to the host.

Taxonomy

For many years, the genus *Trichinella* was thought to be monospecific. Although the taxonomy of the genus continues to evolve, based on the findings using molecular tools, eight species and three genotypes of *Trichinella* are currently recognized. Of these, five species and three genotypes are found encapsulated in the host, whereas three species are non-encapsulating. All species and genotypes cause disease in humans.

Trichinella spiralis (also called T-1) is distributed in temperate regions worldwide and is most commonly associated

with a domestic pig cycle. It is the primary species found in pigs, wild boar, horses, and synanthropic rats, but may also be found in wildlife, especially in the vicinity of pig farms where transmission is ongoing. This species is highly infective for pigs, mice, and rats.

Trichinella nativa (T-2) is a cold climate adapted species. It has very limited infectivity for pigs, is found most commonly in wild canids, bear, and walrus. It is further distinguished by its resistance to freezing. *Trichinella nativa* causes human disease in arctic and subarctic regions.

Trichinella britovi (T-3) is found predominantly in wild animals, although it may occasionally be found in pigs or horses. It is widespread in temperate regions of Europe and Asia and has been associated with human disease resulting from the ingestion of undercooked game meats.

Trichinella murrelli (T-5) has been reported in wild animals from the US and parts of Canada. It has not been reported in domestic pigs, but has been reported in a horse; human infections have resulted from the ingestion of undercooked game meats.

Trichinella nelsoni (T-7) has been isolated sporadically from wildlife in Africa. It is characterized by greater resistance to elevated temperatures (2–3 °C higher) as compared with other species of *Trichinella*.

Three genotypes of encapsulating *Trichinella*, designated T-6, T-8, and T-9 have also been described. T-6 is found in carnivores in North America and parts of Canada. It is similar to *T. nativa* in its resistance to freezing in animal tissues, but does not extend as far north in its range. It is distinguished from *T. nativa* by both biochemical and molecular characters. *Trichinella* T-8 has only been reported in Africa. It is similar to *T. britovi*, but again, may be distinguished by both biochemical and molecular characters. *Trichinella* T-9 occurs in Japanese wildlife and may be differentiated from *T. britovi* by molecular methods. No human cases have been attributed to infection with *Trichinella* T-8 or T-9.

Three species of *Trichinella* do not form capsules in the host (Figure 3). These are *Trichinella pseudospiralis*, *Trichinella papuae*, and *Trichinella zimbabwensis*. *Trichinella pseudospiralis* has been recovered from raptorial birds, wild carnivores including wild boar, rats, and marsupials in Europe, Asia, North America, and the Australian subcontinent. Several human outbreaks due to *T. pseudospiralis* have been reported. *T. papuae* has been found in domestic and wild pigs as well as saltwater crocodiles (fed pig meat) in Papua New Guinea. Owing to its ability to infect reptiles, it is the suspected agent of human trichinellosis resulting from the ingestion of turtle and lizard meat. *Trichinella zimbabwensis* is similar to *T. papuae* in its ability to infect reptiles and wild carnivores. It has been reported in several parts of Africa but has not been implicated in human disease.

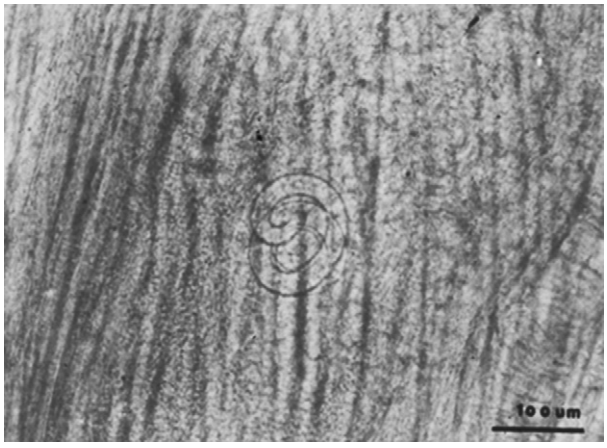

Figure 3 *Trichinella pseudospiralis* larva in muscle tissue. The larva is not encapsulated (compare to Figure 2).

Clinical Manifestations

Trichinellosis (the disease caused by *Trichinella* in humans) is manifested by symptoms associated with worms developing in the intestine (enteral phase) and in the musculature (parenteral phase). The occurrence of symptoms varies depending on the infecting dose, the susceptibility of the individual and possibly the species of *Trichinella*. In heavy infections, intestinal symptoms may develop as early as 1 week following ingestion of infective larvae. Intestinal symptoms, caused by worms invading and growing in the intestinal villi, typically include abdominal discomfort and diarrhea. The enteral phase generally only lasts a few days, but prolonged diarrhea has been observed in outbreaks in Arctic regions where *T. nativa* is the etiological agent, suggesting some clinical differences in the infecting species.

The onset of symptoms in the parenteral phase may occur within 1–2 weeks following infection or may be much later (4–6 weeks) as larger numbers of worms migrate and accumulate in the musculature. The clinical picture of the parenteral phase is much better defined although no signs or symptoms are pathognomonic. Symptoms include facial, and especially periorbital, edema, myalgia, headache, fever, and in some cases a macropapular rash. Conjunctival and subungual hemorrhages are also frequently observed. Complications principally include myocarditis and encephalitis which result from larvae migrating with heart musculature or the central nervous system.

The most common laboratory findings in trichinellosis are high eosinophil counts and increased levels of muscle enzymes including creatine phosphokinase and lactate dehydrogenase. Eosinophil counts may reach 10 000 cell mm^{-3} and elevated levels may persist for several weeks to several months following infection. Muscle enzymes in serum typically increase several fold during active infection (larval migration and muscle cell penetration).

Direct and indirect tests may be used to support the diagnosis of trichinellosis. Muscle biopsy is the only method for the direct verification of infection with *Trichinella* spp. A biopsy of 0.5–1.0 g of muscle from the deltoid or gastrocnemius may be compressed between glass slides and examined microscopically for the presence of larvae. Alternatively, the muscle sample may be digested in acidified pepsin and larvae recovered for enumeration. Muscle larvae may be detected approximately 2 weeks after infection and in numbers of larvae found in the musculature will increase over a period of several weeks. Microscopic detection of larvae in muscle biopsy compressions is more difficult when the infection is due to nonencapsulating species of *Trichinella* and therefore digestion of biopsy samples is the preferred method.

Detection of antibodies to *Trichinella* spp. is useful in confirming infection. The most commonly used test is the enzyme-linked immunoassay (ELISA), with an excretory-secretory antigen. This test is available in commercial kits or may be performed according to various published protocols.

The case definition for trichinellosis, including classification of suspected, probable, or confirmed cases, is based on a combination of signs and symptoms, laboratory findings, including biopsy (if performed), and epidemiological criteria. Several rubrics for case definition have been developed (see Dupouy-Camet *et al*. in Further Reading).

The severity of disease depends on the size of the infecting dose, whether the ingested meat was raw or partially cooked (affecting larval viability), the species of *Trichinella* involved, and the susceptibility of the person infected, including their immune status. It is generally believed that a dose of 1 larva per gram (LPG) of meat consumed in a typical meal (i.e., 150 larvae total) is sufficient to cause clinical disease. Although knowledge of the infecting species on the course of the disease is not well understood, in general it is believed that *T. spiralis* causes more severe disease than *T. britovi* and infection with *T. pseudospiralis* causes a more prolonged clinical disease.

Trichinellosis has been broken into categories of severity including: severe, moderate, mild or benign, and asymptomatic. In severe trichinellosis most or all the signs and symptoms are displayed. Hospitalization is often required and complications are frequently observed. Moderate disease is also characterized by expression of most signs and symptoms, but recovery is more rapid (5–7 weeks). Hospitalization is often not necessary and complications are less likely to occur. In mild disease, signs and symptoms are transient and recovery is rapid (3 weeks or less). Asymptomatic trichinellosis is only identified by a history of exposure and positive serology or biopsy.

Misdiagnosis and subclinical infections contribute to a dramatic underreporting of human trichinellosis. Misdiagnosis results from the general nature of the symptoms and the relatively rare occurrence of the disease in many countries. Patients from group outbreaks are more easily identified as compared to isolated cases. As an example of underreporting, autopsy studies in the US published in 1943 found 16.1% of the US population to be infected, whereas only approximately 400 clinical cases were reported each year between 1947 and 1950.

Treatment of trichinellosis depends on the severity of the disease and may include the administration of anthelmintics (typically mebendazole or albendazole) to kill worms in combination with glucocorticosteroids to reduce inflammation. If the disease is detected early, a primary goal of treatment is to eliminate adult worms in the intestine and thus reduce further accumulation of muscle larvae. For this purpose, anthelmintic treatment is recommended up to 4–6 weeks after exposure. Although anthelmintics also have some efficacy against *Trichinella* larvae in muscle tissue, the resulting inflammation caused by dying worms often creates allergic reactions due to the release of worm antigens. Thus, patients receiving anthelmintics to kill muscle larvae should also be treated with anti-inflammatory drugs (glucocorticosteroids) and monitored during treatment. Recovery is often complete, although muscle pain and weakness may persist. For prolonged muscle pain, glucocorticosteroids may be used for short periods of time to reduce discomfort.

Epidemiology

Prevalence in Humans

Humans acquire infection by ingesting raw or undercooked meat containing infective stages of the parasite. Human infections with *Trichinella* spp. have been reported from numerous countries on five continents – North and South America, Europe, Asia, and Africa. Human infections have not been reported from the mainland of Australia, but have been reported from Papua New Guinea and New Zealand.

As reported by Pozio (2007), the average yearly incidence of the disease in humans worldwide is probably close to 10 000 cases, but with a low mortality rate (approximately 0.2%). It is likely that only a fraction of actual cases are reported due to the nonspecific symptoms exhibited by this disease, as well as a lack of appropriate serological tests and a lack of knowledge of the disease on the part of physicians.

The prevalence of *Trichinella* infection in humans and the source of these infections vary greatly from country to country. Where pork production is tightly regulated and testing of pork for *Trichinella* infection has been required for many years, infections from pork are not found.

However, in countries where pigs are raised by traditional methods (outdoors), human infections occur regularly. Human trichinellosis linked to consumption of infected pork occurs in Central (Mexico) and South America (Argentina and Chile), in Asia (the People's Republic of China, Laos, Myanmar, Thailand, and Vietnam), and Europe (Bosnia-Herzegovina, Bulgaria, Byelorussia, Croatia, Georgia, Latvia, Lithuania, Poland, Romania, Russia, Serbia, and the Ukraine).

Even in countries where *Trichinella* infection has been absent from the domestic pork supply for many years, human infections may still occur from imported meat products which have not been properly inspected. For example, more than a dozen large outbreaks involving more than 3000 people occurred in France and Italy between 1975 and 2005, resulting from the consumption of horsemeat imported from East European and North American countries.

Game animals remain an important source of exposure to *Trichinella* spp. in all countries because infection cannot be controlled in wildlife. For this reason, hunters and consumers of game meats should either assure the safety of the meat through inspection methods, or prepare meat by methods that are known to inactivate the parasites.

Prevalence in Pigs

Trichinella prevalence in pigs varies from country to country, and regionally within countries. The lowest prevalence rates in domestic swine are found in countries where meat inspection programs have been in place for many years (including, in particular, countries of the European Union (EU)). In some instances, countries with long-standing inspection programs consider themselves free from *Trichinella* in domestic swine. In

countries of eastern Europe, higher prevalence rates of *Trichinella* have been reported in pigs and this is supported by higher numbers of cases of human trichinellosis. Increased prevalence of *Trichinella* infection in pigs in some of the Balkan countries is the result of changes from large government run farms to small holdings where pigs are raised outdoors. In the US, no formal inspection programs have been used to control *Trichinella* in pigs. However, changes in the pork industry which focus on confinement housing and other measures of biosecurity have essentially eliminated this infection from the domestic pork supply.

Only sporadic information is available on the prevalence of trichinellosis in South America, Africa, and Asia, but these limited reports suggest high infection rates occur in pigs in some countries. For example, in rural areas of China where pigs are raised outdoors in uncontrolled environments, pig infection rates can be 50% or higher.

Prevalence in Horses

Most evidence for infection in horses comes from the implication of horsemeat in human disease outbreaks. Despite widespread testing, detection of natural infections in horses has been rare – only a few naturally infected horses have been identified, from Mexico, Romania, the former Yugoslavia, and Poland. However, due to the habit of eating horsemeat without thorough cooking, a single infected horse can cause a large outbreak in humans. Horses implicated in human infections have generally been imported from countries where high infection rates occur in pigs. Further, epidemiological studies suggest that horses may be deliberately fed pork scraps or other meat scraps (from wild animals) to increase their weight before sale.

Prevalence in Wild Animals

Trichinella infection in wildlife varies tremendously from region to region, but it is safe to say that no area in nature is completely free from this parasite. The highest rates of infection are found in foxes, wolves, and bear, where infection rates can reach 85–90% of the population. It should be noted that infection rates in wildlife tend to increase in colder climates. In the domestic pig cycle, rats, skunks, raccoons, and other small mammals play an important role and are often found to have high infection rates.

Transmission of *Trichinella* in the Domestic and Sylvatic Cycles

Transmission patterns of *Trichinella* spp. may be grouped loosely into a domestic cycle and a sylvatic cycle (Figure 4). The domestic cycle involves primarily pigs and rats, whereas in the sylvatic cycle *Trichinella* spp. infection may be found in more than 100 species of mammals, as well as several species of birds (*T. pseudospiralis*) and reptiles (*T. zimbabwensis* and *T. papuae*). Although *T. spiralis* is the most common species found in domestic pigs, *T. britovi*, *T. murrelli*, and all three nonencapsulating species have also been reported from domestic pigs. Other species (*T. nativa*, *Trichinella* T-6) have extremely low infectivity for pigs and are not considered a risk for transmission to domestic pigs.

The geographic distribution of the various species is only partially documented due to lack of research in some areas of the globe. It is known that *T. spiralis* is cosmopolitan and this species has been widely reported from North and South America, Europe, and Asia, with localized reports from Africa (Egypt) and New Zealand. Other species have a more restricted distribution, in some cases based on certain

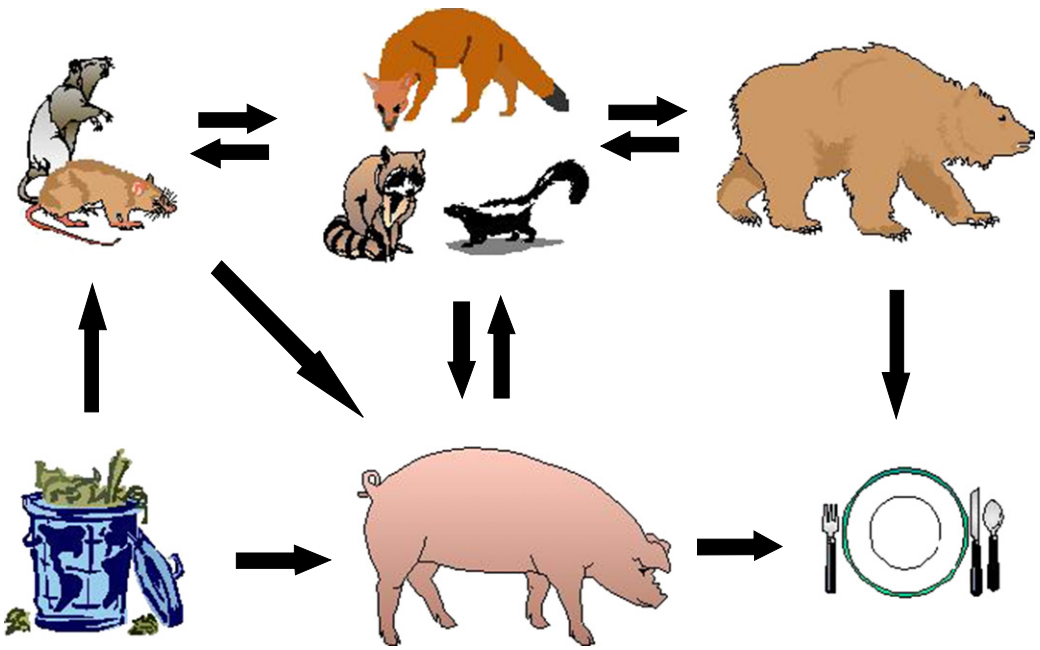

Figure 4 Transmission patterns of *Trichinella* spp. in the domestic and sylvatic (wildlife) cycles.

adaptations. The freeze resistant species (notably *T. nativa* and *Trichinella* T-6) are restricted to arctic and subarctic regions in the northern hemisphere and North America, respectively. *Trichinella britovi* occurs in temperate regions of Europe and Asia and in western Africa, whereas *T. murrelli* occurs in temperate regions of North America. These species overlap in range with *T. nativa* and *Trichinella* T-6. *T. nelsoni* and *Trichinella* T-8 have only been reported from Africa, whereas *Trichinella* T-9 has only been reported from Japan. Among the nonencapsulating species, *T. pseudospiralis* is cosmopolitan in distribution, whereas *T. papuae* is only currently known in Papua New Guinea and *T. zimbabwensis* has only been reported in East Africa.

Transmission of *Trichinella* in the domestic cycles is primarily influenced by animal management practices involving pigs. Exposure of domestic pigs to *Trichinella* spp. is limited to a few possibilities including: (1) feeding of animal waste products or other feed contaminated with parasites; (2) exposure to rodents or other wildlife infected with *Trichinella*; or (3) cannibalism within an infected herd. The use of good production/management practices for swine husbandry will preclude most risks for exposure to *Trichinella* in the environment.

Transmission of *Trichinella* in the sylvatic (wildlife) cycle relies on predation and carrion feeding. Generally, prevalence rates among carnivores increase up through the food chain. Sylvatic *Trichinella* infection affects public health in two ways. As a direct source, game meats pose a significant risk for human exposure to this parasite. Sources of documented human infection include wild boar, bear, walrus, fox, and cougar. Reducing exposure to *Trichinella* from these sources relies on education of hunters regarding the risks associated with eating raw or undercooked game meats. Sylvatic *Trichinella* infection also poses a risk as a source of infection to pigs. This is particularly true for *T. spiralis* and other species/genotypes which can infect pigs. Limiting the contact of pigs with wildlife is part of an overall risk reduction program for control of *Trichinella* infection in the domestic pig cycle.

Human Outbreaks

Human exposure to *Trichinella* spp. can only occur by ingestion of raw or undercooked meat containing infective larvae. Although pork has been the historical source of human infection, and continues to be an important source in some countries, in many countries effective control programs have dramatically reduced or eliminated pork as a risk for exposure. However, a variety of game meats continue to be a potential risk to humans if not prepared properly. These sources commonly include, bear and other carnivores that are high in the food chain (e.g., fox, cougar), feral pig/wild boar, and walrus, and less commonly, smaller mammals (e.g., opossum, badger, and squirrel), turtles, and lizards. Hunter/consumer education and inspection of these game meats are necessary to prevent infection from these sources. Other sources of infection include horses, for outbreaks in France and Italy, and dogs, for outbreaks in China, Russia, and elsewhere.

Because trichinellosis is not a frequent diagnosis in most countries, common source outbreaks are the most recognized occurrence of this disease. Outbreaks often occur during group events – holidays, weddings, etc. – where special or traditional dishes may be prepared that are not thoroughly cooked. Common source outbreaks also occur when *Trichinella*-infected game meats are distributed among a number of people and insufficiently cooked before being eaten. In common source outbreaks, identifying the meat which caused the infection may be used to confirm the presence of *Trichinella* larvae. Additional cases may then be identified from information on those exposed to the infected source meat.

Analytical Methods

Trichinella spp. may be identified by direct or indirect methods. The oldest method of direct detection of *Trichinella*, and one which is still frequently used, is the compression method. Small pieces of pork, or tissue from other animals, collected from the pillars (crus muscle) of the diaphragm, or alternative sites, are compressed between two thick glass slides (a compressorium) and examined microscopically. A minimum of 1 g must be examined to allow a theoretical sensitivity of 1 LPG (a number frequently cited as the threshold of infection posing a public health risk). In practice, the compression method has an approximate sensitivity of >3 LPG of tissue. The compression method is suitable for testing small numbers of samples and should be used to test carcasses of wild carnivores destined for human consumption.

An improvement in direct testing methods for *Trichinella* infection is the digestion method. Samples of tissue collected from sites of parasite predilection (diaphragm, cheek muscle, and tongue) are subjected to digestion in acidified pepsin. Larvae, freed from their muscle cell capsules, are recovered by a series of sedimentation steps, then visualized and enumerated under a microscope. Requirements for performing the digestion test are found in the Directives of the European Union (SANCO 2075/2005), in the US Code of Federal Regulations (9CFR 318.10), and in the OIE Manual of Standards for Diagnostic Tests and Vaccines.

Using methods of inspection testing as practiced on pig carcasses, the sensitivity of the digestion method is approximately 3 LPG. This level of detection is considered effective for identifying pork that poses a significant public health risk. Although there is insufficient information to determine the exact number of larvae which are necessary to cause clinical human disease (and these figures will be affected by the type of *Trichinella*, the amount of meat eaten, and the health of the individual), it is generally considered that infections >1 LPG are a public health risk. Thus, most infections which could cause clinical human disease would be detected by currently employed direct testing methods.

Trichinella spp. infection can also be detected by the presence of antibodies to the parasites in serum, blood, or tissue fluid samples. The ELISA has been used extensively for testing in both pre- and postslaughter applications. Based on the use of an excretory-secretory antigen collected from short-term *in vitro* cultivation of *T. spiralis*, the ELISA has proven to be highly sensitive and specific; no known cross-reactions occur using this test. Because the ELISA is not in widespread use for the detection of *Trichinella* in pigs at slaughter, the reader is referred to the OIE Manual of Standards for Diagnostic Test and Vaccines for specific methodologies involving this test.

Control and Prevention

Many countries require that pigs (and horses) sold as food animals be tested for *Trichinella* infection. These requirements are in the form of regulations governing slaughter inspection. The EU outlines these requirements in SANCO 2075/2005. Other countries have similar regulations and the proof of freedom from *Trichinella* infection must accompany products sold for interstate commerce. As stated in the OIE Terrestrial Animal Health Code, importing countries should mandate that an international health certificate accompany imported pork products. The sanitary certificate should attest that the product has: (1) been tested for *Trichinella* infection at slaughter and was shown to be negative; (2) originated from a *Trichinella*-free country or territory; or (3) been processed to ensure destruction of *Trichinella* larvae. In the same document, it is specified that horsemeat sold for human consumption should be submitted to slaughter inspection or be processed by methods known to kill *Trichinella* larvae.

Some countries, including the US, have relied primarily on further processing (cooking, freezing, and curing) of ready-to-eat pork products and advice to consumers regarding the safe preparation of fresh pork. Commercial processing methods which have been proven experimentally to render pork free from infective *Trichinella* larvae are described in the US Code of Federal Regulations (9CFR 318.10). Pork meat must be heated to 58 °C or frozen at one of several time temperature combinations. For flash freezing, *Trichinella* larvae are killed instantaneously at −35 °C. The effectiveness of curing depends on a combination of salt concentration, temperature, and time. Each method should be tested experimentally to determine effectiveness as no model for curing conditions has been devised. Consumers of fresh product are advised to cook pork, and other meat products that might serve as a source of *Trichinella*, to an internal temperature of 63 °C.

Preharvest Prevention of *Trichinella* Infection in Pigs

Prevention of exposure of domestic pigs to *Trichinella*, requires the implementation of sanitary management practices which: (1) prohibit feeding of animal products (without proper cooking); and (2) preventing exposure to rodents or other potentially infected mammals either directly, or through contamination of feed. Production practices which are free from risk or have minimal risk for exposure to *Trichinella* should be monitored periodically (by testing blood samples from live animals or by sampling slaughtered animals) to verify the absence of infection.

Several regulations support programs for certifying pigs free from risk of exposure to *Trichinella* including, SANCO 2075/2005 in the EU and, the National Trichinae Herd Certification Program in the US.

The OIE Terrestrial Animal Health Code specifies that absence of *Trichinella* infection in pigs can be documented by declaring a country or territory as '*Trichinella*-free' based on certain criteria. These criteria include: (1) the disease is compulsorily notifiable; (2) waste food feeding is officially regulated; (3) a surveillance program is in place to detect *Trichinella* infection at a very low prevalence in the disease free area; (4) surveillance is intensified where infection was last reported; and (5) any outbreaks of infection in swine or humans are fully investigated to determine the source. Establishing a country or region as free from *Trichinella* is difficult because the parasite circulates in so many species of wildlife.

See also: Disciplines Associated with Food Safety: Parasitology. Foodborne Diseases: Overview of Biological Hazards and Foodborne Diseases. Protozoa: *Toxoplasma gondii*

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Trichuris trichiura

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Glossary

Embryonation Development process of the ova from a disorganized mass when laid to a fully developed larva.
Nematodes Worms of the phylum Nematoda, both free living or parasitic in almost all vertebrate and nonvertebrate animal species.
Operculum Openings in the ova through which the larvae escape.
Preventive chemotherapy Mass drug administration to the whole community irrespective of the infection status to reduce the prevalence and intensity of infections.
Soil-transmitted nematodes Generally considered to be whipworm (*Trichuris*), roundworm (*Ascaris*), and the two human hookworms, all of which are transmitted in a fecally contaminated environment (also known as geohelminths).
Trichuriasis Disease caused by infection with *Trichuris trichiura*.

Background

Nematodes (roundworms) of the genus *Trichuris* are found as parasites of vertebrates, including many domestic and wild mammals and have a global distribution. The human whipworm, *Trichuris trichiura*, only affects humans and primates, and has a direct life cycle without intermediate hosts. Infection in humans with closely related species such as *Trichuris suis* (from pigs) or *Trichuris muris* (from mice) may occur, but the infection is short lived and self-curing, and the parasites are unable to complete their life cycle. The World Health Organization estimates that more than 700 million people are infected worldwide, the majority being in Asia, although all tropical and subtropical regions are endemic. Evidence of human infection dates back to prehistoric times, with the demonstration of *Trichuris* eggs at archeological sites and in mummified human remains. The Alpine 'ice man' (approximately 3300 BC) had *Trichuris* eggs in his rectum. Morgani (1740) provided the first written evidence of infection in the human colon, and Roedere and Wagler (1761) gave a complete description and drawings from which Linnaeus derived its taxonomic classification in 1771 and originally called it *Ascaris trichiura*, the current name being finalized in a monograph by Stiles in 1901.

Characteristics

The genus *Trichuris* belongs to the family Trichuridae (syn. Trichocephaloidea), order Enoplida, class Secernentea (formerly Phasmidia) and phylum Nematoda. It is more correctly known as *Trichocephalus*, but the 1941 Committee on Nomenclature of the American Society of Parasitologists opted for *Trichuris* as the commonly accepted name at the time. More than 70 *Trichuris* species have been described; many are from small rodents, but the most important are those of domestic animals, such as *T. suis* (pig); *Trichuris vulpis* (dog); *T. muris* (mouse), *Trichuris ovis* (cattle); and *Trichuris felis*, *Trichuris campanula*, and *Trichuris serrata* (cats). However, *T. trichiura* has only been described from humans and some primates, such as chimpanzees and rhesus and colobus monkeys. *Trichuris suis* was at one time thought to be the same species, being indistinguishable on morphological grounds but has recently been shown to be genetically distinct, and is unable to develop in the human intestine and produce viable eggs. Infections with *T. vulpis* have been described rarely in humans, but again it is doubtful whether they are able to complete their life cycle.

Trichuris trichiura has a simple, direct, life cycle, which does not involve any intermediate hosts (Figure 1). Both male (30–45 mm) and female (30–50 mm) adults inhabit the cecum and much of the large intestine. They have a characteristic narrow anterior portion (hence the popular name of whipworm), which is inserted into the mucosa of the intestine, and a thicker posterior portion, which contains the intestine and the reproductive organs. Adults feed on the host's body fluids, which are obtained through the mouth at the extreme anterior of the worm. Adult females release between 3000 and 10 000 unembryonated ova a day into the feces. The ova are barrel shaped with plugs at the either ends measuring approximately 55 μm × 25 μm and gain a brownish coloration from bile impregnation as they pass through the intestine. They are passed in the stool and require 2–4 weeks to develop into the first-stage larva under optimal conditions in warm damp soil. Development rate is dependent on temperature and humidity (4–6 months at 15 °C, 3–4 weeks at

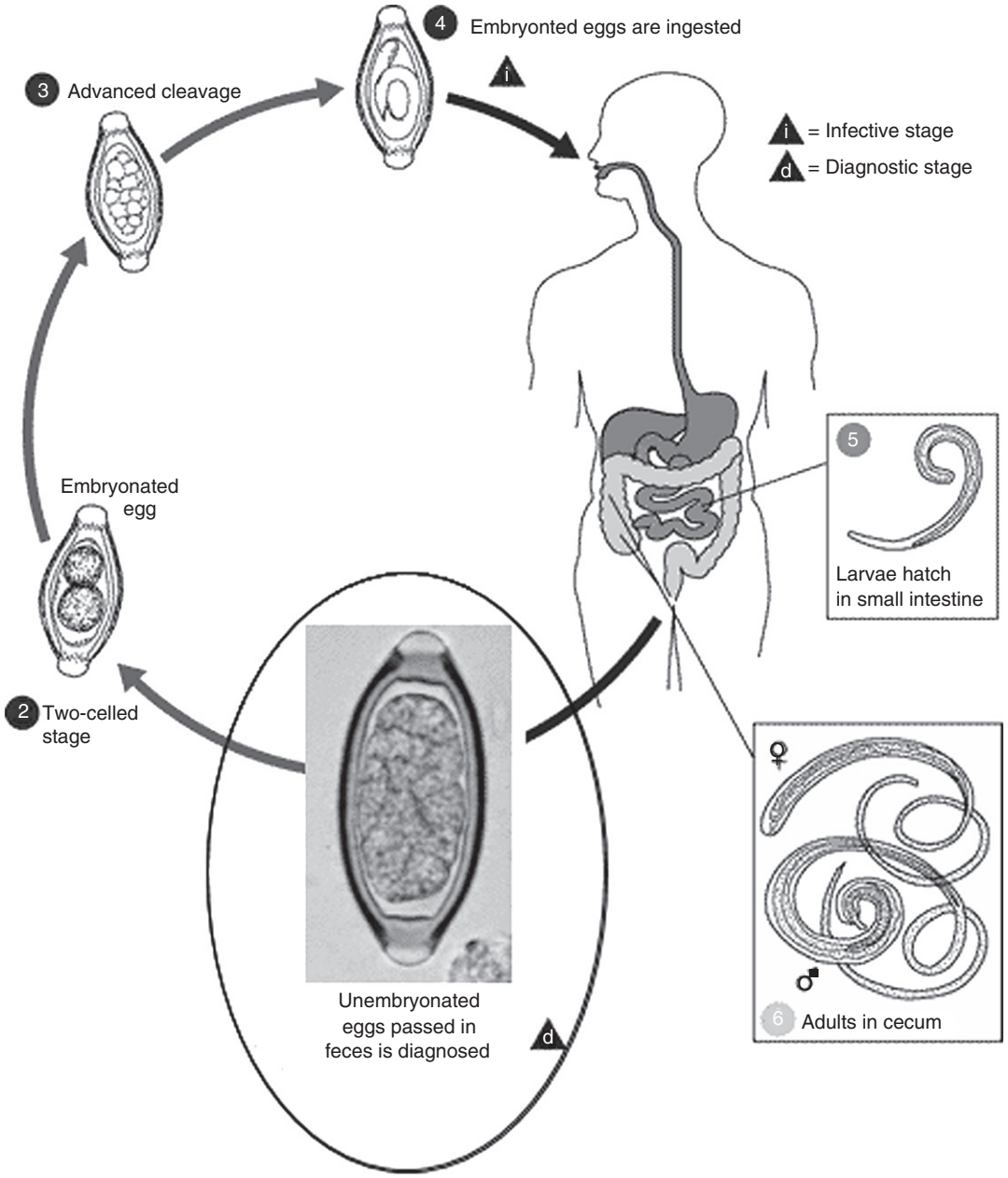

Figure 1 Life cycle of *Trichuris trichiura*. (1) Unembryonated eggs are shed in the feces. Their presence is the principal method of diagnosis. (2 and 3) Eggs develop in the soil. (4) Fully developed eggs containing larvae are ingested with food. (5) Eggs hatch in the intestine and undergo four molts to become adults. (6) Male and female adults are found in the large intestine, from the cecum to the rectum. Adults may be detected on colonoscopy/proctoscopy. www.dpd.cdc.gov/dpdx/images/ParasiteImages/S-Z/Trichuriasis/Trichuris_LifeCycle.gif

26 °C, 17 days at 30 °C, and 11 days at 35 °C). Ova may remain dormant in the soil for up to 5 years and are infective for at least 2 years. Embryonated ova containing infective larvae may subsequently be ingested from contaminated soil, food, or water. After ingestion, the ova wall is weakened, and the first-stage larva hatches and attaches to the intestinal wall, where it undergoes four molts to become an adult after 2–3 months, residing in the large intestine for 1–2 years. Copulation between male and female worms occurs in the intestine, giving rise to fertile females who begin to release ova.

Clinical Manifestations

In areas where *T. trichiura* is endemic, a large proportion of the population may be infected. Coinfection with other soil-transmitted nematodes, such as *Ascaris lumbricoides* and the hookworms *Necator americanus* and *Ancylostoma duodenale*, is common. Significant infections occur in infancy, with prevalence peaking in school-age and teenage children, and reducing in adult life. In most cases (at least 90% of those infected), infections are light and cause little or no obvious

symptoms other than mild abdominal discomfort and other vague symptoms. However, in heavily infected persons, especially children, significant symptoms may be present. Diarrhea, often with blood and mucus with cramping abdominal pains is a sign of the *Trichuris* dysentery syndrome, which when left untreated, may last for 6 months or more and mimics other forms of inflammatory bowel disease. Especially in children, this may be accompanied by rectal prolapse, which can be massive, with 10 cm or more of the rectum being exposed and adult worms visible on its surface. Rectal prolapse usually corrects itself once the underlying infection is treated. As a result of the chronic diarrhea, there is associated anorexia and weight loss, and a microcytic iron-deficiency anemia is also commonly seen in heavier infections. Such severe symptoms result in growth retardation and stunting, with growth rates well below the normal rate. Heavily infected children may look several years smaller than their peers and undergo a massive growth spurt when the infection is removed. In studies of such children, linear growth rates in excess of those seen using growth hormone replacement in deficient children have been seen.

Despite the apparent mild nature of the symptoms in all but a minority, it may be thought that trichuriasis is a minor problem. However, recent studies of children with mild-to-moderate infection have shown that, even in these, there is evidence of an effect on growth, physical fitness, and, more importantly, on cognitive ability. It has been shown that although it is difficult to measure the effects of mild infection directly, there is ample evidence of accelerated growth, improved physical fitness, and improvements in cognition scores following treatment of these mild infections. Thus, although it is a generally mild disease and does not account for much mortality in those affected, it does cause considerable low-level morbidity that affects the individual's functioning and inhibits community social and economic development.

Treatment is with a benzimidazole anthelmintic, such as albendazole (Zentel – GSK) 400 mg as a single dose, although a daily dose for 3 days is probably more effective, or mebendazole (Vermox – J&J) 500 mg daily for 3 days. Neither is fully effective, at least for heavy infections and further treatment may be necessary based on diagnostic stool tests. The addition of ivermectin (Mectizan – Merck) has been shown to increase cure rates.

Epidemiology

Trichuris trichiura has a worldwide distribution, principally within the tropical and subtropical zones, although it may have been more widely distributed in the past. However, with improvements in sanitation and public health in the richer countries, it has been largely eliminated from areas such as Southern USA, Japan, and much of the Mediterranean basin. Nonetheless, global prevalence has been estimated as at least 700 million infections by the World Health Organization and possibly as many as 1000 million by others. Alongside the other soil-transmitted nematodes, it is therefore one of the commonest parasitic infections on the planet. Before the advent of preventive chemotherapy programs with anthelmintics, the infection rates were estimated to be 25% in South and Central America, 31% in Africa, 12% in the Middle East, 36% in Bangladesh, and 58% in Southeast Asia. In Japan where the infection rates were similar historically, they have been reduced to 0.01%. It is likely that the current prevalence is much lower globally due to the effect of preventive chemotherapy, but accurate figures are almost impossible to obtain.

Transmission is direct through contamination of the environment as a result of promiscuous defecation around the living and work areas. Trichuriasis is therefore commonest in areas of poverty where there is little or no sanitation, and there is a tendency for heavier infection to cluster within families. Whether this is due to specific susceptibility factors within families or merely due to high levels of recurrent exposure to the contaminated environment is unknown. An additional factor encouraging higher levels of infection is the practice of using 'night soil' for fertilizing fields, thus directly contaminating foodstuffs. Although this is a practice seen throughout the world, it was a particular problem in the rice growing areas of Southeast Asia and probably drove the high prevalence there. Unfortunately, composting of night soil, effective for many other intestinal pathogens, does not destroy *Trichuris* ova. Children become infected at an early stage by playing in infected areas, and heavy infections are particularly noted in regions where there are micronutrient deficiencies leading to pica (soil eating) in children. Water sources may also be contaminated and add to general infection. In adults, infection, or reinfection, comes chiefly through the consumption of contaminated vegetables. The role of pigs as a potential source of infection, although often quoted, can now be discounted following the demonstration of the genetic differences between the human (primate) *T. trichiura* and the pig species *T. suis*.

Diagnosis

The principal method of diagnosis of infection is by examination of stool samples under the microscope, looking for the typical double plugged ova of *T. trichiura*. This appearance is clearly different from the ova of *Ascaris* and other soil-transmitted nematodes that are often present at the same time in coendemic areas. There are a number of techniques employed, all of which use small samples of stool that are treated and then applied to a slide for examination. The simplest approach is the iodine-stained smear, which can identify heavy infections, but it is hampered by the presence of food debris in lighter infections. The most commonly employed method is the Kato–Katz test, which uses a measured sample stained with malachite green that stains the ova of all soil-transmitted nematodes and provides a semiquantitative measure of the intensity of infection. An alternative is to use one of the flotation and concentration techniques that remove much of the fecal debris and both allow identification of very-low intensity infections and provide a measure of infection intensity. These latter tests are useful for the follow-up of treated individuals because of their sensitivity. Blood tests do not reveal much, other than anemia in chronically infected individuals and an eosinophilia, which may be found in any

case of heavy helminth infection. As has been noted for cases of rectal prolapse, the adult worms may be visualized on the prolapsed mucosa. In the absence of prolapse, adults may occasionally be seen on proctoscopy or colonoscopy when used for the investigation of chronic cases. The identification of adults by these methods is a rarity, but if a parasitologist confirms their identity, it is pathognomonic.

Because only *T. trichiura* commonly infects humans, and the stool identification techniques have proved their worth over many decades, it has not been necessary to resort to molecular techniques. However, comparative analysis of the *Trichuris ITS1* gene has been useful in differentiating *T. trichiura* of primates from *T. suis* of pigs, thus removing the potential of pigs as a reservoir host. As further work is done in this field, it is possible that such analysis may ultimately show that accidental infection with other specific animal species does occur. Of particular interest in this respect is the recent use of laboratory strains of both *T. suis* and *T. muris* as treatment for chronic inflammatory bowel conditions, such as Crohn's disease. In both cases, the infections are only established for a short time (a few weeks at most), which suggests that, for these species at least, sustainable human infection is unlikely.

Control and Prevention

Because of the mode of transmission, it is clear that the principal approach to prevention is sanitary improvement, both at personal and community level. It also applies to all the soil-transmitted nematodes, rather than just to *Trichuris*, although in some cases, other measures, such as wearing shoes, to prevent hookworm infection are also relevant. Education plays a part in ensuring that there is an understanding of the risks of eating potentially contaminated food and explaining the importance of proper personal hygiene for all members of the households. Hand washing, washing of salad vegetables in clean water, and adequate cooking of other vegetables are all important for the prevention of primary infection and reinfection in endemic communities. However, because it is the contamination of the environment with infected fecal material by promiscuous defecation, the proper use of toilets and pit latrines for disposal of feces is of paramount importance. In many areas where trichuriasis is a public health problem, clean water through deep tube wells has been provided, whereas the provision of adequate toilet facilities lags far behind. However, it is not only important to provide good deep pit latrines (or flush toilets where effluent can be treated) but also to ensure that these are used all the time. The main problem here is providing sufficient educational backup to support change in behavior. In endemic communities, chemotherapy with anthelmintic drugs should be considered to reduce the prevalence in the community, and thereby the risk of reinfection. This has been shown to be effective in controlled studies in the Caribbean and elsewhere, and in more extended mass treatment programs involving whole communities or even countries. Preventive chemotherapy can be effectively delivered to schoolchildren through their schools or in high prevalence areas by mass treatment campaigns. However, reducing community infection levels and thus the risk to individuals will take time and requires the combined intervention of chemotherapy, sanitary improvement, and behavioral change through education. The reduction in prevalence or disappearance of infection as a result of improved sanitation alone took many years to achieve in countries such as Japan or Italy.

Conclusion and Future Perspectives

Trichuriasis, alongside the other soil-transmitted nematodes, will remain a public health issue for many years to come, purely as a result of the massive scale of the problem. Within the broad tropical and subtropical belt, infection and reinfection will continue despite large-scale public health programs. Therefore, the key preventive measures to protect the individual is educating them to ensure proper hygiene and food cleaning and handling together with improvements in disposal of fecal material. It is hoped that the use of night soil as a fertilizer will ultimately be eliminated as this was the reason for the extreme prevalence seen in Southeast Asia. Identification and treatment of infected individuals is an impossible task, but effective reductions can be achieved by preventive chemotherapy. Currently, the drugs available are only partially effective for individual treatments, and it is hoped that newer drugs or combinations may become available that can produce a complete cure. This is of particular importance because hundreds of millions of doses of anthelmintic drugs are being used every year in the areas endemic for *Trichuris* and the other soil-transmitted nematodes, thus raising the threat of drug resistance.

See also: Food Safety Assurance Systems: Essentials of Crisis Management. Foodborne Diseases: Foodborne Diseases in Travelers. Helminth-Cestode: *Taenia saginata* and *Taenia solium*. Helminth-Nematode: *Ascaris*. Helminth-Trematode: *Fasciola hepatica* and *Fasciola gigantica*. Protozoa: *Cryptosporidium* spp.. Public Health Measures: Challenges of Developing Countries in Management of Food Safety; Safe Use of Wastewater for Agricultural Production

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HELMINTH-TREMATODE

Contents

Clonorchis sinensis

S-J Hong, Chung-Ang University College of Medicine, Seoul, Korea

Glossary

Cellophane-thick smear Fecal sample is spread on a slide glass and covered with a sheet of cellophane presoaked in glycerol.
Cercaria A larval form with a long tail, swimming in water and transforming into metacercaria.
Cholangi Denote bile duct.
Jaundice Yellowish coloring of the eyes and skin due to pigmentation of bilirubin.
Metacercaria A larval form occurring in fresh water fish and infective to human.
Reservoir host Animals that have parasites which also infect human and act as source of human infection.
Sashimi Thin slice or fillet of fish meat.

Background

Clonorchis sinensis was first discovered in a Chinese patient in 1875 and accepted as a new species of liver fluke in 1907. *Clonorchis sinensis* is a member of the family Opisthorchiidae. Its morphological characters are a spatula-like shape, thin, and translucent body, revealing internal organs, a convoluted uterus in middle of its body, and branched testes. Freshwater fish were identified as its second intermediate host in 1911 and a freshwater snail as its first intermediate host in 1917. Clonorchiasis, human infection caused by *C. sinensis* adults, is prevalent in southeastern and northeastern China, Korea, and northern Vietnam.

Geographical Distribution

Globally, it has been estimated 15–20 million people are infected with *C. sinensis*, and that more than 200 million people are at risk of infection. *Clonorchis sinensis* is distributed in East Asia including China, Korea, eastern Russia, northern Vietnam, and Taiwan. Formerly prevalent in Japan, it has been under control since 1960. In China, Guangdong Province has the highest infection rate, followed by Guangxi, Heilongjiang, and Sichuan provinces. Sporadic foci of high prevalence were reported from Hunan, Jilin, and Liaoning provinces. It has been estimated that 12.5–15 million of the Chinese population are infected with *C. sinensis*. In Korea, clonorchiasis is endemic in river basins in southern areas and 1.3 million people are estimated to be infected. In Russia, clonorchiasis is endemic in the Amur River basin, infecting one million people. In Vietnam, two human liver fluke species have been identified, *C. sinensis* in northern Vietnam and *Opisthorchis viverrini* in the south; approximately one million people are believed to be infected with *C. sinensis*.

Morphology and Life Cycle

Adult Fluke

When live encysted metacercariae infecting fish are ingested by humans, they excyst in the duodenum and migrate under bile-chemotaxis to the intrahepatic bile ducts. Juvenile worms grow

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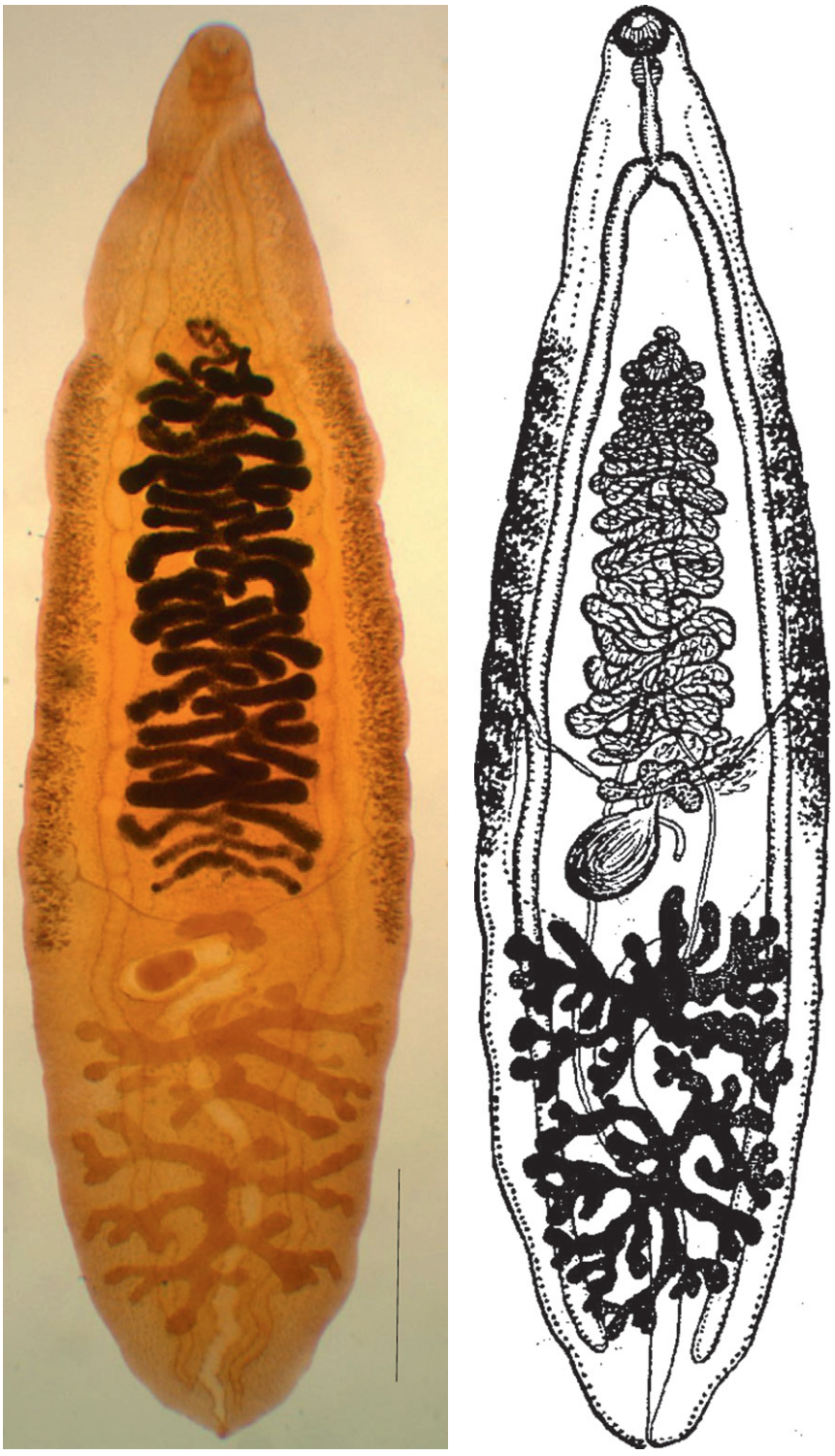

Figure 1 *Clonorchis sinensis* adult worm: (a) An adult worm unstained. Bar = 150 μm. (b) Drawing of a worm (Courtesy of Dr. J.Y. Chai).

to the adult form and produce eggs in the human bile duct 4 weeks after infection.

The adult *C. sinensis* is dorsoventrally flat, elongated, translucent, with a yellowish color due to the presence of lipofuscin pigments (Figure 1). The adult fluke measures 10–25 mm long and 1.5–4 mm wide. Its oral sucker is subterminal anteriorly and its ventral sucker is located at the anterior one-fourth of the body. The oral sucker serves as a mouth which connects a globular pharynx and a short esophagus that bifurcates into two intestinal branches that

extend to the posterior end of the body, terminating blindly. The two testes, branch-like and deeply lobulated, lie tandem in the posterior one-third of the body. The small lobular ovary is at the mid-median anterior to the anterior testis. A large sac-shapes seminal receptacle lies behind the ovary at the equator level. The uterus, full of eggs, convolutes up between the intestines and opens in front of the ventral sucker. The vitelline follicles occupy both sides lateral to the uterus and the intestines (Figure 1).

Egg and Larval Development in the First Intermediate Snail Host

The eggs of *C. sinensis* escape the infected human in the feces (Figure 2). They are ellipsoid, yellowish brown in color, more pointed at one end, and operculate. They measure 26–30 μm in length and 14–15 μm in breadth, and have a wrinkled surface reminiscent of that of a muskmelon. The egg contains the initial larval stage of the parasite, a miracidium.

Miracidia hatch out of egg after ingestion by a suitable aquatic snail host, in which the parasite undergoes several asexual multiplication stages. Among freshwater snail species, *Parafossarulus manchuricus* is the most widely distributed species. Other potential host species are *P. anomalospiralis*, *Alocinma longicornis*, *Bithynia fushiana*, *Bithynia misella*, *Melanoides tuberculata*, *Assiminea lutea*, and *Thiara granifera*. The snails are found in water bodies that have poor flow or are stagnant with a stratum composed of mud rich in organic materials and water vegetation. In southern South Korea, snails appear in water bodies in April, achieve a peak population density in June, and disappear from water in November.

In the snails gastrointestinal tract, the miracidium penetrates the intestinal wall and then develops into sporocysts in peri-intestinal tissues and organs. The sporocysts are sac-like and contain several developing rediae. The rediae eventually yield the cercariae. Mature cercaria posses a body and a long tail. The cercaria, because of unique morphological features, is called a pleurolophocercous cercaria. In the snail host, a single

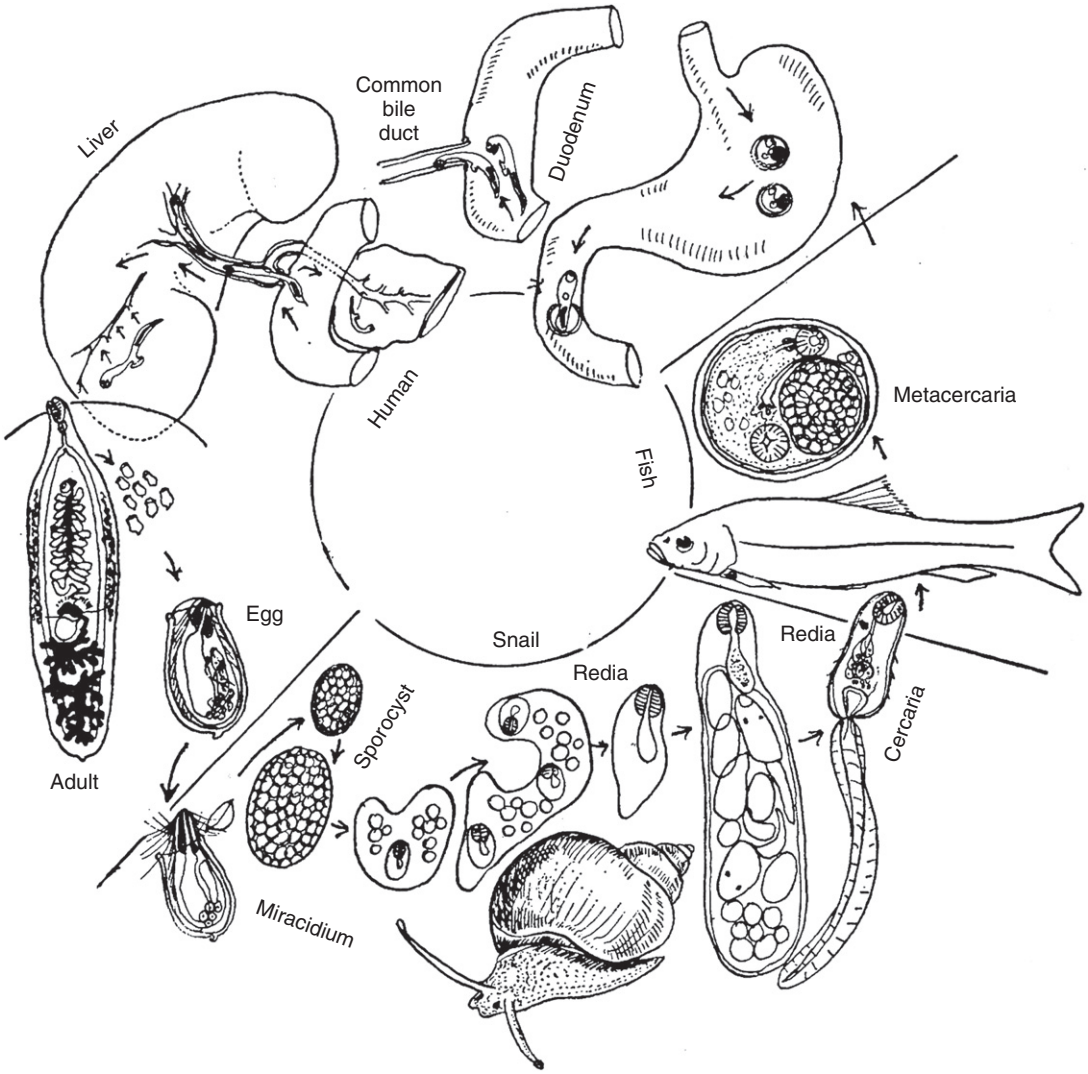

Figure 2 Life cycle of *Clonorchis sinensis* (Courtesy of Dr. J.Y. Chai).

miracidium can, because of this asexual multiplication, yield 3200–10 000 cercariae. The cercariae emerge from snails and swim in fresh water seeking a fish host; although very active for the first 12 h, they lose this swimming activity after 72 h if they do not find a fish host (Figure 2).

Metacercaria and the Second Intermediate Hosts

When cercariae find a suitable fish host, they attach and penetrate the skin between scales to enter subcutaneous tissues in 15–20 min. In the fish tissue, the cercarial body secrets cystogenic materials from its cystogenous glands and become encysted to form metacercaria within 24 h. The metacercaria is round to oval and measures 135–145 μm by 90–100 μm. The cyst wall is thin and clear. The cysts are surrounded by a capsule formed by a local tissue reaction in the fish host. After 10–15 days, the metacercariae have a ventral sucker larger than the oral sucker, an excretory vesicle full of dark excretory granules, and no eyespots (Figure 3). The metacercariae in freshwater fish attain infectivity to rabbits and rats 23–30 days after cercarial infection. The fish hosts of *C. sinensis* include numerous species of freshwater fish, predominantly of the family Cyprinidae. Metacercariae remain viable and infective to definitive hosts in the flesh of freshwater fish for approximately 1.5 years, but some die as early as 30 days after invasion.

When ingested by the final hosts, including man, the metacercariae excyst in the duodenum and migrate to the bile ducts (Figure 2).

Definitive and Reservoir Hosts

Man is the most suitable definitive host of *C. sinensis*, though in endemic areas dogs, cats, rats, pigs, buffaloes, weasels, and foxes have been reported to be animal reservoir hosts. Laboratory animals, rabbits, guinea pigs, hamsters, gerbils, and mice are susceptible to *C. sinensis*. In China, dogs and cats are considered major sources of *C. sinensis* eggs and these contaminate bodies of freshwater, such as ponds, puddles, streams, wetlands, rivers, lakes, and canals. These animals are free to roam in villages and are usually not restrained as pets in homes, and thus, they have access to raw or undercooked foods in household waste. In some endemic areas, cats are reported to have a high infection rate and high intensity infections. In Korea, feral cats and stray dogs infected with *C. sinensis* are considered important sources of the eggs that contaminate the environment. Reservoir hosts play an important role in the *C. sinensis* ecosystem, as a source of *C. sinensis* eggs, which when they contaminate, bodies of freshwater infect the freshwater snail hosts.

Pathology and Clinical Symptoms

Infection with *C. sinensis* provokes cholangitis in the hepatobiliary system of infected animals. During the early infection stage, juvenile worms cause proliferation and inflammation of biliary epithelium, and during late stage of infection, they provoke desquamation, glandular proliferation, and

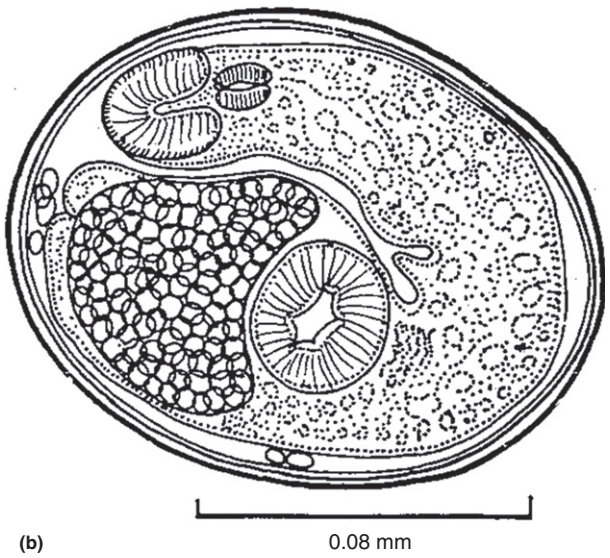

Figure 3 Metacercaria of *Clonorchis sinensis*. (a) A metacercaria isolated from a freshwater fish. (b) Drawing of a metacercaria (Courtesy of Dr. J.Y. Chai).

adenomatous hyperplasia in biliary epithelium (Figure 4), which exhibits high levels of goblet cells. In cases of heavy infection, periductal fibrosis develops and extends to bile capillaries
and the portal area, and the bile duct wall is infiltrated by lymphocytes, plasma cells, and eosinophils. In cases of chronic infection, hepatobiliary changes frequently progress to biliary cirrhosis. Pathologic changes in the biliary system progress chronically in clinical cases with a heavy worm burden due to the fluke accumulation caused by repeated infection.

Incidence of cholangiocarcinoma is higher in clonorchiasis endemic areas. In experimental animals, *C. sinensis* infection has been shown to provoke the production of reactive oxygen radicals and nitrosocompounds, to damage cellular DNA, and

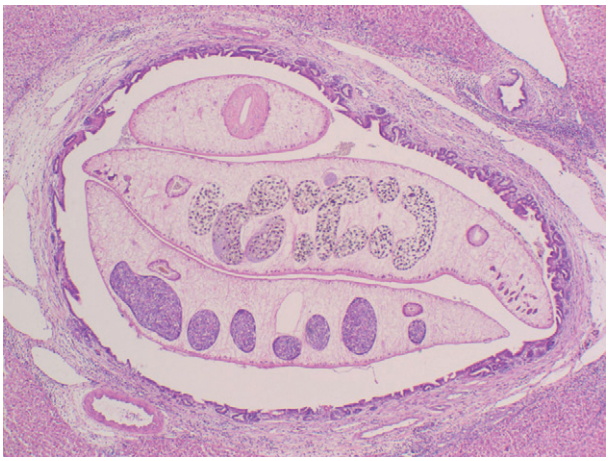

Figure 4 Histopathology of a rabbit liver 2 months after infection with *C. sinensis*. Hematoxylin-eosin stained (original magnification, 100 ×).

to progress to cholangiocarcinoma. In fact, *C. sinensis* infection is recognized to present a high risk of cholangiocarcinoma, and *C. sinensis* was classified as a Group 1 biological carcinogenic agent by the International Agency for Research on Cancer in 2009.

During early stage disease, *C. sinensis* causes mild cholangitis, and infected humans exhibit symptoms (if present) such as mild fever, epigastric tenderness, abdominal discomfort, malaise, and anorexia. In chronic infections with a heavy worm burden, the symptoms manifested are obstructive jaundice, fever, epigastric pain and tenderness, hepatomegaly, diarrhea, and ascites. Peripheral blood exhibits mild eosinophilia and IgG and IgE antibodies.

Diagnosis and Treatment of Human Infections

Human infections are diagnosed by the microscopic detection of eggs in fecal specimens. *Clonorchis sinensis* eggs are differentiated from those of heterophyid flukes by morphological features, such as shouldering around the operculum, wrinkles on the outer surface, and an abopercular knob. However, it is difficult to differentiate *C. sinensis* eggs from those of *Opisthorchis* spp. under a microscope. A cellophane-thick smear method (the Kato–Katz method) is widely employed in the field and the formalin-ether centrifugal sedimentation method is used with higher sensitivity and accuracy in clinical laboratories. Diagnosis can be made by detecting the DNA or proteins of *C. sinensis* in feces using specialized diagnostic kits. Enzyme-linked immunosorbent assay is a common serodiagnostic method for clonorchiasis. The antigen used is a crude antigenic preparation such as a soluble extract of adult *C. sinensis*.

Praziquantel is a potent anthelmintic for clonorchiasis, and at 75 $mgkg^{-1}$ of body weight provides high cure and egg reduction rates. Furthermore, praziquantel is well tolerated; its subjective side effects are mild or transient dizziness, headache, abdominal distress, and nausea.

Epidemiology of the Parasite

Susceptibility of the Fish Hosts

In *Pseudorasbora parva*, *C. sinensis* metacercarial infection and density show substantial seasonal fluctuations, that is, infections are maintained from May to September, but are relatively low from November to April. Freshwater fish become infected during spring and summer, because *P. manchuricus* sheds cercariae at this time. Furthermore, *C. sinensis* metacercariae in *P. parva* survive in cold water during winter, accumulate, and maintain viability until the summer.

The susceptibility of freshwater fish to metacercariae, in terms of infection rate and metacercarial burden, varies widely between species and from water body to water body – even within species. In China, small fish such as *P. parva* and *Parapelecus argenteus* are wild-caught, not raised in aquaculture systems, and show high infection rates and heavy metacercarial burdens. However, large fish, such as *Cyprinus carpio*, *Hypophthalmichthys molitrix*, and *Parabramis pekinesis*, are farmed in ponds and aquaculture systems and exhibit low intensity of metacercarial infections. The difference of the metacercarial infection statuses of large and small fish is understandable from the ecological point of view, as metacercarial infection of small fish is synchronized with the development of snails and of the parasite in a given ecosystem, but large fish in an aquaculture system have a narrow exposure window to cercarial infection.

The occurrence of clavate cells in the epidermis of freshwater fish is believed to be closely related to their susceptibilities to *C. sinensis* cercarial infection. Clavate cells are found in the epidermis of freshwater fish and are characterized by Oxner's dark halo around the nucleus. Small fish, such as *P. parva*, *Zacco platypus*, *Microphysgobio koreensis*, *Gnathopogon majimae*, *Acheilognathus signifier*, *Rhodeus ocellatus*, and *Sarcocheilichthys sinensis*, have no clavate cells in the skin and exhibit moderate to heavy *C. sinensis* metacercarial infection intensities, perhaps reflecting a greater susceptibility to metacercarial infection.

Research has also shown that mucus on the epidermis contains substances that adversely affect invading *C. sinensis* cercariae in large fish. An important cercariacidal substance is an ethyl ester of an unsaturated fatty acid, possibly linoleic acid. Furthermore, it has been reported that concentrations of linoleic acid in mucus vary between freshwater fish species and modulate susceptibility to *C. sinensis* cercariae.

Freshwater Fish as Sources of Human Infection

The Cyprinoid freshwater fish, which constitute approximately 11 families, play a role as second intermediate hosts for *C. sinensis*. Most of the fish species belong to the family Cyprinidae. In clonorchiasis endemic areas, *P. parva* (a small freshwater fish of weight 0.5–1.5 g) is the most common second intermediate host. Small fish, such as *P. parva*, are not raised in aquaculture systems but are caught in the wild, and small fish in the wild tend to show high infection rate and heavy burdens of *C. sinensis* metacercariae. However, large fish, such as the common carp *Cyprinus carpio*, the silver carp *Hypophthalmichthys molitrix*, the goldfish *Carassius auratus*, and

Table 1 Freshwater fish that are major sources of *Clonorchis sinensis* in China, Korea, and Vietnam

Region	*Fish species*
Northeastern China	*Carassius auratus, Hemiculter leucisculus, Misgurnus anguillicaudatus, Oryzias latipes, Pseudorasbora, Perccottus glenii, Phoxinus percurus, Rhodeus seroceus, Saurogobio dabryi, Abbottina sinensis*
Middle China	*Cambarus clarkia, C. auratus, Parabramis pekinesis, Parapelecus argenteus, P. parva, Rhodeus sinensis, Ctenopharyngoden idellus, S. dabryi, Cyprinus carpio, Cirrhinus molitorella, Hypophthalmichthys molitrix, H. nobilis, Hypseleotris swinhonis, Gnathopogon imberbis, Leuciscus leuciscus, Silurus asotus*
Southeastern China	*C. molitorella, C. idellus, C. carpio, Megalobrama hoffmanni, P. parva, P. pekinesis, C. auratus, Ephippus orbis, Tilapia mossambica*
Southwestern China	*C. auratus, C. idellus, C. molitorella, Macropodus chinensis, P. parva, P. pekinesis, R. sinensis, Rhodeus ocellatus, A. sinensis, Aphyocypris chinensis, G. timberbis, H. leucisculus, H. molitrix, H. swinhonis, Hemibarbus maculates, Macropodus opercularis, O. latipes, S. dabryi, Sarcocheilichthys nigripinnis, Silurus meridionalis, Sinilabeo endahli, Siniperca chuatsi, Squalidus argentatus*
Korea	*P. parva, Pungtungia herzi, Pseudogobio esocinus, Acheilognathus intermedia, Odontobutis interrupta, Zacco temmincki, Zacco platypus, Hemibarbus labeo*
Vietnam	*H. molitrix*

P. pekinensis, are usually raised in aquaculture systems, and thus, have lower metacercarial infection rates and intensities. Thus, people who eat raw large fish become infected with fewer *C. sinensis* adults than those who eat small fish. However, those who routinely eat raw freshwater fish accumulate adult *C. sinensis* in the bile duct, which can lead to chronic infection and a heavy worm burden (Table 1).

In mid and southern China, large freshwater fish, such as the common and silver carp and *P. pekinensis*, are farmed in aquaculture systems and supplied to fish markets, and these fish have a light metacercarial burden. The grass carp (*Ctenopharyngodon idellus*) weighs 2–5 kg and is commonly sold in fish markets, and thus, is considered a species of public health importance. This fish has been reported to be infected with *C. sinensis* metacercariae in endemic areas in several Chinese provinces. Small freshwater fish, such as *P. parva*, *Oryzias latipes*, and *Abbottina sinensis*, are recognized as important fish hosts of the metacercariae and act as major sources of human clonorchiasis in northern endemic Chinese provinces. Other small fish, such as *Parapelecus argenteus* and *Gnathopogon imberbis*, have been reported to exhibit high *C. sinensis* metacercariae infection rates in Anhui, Hubei, Hunan, and Sichuan provinces in central China, which are moderately endemic areas (Table 1).

In Korea, most fish hosts belong to the family Cyprinidae and some to the families Bagridae and Clupeidae. The small freshwater fish, *P. parva* and *Pungtungia herzi*, have been reported to be heavily infected by *C. sinensis* metacercariae, whereas other small fish, such as *Pseudogobius esocinus*, *Hemibarbus labeo*, *Acheilognathus intermedia*, and *Zacco* spp., show high metacercarial infection rates but low infection intensities. However, large fish, such as *C. carpio* and *C. carassius*, which are frequently eaten raw, have low infection rates.

In northern Vietnam, seven species of freshwater fish have been reported to act as second intermediate hosts for *C. sinensis* and *H. molitrix* has been found to be the most infected with *C. sinensis* metacercariae.

The major mode of human clonorchiasis infection is the eating of raw or undercooked freshwater fish or crustaceans. In *C. sinensis* endemic areas, typically, more than 70% of inhabitants have eaten raw freshwater fish and in some endemic areas in East Asia, freshwater fish are served as raw fish congee or as 'sushi.' Consumers believe that raw fish is an excellent health food that is free of contamination. Large freshwater fish are favored for raw consumption in restaurants. In fact, in restaurants, small freshwater fish have been replaced by larger species, such as *C. carpio*, *C. capio nudus*, and *Ct. idellus*. Although large fish are less frequently and lightly infected with *C. sinensis* metacercariae, adult *C. sinensis* accumulate in the human bile duct for more than 10 years, and thus, the routine consumption of freshwater fish leads to the accumulation of adult flukes in the liver and heavy infections. However, although small fish like *P. parva* are infected heavily with *C. sinensis* metacercaria, they are rarely consumed raw, and thus are of little medical or public health importance in endemic areas.

In endemic areas, *C. sinensis* metacercariae can contaminate utensils used to prepare raw freshwater fish. Thus, in households where only men eat raw freshwater fish, women and children can become infected due to the contamination of utensils. Furthermore, hands accidentally contaminated with metacercariae, by not washing properly after preparing food or after catching freshwater fish can cause infections.

Detection of Metacercariae in Fish

Microscopy

In small freshwater fish, almost all metacercariae encyst in the flesh of the trunk, and more metacercariae encyst in supraspinal than in infraspinal flesh. In some heavily infected fish, the metacercariae are found in gill and scales, and rarely in viscera.

Clonorchis sinensis metacercariae can be directly detected in the flesh of freshwater fish, as follows. First, prepare thin slices of fish muscle, compress slices, between two slide glasses, and place on the mechanical stage of a microscope. Observe the specimen under bright field at low magnification. To delineate metacercariae, adjust the contrast of the bright field to moderate/high because metacercariae are translucent like fish muscle. *C. sinensis* metacercariae can be differentiated from those of other species by cyst size and shape, the presence of oral and ventral suckers of equal size, and a dark triangular excretory bladder.

To more clearly view and identify metacercariae, fish muscle can be removed by artificial digestion. Briefly, fish muscle is ground in a mortar and pestle and mixed with approximately 10 times its volume of artificial gastric juice (6 g of pepsin (1:10 000, Sigma Co.), 10 ml of concentrated HCl, distilled water made up to 1 l). The mix is incubated at 37 °C for 1 h and stirred intermittently with a glass rod. The digest is filtered through a 212 μm mesh sieve and sediment in the filtrate is washed until clear with physiological saline. *Clonorchis sinensis* metacercariae can be collected from the particulate sediment under a dissecting microscope, mounted on a slide and observed in detail at higher magnification under a light microscope.

Nucleic Acid Based Methods

It is difficult to detect *C. sinensis* metacercaria in fish muscle by conventional light microscopy, and to differentiate them morphologically from those of other digenean trematodes. DNA detection methods, that is, polymerase chain reaction (PCR) and loop-mediated isothermal PCR (LAMP), can be used. Metacercarial genomic DNA can be extracted from freshwater fish after artificial digestion or can be extracted with fish DNA by treating a fish flesh with SDS/proteinase K and following this with spin column chromatography. A PCR method, targeting mitochondrial ITS2 and employing *C. sinensis*-specific primer pairs, detected one metacercaria in 3 g of flesh without cross-reaction with ITS2 of the genera *Opisthorchis* and *Heterophyes*. A LAMP method targeting the cathepsin B3 gene has also been reported to be able to detect 9 metacercariae per g of flesh. LAMP assay is cost-effective, rapid, and can be used to detect *C. sinensis* metacercariae accurately in the field in clonorchiasis endemic areas.

Deactivation Methods

When frozen in liquid nitrogen for 10 s, *C. sinensis* metacercariae in the flesh of *P. parva* remain infective, but all are killed by complete freezing for 30 s. It takes 4 min 50 s to freeze *Cyprinus carpio* (35 cm long) completely in liquid nitrogen and 2 min 4 s for *C. carassius* (15 cm long).

The *C. sinensis* metacercariae in *P. parva*, frozen at −12 °C for 10–18 days or at −20 °C for 3–7 days remain viable and infective to experimental animals. However, when stored at −12 °C for 20 days, metacercariae lose their infectivity. *C. sinensis* metacercaria in the flesh of *P. parva* kept at a heavy salt concentration (fish/salt = 10 g/3 g) for 5–7 days remain viable and infective. However, storage in this concentration for longer than 8 days reduced metacercariae survival in freshwater fish. Accordingly, freezing or storing infected freshwater fish in salt for long periods seems to inactivate *C. sinensis* metacercaria effectively.

The metacercariae in the decaying flesh of dead fish remain infective to experimental animals for 20 days and loose viability after 30 days. Furthermore, nitrogen compounds, mainly ammonia, produced endogenously in decaying fish can be lethal to *C. sinensis* metacercariae. In addition, *C. sinensis* metacercariae isolated from fish flesh can survive in water for longer than 1 week and can retain infectivity in PBS at 4 °C for 3 months. Thus, it seems that liberated metacercariae can contaminate food and utensils and be a source of contamination for family members.

Gamma-ray irradiation at high doses adversely affects the survival of all organisms. Isolated *C. sinensis* metacercariae irradiated with 50 Gy all failed to survive in rats (ID_{50}=16.5 Gy), and irradiation of metacercariae in the flesh of a small fish, *P. parva*, with 100 Gy killed 99% of metacercariae with an LD_{50} of 47.5 Gy.

Food Safety and Control Measures

In endemic areas, human infections are contracted by eating the raw or undercooked flesh of freshwater fish. The most practical measure for prevention, therefore, is not to eat raw or undercooked fish. However, food consumption governed by traditional customs and cultures, and thus, to change the food habits of a community, health education should be well organized and persistent. Health education should include topics, such as modes of infection, infectivity of *C. sinensis* metacercariae, freshwater fish harboring metacercariae, safe cooking procedures, hazards and illnesses associated with infection, diagnosis and treatment, and prevention measures. Control programs against clonorchiasis can be run in endemic areas with the cooperation of regional health staff.

The only source of human infection is freshwater fish infested with *C. sinensis* metacercariae. To prevent contamination of the snail host by eggs, sources of infection should be reduced or eliminated by chemotherapy of infected humans and reservoir hosts. In particular, aquaculture facilities and fishponds must be protected from contact with human and animal feces. Control measure includes sanitary treatment and disposal of feces, and regulation regarding the use of fertilizers.

See also: Helminth-Trematode: *Echinostoma*; *Metagonimus yokogawai*; *Opisthorchis viverrini* and *Opisthorchis felineus*

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Relevant Website

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The Korean Society for Parasitology and Tropical Medicine.

HELMINTH-TREMATODE

Dicrocoelium dendriticum

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Glossary

Cercaria Term derived from the Old Greek word *kérkos*, which means tail, referring to a larval stage of Digenea, which is provided with a tail (cercariae are extruded from the snails in slime balls and ingested by the ants in the case of *Dicrocoelium dendriticum*).

Definitive host The host, usually a vertebrate, in which the parasite reaches maturity and reproduces sexually (mainly ruminants in the case of *D. dendriticum*).

Digenea Term derived from the Old Greek words *dis* (double) and *ghénos* (generation), referring to trematodes characterized by a life cycle with alternation of sexual and asexual reproductive phases.

Fluke Term derived from the Old English name for *flounder*, referring to the flattened shape of trematodes.

Intermediate host The host, usually an invertebrate, in which the parasite reproduces asexually through different larval stages (*D. dendriticum* has two intermediate hosts, i.e., land snails and ants).

Metacercaria Term derived from the Old Greek words *metá* (after) and *kérkos* (tail), which means the next stage of the cercaria, referring to the larval stage of trematodes that is encysted (within the ants in the case of *D. dendriticum*).

Miracidium Term derived from the Old Greek word *meirákion*, which means young, referring to the first larval stage of Digenea, pyriform, and ciliated (it is located in the egg ingested by the first intermediate host, land snails, in the case of *D. dendriticum*).

Prepatent period Time taken for development from infection (ingestion of ants with metacercariae in the case of *D. dendriticum*) until mature parasites reproduce sexually and produce parasitic elements (eggs in the case of *D. dendriticum*).

Sporocyst Term derived from the Old Greek words *sporá* (seed) and *kýstis* (cyst), which means a cyst containing a number of germinal cells, referring to a larval stage of Digenea that develops into the intermediate host (land snail in the case of *D. dendriticum*).

Spurious infection A case in which a parasite or parasitic elements (e.g., eggs) invade a host but do not infect it, for example merely passing through the digestive tract.

This article focuses on *Dicrocoelium dendriticum*, a small lanceolate trematode parasite (fluke) located in the biliary tract of animals (mainly ruminants) and humans. The life cycle is complex with two intermediate hosts, land snails and ants. Infection (foodborne zoonosis) occurs by passive ingestion of ants containing the infective stage metacercaria. Dicrocoeliosis, although very common and prevalent in ruminants, is considered rare in humans or sporadically reported. Misdiagnosis is frequent due to spurious infections as a consequence of eating raw or undercooked animal liver. The characteristics, clinical manifestation, epidemiology, diagnosis, and control of *D. dendriticum* are presented.

D. dendriticum (from the Old Greek words *dicròos*: double, *choilìa*: gut, and *dentrìticon*: branched) was firstly described in 1819 by the Swedish naturalist Karl Asmund Rudolphi, who is also considered the father of helminthology.

This parasite is located in the bile ducts and gall bladders of numerous species of mammals, mainly domestic and wild ruminants (e.g., sheep, goat, cattle, buffalo, deer), rabbits, and occasionally horses, pigs, dogs, and humans. Infection by *D. dendriticum* is a foodborne zoonosis of the biliary tract, considered to be very rare in humans or at least under-reported.

D. dendriticum (synonym *D. lanceolatum*) belongs to the phylum Platyhelminthes (flatworms commonly referred to as flukes), class Trematoda, subclass Digenea, order Plagiorchiida, and family Dicrocoelidae.

D. dendriticum is a small lanceolate fluke, 0.6–1.0 cm long and 1.5–2.5 mm wide, characterized by a dorsoventral, flattened, bilaterally symmetrical body. Like other Digenea, the body surface is covered by the tegument, i.e., a syncytial epithelium with sensory functions, implicated in nutrient absorption, synthesis, secretion, and osmoregulation; it also protects the parasite from the immune system of the host. The tegument of *D. dendriticum* is typically smooth without spines; therefore this fluke is typically semitransparent, with a black uterus and white testes visible to the naked eye (**Figure 1**). Adult *D. dendriticum* has two muscular suckers: the oral sucker (300–400 μm in diameter) at the anterior end surrounding the oral opening, and the ventral sucker (500–600 μm in diameter) also named *acetabulum*, located in pre-equatorial position, used by the fluke to attach to the wall of the small bile ducts of the definitive host (**Figure 2**). The digestive system is simple and contains the oral opening, pharynx, esophagus, and two branched intestinal ceca, which end

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blindly. Excretory and nervous systems are simple; respiratory and circulatory systems are absent. As most trematoda, *D. dendriticum* is hermaphrodite with both the male and female reproductive systems. The male system is composed of two testes arranged in tandem, two vas deferens, a seminal vesicle, and a primitive penis called cirrus that terminates at the common genital pore; the female one is composed of a single ovary, oviduct, ootype, vitelline glands and ducts, uterus, and a common genital pore.

The egg of *D. dendriticum* (Figure 3) is small, 35–40 µm long and 25–30 µm wide, thick-walled, dark brown and operculate, oval, and slightly flattened on one side, and contains a miracidium (having 2 typical germ balls similar to two little eyes) when excreted with the feces of the host.

Variability of adult *D. dendriticum* specimens has been observed both upon morphoanatomic parameters and genetic – using random amplified polymorphic DNA. However, a lower intraspecific variation has been observed when using the second internal transcribed spacer (ITS-2) rDNA, a reliable genetic marker already used for molecular systematic studies

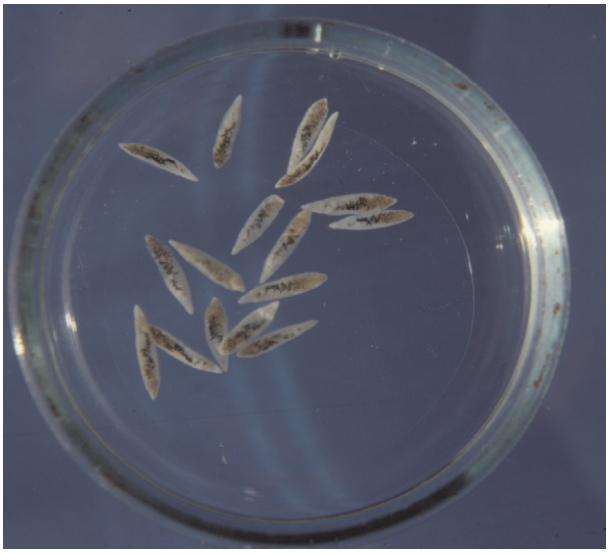


Figure 1 Adults of *D. dendriticum*.

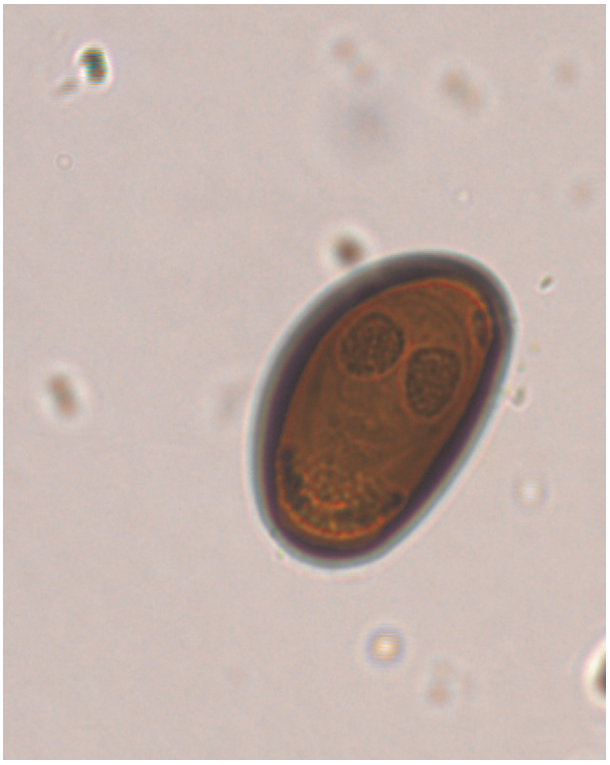
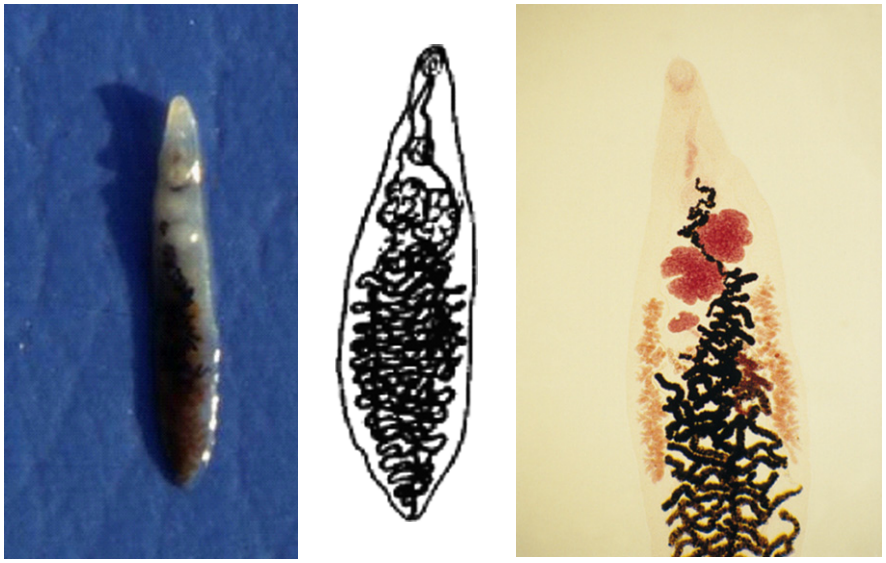


Figure 3 Egg of *D. dendriticum*.



(a) (b) (c)

Figure 2 Adults of *D. dendriticum* (a), scheme (b), and under the microscope (c).

of platyhelminthes. Nevertheless, genetic factors have not yet been associated to *D. dendriticum* virulence so far.

The entire life cycle of *D. dendriticum* (Figure 4) was elucidated between 1952–53 by the naturalists Krull and Mapes. It is indirect and very complex, with two intermediate hosts. The first intermediate hosts are land snails (Figure 5) of at least 30 genera (e.g., *Zebrina*, *Cionella*, *Helicella*, *Abida*); the second intermediate hosts are brown ants (Figure 6) of the genus *Formica* (e.g., *Formica fusca*, *Formica rufa*, *Formica rufibarbis*, *Formica pratensis*).

Like other Digenea there is alternation of sexual and asexual reproductive phases in the definitive hosts and the intermediate hosts, respectively, with four larval stages (miracidium, sporocysts, cercaria, and metacercaria).

In the bile ducts of the definitive host, adults *D. dendriticum* reproduce sexually by self- or cross-fertilization. Then they release eggs containing a miracidium into the environment through the host feces. After being ingested by a snail, these eggs hatch and release the miracidium, which migrates to the hepatopancreas of the snail. Then, two generations of sporocysts (elongated sacs containing a number of germinal cells) develop by asexual multiplication. From the germinal cells of the sporocyst (the redia stage is absent unlike other trematodes) arise the cercaria, a stage provided with a long tail. Numerous mature cercariae migrate to the respiratory chambers of the snail and are extruded from the snails in masses cemented together in mucilaginous slime balls. These slime balls are then ingested by ants, in which they develop to the encysted stage, called metacercaria, mainly in the body cavity and occasionally in the brain of the insect. The presence of a brain lesion in the ant, induced by one or few metacercariae, causes a cataleptic cramp (especially in early morning), which 'paralyzes' the ant on the tips of herbage and grass, thus increasing the chance of infection by the definitive host.

Infection of the definitive host is by passive ingestion of ants containing metacercariae. The metacercariae hatch in the small intestine and the young flukes (adolescariae) migrate to the small bile ducts, to the larger bile ducts and then to the gall bladder via the choledochus without any parenchimal migration into the liver.

The total life cycle (from egg to egg) of *D. dendriticum* requires *circa* 6 months. The prepatent period is 10–12 weeks. Adults flukes are long-lived in the bile ducts and gall bladder and can survive for several years.

Food accidentally contaminated with ants containing *D. dendriticum* metacercariae is the source of infection to humans. Metacercaria is a very resistant stage and has great potential for survival extending to months. Indeed, metacercariae in the ant live as long as the ant itself. Once ingested, the opening of the metacercaria occurs in the intestine of the host and depends on enzymatic factors as well as phenomena of oxidation–reduction and the presence of carbon dioxide.

The duration of clinical manifestation varies mainly according to the status of the patient.

In humans, symptoms of true *D. dendriticum* infection can vary from null to mild, but may also include chronic diarrhea, constipation, right upper abdominal pain, vomiting,

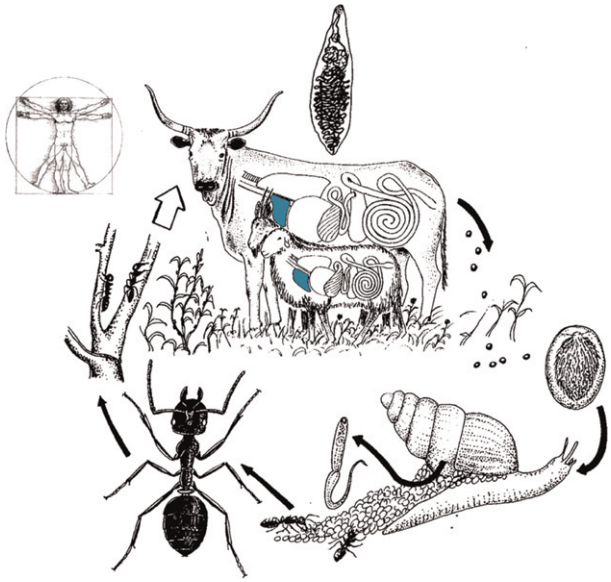


Figure 4 Life cycle of *D. dendriticum*.

Figure 5 Land snails, first intermediate hosts of *D. dendriticum*.

Figure 6 Ants, second intermediate hosts of *D. dendriticum*.

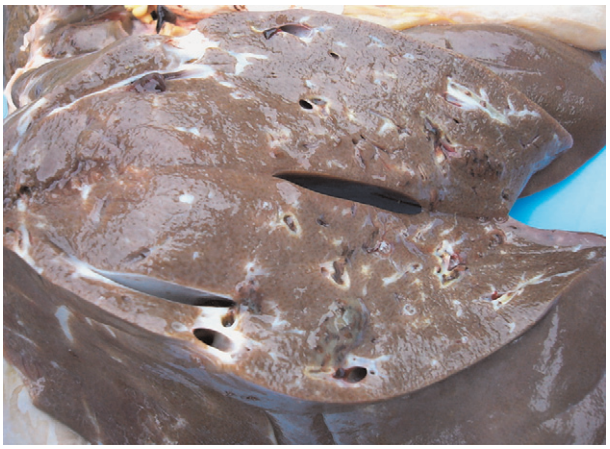


Figure 7 Sheep liver infected by *D. dendriticum*.

weight loss, and fatigue. Hepatomegaly, peripheral eosinophilia, biliary obstruction, and cholangitis may also occur. The pathogenesis is similar to other infections caused by liver trematodes such as *Fasciola*, *Opistorchis*, and *Clonorchis*. In ruminants, in the case of heavy infections, fibrosis of the small bile ducts and extensive cirrhosis can occur (Figure 7).

Pathological consequences of infection by *D. dendriticum* vary with host species, and are generally dose-dependent and attributed to complications arising from chronic inflammation of the mucosal epithelium. The infective dose is unknown. Infected ants may contain several metacercariae (up to 200) and a high percentage of ants may be infected. In infected humans, complications may be related to immunesuppression; indeed cases of patients infected with human immunodeficiency virus (HIV) and *D. dendriticum* have been reported. In addition, acute urticaria associated with *D. dendriticum* has also been reported as complication of the infection.

In animals, especially ruminants, dicrocoeliosis is an important parasitic infection from an economic and health point of view. This infection causes severe economic losses, in terms of milk and meat production in many countries, due to liver function impairment. The disease can be fatal on rare occasions. The economic significance of infection by *D. dendriticum* is due to the direct losses occasioned by the confiscation of altered livers and also to the indirect ones caused by the digestive disorders derived from the hepatobiliary alterations, such as decreased animal weight, growth delay, and reduced milk production, as well as by the cost of anthelminthic treatments.

Risks of misdiagnosis are very high in humans. Indeed, spurious infection (pseudoparasitism) by *D. dendriticum* is very frequent as a consequence of eating raw or undercooked animal liver; in such a case eggs pass unchanged through the digestive tract of an uninfected patient.

The only reliable methods to differentiate true from spurious infections are: (1) repeated coprological examinations (consecutively over 3 days) from patients with a diet from which liver is excluded and (2) examination of duodenal or biliary fluid to detect *D. dendriticum* eggs.

Palaeoparasitology studies show that the presence of *D. dendriticum* in Western Europe was attested from 550 000 years Before Present (BP) to the sixteenth century AD. Moreover, the parasite was identified in the New World and approximately the seventeenth century AD following the colonization of Canada by Europeans. Geographic distribution of *D. dendriticum* covers Europe, Asia (in particular China, Japan, Malaysia, Indonesia, and ex-USSR), North Africa, South America, and some focal points of North America.

Up to now, the importance of *D. dendriticum* as a food safety problem is quite low due to the rarity of human clinical cases of dicrocoeliosis. These few clinical cases, however, could represent only the tip of the iceberg.

Clinical cases of true human dicrocoeliosis have been reported sporadically in various parts of the world. Most reports of human infection by *D. dendriticum* originate in Europe and the Middle East; however the lanceolate liver fluke has been also reported in humans in Canada.

Nevertheless, statistical data on prevalence and/or incidence of dicrocoeliosis in humans are limited and scarce, contrary to what occurs with other foodborne trematodes (e.g., *Clonorchis*, *Opistorchis*, *Fasciola*, *Paragonimus*, *Fasciolopsis*, *Echinostoma*, and Heterophydae). For these latter trematodes, indeed, even if they are still neglected, at-risk population, number of infections, and global burden have been estimated in humans.

This situation in humans contrasts sharply with *D. dendriticum* infection in ruminants. Indeed, dicrocoeliosis is typically considered a 'disease of sheep and cattle' distributed worldwide in lowland or mountain pastures, which provide adequate conditions for the survival and development of terrestrial snails and ants. Prevalence values are usually higher in sheep and goats (up to 100% in many Mediterranean and Middle Eastern areas) than in cattle and buffaloes.

Therefore, the main reservoirs of *D. dendriticum* are ruminants, especially grazing sheep, which eliminate the eggs of the parasite that may overwinter and remain infective for up to 20 months on pastures. Eggs can be found in raw and treated wastewater.

The only vehicle of transmission to humans are field ants infected with metacercariae of *D. dendriticum*. The soil is the reservoir of these infected ants that can be found everywhere by chance, example, on grass, herbs, raw fruit, vegetables, cereals, foodstuffs, and drinking water. Therefore, swallowing infected ants, example, in unwashed fruits and greens or while playing in grass (children) is the main way of infection to human. Furthermore, other sources of ants potentially infected by *D. dendriticum* metacercariae are large warehouse supplies or even supplies outdoors without any barriers to prevent any invasions such as in refugee camps or food banks. Bottled water contaminated by infected ants has also been demonstrated as source of *D. dendriticum* infection. The possibility of transmission through ants in households invading kitchens has not been studied until know.

D. dendriticum is usually found where dry and calcareous or alkaline soils are present, because they represent a favorable biotope for its intermediate hosts, land snails, and ants. A study performed by multivariate spatial analysis of *D. dendriticum* infection in sheep in Italy showed that wood, rocks, and arable with sparse trees explained the spatial

distribution of the fluke. In another study, mountainous pastures with a mean slope of higher than 25%, situated over 600 m, where precipitation was high and temperature low, had involved the highest risk of infection by *D. dendriticum* in cattle in Spain.

However, impact of climatic change seems to be limited on the spatial and temporal distribution of *D. dendriticum* compared to other liver flukes such as *Fasciola hepatica*, whose rise in recent years has been attributed to climate change.

Dicrocoeliosis often remains clinically undetected or undiagnosed, most likely because of its subclinical nature. Its diagnosis in animals is mainly due to recovering adults in the liver at necropsy or detecting eggs at coprological examination. Flotation techniques using jodomercurate-based or zinc-sulfate-based solutions (specific gravity = 1.350–1.450) perform better than sedimentation techniques. As with other foodborne trematode infections, diagnosis is often unsatisfactory when using the current copromicroscopic techniques (e.g., Kato–Katz, ether sedimentation, McMaster technique). Recently, new multivalent techniques for qualitative and quantitative copromicroscopic diagnosis of parasites in animals and humans, the FLOTAC techniques, showed a higher sensitivity and efficiency for detecting *D. dendriticum* eggs in fecal samples from animals and humans.

Immunological and molecular techniques have been developed for the detection of dicrocoeliosis in animals.

As with other foodborne trematodes, there are only two drugs available for treatment and control of *D. dendriticum* in humans: praziquantel and triclabendazole. In the case of a spurious infection, treatment is not recommended. Prevention of contamination by *D. dendriticum* in the environment is difficult because of the longevity and resistance of the eggs in the environment, the number of reservoir hosts, and the wide distribution of intermediate hosts. Methods against the intermediate hosts (e.g., using calcium cyanamide molluscicide and chemical fertilizers) are not feasible because of their high costs and ecological unsustainability. Education measures becomes important especially for young children. Prevention of contamination in the environment depends almost entirely on regular anthelmintic treatments of ruminants.

As it happens for other parasites, *D. dendriticum* is often forgotten in the food safety and hygiene community. Therefore, regulatory measures are still unavailable, and only some practical commonsense preventive measures (e.g., keeping ants away from all food supplies) may be useful to reduce the risk of infection. However, regulatory and education measures should be strengthened because the high prevalence in animals and the high number of human spurious infection detected show that true infection is likely to occur in humans. Improved access to clean water, appropriate sewage disposal and treatments, enhanced food safety measures, and collaboration between public health and veterinary medicine are important integrated measures to reduce transmission to humans through food.

Regarding public health, the global burden of *D. dendriticum* infection is yet to be determined as it is for other foodborne trematode infections, considered an important group of neglected tropical diseases. However, since 2007, *D. dendriticum* has been included in the list of causative agents for which burden of disease estimates are to be derived according to Task Force 1 of the Foodborne Disease Burden Epidemiology Reference Group (FERG) of the World Health Organization.

See also: Disciplines Associated with Food Safety: Epidemiology; Parasitology. Foodborne Diseases: Foodborne Diseases in Travelers; Prevalence of Foodborne Diseases in Europe; Prevalence of Foodborne Diseases in North America; Prevalence of Foodborne Diseases in South East and Central Asia; Prevalence of Foodborne Diseases in Western Pacific Region. Helminth-Trematode: *Fasciola hepatica* and *Fasciola gigantica*; *Opisthorchis viverrini* and *Opisthorchis felineus*. History of Food Safety and Related Sciences: History of Foodborne Disease in Asia – Examples from China, India, and Japan. Public Health Measures: Surveillance of Foodborne Diseases. Safety of Food and Beverages: Fruits and Vegetables. Veterinary Drugs Residues: Anthelmintics

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Diphyllobothrium

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Glossary

Cestod A class of parasitic flatworms, commonly called tapeworms, of the phylum Platyhelminthes. Several species parasitize humans after being consumed in underprepared meat such as pork (*Taenia solium*), beef (*Taenia saginata*), and fish (*Diphyllobothrium* spp.).
Copepods Microscopic crustacean, part of the zoonotic plankton.
Coracidium Ciliated swimming larvae of the genus *Diphyllobothrium*, exiting the egg through the operculum, that need to be ingested by a copepod to develop in a procercoid.
Diphyllobothriosis A disease caused by the cestode species of the genus *Diphyllobothrium*.
Plerocercoid Larval stage of parasitic species of the genus *Diphyllobothrium* found in fish.
Procercoid Larval stage of parasitic species of the genus *Diphyllobothrium* found in the copepod.
Proglottids Part of the adult worm composed of different segments containing eggs and passed through the feces.

Background

Tapeworms of the genus *Diphyllobothrium* are widely distributed all around the world, with a dozen species that are known to be agents of human diphyllobothriosis, one of the most important fish-borne zoonoses caused by a cestode parasite. Intermediate hosts include both freshwater and marine fish, mainly the anadromous species. Piscivorous birds and mammals (including humans) are definitive hosts and contract these parasites by eating raw or poorly cooked fish containing plerocercoid larvae. *Diphyllobothrium* cestodes are commonly present in the wildlife including fish, mammals, and birds which are important reservoir hosts. This parasitosis is known since antiquity: in Europe, eggs of *Diphyllobothrium latum* were discovered by the archeologists in lake villages dating from the Neolithic era in France and Germany. The parasite was first described as *Taenia lata* in 1592 by Dunus, a physician established in Locarno (Switzerland). Nearly 300 years later, Parona working in the subalpine lake regions of the north of Italy, and Janicki and Rosen in Neuchâtel, definitively described the parasitic cycle, which proved to be one of the most complex known at the time.

Characteristics

The genus *Diphyllobothrium* belongs to the family Diphyllobothriidae, order Diphyllobothriidea (formerly Pseudophyllidea), class Cestoda, and phylum Platyhelminthes. To date, approximately 50 species have been described but only 12 have been reported as human pathogens (Table 1). The most frequent are *D. latum* (cosmopolitan), *Diphyllobothrium nihonkaiense* (Japan, South Korea, Eastern Russia, and probably North America), *Diphyllobothrium dendriticum* (northern part of the Northern Hemisphere), and *Diphyllobothrium pacificum* (Argentina, Chile, Peru). Infections with *Diplogonoporus balaenopterae* (syn. *Diphyllobothrium grandis*) have been reported in Japan and recent molecular studies revealed a close relationship with the cetacean tapeworm *Diphyllobothrium stemmacephalum*, which in turn is probably synonym of *Diphyllobothrium yonagoense*. Other species (*Diphyllobothrium alascense*, *Diphyllobothrium cameroni*, *Diphyllobothrium cordatum*, *Diphyllobothrium dalliae*, *Diphyllobothrium hians*, *Diphyllobothrium orcini*, *Diphyllobothrium ursi*, *Diphyllobothrium scoticum*, and *Diphyllobothrium lanceolatum*) have also been described in humans, but very few details are available in the scientific literature.

The life cycle of *D. latum*, probably the most frequent species in humans, is complex and involves several hosts (Figure 1). Released in freshwater, the eggs mature within 8–12 days at a water temperature of 16–20 °C, and yield coracidium larvae that are ingested by zooplanktonic crustaceans; 40 copepod species (Copepoda: Diaptomidae and Cyclopidae) are likely to serve as the first intermediate hosts. The coracidium develops into a procercoid larva within the general cavity of the copepod. When a carnivore fish ingests planktonic crustaceans, the procercoid develops into a plerocercoid larva (Figure 2), a few millimeters long. It migrates into the host musculature or to the viscera, where it can remain inactive in a cyst for several years, but can excyst and re-encyst repeatedly if an infected fish is ingested by other predatory fishes (paratenic hosts). In Europe, fish species recorded as susceptible *D. latum* plerocercoids are perch (*Perca fluviatilis*), pike (*Esox lucius*), Arctic charr (*Salvelinus alpinus*), and burbot (*Lota lota*). Coregonidae and probably Salmonidae

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Table 1 Species of *Diphyllobothrium* reported from humans

Species	*Fish hosts*	*Other hosts*	*Distribution*
D. alascense (Rausch and Williamson, 1958)	Burbot, smelt	Dog	Alaska
D. cameroni[a] (Rausch, 1969)	Marine fish	Monk seal	Japan
D. cordatum (Cobbold, 1869)	Marine fish	Seals, walruses	North Atlantic
D. dalliae (Rausch, 1956)	Alaska blackfish, Dolly Varden	Gulls, dog	Alaska
D. dendriticum (Nitzsch, 1824)	Salmonids, coregonids	Fish-eating birds	Circumpolar
D. hians[a] (Diesing, 1850)	Marine fish	Arctic seals	North Atlantic
D. lanceolatum (Krabbe, 1865)	*Coregonus*	Fur seals	North Pacific
D. latum (L, 1758)	Pike, burbot, perch, Arctic char, other percids	Terrestrial mammals	Cosmopolitan
D. nihonkaiense[a] (Yamane, 1986)	Pacific salmons	Brown bear	Eastern Eurasia, Japan
D. pacificum (Nybelin, 1931)	Marine fish	Sea lions, eared seals	South America
D. ursi (Rausch, 1956)	Red salmon	Bears	Alaska, British Columbia
D. grandis[b] (Blanchard, 1889)	Anchovy, sardine	Cetaceans	Circumpolar, Japan

[a]*D. nihonkaiense* and *klebanovskii* (Muratov and Psokhov, 1988) are synonymous.
[b]This could be synonymous with *Diphyllobothrium stemmacephalum*.

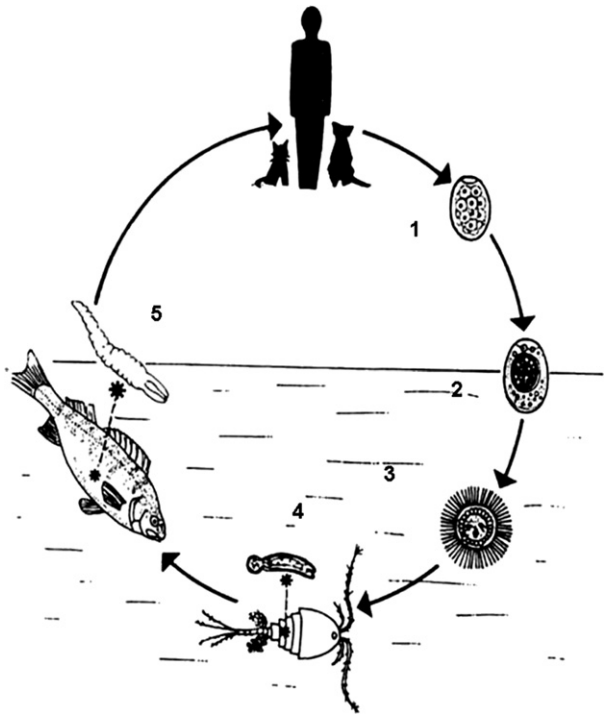

Figure 1 Life cycle of *Diphyllobothrium latum*: 1, egg; 2, embryonated egg; 3, coracidium; 4, procercoid larva in a copepod; 5, plerocercoid larva in a fish.

of the *Salmo* genus do not host *D. latum* larvae, but they can serve as a host for *D. dendriticum*. Pacific salmon of the genus *Oncorhynchus* are the usual hosts of *D. nihonkaiense*. Marine fish are the intermediate hosts for *D. pacificum* and *D. balaenopterae*. Humans and other ichthyophagous animals become infected by ingesting raw infected fish. The plerocercoid larva then develops rapidly into an adult (Figure 3) in the intestine that yields its first eggs 2–6 weeks after the infection.

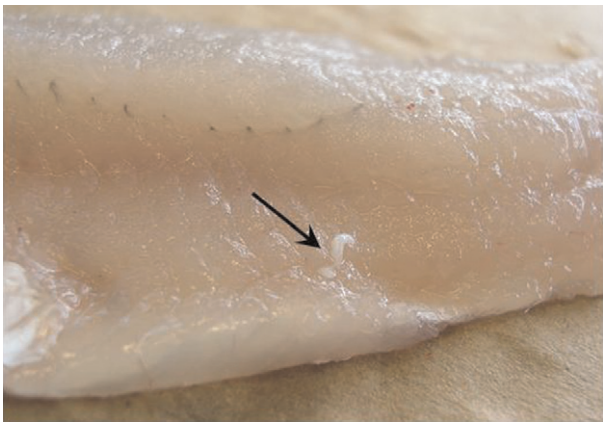

Figure 2 Plerocercoid of *Diphyllobothrium latum* in a perch fillet (*Perca fluviatilis*) from Lake Maggiore, Switzerland (picture: B Wicht).

Figure 3 Proglottids of *Diphyllobothrium latum* obtained from a patient contaminated after eating raw fish from Lake Geneva. Note the ventral opening of the genital pore (picture: J Dupouy-Camet).

Clinical Manifestation

Diphyllobothrium tapeworms are the longest human parasites known (2–15 m long) and can live for several years. The ingestion of a single plerocercoid larva can lead to infection. The larva will grow 1–2 cm per day and 3 weeks after ingestion will provoke the occurrence of symptoms. The symptoms of human diphyllobothriosis, although limited, are polymorphous and may include abdominal discomfort, abdominal pain, diarrhea, weight loss, asthenia, and vertigo. All these symptoms are vague and are quite common among the general population. A study performed 50 years ago during an extensive field investigation in Finnish Karelia compared 295 parasitized persons with 1127 controls. Statistical analysis showed that the worm carriers had a significantly higher frequency of fatigue, weakness, diarrhea, and numbness of extremities. A sensation of hunger and craving for salt was also associated with the worm infection. Some carriers experience very little discomfort and become alerted when proglottids are passed in the feces. The parasite can also be found accidentally when performing a colonoscopy. Megaloblastic anemia due to vitamin B_{12} deficiency has been described in cases of prolonged infection in a malnourished Finnish population after World-War II, but it is only rarely reported nowadays. Most of the time, the parasitosis is mistaken for functional colopathy. *Diphyllobothrium* parasites are sensitive to praziquantel (25 mg kg^{-1} day^{-1} in one dose is effective against most human pathogen species) or to niclosamide (2 g on an empty stomach in two doses an hour apart).

Epidemiology

In the early 1970s, the worldwide prevalence of human diphyllobothriosis was estimated to be 9 million cases. More recent data indicate that 20 million people are infected worldwide, but many more are at risk; estimations of global prevalence have not been adequately performed. The parasitosis is frequently reported from Japan, South Korea, the Baltic countries, Scandinavia, Western and Eastern Russia, and North America (Pacific Northwest). Only sporadic cases have been reported in South America.

Diphyllobothrium latum is present in America (Canada, Chile, Brazil), but recent surveys have indicated that this parasitosis was emerging or re-emerging in some countries, particularly in Italian and French-speaking subalpine regions. This species is especially encountered in Scandinavia, the Baltic States, around the Lake Leman, in France, and Switzerland and in the north of Italy around the Lakes Maggiore and of Como. In Switzerland, between 2002 and 2007, more than 250 cases of diphyllobothriosis were reported in the canton of Geneva (230 cases), in Tessin (11 cases), and in the cantons of Vaud (10 cases). In Geneva, a small epidemic was reported in June 2006 after a wedding banquet where 8 people out of 26 were infested by *D. latum* after consumption of marinated raw perch. Over the same period (2002–2007), 44 cases were reported in the department of Haute-Savoie (France) exclusively in towns located on the shores of Lake Leman. The average prevalence of the parasite (pleurocercoids) in perch filets from the lakes of the subalpine region ranges from 5% to 14%. *D. nihonkaiense* cases are often reported in Japan, in South Korea, and Russia, but this species was also reported in France and in Switzerland after the consumption of imported Pacific salmon (*Onchorhynchus* sp.). *D. pacificum* is frequently found on the Pacific coasts of Chile and Peru. Diphyllobothriosis is a zoonosis and infected piscivorous mammals (cat, dog, fox, etc.) can serve as reservoirs for the parasite and maintain the cycle in the lake ecosystems.

Analytical Methods

Conventional Techniques

All members of the genus *Diphyllobothrium* are morphologically very similar and are sometimes distinguished by hosts and geographical origin. Human infective species can be identified by skillful parasitologists using morpho-anatomical criteria, which, are relative, however, as they vary with the age and the degree of development, and are influenced by physiological modifications. Diagnosis is possible on the whole adult parasite (rarely obtained) showing a medium-sized to large tapeworm with strobila that is usually segmented. Genital apertures are on the ventral face and the scolex is unarmed with a pair of dorsal and longitudinal grooves, named bothria. Segments (proglottids) are wider than long with genital pores opening medioventrally (Figure 3). Each segment measures 2–7 mm × 10–12 mm. Eggs are oval and operculated. The eggs of *D. latum* are grayish brown with a 50–85 × 40–60 µm average size, whereas those of *D. nihonkaiense* are smaller (53–59 × 35–45 µm). However, sizes vary considerably among individual worms and could decrease with the intensity of infection. Eggs can be mistaken with those of *Paragonimus* spp. or *Fasciola hepatica*, but their sizes are larger.

Plerocercoid larvae can be searched for in the host tissues by the candling of thin fillets or by cutting fillets in thin slices and then direct observation with magnifying glasses. The compression method and digestion have not been evaluated but have a major drawback that such treatment could destroy the fish. The plerocercoid larvae, obtained from fish, range from a few millimeters to a few centimeters long and their identification has always been the job of an expert. They appear as white elongated masses in the skeletal muscle of certain fish families (Figure 2). They can be confounded by non-experts with acanthocephalans (easily distinguishable by the presence of an evertable proboscis armed with spines) or with cestodes of the genus *Proteocephalus*. Identification keys are based on the features of gross-morphology visible under a light or a scanning electron microscope, on body-length and site within the host, and features visible in the histological sections.

Molecular Techniques

Biochemical techniques (isoenzymatic assay or immuno-electrophoresis) have been used as alternatives to the traditional tools for species identification, but the development of molecular biology resulted in a better knowledge of the *Diphyllobothrium* genus. For example, Matsuura *et al*. utilized restriction fragment length polymorphisms (RFLPs) of ribosomal DNA (rDNA) to distinguish between *D. latum* and *D. nihonkaiense*, as

the profiles generated with restriction enzymes *Sma*I, *Hinf*1, and *Hha*I provided valuable species-specific markers. Since 1998, important systematic data on the *Diphyllobothrium* genus have been obtained by sequence analysis of the 18S ribosomal RNA (18S rRNA) and cytochrome *c* oxidase subunit 1 (cox1 or COI), NADH dehydrogenase subunit 3 (NADH3) genes, and of the partial internal transcribed spacer 2 (ITS2) region. Recently, 18S rRNA, internal transcribed spacer 1 (ITS1), cox1, and cob (cytochrome *b*) sequences were used to differentiate between *Diphyllobothrium* species isolates. Sequences from samples of plerocercoids, adults or eggs of *D. latum*, *D. nihonkaiense*, *D. ditremum*, *D. dendriticum*, *Diphyllobothrium stemmacephalum*, and *D. pacificum* have been examined. Cox1 and cob sequences analyses were clearly more discriminative than those of the ITS1 and 18S rRNA, therefore representing a useful tool for identifying specimens.

Control and Preventive Measures

Information campaigns directed at consumers must make them aware of the dangers of raw fish consumption. In some countries (e.g., France and Switzerland), legislation requires that any fish intended to be consumed raw must be frozen at a temperature lower than −20 °C, for at least 24 h. But these measures are not reliably performed by individual consumers. In practice, the freezing of fish in the standard home freezers for a week seems to be the simplest precautionary measure. Cooking to 55 °C for at least 10 min kills the parasite. These measures also have the advantage of protecting infection by other zoonotic parasites in fish, such as anisakids and the opistorchids. Salting, smoking, and pickling have not been demonstrated to be effective against the plerocercoids larvae.

Conclusions and Future Perspectives

The growing popularity of raw fish specialties has probably favored the increase of diphyllobothriosis in subalpine regions and elsewhere, and importation of Pacific salmons, or travels to endemic countries, have led to the occurrence of cases of allochtonous *D. nihonkaiense* and *D. dendriticum* in France, Switzerland, Finland, and New Zealand. In the light of these considerations, infections with allocthonous species of *Diphyllobothrium* are probably more widespread than has been recognized. In addition, these parasites sometimes show a high colonization potential, as was the case in South America for introduced *D. latum* and *D. dendriticum*. Although human-infecting species can be easily treated with the same anthelminthic (praziquantel), their correct diagnosis by laboratories and physicians is of great importance from an epidemiological perspective and must be improved. The genetic identification of broad tapeworms and the analysis of anamnestic data of the patients can contribute to clarify the distribution of such parasites and help in detecting the sources of the infections.

See also: Food Technologies: Freezing. Safety of Food and Beverages: Seafood

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WoRMS: World Register of Marine Species.

Echinostoma

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Glossary

Cercaria A free-swimming trematode larva that emerges from the first intermediate host (snail); it may penetrate the skin of a final host, encyst on vegetation, in or on fish, or penetrate and encyst in various amphibian hosts.
Echinostomatidae A family of flukes (Trematoda) characterized by a collar of spines at their anterior end. The body is elongated and is covered with spines, and the two suckers are usually close together.
Echinostomiasis Intestinal parasitic disease caused by trematode flukes from the family Echinostomatidae.
Food-borne trematodiasis Parasitic disease caused by members of the class Trematoda transmitted by food.
Metacercaria The encysted maturing stage of a trematode in the second intermediate host before transfer to the definitive host, usually representing the organism's infectious stage.
Miracidium A small free-living larval stage of parasitic flatworms in the class Trematoda. It is released from eggs which are usually shed in the feces of its vertebrate host and infects the first intermediate host.
Redia Intramolluscan larval stage of trematodes that produces more rediae or cercariae in the first intermediate host.
Sporocyst Intramolluscan larval stage of trematodes that produces more sporocysts or rediae in the first intermediate host.

Background

The term echinostomes includes the trematodes belonging to the family Echinostomatidae. These digeneans are characterized by the presence of a prominent cephalic collar of spines. Echinostomes are a rather heterogeneous group of cosmopolitan hermaphroditic digeneans that, as adults, inhabit the intestine of a great spectrum of vertebrate hosts such as birds, mammals and, occasionally, reptiles and fishes. They also are able to parasitize humans causing the food-borne infection called echinostomiasis. Humans become infected after ingestion of the second intermediate host harboring the encysted metacercariae. Infections are associated with common sociocultural practices of eating raw or insufficiently cooked mollusks, fish, crustaceans, and amphibians, promiscuous defecation, and the use of night soil (human excrement collected from latrines) for fertilization of fish ponds. Echinostomiasis is aggravated by socioeconomic factors such as poverty, malnutrition, a lack of supervised food inspection, poor or insufficient sanitation, and other helminthiases. In the present article, the authors review the main features of these trematodes with emphasis on their food-borne transmission and control.

Morphology and Classification

The morphology of several stages of echinostomes is shown in Figure 1. Adult worms of the family Echinostomatidae are morphologically characterized by the presence of a head collar with collar spines around the oral sucker (Figure 1(a)). The number and arrangement of collar spines is an important feature for taxonomic purposes. Considerable variation exists in size of echinostomes depending on species, fixation procedures, definitive host, and crowding effect. Echinostomes are referred to as small if up to 5 mm in length, medium if 5–10 mm, and large if longer than 10 mm. The spines of the cephalic collar may be arranged in one or two circles and the number of spines is constant within the species. The tegument contains scale-like spines on both dorsal and ventral surfaces, though the number and size of the spines is reduced in the posterior half of the body (Figure 1(b)). The oral and ventral suckers are close to each other. The two testes, usually in tandem, are posterior to the ovary. There is considerable confusion in relation to the systematics of the members of the family Echinostomatidae, particularly within the genus *Echinostoma*. This is attributable to a number of factors, including misidentified species or species that have been insufficiently described, the existence of substantial interspecific homogeneity of the morphological characteristics of the adult stages, as well as the broad range of final vertebrate hosts and the wide geographical distribution. The Echinostomatidae has been viewed as a monophyletic taxon, though the morphology and the diversity of the criteria adopted by different authors have led to its division into an impressive number of taxa (i.e., a total of 21 nominal subfamilies).

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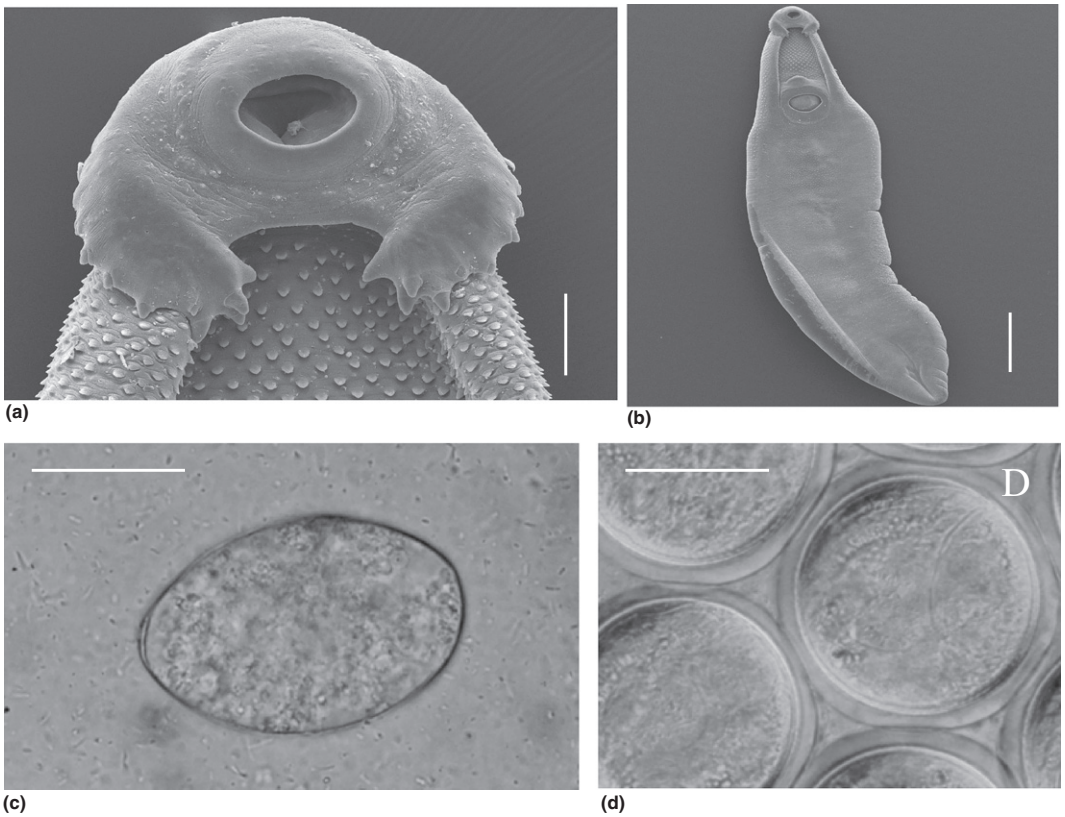

Figure 1 Microscopy of *Echinostoma*: (a) low-magnification scanning electron micrograph of the cephalic collar of spines of an adult worm of *E. caproni* (scale bar: 200 μm); (b) low-magnification scanning electron micrograph of an adult worm of E. caproni showing the spined collar (scale bar: 1 mm); (c) egg of E. sp. in human feces (scale bar: 50 μm); and (d) metacercariae of E. sp. collected from specimens of *Biomphalaria glabrata* (scale bar: 75 μm).

Life Cycle

Echinostome adults are hermaphroditic digeneans that live in the intestine and bile ducts of numerous vertebrate hosts, particularly aquatic or semi-aquatic birds and mammals, including humans. In the wild, the life cycle of an echinostome is maintained when a definitive host releases eggs (Figure 1(c)) into ponds, streams, and lakes. The fertilized eggs are undeveloped when laid and take approximately 2–3 weeks at 22 °C to reach the fully developed miracidial stage. Miracidia hatch from eggs and actively locate the first intermediate snail host in response to host signals and emitted products. Several species of planorbids, lymnaeids, and bulinids have been recorded as first intermediate hosts of echinostomes. Miracidia usually enter the head foot region of the snail and transform into sporocysts in the heart. In the snail first intermediate host, two generations (mother and daughter) of rediae occur. Cercariae develop within daughter rediae and begin to emerge from infected snails 4–6 weeks postinfection. In an aquatic environment, cercariae actively search for the second intermediate host. Echinostome cercariae show a low degree of host specificity, and several species of snails, frogs, tapdoles, and fish may serve as second intermediate hosts. Cercariae enter the second intermediate host via the cloacal opening of a tapdole or the excretory pore of a snail and encyst mainly in the tadpole kidney and kidney/pericardial cavity of the snail; cercariae may also penetrate into the musculature of fish and encyst there.

Definitive hosts become infected after ingestion of the second intermediate host-harboring encysted metacercariae (Figure 1(d)). Echinostomes do not undergo tissue migration in the definitive host. Following infection of the definitive host, the metacercariae excyst in the duodenum and the juvenile parasites migrate to the small intestine where they attach to the mucosa by the ventral sucker. Numerous vertebrates including waterfowl, rodents, and humans, among others, can serve as definitive hosts for echinostomes. The release of eggs from the definitive host usually begins 10–16 days postinfection. Although the release of eggs is continuous from the first day of patency, significant variations over the course of the infection in egg output have been documented. The duration of the infection varies from one to two weeks to more than one year depending on the echinostome species and the host species (Figure 2).

Clinical Manifestations and Pathology

Major clinical symptoms due to echinostome infection may include abdominal pain, diarrhea, easy fatigue, and loss of body weight. The severity of the symptoms depends on the parasite load. Heavy infections are associated with local

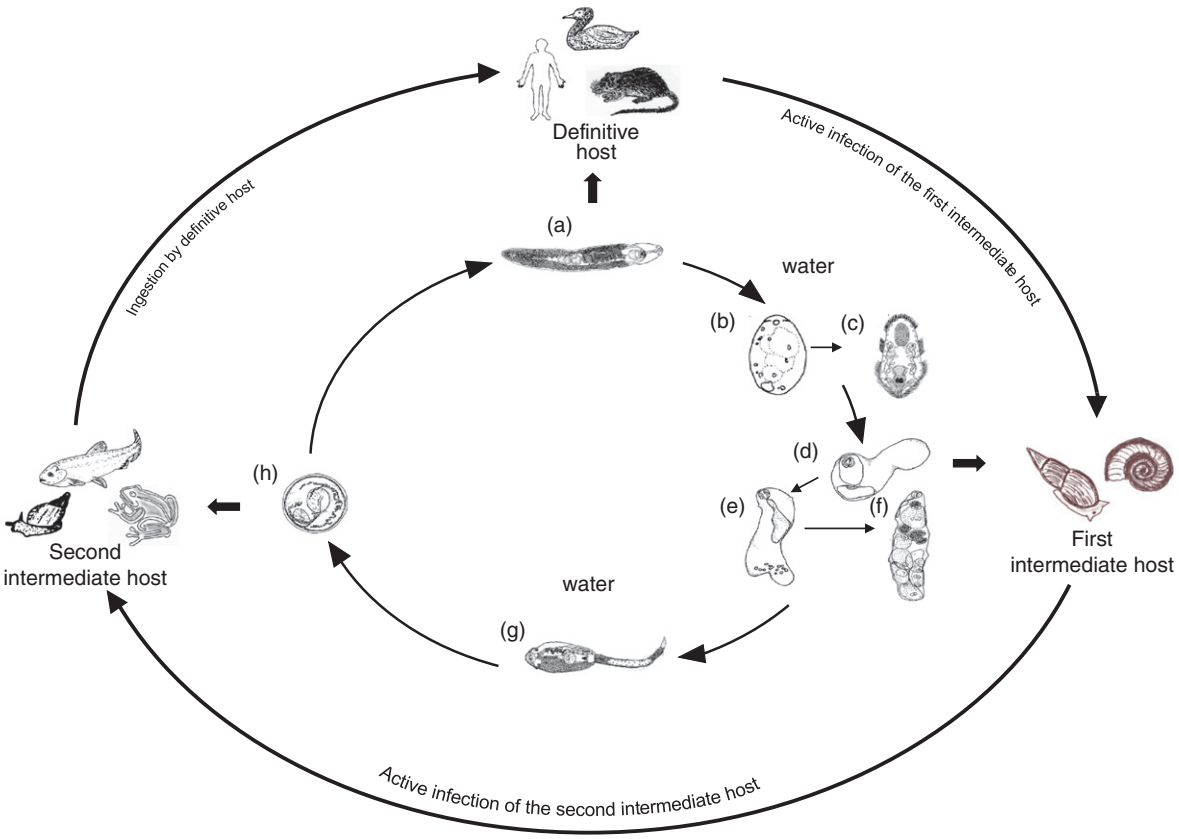

Figure 2 Generalized life cycle of *Echinostoma* spp.: (a) adult worms inhabit the small intestine of several vertebrate hosts, including humans; (b) eggs are voided with the host feces; (c) miracidia hatch in fresh water and actively infect snails; (d) sporocysts, (e) mother rediae, and (f) daughter rediae, are the intramolluscan stages; (g) cercariae are released and swim to locate the second intermediate host (snails, amphibians, bivalves, fishes) in which they encyst to become metacercariae; (h) metacercariae are ingested by the definitive host and excyst to become adults. Reproduced with permission, from Toledo and Fried (2005).

eosinophilia, abdominal pain, watery diarrhea, anemia, edema, and anorexia, and pathological features include catharral inflammation, erosion, and even ulceration. Peripheral blood eosinophilia is commonly observed. In *Echinostoma hortense* infections, the level of peripheral blood eosinophilia was dependent on the worm burden; 11–24% in patients with more than 100 worms; 4–21% among those with 51–100 worms; and 2–14% among those with less than 50 worms. Several studies on *E. hortense* have shown that patients suffered epigastric and abdominal pain, acid belching, weight loss discomfort, anorexia, headache, nausea, and vomiting. Patients infected with *Echinostoma ilocanum* suffered intestinal colic and diarrhea.

The intestinal histopathology in echinostome infections was studied using laboratory rodents. The pathology of echinostomes represents a complex set of reactions and the complexity is dependent on a wide variety of factors including characteristics of the echinostome species, the host species, and intensity of the infection. Pathological changes are often seen at worm attachment sites. Such areas may show marked dilation, erosion of the villi, and lymphocytic infiltration. Moreover, increases in goblet cells, mucosal neutrophils, mononuclear inflammatory cells in the mesentery, and crypt hyperplasia have been observed. Cellular infiltration of lymphocytes, eosinophils, and plasma cells was observed in the lamina propria and submucosa.

Epidemiology

Echinostomes are commonly found in birds (waterfowl) and mammals associated with freshwater habitats, and their distribution is ubiquitous. In general, their specificity toward the definitive host is low and a single echinostome species is able to infect several species of vertebrate hosts. The distribution of echinostomes is mainly dependent on the presence of snail first intermediate hosts because these parasites are more restrictive in their specificity toward this host.

Current incidence of human echinostomiasis is difficult to determine accurately because of the unavailability of epidemiological surveys and most of the available information is based on the occasional case reports. Table 1 summarizes the species of Echinostomatidae infecting humans, their geographical distribution, and the source of infection. Distribution of human echinostomiasis is strongly determined by dietary habits. Humans become infected when they eat raw or inadequately cooked food, especially fish, snakes, amphibians, clams, and snails containing encysted echinostome

Table 1 Geographical distribution and possible source of infection of species of Echinostomatidae infecting humans.

	Echinostome species	*Geographical distribution*	*Possible source of infection*
Africa	*Echinochasmus liliputanus*	Egypt	Freshwater fishes
	Echinochasmus perfoliatus	Egypt	Freshwater fishes
	Echinoparyphium recurvatum	Egypt	Freshwater snails, tadpoles and frogs
	Echinostoma revolutum	Egypt	Snails, tadpoles and clams
America	*Himasthla muehlensi*	Colombia, USA	Clams
	Echinostoma echinatum	Brazil	Mussels
	Isthmiophora melis	USA	Tadpoles
Asia	*Acanthoparyphium tyonense*	Japan, Korea	Bivalves
	Artyfechinostomum malayanum	China, India, Indonesia, Malaysia, Philippines, Singapore, Thailand	Snails
	Artyfechinostomum mehrai	India	Snails
	Artyfechinostomum oraoni	India	Snails
	Echinochasmus angustitestis	China	Freshwater fishes
	Echinochasmus fujianensis	China	Freshwater fishes
	Echinochasmus japonicus	China, Korea, Japan	Freshwater fishes
	Echinochasmus jiufoensis	China	Freshwater fishes
	Echinochasmus liliputanus	China, Palestina, Syria	Freshwater fishes
	Echinochasmus perfoliatus	China, Japan, Taiwan	Freshwater fishes
	Echinoparyphium recurvatum	Indonesia, Taiwan	Freshwater snails, tadpoles and frogs
	Echinostoma cinetorchis	China, Japan, Korea, Taiwan	Freshwater fishes
	Echinostoma echinatum	Indonesia, Japan, Thailand	Mussels
	Echinostoma hortense	China, Japan, Korea	Freshwater fishes
	Echinostoma ilocanum	China, India, Indonesia, Malaysia, Philippines, Thailand	Snails
	Echinostoma japonicum	Japan, Korea	Freshwater fishes
	Echinostoma macrorchis	Indonesia, Japan, Korea, Taiwan	Snails and frogs
	Echinostoma revolutum	Thailand	Snails, tadpoles and clams
	Episthmium caninum	India, Thailand	Freshwater fishes
	Hypoderaeum conoideum	Thailand	Snails and tadpoles
	Isthmiophora melis	China, Taiwan	Tadpoles and loaches
	Paryphostomum sufrartyfex	Thailand	Snails
Europe	*Echinochasmus perfoliatus*	Denmark, Hungary, Italy, Romania, Russia	Freshwater fishes
	Echinostoma echinatum	All Europe	Mussels
	Echinostoma paraulum	Russia	Unknown
	Echinostoma revolutum	All Europe	Snails or clams
	Hypoderaeum conoideum	All Europe	Snails and tadpoles
	Isthmiophora melis	All Europe	Tadpoles and loaches
Oceania	*Echinostoma revolutum*	Australia	Snails, tadpoles and clams

metacercariae. Infections are thus most prevalent in areas where traditional cultural practices encourage ingestion of raw or undercooked fish, frogs, snakes, or snails and bivalves. In the Philippines, human echinostome infections are due to eating raw fish dipped in a salt and vinegar mixture, known as *kinilaw*. Other methods of fish preparation are *tinola* (boiled), *ginataan* (stewed in coconut milk), and *sinugba* (charcoal-grilled). All echinostome-infected patients had a history of having eaten snails, *kuhol*, and *kiambu-ay*, prepared raw with coconut milk and lime juice (kinilaw), especially when found in greater abundance during the rainy season. This suggests that various types of marinades and food preparations may not affect the viability of echinostome metacercariae. Moreover, it has been suggested that drinking untreated water containing echinostome cercariae can be a source of human infection.

Human echinostome infections are limited to areas where both intermediate hosts live together in the same habitat and suitable climatic factors are present. Hence, distribution is highly focal. Most human infections are reported from foci in East and Southeast Asian countries such as China, India, Indonesia, Korea, Malaysia, Philippines, Russia, Taiwan, and Thailand. Moreover, occasional cases have also been reported in other countries (Table 1). Characteristic of several of the food-borne diseases, changing infection patterns have been observed in recent years. Population growth, pollution, poverty, and lack of improved sanitation have contributed to increased infection rates in several areas. In contrast, social and economic advances, combined with health education campaigns and mass drug administration, have reduced prevalence in other settings.

Analytical Methods

The diagnosis of human echinostomiasis is usually based on detection of eggs in fecal examinations. The most frequently used method of diagnosis is the Kato-Katz thick smear, though

other techniques such as the Stoll's dilution or the quantitative formalin acetate concentration can also be used. In the Kato-Katz technique, the fecal sample is processed in a staining procedure and the slide is examined by light microscopy. The eggs are oval in most cases, yellowish, with a thin and refractile shell and with a small inconspicuous operculum located at the anterior end. The operculum may be difficult to see using the Kato-Katz method. The size of echinostome eggs of zoonotic species is in the range of 0.066–0.149 mm in length and 0.043–0.090 mm in width. With careful microscopic observations including egg measurements, specific diagnosis may be possible in known endemic areas with a single or a mixed echinostome species infection. However, recovery and identification of the adult flukes, following anthelmintic treatment and purgation, is recommended if a definite diagnosis is preferred.

Occasionally, human echinostomiasis has been revealed by gastroduodenal endoscopy performed in relation to severe epigastric symptoms and ulcerative lesions in the stomach and duodenum. In Japan, four cases of *E. hortense* infections were reported based on worm recoveries by upper endoscopy.

From a food-safety standpoint, detection of the metacercariae in an intermediate host, especially fish, is vital. The metacercaria is morphologically distinct from other zoonotic and nonzoonotic trematode metacercariae, particularly by the possession of a cephalic collar of spines. The specific diagnosis may be performed on the basis of adult morphology, after metacercarial infection of a laboratory animal, or by polymerase chain reaction (PCR).

Examination for echinostome metacercariae in the second intermediate host, especially in fish, is commonly performed by two methods, i.e., muscle compression and pepsin–HCl artificial digestion. In the compression technique, a sample of flesh from different parts of the fish (e.g., head, gill, muscle, fin, scale, intestine, and other viscera) or other host is compressed between two glass slides and examined under the stereomicroscope with 20- to 100-fold magnification. The artificial digestion method is more complicated. Small pieces of flesh are minced and mixed in a breaker with artificial gastric juice (concentrated HCl 8 ml + pepsin 1:10 000 6 g + distilled water 1000 ml) and incubated at 37 °C for 2 h with occasional stirring. After removal of the larger particles by filtration (1 mm × 1 mm of mesh), 0.85% saline is added. The supernatant is discarded and the sediment retained. This procedure is repeated until the supernatant becomes clear. Finally, the sediment is examined under a steromicroscope for the presence of metacercriae.

Immunological methods have been developed but their application is presently limited to experimental infections in animals. The application of molecular methods for the diagnosis of echinostomes is still very limited. However, in recent years a number of species-specific PCR-based methods have been developed that are capable of detecting and differentiating trematode species from environmental and clinical samples obtained from definitive and intermediate hosts. These methods are rapid, provide increased discriminatory power, and have the ability to analyze small amounts of sample. Moreover, they negate the need for laborious morphological examination of the individual adult flukes following anthemintic purging (in humans) or morphological investigation of the intermediate host for the presence of metacercariae. For example, a tandem repeated DNA sequence has been recently described and used to detect metacercariae of *Echinostoma caproni* from snail tissues. This methodology could be used in other intermediate hosts such as fish.

Treatment and Control Measures

In human infections, praziquantel is the drug of choice for echinostomiasis. Mebendazole and albendazole have also been shown to have an effect against echinostomiasis.

Eating raw or improperly cooked freshwater fish and fresh or brackish water snails should be avoided to prevent echinostome metacercarial infections. There is evidence that various types of marinades and food preparations commonly used may not affect the viability of echinostome metacercariae. In a local market in Thailand, the fish (*Cyclocheilichthys armatus*) were infected with large numbers of *Echinostoma* metacercariae and were used to evaluate the effect of traditional food preparation on the viability of the metacercariae. The metacercariae *in situ* were evaluated using the following preparations: (1) left to dry at room temperature; (2) frozen; (3) refrigerated; (4) marinated in saline; and (5) marinated in 5% acetic acid solution. It was found that degeneration of the metacercariae was slowed by cooling: complete degeneration of metacercariae required approximately 10 h in the refrigerated or frozen fish, compared with only 4 h in fish left at room temperature or marinaded in saline or acetic acid solution.

Various physical and chemical factors have been studied to determine their effects on the viability of encysted metacercariae of *E. caproni*. Viability was determined by the ability of the metacercriae to excyst in an alkaline trypsin–bile salts medium. Of numerous marinades tested, vinegar was the most harmful to cysts. Concentrated solutions of NaCl and sucrose had no effect on the viability, suggesting that their use in food preparations would not be effective in killing echinostomatid cysts, especially those in freshwater snails.

The nature of echinostomiasis does not justify the establishment of a separate control program because it can be controlled along with other food-borne diseases for which there are World Health Organization control programs. The control of human echinostomiasis via blocking or interruption of the life cycle can be achieved through proper diagnosis, followed by pharmacologic treatment and prevention of reinfection. The control of echinostomiasis should be focused predominantly on a reduction or elimination of the transmission of the disease. In theory, the means of control in endemic regions can include: a reduction of the sources of infection, particularly humans, through effective treatment; the protection of fish ponds and aquaculture systems from contamination with feces from people and other definitive hosts; the treatment or sterilization of feces; the control of snail host populations; and the implementation of education campaigns. Because infection of the definitive host is only contracted through the ingestion of metaceracriae harbored by the second intermediate hosts, the most practical measure for preventing and controlling human infection is to eliminate the consumption of raw, undercooked, or freshly pickled fish and shrimp flesh and to instill the principle that only

adequate cooking will render such fish safe for human consumption. However, this strategy may be difficult to implement in some endemic regions because of the ancient tradition of eating raw fish. Nonetheless, together with education awareness programs focused particularly on teaching young children about the parasite, its life cycle, and the disease it causes, prevention and control could be successful.

Although a reduced number of cases in other trematode infections have been reported in endemic regions, the prevalence of echinostomiasis has not changed, probably in relation to the extensive zoonotic reservoir hosts of echinostomes. Reservoir hosts other than humans may play a major role in sustaining transmission of echinostomes. There are a wide range of potential definitive hosts in the life cycle of echinostomes, particularly wild and domestic animals and fish-eating birds. Recent studies have shown that domestic dogs, cats, pigs, ducks, and chickens may act as reservoir hosts of echinostomes and other food-borne trematode infections. The reservoir hosts can become contaminated with eggs in water bodies and infect snail intermediate hosts. Because of these conditions, the prevention of echinostome infections in domestic animals must be included in any public health strategy. This can be accomplished by measures such as educating farmers about the risks associated with feeding animals with snails or raw fish or placing the animal houses as far away as possible from water bodies. However, the most effective measure may be the inclusion of these potential reservoir hosts in drug-treatment programs aimed at their human owners. A praziquantel dose of 40 mg kg^{-1} has been shown to be effective for the treatment of dogs and cats. Information on the optimal doses to treat other reservoir hosts should be further evaluated, as only little information is available on this topic.

See also: Disciplines Associated with Food Safety: Parasitology. Helminth-Trematode: *Fasciolopsis buski*; *Heterophyes heterophyes*; *Metagonimus yokogawai*

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Fasciola hepatica and *Fasciola gigantica*

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Glossary

Contamination The introduction or occurrence of a contaminant in the food or food environment.

Control measure Any action and activity that can be used to prevent or eliminate a food safety hazard or reduce it to an acceptable level.

Food Any substance, whether processed, semi-processed, or raw, which is intended for human consumption, including drink, chewing gum, and any substance which has been used in the manufacture, preparation, or treatment of 'food'; it does not including cosmetics, tobacco, or substances used only as drugs. (In the context of this topic-level contribution, drinking water is food).

Foodborne disease Any disease of an infectious or toxin nature caused by or thought to be caused by consumption of food or water.

Food safety Assurance that food will not cause harm to the consumer when it is prepared or eaten according to its intended use.

Risk A function of the probability of an adverse health effect and the severity of that effect, consequential to hazard(s) in food.

Background

Fascioliasis or fasciolosis is a disease caused by two trematode parasites of the genus *Fasciola*, *Fasciola hepatica* and *Fasciola gigantica*. The former is reported from throughout the world whereas the latter occurs mainly in tropical regions of Africa, South and East Asia, and the Middle East. Two different reports have estimated the rate of global human fasciolosis as 2.4–17 million, whereas the population at risk was estimated to be 180 million (World Health Organization (WHO), 1995). Human beings together with at least 46 other species of both domestic and wild mammals are reported to be suitable as final hosts, either naturally or experimentally, whereas the intermediate hosts are snails of the family Lymnaeidae.

The earliest mention of *Fasciola* goes back to 1379, when Jean de Brie in France reported the impact of sheep liver rot on sheep management and wool production. Later, in 1547, it was identified in the livers of sheep and goats. The first record of human fasciolosis is that of Pallas in 1760 in Berlin, in a female patient found to be infected on autopsy.

Fasciolosis as a foodborne disease has been included in the list provided by the Institute of Food Technologists' Expert Panel on Food Safety and Nutrition, and by the Parasitic Task Force of The Foodborne Disease Burden Epidemiological Expert Group (FERG) of the WHO. The FERG is an advisory group to the WHO initiative to estimate the global burden of foodborne diseases from enteric, parasitic, and chemical causes.

Characteristics

Classification

Fasciola hepatica and F. gigantica are parasitic flatworms of the phylum Platyhelminthes, class Trematoda, order Digenea, family Fascioloidae, and genus *Fasciola*.

Other members within the family include *Fasciola indica*, *Fasciolopsis buski*, and *Fascioloides magna*.

Morphology

The characteristic trait of the *F. hepatica* adult parasite is that approximately all its internal organs, i.e., reproductive systems besides the two-gut ceca, are branched, so that it is almost unfeasible to differentiate those organs through a microscope (Figure 1). The hermaphroditic adult is as large as 2–3 cm long, and encompasses the characteristics of an anterior cephalic cone, an oral sucker, a ventral sucker, and a covered tegument with tiny spines.

Eggs measuring 130–150 μm × 60–85 μm are all thin shelled, ellipsoid, and quinone colored (bile stained) with an inconspicuous operculum.

Differential Diagnosis Between *F. hepatica* and *F. gigantica*

Before setting up the new molecular diagnostic methods, the differentiation between the two species was mostly based on morphological characters. Normally, *F. gigantica* is longer (Figure 2). Some minor and major characteristics are

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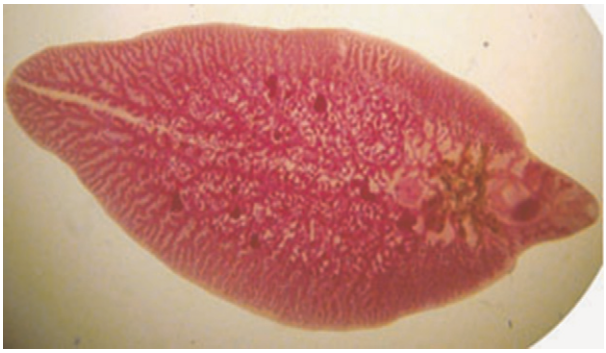

Figure 1 Adult of *Fasciola hepatica*.

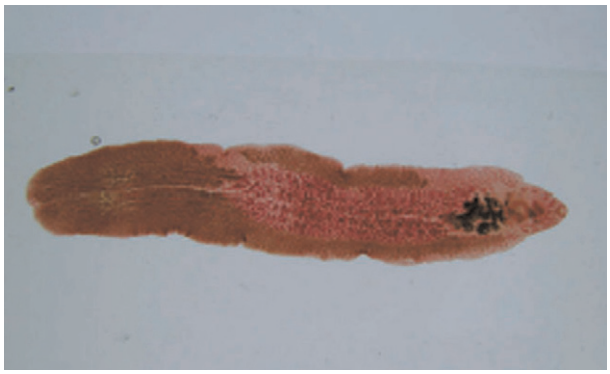

Figure 2 Adult of *Fasciola gigantica*.

Table 1 Comparative morphologic characteristics of *F. hepatica* and *F. gigantica*

Topic	*F. hepatica*	*F. gigantica*
Length	2–3 cm × 1.5 cm	2.5–7.5 cm × 1.2 cm
Cephalic cone	Pronounced	Shorter
Eggs	130–150 μm × 60–85 μm	Larger (160–190 × 70–90 μm)
Testes	–	More anterior
Ventral sucker	–	Larger

elucidated in Table 1. However, frequent variations in these morphological features often cause difficulties for accurate differentiation. In addition, abnormal diploidy, triploidy, and mixploid parthenogenesis as well as hybridization between different genotypes of *Fasciola* give rise to different forms of this genus. In some countries such as Japan, it is often impossible to morphologically differentiate between the two species; in many endemic countries, apparent intermediate forms have been reported.

To overcome these hybridization effects, molecular DNA-based approaches such as PCR-restriction fragment length polymorphism (RFLP) and Randomly amplified polymorphic DNA (RAPD)-derived sequences have been investigated to distinguish between these species. ITS1 and ITS2 sequences from rDNA provide reliable genetic markers for identification. Eventually, the new molecular methods described as reliable, fast, and straightforward devices for differentiating between *F. hepatica* and *F. gigantica*, seem to be acceptable for differential diagnosis. These methods are especially apposite for screening a large number of *Fasciola* isolates.

Life Cycle

Fasciola hepatica is a hermaphrodite and auto-fertilization is achievable, although cross-fertilization between two adult flukes is the most common form of sexual reproduction. *Fasciola hepatica* has two stages of growing in its life cycle: the sexual stage in its adult form and the asexual in the larval or intermediate stages (Figure 3). The normal habitat of the parasite is biliary ducts and gall bladder of the definite host. In these locations, the mature parasite releases its eggs, which escape the host's body via the feces. The undifferentiated ovum in the egg, after spending 9–15 days in the water, develops into miracidium which hatches out of the egg and searches for a snail intermediate host from the family of Lymnaeidae, including species of the *Galba*/*Fossaria* group for *F. hepatica* and species of *Radix* for *F. gigantica*. The life expectancy of miracidium is approximately 8 h, during which time it must find a suitable snail. It then goes through several development stages in the snail (i.e., sporocyst followed by redia), which include considerable asexual multiplication. In the final stage, tailed cercariae, measuring 200–300 μm, emerge from the snail and swim through the water until they locate suitable vegetation on which they encyst, becoming metacercariae.

A single miracidium can produce from 10 to 700 metacercariae. The metacercariae are ingested by the definite host through eating (or sometimes drinking water with floating metacercariae). In the duodenum, the metacercariae unencyst, releasing the juvenile stage, which burrow through the gut mucosa and migrate to the liver parenchyma. After 3–4 months, the juvenile flukes reach the deep-seated bile ducts where they mature sexually and initiate egg production. The adults lay, on average, between 8000 and 25 000 eggs per day.

Survival

Fasciola may live from 9 to 12 months in cattle, 11 years in sheep, and from 9 to 13 years in humans. *F. hepatica* eggs and metacercariae may remain alive for long periods under low temperatures, but they are susceptible to heat and drought. The longevity of metacercariae in the environment varies from 122 days in running water to 95 days in stagnant water. Under optimal conditions, metacercariae may remain viable for as long as 1 year on pasture and can even live for a few months on dry hay.

Diagnosis

Routine methods of stool examination normally are not capable to detect the parasite's eggs and methods such as the Kato-Katz technique, although efficient, are not normally conducted as a routine test particularly in developing countries. It is a specific but not sensitive diagnostic test, and has

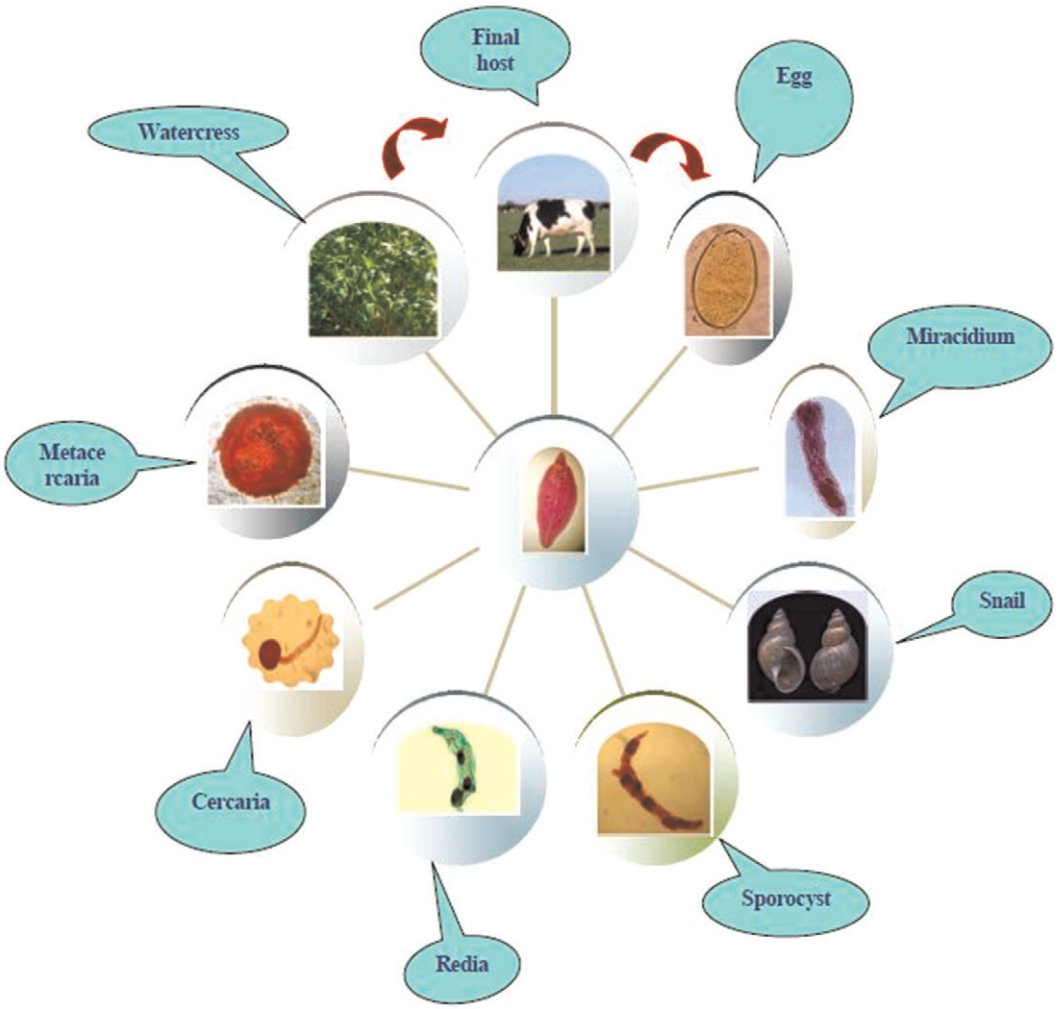

Figure 3 Life cycle of *Fasciola* spp.

been shown to underestimate by 30% the prevalence obtained by Fas2-enzyme-linked immunosorbent assay (Fas2-ELISA). Now, the method of choice is ELISA using excretory–secretory antigens.

Clinical Manifestation

Fasciolosis embraces two important phases in final host: immigrating and residing, or acute and chronic phases. The first phase, which lasts 1–3 months after ingestion of metacercariae, has symptoms including epigastric pain, fatigue, fever, right quadrant abdominal pain, indigestion, weight loss, and malaise. Extrahepatic abnormalities, such as pulmonary infiltrates, pleuropericarditis, meningitis, and lymphadenopathy, are also possible.

After establishment, a variety of signs and symptoms depending on the burden of the disease may be seen comprising hepatomegaly, obstructive jaundice due to biliary obstruction, cholecystitis, cholelithiasis, fibrosis, cirrhosis, liver abscesses, hyperplasia of the ductal epithelium, and subcapsular hemorrhages. Anemia, of normocytic normochromic nature, is likely. The presence of small multiple intrahepatic stones along with cholangitis and cholelithiasis constitute the features of the human chronic phase.

Although the routine habitat of *Fasciola* is the biliary ducts, at times the parasite lodges in abnormal spots of the body, due to deviation of the normal route for unknown reasons. The phenomenon is called ectopic fasciolosis. Almost all parts of the body have been reported as having ectopic fasciolosis, including gastrointestinal tract, subcutaneous tissue, heart, blood vessels, the lung and pleural cavity, brain, orbit, abdominal wall, appendix, pancreas, spleen, inguinal nodes, cervical nodes, skeletal muscle, and epididymis. The usual pathological effects of ectopic lesions are due to the migratory tracks causing tissue damage with inflammation and fibrosis. The prevalence of fasciolosis in Lima, Peru, where the disease is endemic, in these patients with established cirrhosis, was reported as 9.1%. This shows the necessity for more attention in patients with cirrhosis, due to the greater possibility of fasciolosis in these patients with cirrhosis in endemic countries. In endemic areas, due to gradual resistance of the body, half of the chronically infected patients might be asymptomatic or have few complications.

Considering mortality, appraisal literature shows very little documentation. These scanty reports were based on autopsy observations. Thus, nobody could be sure of fasciolosis as the sole cause of the death.

Epidemiology

Geographic Distribution

A typical hyperendemic area of fasciolosis is the Altiplano region of Northern Bolivia. Infection is highest in children and females, and prevalence can exceed 40% in certain communities. In Africa, the Nile Delta region between Cairo and Alexandria has also been reported as another hyperendemic area. In Europe, human fluke infections occur more sporadically. However, significant and recurrent outbreaks of fasciolosis occur in France, Portugal, and Spain. In Iran there have been two important outbreaks, each of which reported up to 10 000 infections in northern parts of the country. In the USA, sporadic cases have been reported but mostly of imported nature through immigrants and tourists.

Although most of the cases of fasciolosis have been ascribed to *F. hepatica*, the exact differentiation between the two species has only been recorded recently; therefore, in time the relative importance of the two species may change. However, there is evidence of a rise in the number of human cases of fasciolosis of *gigantica* origin in Vietnam. The possibility of more cases in some Southeast Asian countries has also arisen. In some countries of Africa and in Hawaii, where only *F. gigantica* occurs, fasciolosis cases can be attributed to this parasite. Apparently, humans are less sensitive to *F. gigantica*, and ectopic cases due to *F. gigantica* are more prevalent compared with *F. hepatica*. Cases of overlap between two species have been demonstrated in some endemic countries such as Egypt and Iran, and hybrids exist in eastern Asia and perhaps elsewhere.

Demographic Factors

Review of literature shows that Bolivia, Peru, France, Russia, Turkey, and Vietnam encompass a similar pattern of showing no significant difference in terms of the patients' sex, whereas in Chile, Spain, Egypt, and Iran, females are infected more than males. A wide age range among patients with fasciolosis is seen in Chile, France, Russia, Turkey, Egypt, and Vietnam; in contrast, in Bolivia, most patients are less than 20 years of age, and in Iran mostly adults are infected.

Epidemiological Classification Concepts

A new epidemiological classification for human fascioliasis has been proposed as follows: imported cases; autochthonous; endemic; and epidemic (Table 2).

Human Risk Factors

Some observations show that it is not necessary to presume a direct relation between the high rates of fasciolosis in humans and similar situation in animals in a given area. For example, in Kermanshah Province, western Iran, there is a high prevalence of fasciolosis in animals, whereas normally there are no reports of human cases, with the exception of a small outbreak (13 cases). It would seem that the factor of culture plays an important role in this regard.

Foodborne Transmission

Plants

The main source of infection is ingestion of metacercariae encysted on water plants such as watercress (*Nasturtium officinale*). Raw watercress in many countries is eaten as salad or in other dishes such as appetizers. However, other cultivated, freshwater plants are also reported as sources. Metacercariae were found in 1% of lettuce sold at a local market in the

Table 2 Proposed new epidemiological classification for human fascioliasis

Class		*Definition*
Imported cases		Human cases diagnosed in a zone lacking *Fasciola* spp.
Autochthonous		Humans who have acquired the infection in the area where they live and where animal fascioliasis is also present
Endemic (three types based on the prevalence)	Hypoendemic	Prevalence less than 1%; arithmetic mean intensity less than 50 epg
	Mesoendemic	Prevalence 1 ± 10%; 5- to 15-year-olds may present higher prevalences; arithmetic mean intensity in human communities usually 50 ± 300 epg
	Hyperendemic	Prevalence more than 10%; 5- to 15-year-old children usually present higher prevalences; arithmetic mean intensity in human communities usually more than 300 epg
Epidemic		A. Epidemics in areas where fascioliasis is endemic in animals but not humans B. Epidemics in human endemic areas: outbreaks in zones where the disease is endemic in humans

Source: Mas-Coma S, Esteban JG, and Bargues MD (1999) Epidemiology of human fascioliasis: A review and proposed new classification. *Bulletin of the World Health Organization* 77: 340–346.

Mantero valley, Peru. In Cuba, all cases of fasciolosis have been associated with consumption of watercress and lettuce. In France, *Taraxacum dens leonis* (dandelion leaves), *Valerianella olitora* (lamb's lettuce), and *Mentha viridis* (spearmint) are implicated. In Iran, *Nasturtium* spp. and *Mentha* spp. are important sources. In the Bolivian Altiplano, *Juncus andicola* (Juncaceae), *Juncus ebracteatus* (Juncaceae), *Mimulus glabratus* (Scrophulariaceae), and *Nostoc* sp. (Cianofitas) have all been reported as sources.

In Limousin, France, snails infected with *F. hepatica* were found in 14 watercress beds. Five percent of examined vegetables in Iran were contaminated with *Fasciola* eggs. *F. hepatica* eggs were isolated from irrigation water sources 2–5 egg l^{-1} in Ghana. Moreover, vegetables irrigated with water had 2–4 eggs 100 g^{-1} wet weight contamination.

Two of the most important sources of fasciolosis in endemic regions of Iran, i.e., the northern parts, are green salt (local name: *dalar*) and elaborated olive (local name: *zeitoon parvardeh*) (**Figure 4(a)** and **(b)**). Green salt is made from a mixture of ground aquatic plants such as *Mentha pulegium* (local name: khlivash) as well as *Mentha piperita* (bineh) and 30–40% salt. The final pH is 5. It is usually eaten with cucumber, prunes, yogurt, etc. *Zeitoone parvardeh*, an appetizer, is a mixture of stone-free olive, ground aquatic plants, mostly *Eryngium coucasicum* (local name: *choochagh*), walnuts, various spices, garlic, and sour-pomegranate juice. Regarding *zeitoon parvardeh* and *dalar*, 66.6% and 57.8% of the metacercariae, respectively, can be alive for 2 weeks after the preparation of the food.

Water

Normally *Fasciola* spp. use water plants as a matrix on to which they affix their metacercariae, but sometimes they release the metacercariae into water, hence contaminated water can be another source of infection. In some endemic areas, drinking contaminated water has been reported as the main source of infection. For example, in the Americas and Peruvian Altiplano, watercress is not normally consumed as in other nations. However, in the Bolivian Altiplano, 13% of the metacercariae of *Fasciola* isolates were floating on the water.

Beverages

In some parts of the world, people drink beverages made of local plants, for example, stalks of cane in Pakistan, roots of carrot in Iran, and alfalfa and watercress in Peru, and elsewhere. These plants have a high possibility of contamination with metacercaria due to the irrigation by contaminated water.

In Peru, people infected with fasciolosis mentioned a history of consuming watercress (45.6%), lettuce (31.6%), alfalfa (10.5%), spinach (5.3%), and drinking water from *puquiales* (10.5%) or emolientes (the warm beverages made from various plants, chiefly alfalfa and watercress) (5.3%).

(a) (b)

Figure 4 Two traditional dishes made from local aqua plants in endemic parts of fasciolosis in Iran: (a) elaborated olive (local name: *zeitoon parvardeh*); (b) green salt (local name: *dalar*).

Kitchen Utensils

Another source is kitchen utensils, which may be washed by contaminated water and used for preparing food.

Raw Liver

Raw liver as a potential source of infection of has recently come to light. It is generally considered that eating liver contaminated with *Fasciola* results only in transitory infection, whereby a lot of *Fasciola* eggs are released in the stool but the consumer is not infected and only acts as an egg passer. However, some researchers believe that eating raw fresh liver contaminated with *Fasciola* may establish the real infection. In fact, animal-based studies have demonstrated that consuming fresh raw liver infected with immature forms of *Fasciola* might cause fasciolosis. Definitely, this subject needs more clarification.

Analytical Methods

Verification of Metacercarial Viability and Infectivity

Metacercarial viability can be assessed by checking the refractile appearance of secretory granules through microscopy and the movements of juveniles within cysts. Metacercariae are inserted on a glass slide covered by a coverslip and examined under a light microscope. It can be verified by *in vitro* excystation capability of metacercariae as well.

Infectivity of metacercariae is measurable through animal infection assays. However, the mouse is not a suitable host for *F. gigantica*; instead, hamster and rabbit are considered appropriate.

Control and Preventive Measures

The life cycle of *Fasciola* contains three important key elements including the final host, the intermediate host, and the water plants. A successful preventive strategic planning must embrace all these factors.

Public Health Education

As other parasitoses, public health education should be placed in the front. People should learn how to wash vegetables so that the metacercariae can be separated from the surface of the plants. In many fasciolosis endemic areas, domestic animals, especially cows, roam freely and launch the start point of parasite life cycle, i.e., releasing parasite's eggs. In endemic parts of Iran, cows leave their owner's house for 3–4 months and return after spreading many parasite's eggs. Making people familiar with the hazard of watercress, the different ways

metacercariae are transferred, and modifying the processes of making certain domestic dishes, instead of halting the consumption, are some other possible strategies.

Control of Environmental Factors

Horizontal and downflow roughing filters could remove *Fasciola* eggs from drinking water and are advised for water and secondary effluent treatment. Another scheme, as a forecast model to strategic control of fasciolosis, is geographic information system (GIS). In Ethiopia, this model was created and managed based on moisture and thermal regime and the results were labeled as promising. It was shown that thermal and soil moisture regimes were particularly important in determining the patterns of seasonal infection in a region due to its adverse effects on intra-molluscan larval development, extra-lymneaid phases, and activity of the snail vectors. Regarding mapping and prediction devises, a model called the normalized difference vegetation index (NDVI) has been used in northern Bolivian Altiplano and has been branded as an advantageous data component in monitoring the fasciolosis control and management.

Control of the Intermediate Host

The snail, which is the intermediate host, is amphibious; hence using molluscicides is futile. Besides, the distribution of the snail is such that it is worthless to use this method. The snail is widespread throughout the affected regions, even in small holes filled with water, so there is the risk of hampering the ecology of these areas. Nevetheless, this method has been used mostly to target more than one parasite simultaneously.

Chemotherapy

Although many trials have not been able to establish a successful vaccine against fasciolosis, chemotherapy has remained the mainstay of treatment and a plan for the control of the disease. Chemotherapy of animal populations has been used effectively in many countries so far.

Vaccination

To date, there are no successful or commercial vaccines against fasciolosis. There are some reports of immunization of mice, rats, sheep, and some other animals against fasciolosis, but not in humans. Some antigens of *Fasciola* including leucine aminopeptidase, fatty acid-binding protein, glutathione S-transferase, and cysteine protease have been challenged with protection rates of 65–89.6%. Further, an Australian group has used a multivalent vaccine derived from E/S antigens to generate immune responses in the rat fascioliasis model.

Economic Damage

The economic losses due to fasciolosis are estimated at US $2000–3000 million annually, and to the livestock industry at more than US $3 billion per annum.

The most important economic effect of fasciolosis in terms of nutritional cycle is the daily demolition of infected livers as well as lost productivity, such as reduction of milk and meat yield.

See also: Disciplines Associated with Food Safety: Parasitology. Foodborne Diseases: Overview of Biological Hazards and Foodborne Diseases. Helminth-Trematode: *Dicrocoelium dendriticum*; *Fasciolopsis buski*; *Opisthorchis viverrini* and *Opisthorchis felineus*. Safety of Food and Beverages: Fruits and Vegetables

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HELMINTH-TREMATODE

Fasciolopsis buski

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Glossary

Cercaria Fourth larval stage of a digenean trematode, which, owing to its tail, swims out from the snail in search for a support (e.g., a freshwater plant) or a second intermediate host to produce a metacercaria.
Cirrus pouch Thick-walled bursa present in the terminal part of the male genital apparatus of a fluke, which contains an internal seminal vesicle, pars prostatica, and prostate glands, and a usually evaginable male copulative organ called a cirrus.
Daugther redia Second generation of the third larval stage of a digenean trematode, which develops inside the intermediate snail host and produces cercariae.
Gymnocephalous cercaria Morphological type of digenean cercaria characterized by a body presenting two suckers and a long, simple, swimming tail.
Metacercaria Usually encysted fifth larval stage of a digenean trematode which attaches to a support (e.g., a freshwater plant) or inside a second intermediate host, to await the occasion to infect the definitive host by ingestion.
Miracidium First larval stage of a digenean trematode, which develops inside the egg, escapes by opening the operculum of the egg, and swims to infect the first intermediate snail host or vector host.
Mother redia First generation of the third larval stage of a digenean trematode, which develops inside the intermediate snail host and produces daugther rediae.
Planorbid Snail belonging to the family Planorbidae, a group of freshwater, mostly discoidal, snails within the molluscan class Gastropoda, which is of great medical and veterinary importance due to the parasitic diseases of humans and animals they transmit.
Sporocyst Second larval stage of a digenean trematode, which develops inside the intermediate snail host and produces rediae.
Vitelline glands Glandular follicles distributed into two bilateral fields in fluke adults and which secrete egg precursors.

Etiology: The Parasite

Fasciolopsis buski is the only species included in the genus *Fasciolopsis*. It is a trematode parasite commonly known as the giant Asian intestinal fluke, due to its large size, its geographical distribution restricted to Asia, and its location within the host's body confined to the intestine. Together with *Fasciola gigantica*, it is the largest of the digeneans to infect humans.

Fasciolopsis buski is usually elongated, oval in shape, without a cephalic cone. Its size varies depending on the host species, and is between 2 and 10 cm in length and 0.8 and 3 cm in width. The oral sucker is subterminal, small and approximately one-fourth the size of the ventral sucker, which is situated not far behind the former. An oval pharynx is present and the short esophagus leads to the caeca which are unbranched and terminate near the posterior end of the body. The two testes are highly branched, tandem, and situated in the posterior half of the body. The branched ovary is pretesticular situated in the middle of the body slightly to the right of the midline. A large cirrus pouch is present and opens at a genital pore immediately anterior to the acetabulum. The vitelline glands are numerous and small-sized, and extend from the level of the ventral sucker along the two lateral fields up to the posterior end of the body, where bilateral glands join together (Figure 1).

The eggs are ellipsoidal, rounded at both ends, yellow and unembryonated, and have a clear thin shell with a delicate operculum at one end (mean diameter of operculum 27 μm). The eggs measure 120–140/70–90 μm (mean 138/82 μm) (Figure 2).

Parasite Biology and Life Cycle

The giant Asian intestinal fluke is a parasite which, in humans, inhabits the duodenum and jejunum in light infections, either attached by their suckers to the mucosal epithelium or buried in the mucous secretions, and can also be found in much of the intestinal tract, including the stomach, in moderate and heavy infections.

In most endemic areas, pigs appear to be the only animal reservoir of any significance. Interestingly, pigs generally harbor fewer adult worms and egg production per worm is lower than in human beings. The pig has also proved to be a good experimental model.

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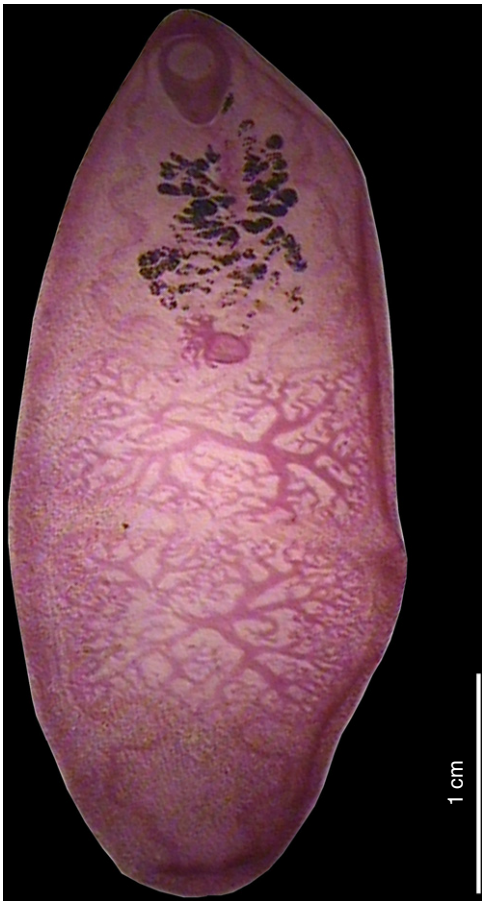

Figure 1 Adult stage of *Fasciolopsis buski*, in ventral view. Note the unbranched bilaterally descending caeca, two pronouncedly branched testes in longitudinal tandem, slightly dextral branched ovary, and numerous brown eggs between ovary and large ventral sucker or acetabulum.

This parasite shows susceptibility differences in experimental tests when using different animal species: worms remain immature in dogs; both immature and mature worms were recovered from rabbits; immature flukes were recovered from young buffalo; mice, rats, monkeys, and dogs were completely refractory; and guinea-pigs were only partially susceptible. However, young rabbits (6–8 weeks old) were found to be susceptible and can be used as an animal model for experimental work. The parasite may establish temporarily in rabbits or the squirrel monkey *Saimiri sciureus petrinus*. It has been very rarely reported in the rhesus monkey of the species *Macaca mulatta*, but there is no report incriminating monkeys in endemic areas. It is worth mentioning that field studies showed no infection in cows, buffaloes, dogs, and horses where pigs were harboring the parasite.

The development of this trematode species follows a two-host life cycle, including a freshwater gastropod snail as intermediate or vector host and humans or pigs as definitive host (Figure 3). The adult stage of *F. buski* produces a large number of eggs in humans, counts showing between 13 000 and 26 000 (mean 16 000) per worm per day. Eggs are undeveloped when they leave the host with the feces and must reach freshwater to continue the cycle. The miracidium development period is 16–77 days, with a mean of 22 days, the optimum conditions being a water temperature of 27–30 °C and 6.5–7.2 as the pH range.

Intermediate hosts are limited to small planorbid snails (Figure 4) of the species *Segmentina hemisphaerula* (syn. *S. coenosus*, *S. nitidella*, *S. calathus*, *S. largillierti*), *Hippeutis cantori* (syn. *H. smackeri*), *H. umbilicalis*, and *Gyraulus convexiusculus* in China, Vietnam, and Thailand. In India (Assam), the snail host includes *S. trochoideus*, *S. hemisphaerula*, and probably also *H. umbilicalis*. In Thailand, the species *S. hemisphaerula*, *S. trochoideus*, and *G. chinensis* appear to be involved in transmission, whereas in Thailand it is *S. hemisphaerula*, in Laos *H. umbilicalis*, and in Bangladesh *S. trochoideus* and *H. umbilicalis*. The species *Indoplanorbis exustus* has also been recently involved in India.

Segmentina hemisphaerula and *H. cantori* live in similar habitats. The first one is extremely sensitive to dessication, the survival limit of snails kept out of water at 25 °C and 50% humidity being 25 h. Exposure of 2 h to real drying usually kills them, but they are able to live for some time in slightly moist mud.

The prevalence of infection in snails is usually very low (less than 2%), although local monthly prevalences of up to 20% have been found. Snail prevalence appears related to the distance of the snail habitat from a *F. buski* source: when the distance was 25 m, 26% of snails were infected, at 100 m it was 8%, and at 300 m only 6%.

The larval stages of sporocyst, mother and daughter rediae and cercariae develop in the intermediate snail host. Sporocysts are elliptical, and of 131–169/34–83 mm in size. Mother rediae develop rapidly inside the sporocyst, already emerging in days 9–10 after miracidium penetration into the snail. Mother rediae migrate to the ovotestis and are 701/159 μm in size. Daughter rediae may be up to 2.8 mm when mature and harbor up to 45 cercariae at one time. Gymnocephalous cercariae include a body of 195/145 μm and a tail of 498/57 μm, for a total length of the cercaria of 693 μm. Cercariae leave the rediae after 25–30 days but emerge from the snail after an average of 49 days, because they require a maturation period in the snail tissues. Other incubation periods recorded are 46–59 days at 22–24 °C, and 85–100 days at 18–22 °C, and beginning at 31 days after exposure to the miracidia. A very short prepatent period of only 21 days has been found in *S. trochoideus* and *H. umbilicalis*, in which *F. buski* causes mortality in 100% of the snails because of mechanical damage to the ovotestis.

Cercarial emergence from the snails is remarkably dependent on light. The large sized cercariae appear to be strongly phototactic, and a great variation was noticed in the daily emergence patterns of cercariae. Cercariae swim in water until metacercarial encystment on a substrate, mainly aquatic plants and debris but also on the water surface. The survival time of metacercariae in water varies from 64 to 72 days.

Food-Borne Transmission

Infection of the definitive host takes place by ingestion of plants carrying metacercariae. There is little selective

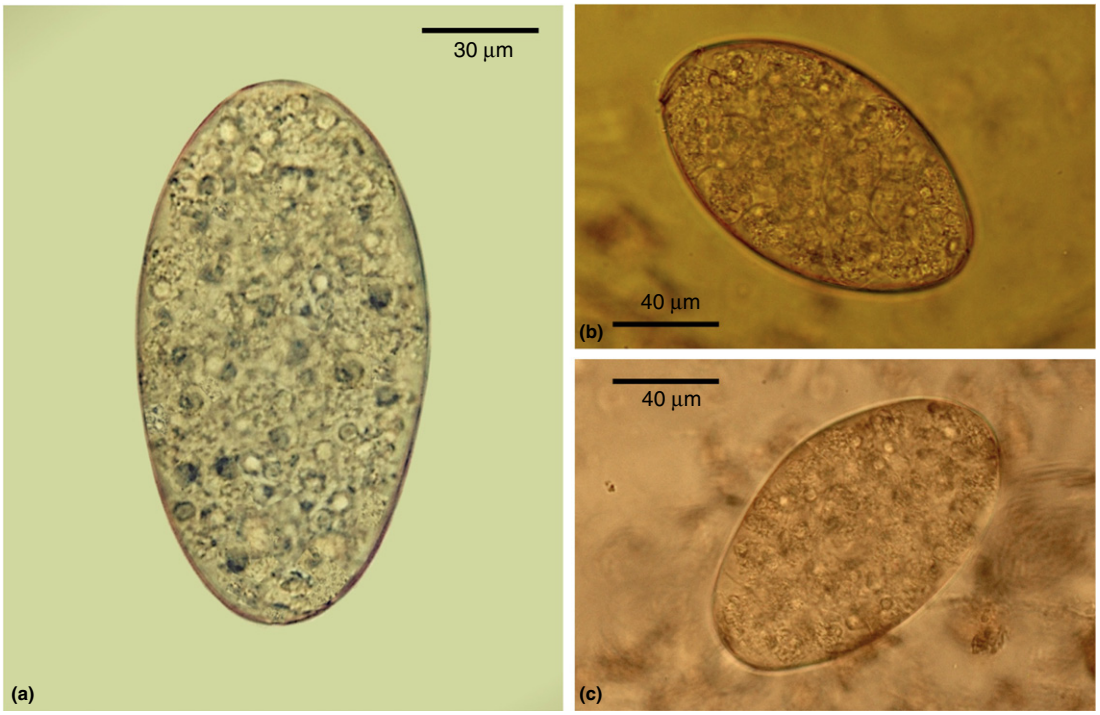

Figure 2 Eggs of *Fasciolopsis buski*: (a) egg isolated from the last part of the uterus showing typical aspect, thin shell, hardly visible operculum on top, refrangible brown-greenish granules equally distributed inside, and without irregular protuberance at abopercular end; (b) and (c) two eggs found in stool sample of Chinese patient (note small operculum microscopically focused in (b)).

specificity, metacercarial cysts attaching to all sorts of aquatic vegetation in stagnant ponds.

Of interest from the food point of view, there are various consumable water plants, among which are water caltrop (*Trapa natans* in China, *T. bispinosa* in Taiwan, and *T. bicornis* in Bangladesh and Thailand), water chestnut (*Eliocharis tuberosa*), water hyacinth (*Eichhornia* sp.), water bamboo (*Zizania* sp.), water lotus (*Nymphaea lotus*), water lily (*Nymphaea* sp.), watercress, gankola (*Otelia* sp.), and water morning glory or water spinach (*Ipomoea aquatica*) (**Figures 5** and **6**).

Encysted metacercariae exist not only on aquatic plants, but also on the surface of the water. This opens the probability of human and pig infection by drinking natural water. The proportion of the encysted metacercariae floating on the water surface is approximately 3.6% of that of the total encysted metacercariae. By inquiring into case histories, it has been found that 10.3–12.8% of patients and 35.1–40% of infested pigs are possibly infected by drinking water contaminated with encysted metacercariae.

Once ingested together with plants or drinking water, the metacercariae excyst in the duodenum and attach to the intestine wall to grow into sexually mature flukes in approximately 3 months.

Geographical Distribution

Human infection by *F. buski* has been reported in central and south China, Hong Kong, Taiwan, Bangladesh, India, Vietnam, Laos, Cambodia, Thailand, Kampuchea, Singapore, Burma, Malaysia, the Philippines, and Indonesia (**Figure 7**).

A summarized overview of the characteristics of fasciolopsiasis in China, Taiwan, Bangladesh, India, Vietnam, Laos, Cambodia, Indonesia, and Thailand is given in **Table 1**. In Vietnam, a curious case of a two and a half-year-old boy who vomited eight live *F. buski* adults has very recently been described from Vinh City, Nghe An Province.

Reports on Korean and Japanese subjects do not seem to be confined to their respective countries. Reports in the USA, Venezuela, Australia, Guatemala, Israel, and Cuba may be due to immigrants from the Far East or to misidentification of the eggs in feces.

Epidemiology

An estimated 10 million people infected by *F. buski* at the beginning of the twenty-first century is the figure now being quoted. Field studies suggest that the disease is underreported in the endemic areas, and is most prevalent in remote rural places and semiurban areas. Prevalences found in the endemic areas of different countries in both humans and pigs in studies and surveys performed during different periods are noted in **Table 1**.

Infection by *F. buski* is most prevalent in school-age children, where the number of worms per child can exceed 800. The highest infection rates were found in children in the age group 10–14 in Taiwan Hsien, southern Taiwan, some time ago. Within foci of parasite transmission, the prevalence of infection in children ranged from 57% in mainland China to 25% in Taiwan, and from 60% in India and 50% in Bangladesh to 10% in Thailand.

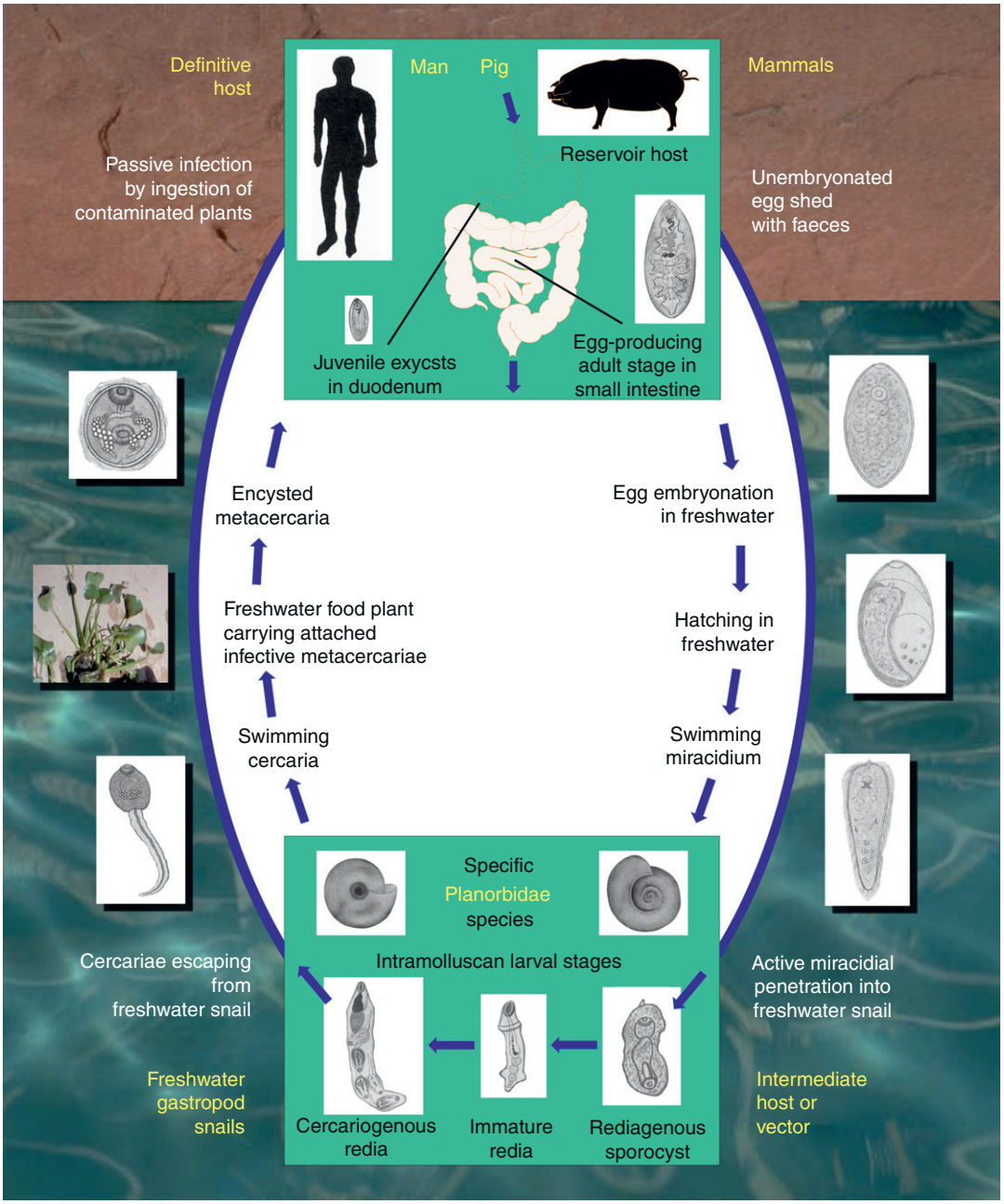

Figure 3 Two-host life cycle and transmission of *Fasciolopsis buski*. Note complete larval development and transmission by snail vector occurring completely in freshwater; only the adult stage develops in a terrestrial mammal (humans and pigs).

A higher prevalence among females (122 or 16%) than males (86 or 11%) was detected in a coprological survey of 1563 people (784 males and 779 females) in the endemic area of Thailand. The prevalence varied from 8% among adults (15 years and over) to 15% in children between 5–14 years old, with an average for all the age groups of 13%. Both in males and females, the severity of infection peaked between 10–14 years of age and decreased in older groups. The difference in prevalence and intensity is related to the activity of the various age groups. The school-age group, i.e., 5–14-year-olds, usually pick up and eat water caltrop on their way to and from school. In the older group, i.e., 15 years and over, the individuals usually do not pick up water caltrop as it would be considered stealing, but they contract the infection when they are engaged in the cultivation, harvesting, and selling of water caltrop.

Another survey in Thailand also found significantly more females than males were infected, the highest prevalence being among the 10- to 20-year age groups. A higher prevalence in females than in males after the age of 7 was also found in Bangladesh.

In endemic areas, the disease occurs focally, is wide-spread, and is linked to freshwater habitats with stagnant or slow-moving waters, is associated both with common social and agricultural practices, and with promiscuous defecation.

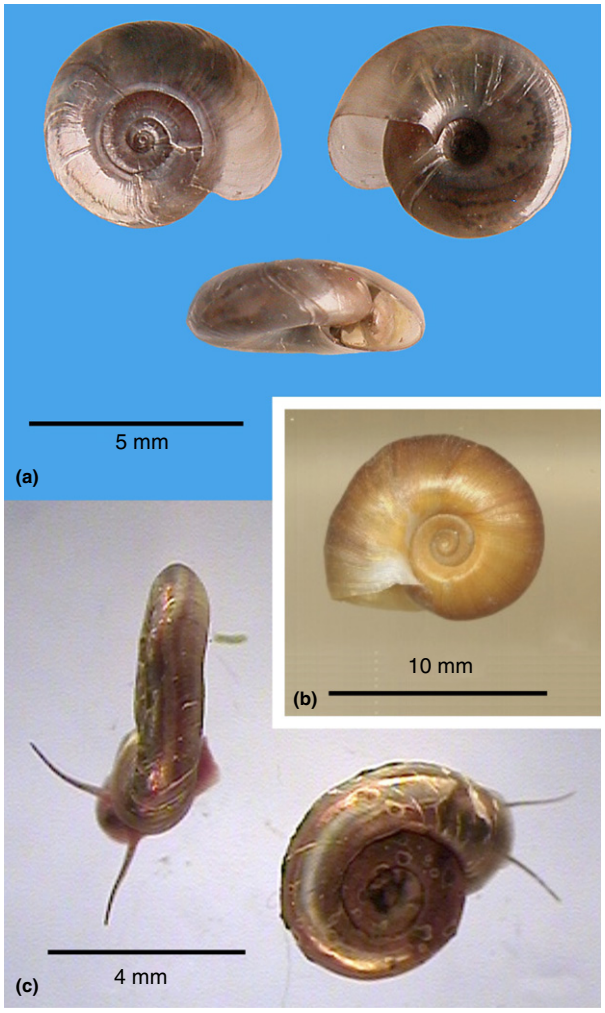

Figure 4 Small freshwater planorbid snail, vector species of *Fasciolopsis buski*: (a) shells of *Segmentina hemisphaerula* (furnished by H. Madsen); (b) shell of *Indoplanorbis exustus* (by M. Kohl); (c) living specimens of *Gyraulus chinensis*.

According to studies in pigs, it appears to follow a seasonal pattern. There is a peak between June and September and cases decline thereafter to a low level during winter and early spring (November–March), being absent during January and February, at least in a humid subtropic hilly city of north-east India where rearing of pigs is a common household practice.

Unnoticed ingestion of metacercarial cysts is the way by which humans and pigs contract the infection. This occurs through the eating of raw or undercooked aquatic plants, drinking or using raw water, and handling or processing water-derived plants, i.e., peeling plants using one's teeth. Infection takes place when peeling off the hull or skin of infected plants between the teeth because cysts are thus freed and are swallowed. Water caltrop is found in the wild, but, together with other plants, it is cultivated for its nuts in basins of stagnant water or in irrigation channels, which also harbor aquatic snails, among them the susceptible intermediate hosts. Cultivation of aquatic, metacercariae-carrying plants for consumption on a large scale and the pollution of the areas in which they are grown with human and animal (mainly pig) excreta are important epidemiological factors in the spread of the disease. The feces of infected pigs and the "night soil" (human excrement collected from latrines) used to fertilize fish ponds and to feed fish enhance the transmission of this parasite.

In Thailand, in the area of the Bang Kun Sri, the prevalence of *F. buski* infection among people living in the immediate vicinity of water caltrop plantations was much higher than in the villages a few hundred yards from plantations. Patterns were detected in children in Taiwan. Given that *F. buski* metacercariae do not withstand drying, plants have to be consumed shortly after they are harvested to cause infection. However, the common habit of dipping water caltrops in water from time to time to retain their freshness could help in the spread of the infection from areas near to where the plants are cultivated to areas where these plants are not grown.

In several countries where *F. buski* is endemic, pollution of the water apparently occurs through the use of human excreta as fertilizer. In Thailand, however, contamination of the fields in which water caltrop and water morning glory are cultivated takes place in areas where there is little or no dry ground and where the people defecate directly into the standing water beneath the houses. During the rainy season (June–October), water plants grow in abundance along the edges of rice fields and waterways throughout the central portion of the country. However, most of the edible plants are cultivated near the houses, that is, where pollution takes place.

In many oriental countries, human *F. buski* infection is aggravated by social and economic factors, such as poverty, malnutrition, and an explosively growing free-food market associated with a lack of food inspection, poor sanitation, other helminthiasis, and declining economic conditions. The differences in incidence in the same area are due to several factors, including economic status, educational background, standard of health, and mode of living.

The only effective reservoir is the pig (**Figure 8**). Under natural conditions, only 3–12 flukes are usually found in the pig. Water plants may serve as the main source of food for this animal. Fresh aquatic green fodder and raw water used to raise pigs are the main sources of *F. buski* metacercarial cysts infecting farm animals. Different pig infection rates have been reported in different areas: 30% in Uttar Pradesh, India; 10% in Kwangtung, China; and 52% in Taiwan Hsien, Taiwan. Interestingly, however, the expected correlation between human and pig prevalences is not always found, so there is still and disagreement as to whether there is only one strain of *F. buski*, or whether more than one local strain exists, with one being better adapted to man and another to animals. This would help to explain how infections among humans and pigs can show completely different epidemiological patterns, in certain areas e.g., (1) there are places in which the parasite is common in humans but absent in pigs, such as around Bombay, (2) there are areas where infection rates among humans and pigs are almost equal, and (3) there are other places with relatively important prevalences in pigs but very rare or even no infection in humans, as in Tonkin area, Vietnam. In Tonkin, the situation may be explained by the fact that inhabitants always cook aquatic plants (mainly water caltrop and water chestnut) and their fruits (the source of infection with the metacercariae) before they are eaten, whereas these plants, often carrying

(a) (b)

Figure 5 Freshwater food plants carrying metacercariae of *Fasciolopsis buski* attached to stems and leaves and thus participating in human infection by ingestion: (a) water caltrop *Trapa natans*; (b) water morning glory or water spinach *Ipomoea aquatica*.

Figure 6 In search of small freshwater planorbid snail vector species of *Fasciolopsis buski* in the water lotus *Nymphae lotus* taken out of a water pond.

infective metacercariae, are used for feeding pigs and other animals, resulting in the infection of pigs. Thus, different factors related to traditions, diet, and customs may go some way to explaining these local differences. Nevertheless, the controversy is still there, and recent studies having again referred to morphological and microtopographical strain variations among *F. buski* originating from different geographical areas.

Present Emergence Risk

Climate and global changes appear to increasingly affect the snail-borne helminthiases, which are pronouncedly dependent on the environment. This has been linked to the emergence of many trematodiases in different regions detected in recent years.

Fasciolopsiasis is a trematodiasis which shares many transmission characteristics (two-host life cycle, similar larval development pattern, freshwater snail vector, zoonosis, plant-borne infection) with fascioliasis, the best example of an emerging–re-emerging parasitic disease in many countries as a consequence of many phenomena related to climate change. However, the reservoir restriction to only the pig, in contrast to the low specificity of *Fasciola*, which is able to develop inside many different livestock species, suggests that the climate change influences on *F. buski* transmission increases (or decreases) may be somewhat buffered and thus perhaps less pronounced (or need more time to become obvious) when compared to fascioliasis.

In fasciolopsiasis, the life-cycle phases which develop in water are extremely susceptible to climate. Temperature and water-related climate variables greatly influence the embryonation of eggs in freshwater. The small planorbid snail vectors involved are extremely sensitive to dessication. Moreover, the parasite larval development within the snail vector is markedly dependent on abiotic factors such as temperature. Additionally, cercarial emergence is dependent on light.

The disease occurs focally, is wide-spread and is linked to freshwater habitats with stagnant or slow moving waters. It is therefore susceptible to climatic changes in precipitation rates as well as rainfall and extreme events such as floods and drought which may modify freshwater bodies and water velocity.

The disease appears moreover to follow a seasonality. A local climate change implying a modification of the current seasonality, by enlarging the yearly transmission window, would pronouncedly affect the epidemiological characteristics

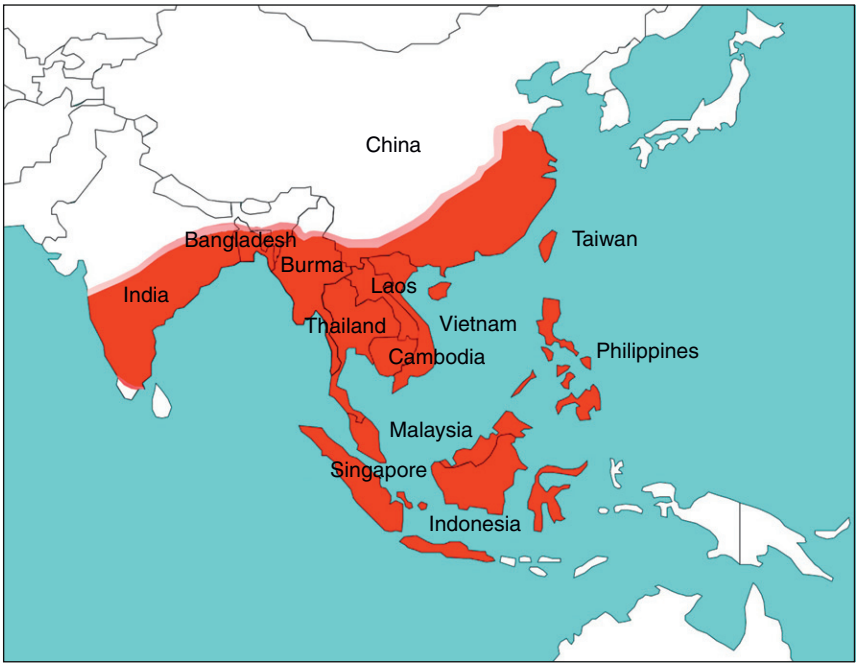

Figure 7 Geographical distribution of fasciolopsiasis in South East Asia, including countries in which human infection by *Fasciolopsis buski* has been reported.

of fasciolopsiasis in the area, with increase in prevalences, intensities, and geographical spread of infection both in humans and pigs.

Although there is still no data indicating a possible infection rate increase of fasciolopsiasis in humans or pigs related to climate change, (1) the absence of sufficient field surveys in the last two decades, (2) a few reports talking about a re-emerging infection in recent years, and (3) the present knowledge on climate-induced effects on fascioliasis, suggest that it would be prudent to undertake appropriate measures in fasciolopsiasis too. Field studies to assess such a potential phenomenon should be performed in endemic areas of Asia where climate change has shown to be more drastic in recent years.

Pathology and Symptomatology

The disease caused by intestinal *F. buski* infection is named fasciolopsiasis, which should not be confused with the similar term fascioliasis, which refers to another worldwide trematode disease caused by liver flukes of the genus *Fasciola*.

In humans, morbidity due to fasciolopsiasis in endemic areas appears to be high. Localized inflammation occurs at the site of worm attachment, which is usually followed by ulceration. When deep erosions take place, hemorrhage from the capillaries of the wall may result. Abscesses may also develop in the wall.

Intestinal obstruction may be caused by the large size of the worms. The worms feed actively not only on the host's intestinal contents but also on the superficial mucosa, causing extensive intestinal and duodenal erosions, ulceration, hemorrhage, abscesses, and catarrhal inflammation. Absorption of toxic and allergic worm metabolites cause ascites and both general and facial edema, for example, cheek and orbital edema. Pathological changes may be traumatic, obstructive, and toxic, especially in heavy infections. In such infections, the worms disturb the secretion of intestinal juices, cause excess mucus secretion, and obstruct the passage of food.

Feces are profuse, yellow-brown in color, and contain pieces of undigested food, due to malabsorption.

Clinical symptoms are related to parasite load (Table 2). Asymptomatic cases may be common, as found in Unnao district, Uttar Pradesh, India, where 61.2% proved asymptomatic among 181 persons shedding eggs in stools. The clinical disease appears to be only evident in cases with sufficient numbers of worms. The serious clinical picture caused by moderate and massive infections clearly demonstrates its public health importance.

Fasciolopsiasis is considered to be the main factor for persistent poor nutritional status of children in endemic areas of developing countries where it is present.

Advanced, heavy infections can be fatal. Mortality has been reported in children in with heavy infections owing to profound intoxication in India, China, and Thailand. In the autopsy of a fatal case, the mucosa of stomach and small and large intestine was hyperemic. Sections of the intestine showed hyperemia of both mucosa and submucosa, with hyperplasia of the lymphoid cells. Lungs revealed small scattered hemorrhagic spots. The liver was small, yellowish, and friable, with degeneration of the liver cells and fat vacuoles in the cytoplasm. Profound intoxication and sensitization are evidently produced by absorption of fluke metabolites into the system.

In pigs, intestinal lesions consist of minute foci of petechial hemorrhages scattered in the mucosal wall. Duodenum lesions show edema and congestion of the mucosa, with blood extravasation in both mucosa and submucosa. The mucosal

Table 1 Prevalences and other data characterizing fasciolopsiasis in the different countries

Country	*Endemic areas*	*Examples of prevalences*		*Other data*	*Year or period when studies were performed*
		In humans	*In pigs*		
China	Infections of humans and pigs reported from 10 provinces	• Up to 85% in certain areas of Chekiang and Kiangsi Provinces • A prevalence of 57% found in schoolchildren • In other areas it varies from less than 1% to 5%.		Recent nationwide surveys suggest a decrease in fasciolopsiasis	Different years
Taiwan	• In many parts of the island • Most highly endemic area appears to be Taiwan Hsieh in southern Taiwan	• 24%, 28%, 48%, and even 61% in Pa-Weng village • 25% were described in schoolchildren	Pigs appear to be also infected in many parts of the island	Intensity of 10 worms per person estimated in the Liuying area Recently also detected in immigrant aiwanese laborers	1960s and 1970s 1980s
Bangladesh		• 39.2% in one village near Dacca • 8.6% in another village • 50% found in schoolchildren in endemic focus		Endemic area in Bangladesh seems to be continuous with areas in India	1975
India	Assam	60%	Contrasting situation emphasized	1–57 worms per patient collected after treatment	First half of last century
	Calcutta	High infections reported	High infections		
	Bombay	29% in the city	Absence highlighted		
	Maharashtra	Human foci detected			
	Unnao district in Uttar Pradesh	22.4% out of 808 studied in six villages			1974
Vietnam		15% in Asians and 3% in Europeans			Old studies
		Positive cases	Positive cases		Up to the end of 1990s
Laos		Human infections reported			1990s
Cambodia	Area of Phnom-Penh	0.04%	5%		
Indonesia	Borneo and Sumatra	• 27.0% in Sei Papuyu village, Kalimantan • 20.3% in schoolchildren outside that area		Highest prevalence in the 5- to 14-year-old age group (56.8%) and decreasing with age	
Thailand	Main problems in central part of the country	• 13% out of 1500 people from three provinces • Estimated 100 000 persons infected among 500 000 persons	Closely parallel prevalence found in man		Mid-last century
	Areas as Pak Hai	Likely that 100% of the indigenous population is infected			
	Northern part	Lower than 10%		Immigrants from other parts of the country seem to be involved	

glands are cystic and in a catarrhal condition, and some mucus exudates may be present in the acini. There are patches of submucosal congestion where worms are attached and edema where worms are detached from the wall.

Figure 8 Free pigs constitute the main reservoirs and assure the transmission of the disease when entering in freshwater ponds inhabited by the planorbid snail vectors.

Diagnosis in Hosts

In endemic areas, the clinical picture becomes highly suggestive although usually not distinctive. Hence, diagnosis is routinely made by examining fecal specimens for the eggs, or occasionally by examination of expelled adult worms vomited or passed in feces.

When diagnosing coprologically, care should be taken with eggs, as they are not easily distinguishable from those of other trematode species usually infecting humans in the same endemic areas of Asia and which also produce unembryonated eggs. Thus, misclassification of eggs by nonspecialists may occur. A detailed comparative differentiation is noted in **Table 3**.

The differentiation with *Fasciola* species and hybrids becomes crucial due to the frequency of human fascioliasis infection worldwide. Therefore, the shape and nature of the granules in yolk cells might help. In *F. buski* eggs, the granules are larger in size, more refrangible showing a light shade of greenish-brown black in their appearance, and appear equally distributed all over the cytoplasm of yolk cells (**Figure 2**). In *Fasciola* eggs, the granules are smaller in size, less refrangible with a light shade of yellowish-brown in color and distributed densely near the yolk cell nuclei and hardly observed in the peripheral part of the cytoplasm.

The sequences corresponding to the 18S rRNA gene and the nuclear ribosomal DNA spacers ITS-1 and ITS-2 are

Table 2 Main characteristics of the clinical picture of fasciolopsiasis according to parasite burden and disease severity

Parasite burden	*Light infection*		*Moderate infection*	*Heavy infection*
	Asymptomatic	*Symptomatic*		
Symptoms	Usually asymptomatic, sometimes abdominal pain	Headache, dizziness, stomach ache, gastric pain	Headache, severe epigastric pain, abdominal pain, mild abdominal colic, acute ileus, anasarca	Common bitemporal headache, severe epigastric pain, common generalized abdominal pain, mild abdominal colic, acute ileus, anasarca, common ascites, giddiness
			Protuberant abdomen, abdominal distention, dysentery	Protuberant abdomen, abdominal distention, dysentery
			Nausea (especially in the morning and resolving after the first meal), vomiting	Nausea (especially in the morning and resolving after the first meal), vomiting
			Fever	Low-grade fever
				Asthenia, pallor
Toxic and allergic symptoms				Generalized toxic and allergic symptoms, usually in the form of edema, particularly of the face, abdominal wall and lower extremities
Liver and spleen				Nonpalpable
Feces	Sometimes diarrhea, alternating with periods of constipation	Loose stools	Diarrhea or bowel obstruction	Diarrhea or bowel obstruction
Blood		Anemia eosinophilia	Anemia marked eosinophilia leucocytosis	Anemia marked eosinophilia leucocytosis
Associations			Poor appetite, malnutrition	Poor appetite, malnutrition, malabsorption, lowering in serum vitamin B_{12} content in children

Table 3 Coprological diagnosis of fasciolopsiasis by morphological differentiation of the eggs of *Fasciolopsis buski*[a] by comparison with similar nonembryonated eggs laid by other human-infecting trematode species present in the same endemic countries of Asia and rest of the world

Characteristics	*Fasciolopsis buski*	*Fasciola hepatica*	*Fasciola gigantica*	*Fasciola hybrids*	*Gastrodiscoides hominis*	*Paragonimus* spp. *of Asia*
Egg length	120–140 μm	100.6–162.2 μm[a]	129.6–204.5 μm	Fh-like 106.5–171.5 μm[a] Fg-like150.9–182.2 μm[a]	127–160 μm	80–120 μm
Egg width	70–90 μm	65.9–104.6 μm[a]	61.6–112.5 μm	Fh-like 63.9–95.4 μm[a] Fg-like15085.1–106.2 μm[a]	62–75 μm	45–70 μm
Shape	Broadly ovoid, although sometimes more ellipsoidal and less slender than in *Fasciola*	Broadly ovoid as in *Fasciolopsis*, but sometimes spindlelike or slightly asymmetrical, with somewhat pointed ends	Broadly ovoid as in *Fasciolopsis*, but sometimes spindlelike or slightly asymmetrical, with somewhat pointed ends	Broadly ovoid as in *Fasciolopsis*, but sometimes spindlelike or slightly asymmetrical, with somewhat pointed ends	Broader, tapering more toward the opercular end	With broad end, several more broadly oval than others
Shell	Thin	Less thin	Less thin	Less thin	Thin	Moderately thick
Operculum	Delicate and difficult to visualize even at high magnification	Relatively well visible	Relatively well visible	Relatively well visible	Relatively less small but not well visible	Prominent, with lateral ledge
Abopercular end	Not blemished	Irregular protuberance	Irregular protuberance	Irregular protuberance	Generally thickened and rarely provided with a spine like elongation	With distinctly thickened shell
Color	Yellow	Yellowish-brown	Yellowish-brown	Yellowish-brown	More pale	Golden-brown
Shedding	Feces	Feces	Feces	Feces	Feces	Feces and sputum

[a]Measurements correspond to eggs found only in humans.

already known for *F. buski*. This may be useful for molecular confirmation of individual diagnosis of patients, whether by sequencing of eggs found in stool specimens or from fluke adults when vomited, as was recently demonstrated in a Vietnamese child in whom only two nucleotide substitutions were found when comparing.

Treatment

Carbon tetrachloride, beta-naphthol, hexylresorcinol, niclosamide, dichlorophen, and (primarily) tetrachloroethylene were the drugs used for the treatment in the early days.

Praziquantel at dosages of 40, 25, and 15 mg kg^{-1} was given to school-age children heavily infected with *F. buski* for the first time in central Thailand. Even administered at the lowest dose of 15 mg kg^{-1}, it showed to be highly efficient. As no side-effects occurred, a single praziquantel dose of 15 mg kg^{-1} at bed time was recommended for children. Praziquantel treatment was also highly effective in school-age children with severe fasciolopsiasis in Taiwan. In Indonesia, infection in children was treated with 30 mg kg^{-1} of praziquantel with minor side effects. However, this drug could not save the life of a heavily infected 20-year old woman.

A single dose of praziquantel at 15 mg kg^{-1} is, therefore, the treatment of choice nowadays. The pharmacological termination of *F. buski* infections are both effective and low-cost. A single-dose of the broad-spectrum anthelminthic praziquantel is given to school-age children in endemic areas, which together with the prevention of reinfection, is aimed at improving the children's health at a crucial time in their growth and development.

The efficacy of triclabendazole, oxyclozanide, and rafoxanide was recently evaluated in naturally infected pigs, based on fecal egg count reduction tests and clinical improvement. On the 28th day after the treatment, triclabendazole showed the highest efficacy (97.12%), followed by oxyclozanide (93.27%) and rafoxanide (83.17%). None of these anthelmintics exhibited any side-effects in the treated animals.

Detection and Management for Food Safety

Attention should be focused on the metacercarial stage. Metacercarial cysts on plants are visible to the naked eye. The cysts average 3.9/2.1 mm. Up to 200 cysts may be found on the skin of one water caltrop, but the usual number is approximately 15–20.

Dried aquatic plants are not dangerous because dessication kills the metacercariae. Metacercariae are very sensitive to dryness, which causes death of metacercariae in 19 h at 27 °C. Metacercariae are killed by direct solar radiation in 20–30 min. People need to be instructed not to soak the fruits in water every once in a while to preserve their freshness. There should be sufficient time between the time the fruits are harvested to the time they are sold in the market to make them safe.

Prevention of infection might also be accomplished if the aquatic plants and their fruits are immersed in boiling water for a few minutes. Aquatic plants such as water chestnut should be boiled for 1–2 min before eating to kill the encysted metacercariae on the plants. Moreover metacercariae are killed after 1 min in boiling water, in 1% HCl in 18 days, by 2% acetic acid in 9 days, by 3% acetic acid in 6 days, by 5% salt solution in 3 h, by soybean sauce in 30 min, and by 10% cane sugar in 3 days.

Peeling the plants and fruits and washing in running water to wash away the freed metacercarial cysts should also be considered as a preventive measure.

Disease Control

The control of *F. buski* infections is theoretically very simple and the most practical method is to avoid eating raw, water-derived food. However it is extremely difficult to enforce such a simple method in face of century-old traditions. Such measures demand fundamental changes in the eating habits, customs, and economic conditions of the people. Infections with *F. buski* follow a familial trend, as food preparation and eating habits are passed from one generation to the next. In addition, water plants are common food source because they are cheap and readily available (**Figure 9**).

The nature of fasciolopsiasis does not justify the establishment of a separate control program, because it can be controlled along with other food-borne parasitoses for which there are sustained control programs, such as those launched by the World Health Organization (WHO). Control of fasciolopsiasis by blocking or interruption of the parasite life-cycle can be achieved through proper diagnosis followed by pharmacological treatment of people and their livestock, and by preventing reinfection. In countries where eating raw plants is customary and treated water is not available, the prevention of reinfection relies on consistent educational propaganda stressing the importance of thoroughly cooking all aquatic plants and boiling all water.

The most easy way to control fasciolopsiasis is by pharmacological treatment and the implementation of modern pig farming. Control measures include prevention of pollution of the ponds where water caltrop, water chestnut, and other aquatic plants are cultivated. Pollution of the ponds happens in different ways in the various endemic areas. Human excreta are used as fertilizer in China, promiscuous

Figure 9 Uncontrolled food plant markets in city streets contribute to the dissemination of the disease to people who do not necessarily live in the countryside.

defecation takes place in the bodies of water near the houses in Thailand, and excreta of pigs are washed into neighboring bodies of water in Taiwan.

Pigs easily acquire this disease because they are often fed with infected raw vegetables. Thus, the plants must always be checked for the presence of metacercariae, the greatest numbers being found from May to October. The use of fermented silage to feed pigs instead of fresh aquatic green fodder has been suggested to prevent infection in the animals.

Eggs cannot survive anaerobic conditions but may resist low temperatures and be maintained at 4 °C for 3–4 months.

Educational efforts should be directed primarily toward school-age children because they are less entrenched in their food and eating habits, behavior, and customs. Significant changes in the control of intestinal helminthiasis rely on the development of effective broad-spectrum anthelminthics and the implementation of control programs in school-aged children with strong community therapy programs.

In Taiwan, fasciolopsiasis was highly endemic in the past, but in the 1980s the number of human cases decreased. The aggressive education programs in Taiwan included lectures and demonstrations of *F. buski* flukes in primary schools and during home visits. Although the reduction in the number of cases was recognized in their endemic regions, this tendency was not stable, because many cultures, particularly in China, still enjoy eating raw food products. In addition, there is a belief that cooking food destroys the flavor and nutritional value. The simplest control measures in endemic areas should include boiling water, thorough cooking or steeping of aquatic plants in boiling water, restraining pigs from having access to ponds and canals, eliminating intermediate host snails, and prohibiting the use of unsterilized "night soil" as fertilizer and aquatic green fodder for pigs. Unfortunately, despite WHO control programs implemented and sustained in the communities, fasciolopsiasis still remains a public health problem in endemic areas. Furthermore, in areas where it was thought to be fully controlled, as in Uttar Pradesh, India, where no case was detected since the 1990s, there are reports of a re-emerging infection in recent years.

Fasciolopsiasis, together with other food-borne trematodiases, have been very recently added to the list of priority helminthiases and food-borne zoonoses with high impact on human development, within the so-called neglected tropical diseases (NTDs) by WHO. This decision assures targeted action against this disease in endemic Asian countries for the years to come.

See also: Disciplines Associated with Food Safety: Parasitology. Foodborne Diseases: Prevalence of Foodborne Diseases in South East and Central Asia. Helminth-Trematode: *Fasciola hepatica* and *Fasciola gigantica*; *Paragonimus westermani* and *Paragonimus* Species

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HELMINTH-TREMATODE

Heterophyes heterophyes

J-Y Chai, Seoul National University College of Medicine, Seoul, Korea

Glossary

Cellophane thick smear technique A method of fecal examination to detect helminth eggs. Approximately 60 mg of feces is placed on a glass slide and a cellophane paper soaked with glycerin and malachite green is put on the feces. The feces under the cellophane are compressed to become flat and dried before microscopic examination.

Kato-Katz thick smear technique A method of fecal examination to detect helminth eggs. The feces are sampled by filtering through a fine mesh and used to fill a small premade hole on a plastic plate which is placed on a glass slide. The plastic plate retaining the feces (41.7 mg) on the slide is carefully removed. A cellophane paper soaked with glycerin and malachite green is used to cover the feces. The feces under the cellophane are compressed to become flat and dried before microscopic examination.

Niclosamide An anthelmintic drug used for the treatment of a wide spectrum of helminth infections, including many species of trematodes and cestodes.

Praziquantel An anthelmintic drug used for the treatment of a wide spectrum of helminth infections, including almost all species of trematodes and many species of cestodes.

Background

The trematode family Heterophyidae (=heterophyids) includes a number of minute intestinal trematodes (or flukes) infecting humans and animals. The genus *Heterophyes*, one of the members, was created by Cobbold in 1866. The adult flukes of *Heterophyes*, together with *Heterophyopsis*, have a peculiar genital apparatus which is called a 'genital sucker,' and this character is comparable with and distinct from other genera of the Heterophyidae. Within the genus *Heterophyes*, at least 17 species or subspecies have been reported (Table 1). However, only eight species, including *Heterophyes aequalis*, *Heterophyes bitorquatus*, *Heterophyes chini*, *Heterophyes dispar*, *Heterophyes heterophyes*, *Heterophyes nocens*, *Heterophyes pleomorphis*, and *Heterophyes superspinatus*, are acknowledged as valid species. The species known to infect humans include *H. heterophyes*, *H. nocens*, and *H. dispar*. Heterophyiasis is a disease caused by infection with species of *Heterophyes*. The disease is prevalent in the Far East, the Middle East, Egypt, Sudan, and southeastern Europe.

Characteristics

Morphologic Characters of *Heterophyes*

Heterophyes flukes are characterized by their minute body (0.4–2.7 mm long), a large and median located ventral sucker, presence of a genital sucker armed with gonotyl, and two adjacent testes near the posterior extremity (Figure 1). *Heterophyes* flukes are morphologically distinguished from other heterophyids, including *Heterophyopsis*, *Metagonimus*, *Pygidiopsis*, *Centrocestus*, *Stellantchasmus*, *Haplorchis*, *Procerovum*, and *Stictodora*, by various features. *Heterophyes* differs from *Heterophyopsis* in that the latter has an elongated body and also two obliquely tandem testes. *Heterophyes* differs from *Metagonimus* in that the latter has a smaller submedian located ventral sucker and the genital sucker is absent. *Pygidiopsis* differs from *Heterophyes* in that the former has a small ventral sucker connected anterolaterally to a ventrogenital apparatus but lacks the genital sucker. *Centrocestus* is armed with small circumoral spines around the oral sucker and lacks the genital sucker. *Stellantchasmus* has a small laterally deviated ventral sucker and an elongated sac-like seminal vesicle connected to a muscular expulsor at the opposite side of the ventral sucker and lacks the genital sucker. *Haplorchis* and *Procerovum* have only one testis, whereas *Heterophyes* and other heterophyids have two testes.

Important Species

H. heterophyes

H. heterophyes was first discovered by Bilharz in 1851 at an autopsy of an Egyptian in Cairo, and is now known to cause human infections along the Nile Delta of Egypt and Sudan, the Middle East, and southeastern Europe. *H. heterophyes* adults are 0.4–2.7 mm long and their unique morphology includes the presence of two side-by-side testes near the posterior end of the body, a large ventral sucker which is located median, and a large submedian genital sucker armed with 70–85 chitinous rodlets on its gonotyl. *H. heterophyes* differs from *H. nocens* in the number of rodlets on the gonotyl; 50–62 in *H. nocens* versus 70–85 in *H. heterophyes*. The adults of *H. dispar* (0.4–1.4 mm) are slightly smaller in body size and

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Table 1 Species of *Heterophyes* reported in the literature

Species	*Human infection*	*Area (country)*	*Reporter (year)*
Valid species			
Heterophyes aequalis	No	Egypt	Looss (1902)
Heterophyes bitorquatus	No	Malaysia (Borneo)	Pearson and Pearson (1981)
Heterophyes chini	No	Malaysia (Borneo)	Pearson and Pearson (1981)
Heterophyes dispar	Yes[a]	Egypt	Looss (1902)
Heterophyes heterophyes	Yes[b]	Middle East, Egypt	von Siebold (1853)
Heterophyes nocens	Yes	Korea, Japan	Onji and Nishio (1916)
Heterophyes pleomorphis	No	Uganda	Bwangamol and Ojok (1977)
Heterophyes superspinatus	No	Kazachstan	Leonov and Belogurov (1965)
Other species (including synonymy)			
Heterophyes aegyptiaca	No	Egypt	Cobbold (1866)
Heterophyes dispar limatus	No	Egypt	Looss (1902)
Heterophyes elliptica	No	Taiwan	Yokogowa (1913)
Heterophyes fraternus	No	Egypt	Looss (1894)
Heterophyes heterophyes sentus	No	Egypt	Looss (1902)
Heterophyes indica	No	India	Rao and Ayyar (1931)
Heterophyes inops	No	Egypt	Looss (1902)
Heterophyes katsuradai[c]	Yes	Japan	Ozaki and Asada (1926)
Heterophyes pallidus	No	Egypt	Looss (1902)
Heterophyes persicus	No	Egypt	Looss (1902)

[a]Human infections were reported from Koreans who traveled to Saudi Arabia or Sudan.
[b]Human infections were reported from inhabitants of Egypt, the Middle East, and the Mediterranean, and also from several Koreans who traveled to Saudi Arabia or Sudan.
[c]Regarded a synonym of *H. nocens*.

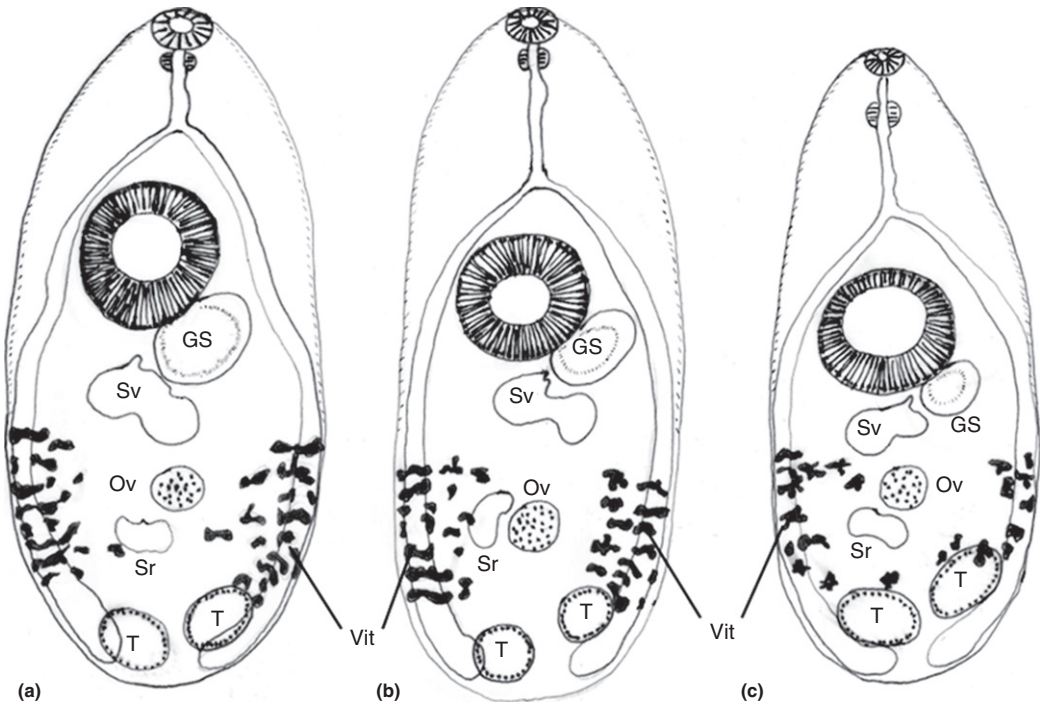

Figure 1 *Heterophyes* spp. infecting humans. (a) *Heterophyes heterophyes*; (b) *Heterophyes nocens*; (c) *Heterophyes dispar*. GS, genital sucker; Ov, ovary; T, testis; Sv, seminal vesicle; Sr, seminal receptable; Vit, vitelline follicles.

have smaller sizes of the genital sucker and smaller numbers of rodlets on the gonotyl (22–33) than *H. heterophyes*. The eggs of *H. heterophyes* are slightly larger (0.024–0.029 mm long) than those of *H. dispar* (0.021–0.023 mm long).

The life cycle of *H. heterophyes* is elucidated in Figure 2. The molluscan intermediate host is a kind of brackish water snail, *Pirenella conica*, in the Mediterranean and Red Sea, and *Cerithidea cingulata* (= *Tympanotonus microptera*) in India. In some

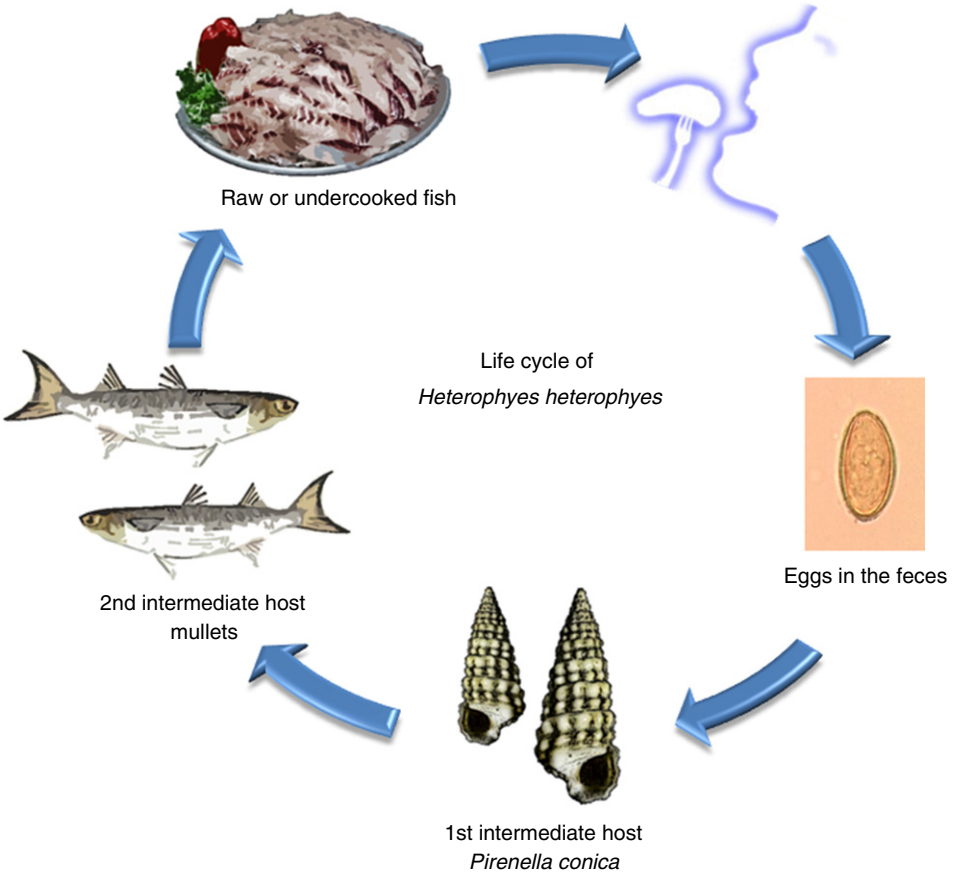

Figure 2 The life cycle of *Heterophyes heterophyes*.

areas, up to 38% of *Pi. conica* shed the cercariae of *H. heterophyes*. The cercariae shed from the snails, swim in water, find fish hosts, and quickly enter between the scales of the fish. They encyst in the muscle of the fish. Brackish water fish, including *Mugil cephalus*, *Mugil capito*, *Mugil auratus*, *Mugil saliens*, *Mugil chelo*, *Tilapia nilotica*, *Tilapia zilli*, *Aphanius fasciatus*, *Barbus canis*, *Sciaena aquilla*, *Solea vulgaris*, and *Acanthogobius* spp., are important fish hosts. Metacercarial cysts are globular or slightly oval, 0.13–0.26 mm in diameter, and usually found in the fish muscles. The larva inside the cyst is usually folded on itself showing the oral sucker, ventral sucker, genital sucker with gonotyl spines, and excretory vesicle filled with refractile globules. The longevity of metacercariae in the fish is unknown, but they may be able to survive for several years or throughout the life span of the fish hosts. In salted fish, they can survive only up to 7 days. A variety of mammals and birds, including dogs, cats, wolves, bats, rats, foxes, sea gulls, and pelicans, take the role of the reservoir hosts. Rats, dogs, cats, foxes, badgers, pigs, macaques, and gulls can be used as experimental definitive hosts. The life span in the canine or feline host is 1–4 months but may be longer in humans.

H. nocens

H. nocens was first reported in Japan by Onji and Nishio in 1916 based on worms recovered from experimental dogs and cats fed with the metacercariae encysted in the mullet, *Mu. cephalus*. The adult flukes are 0.9–1.6 mm long and morphologically close to *H. heterophyes*. Therefore, this species was for sometime called a subspecies of *H. heterophyes*, i.e., *H. heterophyes nocens*. However, *H. nocens* is now acknowledged as a distinct species because it has a consistent morphologic feature, in particular, the smaller number of rodlets (50–62) on the gonotyl in comparison to *H. heterophyes* (70–85). This species is now known to occur as human infections in Korea, Japan, and China. In China, the species described as *H. heterophyes* by Xu and Li in 1979 is presumed to be *H. nocens*. The eggs of *H. nocens* are similar in size (0.024–0.029 mm long) compared with *H. heterophyes* but slightly larger than those of *H. dispar*.

The first intermediate host is a brackish water snail, *C. cingulata* (= *Ty. microptera*). The cercariae are of the ophthalmo-pleuro-lophocercous type and shed from the snails into water. They can survive for as long as 42–54 h in sea water. When the fish hosts are available nearby them, they quickly attach to the fish skin, penetrate between the scales, and enter into the muscles. Important fish hosts include brackish water fish species, including *Mu. cephalus*, *Acanthogobius flavimanus*, *Lateolabrax japonicus*, *Therapogon oxyrhynchus*, and *Liza menada*. Metacercarial cysts are globular or slightly oval, 0.16–0.20 mm in diameter, and usually found in the fish muscles (Figure 3). The larva inside the cyst characteristically reveals its oral sucker, ventral sucker, genital sucker with

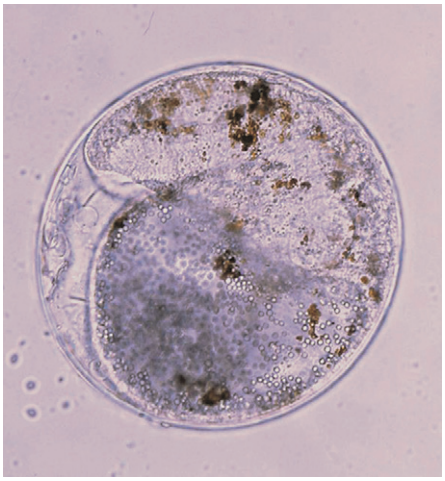

Figure 3 An infectious stage larva (= metacercaria) of *Heterophyes nocens* isolated from the muscle of a mullet, *Mugil cephalus*, slightly flattened. The cyst size is 0.17 mm in diameter.

gonotyl spines, and excretory vesicle filled with refractile granules. The longevity of metacercariae in the fish is unknown, but they may be able to survive for several years or throughout the life span of the fish hosts. In salted fish, they can survive only up to 7 days. Domestic or feral cats, dogs, and rats are natural definitive hosts. Mice and rats are experimental definitive hosts. The life span in animal host is 1–4 months but may be longer in humans.

H. dispar

H. dispar was first discovered in the intestines of dogs and cats in Egypt by Looss in 1902, and then in mammals, including the fox and wolf in northern Africa and the eastern Mediterranean. The geographic distribution is similar to that of *H. heterophyes*. The size of *H. dispar* adults is approximately three-quarters of that of *H. heterophyes* and *H. nocens*. The internal structures are almost the same as *H. heterophyes*; however, the most striking difference is the much smaller number of rodlets on the gonotyl (22–33) in *H. dispar*. There was a debate on the possible synonymy of *H. dispar* with a closely related species, *H. aequalis*. However, *H. aequalis* differs from *H. dispar* in the smaller number of rodlets on the gonotyl (14–25) and short ceca, i.e., ending before the anterior margin of two testes in *H. aequalis* but extending down to the posterior margin of two testes in *H. dispar*.

The whole life cycle is similar to that of *H. heterophyes*. Brackish water snails, *Pi. conica*, serve as the first intermediate hosts, and brackish water fish, including *Mugil* spp., *Tilapia* spp., *So. vulgaris*, and *Sc. aquilla* are the important second intermediate hosts. Metacercariae are morphologically similar to those of *H. heterophyes*. They usually encyst in the fish muscles. The larva shows its oral sucker, ventral sucker, genital sucker with gonotyl spines, and excretory vesicle filled with refractile granules. The longevity of metacercariae in the fish is unknown, but they may be able to survive for several years or throughout the life span of the fish hosts. Natural definitive hosts include dogs, cats, wolves, jackals, foxes, and kites. Pups and rabbits were used as experimental definitive hosts.

Clinical Manifestations

The most frequent clinical manifestations in heterophyiasis patients are mild to moderate degrees of abdominal pain, diarrhea, lethargy, anorexia, and weight loss. However, the severity of symptoms may vary and depend on host-side factors. One factor is the intensity of infection in each patient, i.e., the number of infected worms. Heavier infection cases tend to suffer from more severe illnesses. Another factor is the immune status of the patient, i.e., immunocompetent or immunodeficient. A third factor is the history of previous exposures to infection that can confer acquired immunity of individual patients. A new visitor to an endemic area may suffer from severe illnesses after a primary infection, whereas residents in endemic areas generally complain of milder symptoms. In immunocompromised patients, severe clinical manifestations, including erratic parasitism (= extraintestinal heterophyiasis) in the heart, brain, and spinal cord, may occur as in other heterophyid species infections. *H. heterophyes* and *H. nocens* (presumed) infections are suspected as the causes of cerebral manifestations, including epilepsy, brain abscess, or brain cyst.

Virulence Factors and Pathogenesis

The virulence of *Heterophyes* flukes might be different for different species of parasites. However, there have been no studies supporting this hypothesis. Two principal factors related to virulence are mechanical and chemical irritation by the flukes. Mechanical irritation is chiefly caused by the movement of worms which can have harmful effects on the intestinal mucosa, in particular, the villous and crypt layers of the small intestine. Chemical substances produced by the flukes, which include excretory–secretory proteins (ESPs), can play the role of not only antigens but also toxins to the host. Immune responses of the host against the flukes or their ESPs may be too strong (hypersensitivity) that the host immunity can damage the host itself. The affected mucosa may undergo hypersensitive and allergic reactions, including severe catarrhal inflammation and loss of villi. Pathogenicity is also related to other host–parasite relationships, including the intensity of infection; heavier infections generally undergo severer illnesses.

In human or animal hosts, adult flukes of *Heterophyes* parasitize the middle part of the small intestine, and invade the crypt of Lieberkühn by day 2–3 postinfection, and localize between villi or in the intestinal lumen during later stages. At the site of attachment in the host intestinal mucosa, *Heterophyes* adults can cause mechanical and chemical irritation and elicit inflammatory reactions, ulcers, and superficial necrosis of the mucosa. Villous atrophy and crypt hyperplasia may be the two major histopathologic features, accompanied by inflammatory cell infiltrations. In avian hosts, like sea gulls, the flukes frequently invade extraintestinal or somatic tissues and organs, in particular, the liver, pancreas, and bile duct. Also in

humans, eggs of *H. heterophyes*, and presumably *H. nocens*, were found encapsulated in the brain of patients with neurological symptoms. Therefore, erratic parasitism, as reported by Africa and coworkers in 1940 in *Stellantchamus falcatus*, *Haplorchis* spp., and *Procerovum* spp., seems to occur in patients infected with *Heterophyes* flukes.

Epidemiology

H. heterophyes

The principal mode of human infections with *H. heterophyes* is consuming raw or improperly cooked flesh of fish intermediate hosts, notably mullets and gobies. Endemic areas are scattered along riverside areas of Egypt, Sudan, Iran, Greece, Turkey, Italy, and Tunisia. Also in Asia, several endemic foci were reported but the parasites seem to have been actually *H. nocens*. In Egypt, human infections are prevalent in the northern part of the Nile Delta, particularly around Lakes Menzaleh, Burullus, and Edco, where fishermen and domestic animals frequently consume salted or insufficiently baked fish. In these areas, 36–90% of school children and 22% of adults were infected with this fluke during the 1930s–1950s; however, the prevalence among these people declined to 2.5–10.0% in the 1980s. The population at risk in these areas is approximately 1 million. In Iran, the prevalence in villages of Khuzestan was 8% on average and ranged from 2% to 24%. In postmortem examination of carnivores in the same areas of Iran, 14.2% of jackals, 33.3% of foxes, and 2.5% of dogs were infected with heterophyid flukes, including *H. heterophyes*, *Metagonimus yokogawai*, and *Heterophyes katsuradai* (a synonym of *H. nocens*). Imported human infections were reported in Korea and Japan, among people who returned from Egypt, Saudi Arabia, or Sudan.

H. nocens

After the discovery of *H. nocens* in Japan, the existence of this fluke in Korea was also confirmed by recovery of adult flukes from experimental mice and rats fed the metacercariae in mullets, *Mu. cephalus*. Human infections occur in various parts of Japan and Korea, where mullets and gobies are consumed raw or under improper cooking conditions. In Korea, more than 40% prevalence were noted among residents in southwestern coastal areas, including Jeonranam-do and Gyeongsangnam-do (Provinces). In the coastal village of Muan-gun, Jeonranam-do, 75% of the inhabitants were positive for heterophyid eggs; they were mixed-infected with *H. nocens* (all of 20 worm-recovered cases) and *Pygidiopsis summa* (18 of 20 worm-recovered cases). In these areas, individual worm burdens of *H. nocens* ranged from 3 to 1338 (average 237 worms per person). Many coastal islands in the western and southern seas of Korea were added to the list of endemic areas. In Japan, human infections occur in Kochi, Chiba, Yamaguchi, Chugoku, and Hiroshima Prefectures. Lakeside villages of Mikkabi-cho, north end of Hamana Lake, and Shizuoka Prefecture are new endemic areas with prevalence rates of 7.5% and 10.5% among the people.

H. dispar

Human infections can occur when fish intermediate hosts, including *Mugil* spp., are eaten raw or are inadequately cooked. However, human infections were unknown before 1986 when two Korean men who returned from Saudi Arabia were found to be infected with this fluke together with *H. heterophyes*. Human infections possibly exist in Thailand.

Analytical Methods

Conventional Methods

The detection of heterophyiasis patients is usually based on recovery of eggs in the feces. Smear techniques, including the direct smear, cellophane thick smear, and Kato-Katz thick smear, are applied in field studies. Concentration techniques, including formalin–ether sedimentation and brine flotation, are performed in laboratories equipped with centrifuges. However, in areas of mixed infections with other heterophyid species, specific diagnosis is problematic. In addition, detecting eggs in the feces is often unsuccessful in light infection cases and it is necessary to use immunological or molecular detection techniques.

Heterophyes eggs can be differentiated from those of *Clonorchis*, *Opisthorchis*, and other heterophyid species. However, it is difficult and needs experience. Eggs of *H. heterophyes* and *H. nocens* are similar and characterized by their length of 23–27 and 24–29 mm, respectively, ellipsoid to ovoid shape with length/width ratio of 1.5–2.1, clean shell surface, less prominent operculum, no shoulder rims, and yellowish brown color. The eggs of *H. dispar* are similar to those of *H. heterophyes* or *H. nocens*, with the exception of smaller egg size. There can be false egg-negative results among light infection cases, for example, with less than 100 worms in an infected person. The number of eggs produced per day per *H. heterophyes* or *H. nocens* may be less than 100 in the human host, so the probability of detecting eggs in the feces from such a case is almost negligible. In such cases, serological tests, including ELISA, and genetic techniques, including polymerase chain reaction, may be helpful. The diagnosis of erratic parasitism in the heart, brain, or spinal cord is impossible unless a biopsy or necropsy is done on the affected lesion. In this case, serological diagnosis may be helpful.

Serologic, Molecular, or Numerical Taxonomy Studies

Few studies have been performed in these areas. In dogs, three immunodiagnostic methods, including counter current immunoelectrophoresis, intradermal test, and indirect fluorescent immunoassay, to detect serum antibodies against *H. heterophyes* were attempted. The intradermal test revealed 100% sensitivity and 90% specificity. Molecular studies are not yet available.

Control/Preventive Measures

To control heterophyiasis in endemic areas, detection and chemotherapy of infected people are critically important.

The drug of choice for individual treatment is praziquantel, and the efficacy of a single oral 10–20 mg kg^{-1} dose of praziquantel is satisfactory, with a more than 95% cure rate. Niclosamide can also be used. Proper disposal of human or animal feces can reduce the infection source (*Heterophyes* eggs) to the snail intermediate host. However, control of the snail host is hardly practicable or feasible. Also, prevention of wild fish, including the mullets, from infection is impractical because fish-eating birds and mammals in nature, i.e., reservoir hosts, are implicated in the life cycle. One of the most efficient preventive methods is to persuade people to cook fish before eating. However, public health workers have experienced how difficult it is to persuade people in endemic areas to change their long-held food traditions of eating raw or undercooked fish. Because of this strong food tradition, prevention of heterophyiasis at the consumer level is very difficult.

There are reports that heating fish for 10–15 min at 70 °C could inactivate metacercariae; however, the extent of research and available data are insufficient to determine the exact time–temperature treatment required. A review by WHO in 1995 of available data on processing conditions identified some parameters for heating, pickling, salting, freezing, and irradiating fish for inactivation of parasites. Similar treatment conditions are also expressed in regulatory requirements of the US (FDA) and the EU. For example, freezing and storing at −20 °C or below for 7 days, or freezing at −35 °C or below until solid and storing at −35 °C or below for 15 h, or freezing at −35 °C or below until solid and storing at −20 °C or below for 24 h is sufficient to kill parasites. The FDA's Food Code recommends these freezing conditions to retailers who provide fish intended for raw consumption. Further, heating of fish flesh to a temperature sufficient to kill bacteria is also considered adequate for parasites. Brining and pickling may reduce the parasite hazard in a fish; however, some zoonotic trematode metacercariae require up to 8 days in 30% brine to be killed, as reported by WHO in 1995. The effectiveness of smoking to a temperature of 65 °C is considered to be effective for parasitic nematodes in fish, but there is little research on this process for intestinal trematodes, including *Heterophyes*.

Research Needs

Further studies are required to draw firm conclusions regarding the phylogenetic relationships of different *Heterophyes* species. Clinicopathological significance of *Heterophyes* flukes should be investigated in detail. The diagnostic procedures other than the conventional fecal examination techniques should be investigated further. Further research is needed to determine the exact time–temperature relationships to kill metacercariae in fish for prevention of heterophyiasis.

See also: Disciplines Associated with Food Safety: Parasitology. Foodborne Diseases: Prevalence of Foodborne Diseases in South East and Central Asia. Helminth-Nematode: *Haplorchis*. Helminth-Trematode: *Clonorchis sinensis*; *Metagonimus yokogawai*; *Opisthorchis viverrini* and *Opisthorchis felineus*

Further Reading

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Metagonimus yokogawai

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Glossary

Cellophane thick smear technique A method of fecal examination to detect helminth eggs. Approximately 60 mg of feces are placed on a glass slide and a cellophane paper soaked with glycerin and malachite green is put on the feces. The feces under the cellophane are compressed to become flat and dried before microscopic examination.

Crypt hyperplasia Pathological feature in the small intestine of humans or animals characterized by marked proliferation and increase in the number of crypts.

Kato-Katz thick smear technique A method of fecal examination to detect helminth eggs. The feces are sampled by filtering through a fine mesh and then used to fill a small pre-made hole on a plastic plate, which is placed on a glass slide. The plastic plate retaining the feces (41.7 mg) on the slide is carefully removed and the feces are covered by a cellophane paper soaked with glycerin and malachite green. The feces under the cellophane are compressed to become flat and dried before microscopic examination.

Praziquantel An anthelmintic drug used for treatment of a wide spectrum of helminth infections, including almost all species of trematodes and many species of cestodes.

Villous atrophy A pathological feature in the small intestine of humans or animals characterized by shortened, blunt, edematous, inflammatory, and congestive villi.

Background

The trematode family Heterophyidae (=heterophyids) includes a number of minute intestinal trematodes (or flukes) infecting humans and animals. The genus *Metagonimus*, one of the members, was created in 1912 by Katsurada based on adult flukes recovered from dogs experimentally fed with sweetfish, *Plecoglossus altivelis*, and also from humans who consumed sweetfish raw in Taiwan. Several other *Metagonimus* species have subsequently been reported; *Metagonimus takahashii*, *Metagonimus miyatai*, *Metagonimus katsuradai*, *Metagonimus minutus*, and *Metagonimus otsurui*. Among them, *Metagonimus yokogawai*, *M. takahashii*, and *Metagonimus miyatai* are the major species occurring as human infections. *M. katsuradai* and *M. minutus* are listed among the possible human-infecting species; however, no evidence of natural infections has been demonstrated. Metagonimiasis is caused by one or more species of the genus *Metagonimus* and is prevalent in the Far East, including the Republic of Korea (Korea), China, Japan, and far eastern Russia.

Characteristics

Morphologic Characters of *Metagonimus*

Metagonimus flukes are minute (0.5–1.5 mm in length) and characteristically have a laterally deviated (submedian located) ventral sucker without a ventrogenital apparatus or a genital sucker and two testes near the posterior extremity (Figure 1). *Metagonimus* flukes are morphologically distinguished from other heterophyids, including *Heterophyes*, *Heterophyopsis*, *Pygidiopsis*, *Centrocestus*, *Stellantchasmus*, *Haplorchis*, *Procerovum*, and *Stictodora*, by various features. *Heterophyes* and *Heterophyopsis* differ from *Metagonimus* in that they have a bigger median-located ventral sucker and a prominent genital

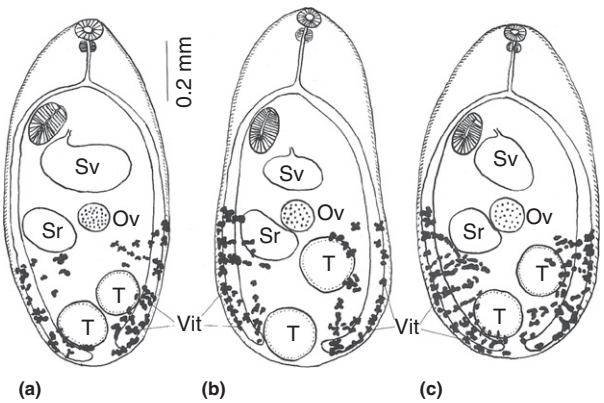

Figure 1 *Metagonimus* species infecting humans in Korea and Japan. (a) *Metagonimus yokogawai*; (b) *Metagonimus miyatai*; (c) *M. takahashii*. Ov, ovary; Sr, seminal receptable; Sv, seminal vesicle; T, testis; Vit, vitelline follicles. Reproduced with permission from Figure 9 in Chai *et al.* (1993) *Korean Journal of Parasitology* 31: 99.

 doi:10.1016/B978-0-12-378612-8.00157-8

sucker. *Pygidiopsis* differs from *Metagonimus* in that the former has a median located ventral sucker connected anterolaterally to a ventrogenital apparatus. *Centrocestus* has a median located ventral sucker and is armed with small circumoral spines around the oral sucker. *Stellantchasmus* is similar to *Metagonimus* in that it has a small, laterally deviated ventral sucker but differs in the presence of an elongated sac-like seminal vesicle connected to a muscular expulsor at the opposite side of the ventral sucker. *Haplorchis* and *Procerovum* have only one testis, whereas *Metagonimus* and other heterophyids have two testes. *Haplorchis, Procerovum,* and *Stictodora* have an armed gonotyl superimposed on a slightly submedian ventral sucker.

Important Species

Metagonimus yokogawai

Metagonimus yokogawai was originally described by Katsurada first reported in 1912 in Taiwan and Japan, and is currently the most prevalent species of *Metagonimus* in the Far East. This species has been reported to be distributed in Japan, Korea, China, Taiwan, Russia, Rumania, Israel, the Balkan states, and Spain. Some of the old literatures on *M. yokogawai* actually dealt with *M.takahashii* or *M. miyatai*. The unique morphologies of *M. yokogawai* include the presence of two testes closely adjacent to each other near the posterior end of the body, whereas in *M. takahashii* and *M. miyatai*, two testes are more or less separated. Another character of *M. yokogawai* is the distribution of vitelline follicles in lateral fields between the level of the ovary and the posterior portion of the posterior testis, whereas *M. takahashii* has abundant distribution of vitellaria beyond the posterior testis, and *M. miyatai* has no vitellaria distribution beyond the posterior testis. In *M. yokogawai*, the uterine tubule does not overlap or cross over the middle portion of the anterior testis, whereas it crosses over and overlaps the whole anterior testis in *M. takahashii* and *M. miyatai*. The eggs of *M. yokogawai* are smaller (0.0260–0.0300 mm long) than those of *M. takahashii* (0.0300–0.0360 mm long) and *M. miyatai* (0.0285–0.0315 mm long), though there are some overlaps. The adults are slightly smaller in *M. yokogawai* (0.800–1.320 mm long) compared with *M. takahashii* (0.863–1.193 mm long) and *M. miyatai* (0.998–1.300 mm long). The adults of *M. yokogawai*, *M. takahashii*, *M. miyatai*, and *M. minutus* differ from those of *M. katsuradai* and *M. otsurui* in that they have a larger ventral sucker than the oral sucker. *M. minutus* differs from *M. yokogawai*, *M. takahashii*, and *M. miyatai* in its smaller body (0.457 mm long) and smaller egg sizes (0.023 mm long).

The life cycle of *M. yokogawai* is elucidated in Figure 2. The molluscan first intermediate host is a kind of freshwater snail, *Semisulcospira coreana* or *Semisulcospira libertina*. The cercariae are ophthalmo-pleuro-lophocercous type and are shed from the snails. They swim in water and infect freshwater fish, the second intermediate host. The sweetfish, *P. altivelis*, is the most important fish host but the dace *Tribolodon hokonensis* or *Tribolodon taczanowskii* and the perch *Lateolabrax japonicus* can also serve as the second intermediate hosts. Metacercarial

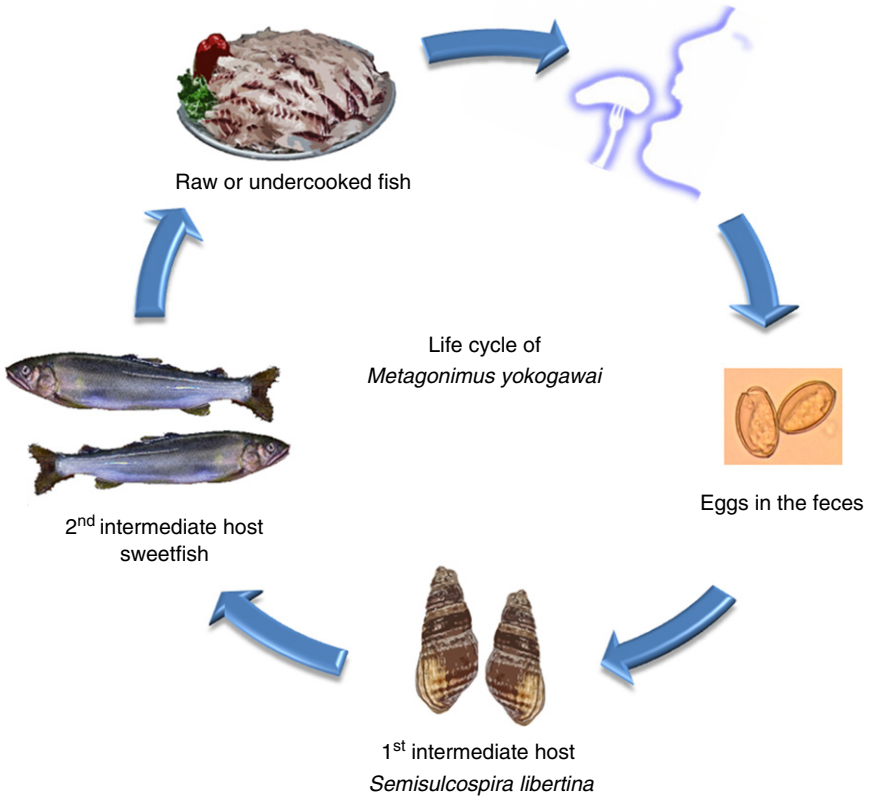

Figure 2 The life cycle of *Metagonimus yokogawai.*

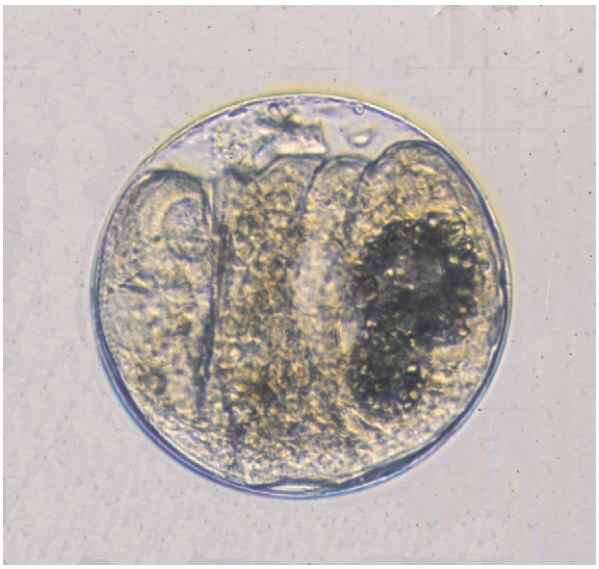

Figure 3 An infective stage larva (metacercaria) of *Metagonimus yokogawai* isolated from the muscle of a sweetfish, *Plecoglossus altivelis*. The cyst size is 0.15 mm in diameter.

cysts are round or slightly elliptical (Figure 3), 0.14–0.16 mm in diameter, and are usually found in the fish muscles. The larva inside the cyst is usually folded on itself showing the oral sucker, ventral sucker, and excretory vesicle filled with refractile granules. The longevity of metacercariae in the fish is unknown, but they may be able to survive for 2.5 years or throughout the life span of the fish hosts. Natural definitive hosts include dogs, rats, cats, and foxes, and birds including kites. However, the significance of each animal host as the source of human infection (i.e., as reservoir hosts) has not been well verified. Mice, rats, cats, and dogs can be experimentally used as the definitive hosts.

Metagonimus takahashii

Metagonimus takahashii was first reported in Japan by Takahashi in 1929 and named by Suzuki in 1930. The worms were recovered in the small intestine of mice and dogs fed with metacercariae encysted in various species of freshwater fish, excluding the sweetfish, which is a principal host for *M. yokogawai*. These worms were initially called a large egg-type *Metagonimus*, but a year later they were reported as a new species. The validity of *M. takahashii* as a distinct species was questioned for some time, and the name was compromised as *M. yokogawai* var. *takahashii*. However, recently, its taxonomic significance became strongly supported not only by the morphology of larval and adult stages, but also by different specificities to fish intermediate hosts. *Metagonimus takahashii* differs from *M. yokogawai* and *M. miyatai* in the position of the two testes, the distribution of vitelline follicles, and the egg size, and from *M. katsuradai* and *M. otsurui* in that the latter two have a smaller ventral sucker than the oral sucker. *Metagonimus takahashii* differs from *M. minutus* in its larger body and larger eggs. The Koga type of *Metagonimus*, which encysts in the dace *T. hokonensis*, is regarded a synonym of *M. takahashii*.

The snail host includes *S. libertina*, *S. coreana*, and *Koreanomelania nodifila*, and freshwater fish, including the crucian carp *Carassius carassius*, carp *Cyprinus carpio*, dace *T. taczanowskii*, and perch *L. japonicus*, harbor the metacercariae. Metacercarial cysts are slightly elliptical or disk shaped, 0.15–0.17 mm in diameter, and usually found inside the scales of the fish. The larva inside the cyst shows the oral sucker, ventral sucker, and excretory vesicle filled with refractile granules. They remain alive in the fish hosts for several months to years. Pelicans, kites, and other species of birds have been reported to be natural definitive hosts. Dogs, cats, mice, and rats are experimental definitive hosts.

Metagonimus miyatai

Metagonimus miyatai flukes were first found in 1941 in Japan but its taxonomic significance had long been questioned. Thus, it had been designated as the *M. yokogawai* Miyata type. A numerical taxonomy study report suggested that *M. miyatai* could be a subspecies of *M. takahashii*. However, its validity was acknowledged in 1997 by Saito and co-workers. The new species description was based on adult flukes from dogs and hamsters experimentally fed with metacercariae encysted in the sweetfish, dace, common fat-minnow *Morocco steindachneri*, pale chub *Zacco platypus*, and dark chub *Zacco temmincki*, and also on specimens from naturally infected humans in Japan and Korea. *Metagonimus miyatai* is morphologically different from *M. yokogawai* and *M. takahashii* in the position of the posterior testis, which is markedly separated from the anterior testis, the distribution of vitelline follicles that never cross over the posterior testis, and the intermediate size of eggs between *M. yokogawai* and *M. takahashii*. *Metagonimus miyatai* differs from *M. katsuradai* and *M. otsurui* in that the latter two have a smaller ventral sucker than the oral sucker, and from *M. minutus* in its larger body and egg size. *Metagonimus miyatai* is genetically distinct from *M. yokogawai* and *M. takahashii*.

Freshwater snails, *Semisulcospira globus*, *S. libertina*, and *Semisulcospira dolorosa*, are the first intermediate hosts. Freshwater fish, *Z. platypus* and *Z. temminckii*, are the important second intermediate hosts. Other freshwater fish, including *P. altivelis*, *T. hokonensis*, *T. taczanowskii*, *Opsariichthys bidens*, and *Morroco steindachneri*, also serve as the second intermediate hosts. Metacercarial cysts are round or slightly elliptical, 0.15–0.18 mm in diameter, and are usually found inside the scale of the fish hosts. The larva inside the cyst shows the oral sucker, ventral sucker, and excretory vesicle filled with refractile granules. The longevity of metacercariae in the fish is unknown, but they may be able to survive for several months to years. Natural definitive hosts include the dog, red fox, raccoon dog, and black-eared kite. Mice, rats, hamsters, and dogs are experimental definitive hosts.

Clinical Manifestations

The most frequent clinical manifestations in metagonimiasis patients are mild to moderate degrees of abdominal pain, diarrhea, lethargy, anorexia, and weight loss. However, the severity of symptoms may vary and depend on host-side

factors. One factor is the intensity of infection in each patient, i.e., the number of infected worms. Heavier infection cases tend to suffer from more severe illnesses. Another factor is the immune status of the patient, i.e., immunocompetent or immunodeficient. A third factor is the history of previous exposures to infection that can confer acquired immunity of individual patients. A new visitor to an endemic area may suffer from severe illnesses after a primary infection, whereas residents in endemic areas generally complain of milder symptoms. In immunocompromised patients, severe clinical manifestations, including erratic parasitism in the heart, brain, and spinal cord, may occur as in other heterophyid species infections. In one patient, multiple intracerebral hemorrhages and diabetes mellitus occurred, though it is unclear whether the patient was immunocompromised or not.

Virulence Factors and Pathogenesis

The virulence of *Metagonimus* flukes might differ for different species of parasites. However, there have not been sufficient studies to support this hypothesis. Two principal factors related to virulence are mechanical and chemical irritations by the flukes. Mechanical irritation is chiefly caused by the movement of worms which can give harmful effects to the intestinal mucosa, in particular, villous and crypt layers of the small intestine. Chemical substances produced by the flukes, which include excretory-secretory proteins (ESPs), can play the role of not only antigens but also toxins to the host. Immune responses of the host against the flukes or their ESPs may be too strong (hypersensitivity) that the host immunity can damage the host itself. The affected mucosa may undergo hypersensitive and allergic reactions, including severe catarrhal inflammation and loss of villi. Pathogenicity is also related to other host–parasite relationships, including the intensity of infection; heavier infections generally lead to severer illnesses.

In animal hosts, adult flukes of *M. yokogawai* parasitize the middle part of the small intestine, and invade the crypt of Lieberkühn by day 2–3 postinfection, and localize between villi or in the intestinal lumen during later stages. The worms can cause mechanical and chemical irritation to the mucosa and the affected mucosa reveals inflammatory and allergic reactions. Villous atrophy and crypt hyperplasia are the two major histopathologic features, accompanied by inflammatory cell infiltrations. The infected villi show blunting and fusion, edema at their tips, congestion and inflammation, and decreased villus/crypt height ratio. In immunocompetent hosts the location of worms is generally confined to the intestinal mucosa. However, in immunosuppressed hosts the worms may invade a deeper mucosal layer and even the submucosa. Immunosuppression also enhances survival of worms and prolongs their life spans. Poor absorption of intestinal secretions from the secretory crypt cells is thought to be an important mechanism of diarrhea. In *M. miyatai* infection, the intestinal histopathology of experimentally infected mice is similar to that seen in *M. yokogawai* infection, although the degree of mucosal damage is less severe. In other species of *Metagonimus*, pathogenicity and intestinal histopathology have not been studied.

It has long been a question whether *Metagonimus* worms parasitize only the intestine of the hosts. In other heterophyid flukes, *Stellantchamus falcatus*, *Haplorchis* spp., and *Procerovum* spp. can invade the submucosa and muscle layer of the intestine. This can cause erratic parasitism in man, which is often fatal, based on reports of Africa and co-workers in 1940. The heart valve, brain, and spinal cord are the three most frequently affected sites, where eggs and adult flukes, originating from the intestinal mucosa, embolize in the blood vessels. Such erratic parasitism may occur in immunocompromised patients due to lowered capacity of immune function against foreign stimuli. With regard to *Metagonimus* spp., no direct evidence of erratic parasitism in humans has been reported. However, in a patient infected with *M. yokogawai*, intracerebral hemorrhage was noticed and this could be an acute complication of metagonimiasis. In experimental mice, *M. yokogawai* adults were found to have invaded the submucosa of the small intestine when the mice were immunosuppressed by prednisolone injection.

Epidemiology

Metagonimus yokogawai

The principal mode of human infection with *M. yokogawai* is consuming raw flesh of the fish intermediate hosts, notably the sweetfish and the dace in the Far Eastern countries. Endemic areas are widely scattered along the riverside where people traditionally eat raw fish dishes. In Korea, approximately 240 000 cases infected with *M. yokogawai* are estimated along the rivers and streams running through the eastern and southern coastal areas. The prevalence of the infection in villagers in these areas can sometimes reache 60–70%. In China, human infections have been found in Guangdong, Anhui, Hubei, and Zhejiang Provinces. Taiwan is also included among the endemic areas. In Japan, a lakeside area around the Hamamatsu Lake, Shizuoka Prefecture, showed a 13.2% egg-positive rate among 4524 lakeside people examined. In Russia, the prevalence among ethnic minority groups in the Amur and Ussuri valleys of Khabarovsk Territory was between 20% and 70%. In the north of Sakhalin Island, the infection rate among Russians was 1.5% and that among ethnic minorities was 1.0%. Sporadic cases were also reported from the Amur District and the Primorye Territory.

Metagonimus takahashii

After its discovery in Japan, the existence of *M. takahashii* was also confirmed in Korea through recovery of adult flukes from experimental rabbits fed with the metacercariae in fish. The presence of human infections was noticed in the riverside people of the Hongcheon River, Gangwon-do, in 1988, and the upper reaches of the Namhan River, Chungcheongbuk-do, in 1993. The inhabitants were mixed-infected with *M. takahashii* and *M. miyatai*, with an egg-positive rate of 9.7% for both species. *Metagonimus takahashii* is now presumed to be prevalent in many small rivers and streams in inland areas of Korea. The major source of human infection is the crucian carp, *C. carassius*.

Metagonimus miyatai

Human infections are known in Korea and Japan. In Korea, it was reported as an undetermined species of *Metagonimus* by detecting eggs that are slightly larger in size than those of *M. yokogawai*. Later, adult worms were recovered from the riverside people living along the Namhan river in Umsong-gun (mixed-infected with *M. takahashii*) and Yongwol-gun (infected only with *M. miyatai*). In Yongwol-gun, the egg-positive rate among 77 riverside people was 48.1%. Adult worms were also recovered from inhabitants along the Hantan River, Chorwon-gun, Gangweon-do and Talchon River, Chungwon-gun, and Chungcheongbu-do. In Japan, the Hiroi River basin, Nagano Prefecture, and many small rivers of Shizuoka Prefecture are endemic areas of *M. miyatai* infection.

Analytical Methods

Conventional Methods

The detection of metagonimiasis patients is usually based on recovery of eggs in the feces. Smear techniques, including the direct smear, cellophane thick smear, and Kato-Katz thick smear, are applied in field studies. Concentration techniques, including formalin–ether sedimentation and brine floatation, are performed in laboratories equipped with centrifuges. However, in areas of mixed infections with different species of *Metagonimus*, specific diagnosis is problematic. In addition, detection of eggs in the feces is often unsuccessful in light infection cases and it is necessary to use immunological or molecular detection techniques.

Metagonimus eggs can be differentiated from those of *Clonorchis, Opisthorchis*, and other heterophyid species. However, it is difficult and needs experience. Eggs of *M. yokogawai* are characterized by their length of 26.9–31.6 mm, elliptical shape with length/width ratio of 1.5–2.1, clean shell surface, less prominent operculum, no shoulder rims, and dark yellow or brown in color. The eggs of *M. takahashii* and *M. miyatai* have similar morphology to those of *M. yokogawai*, with the exception of their larger egg sizes. There could be false egg-negative cases among the light infection cases, for example, with less than 100 worms in an infected person. The number of eggs produced per day per *M. yokogawai* worm is 14–64 eggs in the human host, so the probability of detecting eggs in the feces from such a case is almost negligible. In such cases, serological tests, including enzymze linked immuno sorbent assay (ELISA), are helpful.

Molecular Methods

Adult flukes of *M. yokogawai, M. miyatai,* and *M. takahashii* can be genetically differentiated by the polymerase chain reaction-based restriction fragment length polymorphism (PCR-RFLP) patterns. The target genes are internal transcribed spacer 1 (ITS1) site of ribosomal RNA and mitochondrial cytochrome *c* oxidase I (mCOI). PCR analyses based on random amplification of polymorphic DNA (RAPD) technique also show distinguishable banding patterns between *M. yokogawai* and *M. miyatai*. Analyses of the chromosome number and karyotype are also helpful to discriminate genetic differences between *M. yokogawai* and *M. miyatai*. The sequence comparison of 28S ribosomal DNA and mitochondrial cytochrome *c* oxidase subunit I can support *M. miyatai* as a distinct species. Application of genetic techniques to discriminate eggs of *Metagonimus* species in the human feces has not yet been successful.

Control/Preventive Measures

To control metagonimiasis, the detection and chemotherapy of infected people are critically important. The drug of choice for individual treatment of *Metagonimus* infection is praziquantel, and the efficacy of a single oral dose of 10–20 mg kg^{-1} praziquantel is satisfactory, with up to 95–100% cure rate. Proper disposal of human or animal feces can reduce the infection source (*Metagonimus* eggs) to the snail intermediate host. Control of the snail host is hardly possible or feasible. Also, control of the fish host, including the sweetfish, is impractical. One of the most efficient preventive methods is to persuade people to cook fish before eating. However, public health workers have experienced how difficult it is to persuade people in endemic areas to change their long-held food traditions of eating raw or undercooked fish. Because of this strong food tradition, prevention of metagonimiasis at the consumer level is highly difficult.

There are reports that heating fish, for example, sweetfish, for 10–15 min at 70 °C could inactivate metacercariae; however, the extent of available data is insufficient to determine the exact time–temperature treatment required. A review by World Health Organization (WHO) in 1995 on processing conditions identified some parameters for heating, pickling, salting, freezing, and irradiating fish for inactivation of parasites. Similar treatment conditions are also expressed in the regulatory requirements of the US (FDA) and the EU. For example, freezing and storing at −20°C or below for 7 days, or freezing at −35 °C or below until solid and storing at −35 °C or below for 15 h, or freezing at −35 °C or below until solid and storing at −20 °C or below for 24 h is sufficient to kill parasites. FDA's Food Code recommends these freezing conditions to retailers who provide fish intended for raw consumption. Further, heating of fish flesh to a temperature sufficient to kill bacteria is considered also adequate for parasites. Brining and pickling may reduce the parasite hazard in a fish; however, some zoonotic trematode metacercariae require up to 8 days in 30% brine to be killed as reported by WHO in 1995. The effectiveness of smoking to a temperature of 65 °C is considered to be effective for parasitic nematodes in fish, but there is little research on this process for intestinal trematodes, including *Metagonimus*. The infectivity of metacercariae can be controlled by gamma-irradiation of the fish at 200 Gy but the feasibility of the irradiation technique in the field conditions is low.

Research Needs

Further studies are required to draw firm conclusions regarding the phylogenetic relationships of different *Metagonimus* species.

Clinical significance of *Metagonimus* flukes and diagnostic procedures other than the conventional fecal examination techniques should be further investigated. Further research is needed to determine the exact time–temperature relationships to kill metacercariae in sweetfish for prevention of metagonimiasis.

See also: Disciplines Associated with Food Safety: Parasitology. Foodborne Diseases: Prevalence of Foodborne Diseases in South East and Central Asia. Helminth-Nematode: *Haplorchis*. Helminth-Trematode: *Clonorchis sinensis*. *Heterophyes heterophyes*; *Opisthorchis viverrini* and *Opisthorchis felineus*

Further Reading

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Opisthorchis viverrini and *Opisthorchis felineus*

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Glossary

Flukes Parasitic flat worms.

Geographical Distribution

Current estimated total number of fishborne liver fluke infections is 45 million people in Asia and Europe, of which 9 million are infected with *Opisthorchis viverrini*, 1.2 million with *Opisthorchis felineus* and 35 million with *Clonorchis sinensis*. As many as 680 million people worldwide are at risk of infection. Infection hot spots for *O. viverrini* are northeast Thailand, Lao PDR, Cambodia and to a lesser extent, south Vietnam (Figure 1). As many as 67 million people in Southeast Asia are at risk of infection. *Clonorchis sinensis* is found in North Vietnam, China, Taiwan, and Korea, and previously in Japan. To date, *O. felineus* has been reported to occur in all European countries except Great Britain, Ireland, Finland, and Scandinavian. Among the republics of the former USSR, it is present in the Ukraine, Belarus, Baltic countries, Moldova, and Kazakhstan (Figure 2).

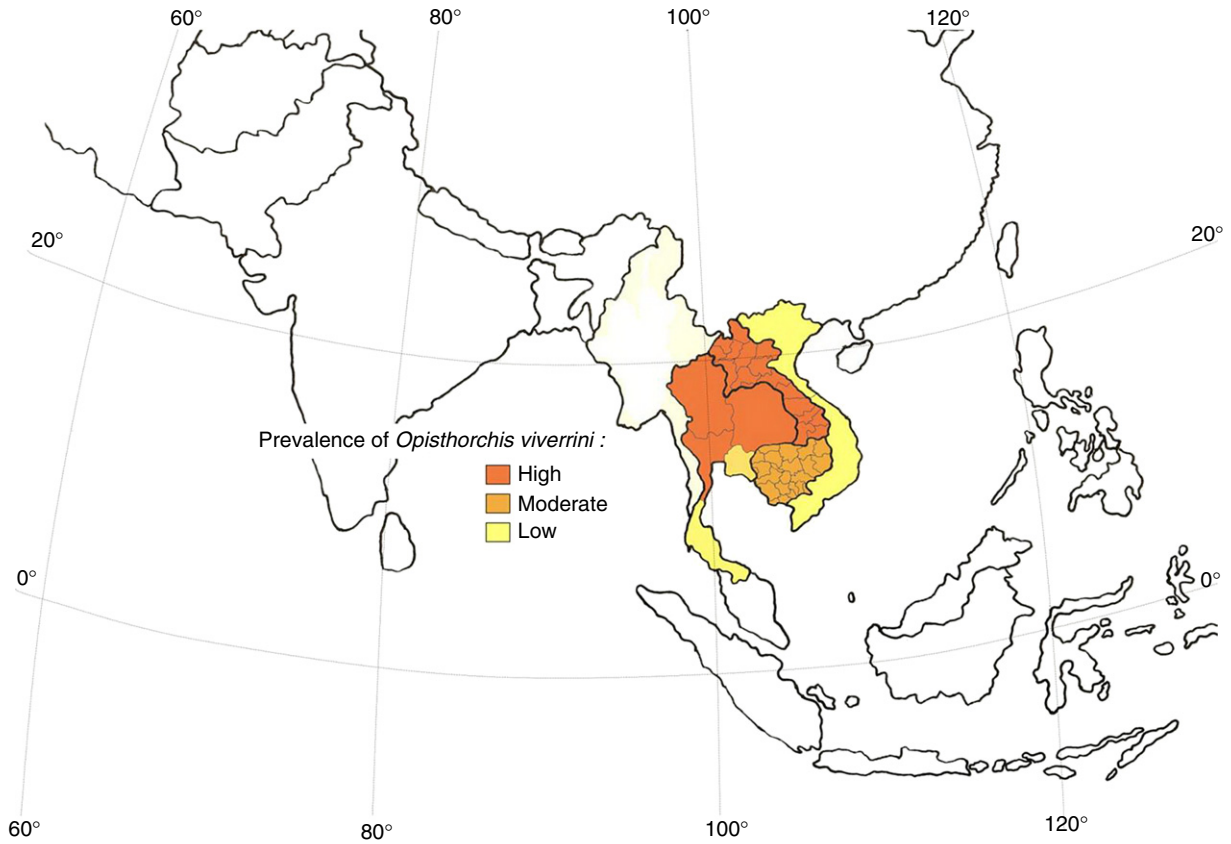

Figure 1 Geographical distribution of *O. viverrini*.

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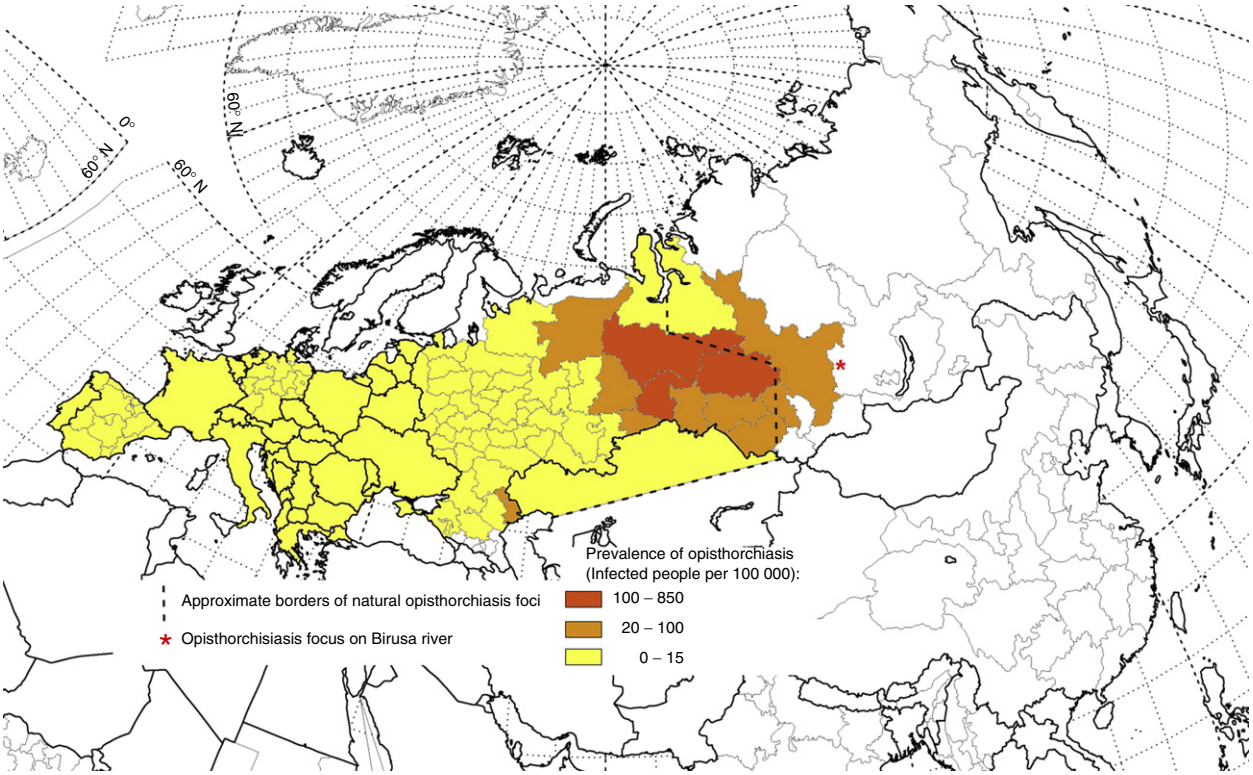

Figure 2 Geographical distribution of *O. felineus*.

It is believed that the presence of suitable snail intermediate hosts dictates the geographical distributions of these liver flukes. However, because of human population migration and extensive traveling plus growing importation of freshwater aquaculture products from endemic countries, it is now increasingly common to detect infected people in nonendemic areas. For example, cases of opisthorchiasis in nonendemic areas, such as USA and Europe, have been reported in immigrants from Asia. The cause of infection was due to the consumption of imported raw or undercooked freshwater fish containing metacercariae.

Taxonomy and Morphology

It is assumed that *O. felineus* was originally discovered and described in 1831 as *Distomum conus*. However, this and a subsequent description in 1836 as *Distomum lanceolatum felis cati* were not valid. In 1884, *O. felineus* isolated from cats and described as a valid species, *Distomum felineus* adhering to rules of zoological nomenclature and in 1895 *O. felineus* was included into the new genus *Opisthorchis*. The first discovery and description of *O. viverrini* was in 1915 from humans in Chiang Mai Thailand and subsequently from a fish-eating cat.

The three liver flukes are morphologically very similar. *Clonorchis sinensis* was isolated into a separate monotypic genus where the main distinctions from the *Opisthorchis* species are the branched shape of its testes (vs. lobed testes in *Opisthorchis*) and the position of testes and vitelline glands. There is, however, an alternate point of view that argues that *O. viverrini* and *C. sinensis* are more closely related to each other than to *O. felineus*. This is based on the number and arrangement of protonephridia (excretory formulae) of cercariae and metacercariae, namely, for *O. viverrini* and *C. sinensis* $2[(3+3)+(3+3+3)]=30$, versus $2[(5+5)+(5+5+5)]=50$ for *O. felineus*. It is generally believed that this character is constant for the representatives of a single genus in trematodes. Another argument is the number of the penetration glands in cercariae: 14 in *O. viverrini* and *C. sinensis* versus 20 in *O. felineus*. In addition, the *O. viverrini*/*C. sinensis* relationship versus *O. felineus* has been supported by phylogenetic molecular genetic analyses.

The liver flukes are hermaphroditic, dorso-ventrally flattened, and leaf like in shape. Adult worms (maritae) of *O. felineus* have a lancet-shaped body narrowed at the front end with a rounded tail. The size of adult flukes measures 5.5–10 × 0.77–1.65 mm with *C. sinensis* largest (10–25 × 3–5 mm) and *O. felineus* smallest (7–12 × 2–3 mm). The body size varies depending on the definitive host species. The body is armed with two muscular suckers, the oral sucker situated anteriorly and the ventral sucker at the mid-body. All three species of liver fluke eggs have distinct opercular shoulders surrounding the operculum at one end and a small knob or comma shape appendage at the abopercular end.

The ovary is smooth-edged or, more rare, weakly lobed, and occurs in front of the testes in the middle of the body. The uterus spans from the ovary to the front edge of the ventral sucker but does not overlap it. The vitelline glands form bunches located in the middle body third behind the ventral

sucker. The seminal receptacle is located somewhat behind the ovary. The testes have four or five lobes and are located diagonally one after another. The genital pores appear at the anterior side of the ventral sucker. The egg shell surface is rough and irregular or seen as musk-melon patterns by electron microscopy. Eggs of the three species of liver flukes share similar morphologies and are difficult to differentiate.

Recent systematic analyses of *O. viverrini* in Southeast Asia has shown that it is in fact a species complex in which adult worms show identical and indistinguishable morphologies but they are genetically very different, hence cryptic species. Whether this is the case for *O. felineus* and *C. sinensis* remains to be determined.

Life Cycle

Adult worms inhabit bile ducts, gall bladder, and to a lesser extent the pancreatic duct. Once sexually mature adult worms cross-fertilize and produce eggs that are discharged with bile fluid into the intestine and the feces and passed into the environment (Figure 3). The number of eggs produced per worm depends on the worm burden. In humans each worm can on average produce 50 egg g^{-1} feces. A common characteristic of all opisthorchiids is high specificity toward the first intermediate host, with usually one snail species or a few closely related species involved. Different species of *Bithynia* snails serve as the first intermediate hosts of *O. viverrini*, namely *Bithynia goniomphalos*, *Bithynia siamensis siamensis*, and

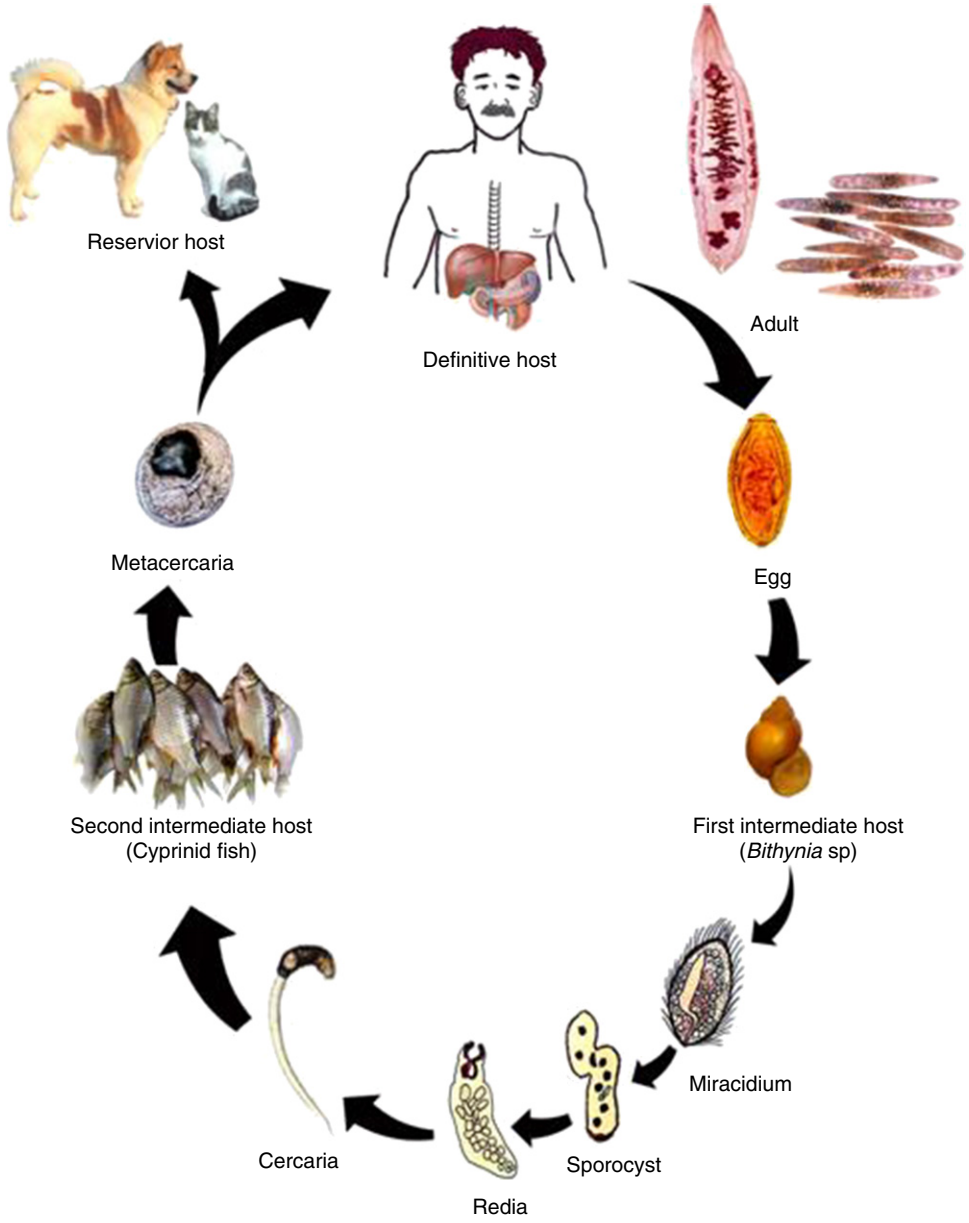

Figure 3 Life cycle of the liver flukes.

Bithynia funiculate. Until the middle of the twentieth century, *Bithynia leachi* was considered the first intermediate host of *O. felineus*. Subsequently, it was divided into four species *B. leachi*, *Bithynia troscheli*, *Bithynia inflata*, and *Bithynia sibirica*. The first three species are susceptible to *O. felineus* as well as *Bithynia tentaculata* and *Boreoelona linholmiana*. When eggs reach a body of freshwater, i.e., small ponds, streams, and rivers, or flooded rice fields and are ingested by specific *Bithynia* spp., miracidium hatch and develop into sporocysts and rediae. The rediae gave rise to cercariae that are released daily approximately 2 months after infection.

Once exposed to appropriate fish intermediate hosts, the free-swimming cercariae shed their tails, penetrate into the tissues or the skin of freshwater fish. Metacercariae penetrate subcutaneous adipose and muscle tissues to a depth of no more than 1–2 mm. Several unusual sites for clustering of metacercaria are known, such as pectoral fins, gills, and gut walls. Development of cercaria into metacercaria takes from 3 weeks to 2 months depending on the fish species and water temperature.

Humans become infected by ingesting the metacercariae in uncooked or partially cooked fish. The ingested metacercariae excyst in the duodenum, enter the common bile duct and migrate to the distal bile duct. Development of juveniles requires approximately 2 months to become the sexually mature stage. In the case of the closely related liver fluke, *C. sinensis*, it has been reported that they can survive up to 26 years in a human host. Based on the pattern of age-intensity profiles, it is anticipated that *O. viverrini* may survive in humans for approximately 10–20 years, which may also be the case for *O. felineus*.

The prevalence of *O. viverrini* infection in *Bithynia* snails is typically less than 1%. Snail population density exhibits strong seasonality and is dependent on rainfall, being highly abundant in the rainy season and distributed extensively in shallow water and rice fields; but disappearing rapidly in the dry season.

Prevalence of infection in fish intermediate hosts is much higher. As high as 90–95% prevalence of *O. viverrini* metacercariae has been recorded in several species of cyprinid fish. The most common species are in the genera *Puntius*, *Cyclocheilichthys*, and *Hampala*. Host finding mechanisms of cercariae is a complex process. Free-swimming cercariae are very efficient in locating the appropriate species of fish in a large volume of water. The intensity of liver fluke infections in fish varies by season, species, individuals, and types of water bodies. Most metacercariae are distributed throughout the body of fish and some are found in the head of the fish. For *O. viverrini*, metacercarial burdens peak in winter (October–February) and become low in the rainy season and summer thus transmission of the parasite from fish to humans is probably seasonal. This seasonal pattern may also occur in *O. felineius*.

In Thailand and Lao PDR, 18 species of fish from seven genera have been reported to serve as intermediate hosts of *O. viverrini* (**Table 1(a)**). Of these, *Cyclocheilichthys apogon*, *Cyclocheilichthys armatus*, *Cyclocheilichthys repasson*, *Puntius leiacanthus*, and *Hampala dispar* are considered to be the most important. The number of metacercariae reported in fish varies from between one to hundreds. The frequency distribution of *O. viverrini* metacercariae in fish is highly dispersed with most fish having few metacercariae whereas a few fish harbor a heavy metacercarial load. Recent findings of trematode metacercariae other than *O. viverrini* in cyprinid fish in Thailand have indicated that the occurrence of mixed species of trematodes in a given fish species is common. **Table 1(b)** lists 23 cyprinid fish that can act as second intermediate hosts for *O. felineus*. Ide is the host most susceptible to *O. felineus* and the most important source of infection, followed by common bream, whereas carp has low infection.

Dogs (*Canis familiaris*) and cats (*Felis catus*) are reservoir hosts in the life cycle of *O. viverrini* but the prevalence of infection in these animals is relatively low. The animal reservoir hosts of *O. felineus* are domestic dog and cat and wild fish-eating animals such as wolf (*Canis lupus*), red fox (*Vulpes vulpes*), corsac fox (*Vulpes corsac*), Arctic fox (*Alopex lagopus*), raccoon dog (*Nyctereutes procyonoides*), brown bear (*Ursus arctos*), raccoon (*Procyon lotor*), ermine (*Mustela erminea*), weasel (*Mustela nivalis*), Siberian weasel (*Mustela sibirica*),

Table 1 Fish intermediate hosts of the liver flukes

(a) Opisthorchis viverrini

Latin name	*Local name*
Cyclocheilichthys apogon	Sai Tan Ta Daeng
Labiobarbus leptocheilus	Sa
Puntius brevis	Taphian Jut
Barbonymus gonionotus	Taphian Khao
Henicorhynchus siamensis	Soi Khao
Henicorhynchus ornatipinnis	Soi Peek Daeng
Hampala dispar	Kra Sup Jut
Hampala macrolepidota	Kra Sup Khit
Osteochilus hasselti	Soi Nokkhao

(b) Opisthorchis felineus

Latin name	*Common name*
Leuciscus idus	Ide
L. cephalus	Chub
L. leuciscus	Dace
Rutilus rutilus	Roach
Blicca bjorkna	Silver bream
Carassius carassius	Crucian carp
C. auratus gibello	Silver crucian carp
Abramis brama	Common bream
A. ballerus	Blue bream
A. sapa	White-eye bream
Chondrostoma nasus	Nase
Scardinius erythrophtalamus	Common rudd
Pelecus cultratus	Ziege
Cyprinus carpio	Common or European carp
Tinca tinca	Tench
Gobio gobio	Gudgeon
Alburnus alburnus	Bleak
Phoxinus phoxinus	Minnow
Ph. chekanowskii	Chekanovsky minnow
Aspius aspius	Asp
Barbus borysthenicus	Barbel
Leucaspius delineatus	Sunbleak
Cobitis taenia	Spined loach

steppe polecat (*Mustela eversmani*), European mink (*Mustela lutriola*), sable (*Martes zibellina*), wolverine (*Gulo gulo*), Eurasian badger (*Meles meles*), and otter (*Lutra lutra*). Chipmunks (*Eutamias sibiricus*) have been found infected as well as beaver (*Castor fiber*), Eurasian water shrew (*Neomys fodiens*), Caspian seal (*Phoca caspica*), wild boar (*Sus scrofa*), domestic pig (*Sus scrofa domestica*), and muskrat (*Ondatra zibellina*). Although fecal contamination from infected animals undoubtedly contributes to parasite transmission to snails in the environment, its actual importance is unclear. Where fecal contamination from humans is reduced or eliminated by mass treatment and/or improved sanitation, infection among domestic cat and dog reservoir hosts could maintain the source of parasite and play a critical role in transmission and viability of the life cycle.

Pathogenesis, Pathology, and Morbidity

Pathological changes in liver fluke infection are confined mainly to bile ducts, liver, and gall bladder in both humans and in animal models. The magnitude of the pathology depends on the intensity, duration, and susceptibility of hosts. For light infections, the liver appears grossly normal, whereas, in heavy infections localized dilation of the thickened peripheral bile ducts can be seen on the surface beneath the fibrotic capsule of the liver. Major microscopic changes are confined to the large- and medium-sized bile duct where the flukes are found. The gross and microscopic characteristics of human opisthorchiasis are well established within 7–15 years after *O. viverrini* infection. Clinical manifestation is infrequent and often nonspecific and related to abdominal discomfort as a result of indigestion. Community based morbidity rates are approximately 5% among infected people. However, preclinical pathology can be detected by ultrasonography which has revealed various hepatobiliary abnormalities as a result of chronic opisthorchiasis. This chronic infection condition leads to bile duct inflammation and may eventually induce cholangiocarcinoma (CCA). Although pathogenesis of opisthorchiasis is complex, recent studies have revealed that the pathogenenic features in opisthorchiasis are largely a consequence of immunomodulation during the acute and chronic phase of infection. For pathogenesis of CCA, chronic inflammation plays a central role in carcinogenesis through oxidative and nitrative DNA damage which initiates tumorigenesis. While CCA is fatal and curative treatment is not forthcoming, opisthorchiasis is effectively cured by treatment with praziquantel. Praziquantel treatment eliminates not only *O. viverrini* but also reduces associated morbidities and risk of CCA.

Liver Flukes and Cancer

CCA accounts for 15% of liver cancers worldwide. In the absence of flukes or endemic infections, development of CCA is quite rare although incidence is increasing in many high-income countries for unknown reasons. The northeast of Thailand has the highest CCA incidence in the world. At present, *O. viverrini* and *C. sinensis* have been classified as type 1 carcinogens. A recent study suggested that host genetic background may play a role in the development of CCA and may help explain the high incidence of CCA in northeast Thailand. Additionally, past exposure to infection in terms of elevated *O. viverrini* antibody levels may be a risk factor for CCA development. Liver cirrhosis, chronic infection with the Hepatitis C virus, heavy alcohol consumption, high fluke egg density in stools, obesity, consumption of nitrate-containing foods, history of familial cancer, and gallstones may also be risk factors.

Epidemiology

Opisthorchiasis due to *O. felineus* in humans was first discovered in Tomsk, Siberia in 1881. A subsequent study of 1535 corpses revealed a high prevalence of opisthorchiasis in Tomsk of 6.91% (106 cases). This was the first time showing that opisthorchiasis presented a major health problem. As limited detailed records and studies are available it is not clear whether *O. felineus* represents a significant health problem in European countries. It apparently does not present an evident health problem in all of these countries except Kazakhstan, where it used to be a significant problem in the northern regions of the country, especially in Pavlodar. Unfortunately, no recent statistics on opisthorchiasis in Kazakhstan are available. It is generally believed that the prevalence of *O. felineus* both in humans and in animals increases from west to east. Opisthorchiasis represents a significant medical problem in the Ob-Irtysh basin, where the prevalence of opisthorchiasis in humans increases dramatically (see **Figure 2**).

Dashed lines in **Figure 3** represent the approximate borders of *O. felineus* geographical range, which is believed to be limited by its first intermediate host. No natural opisthorchiasis foci are believed to be present east of the Ob River, except for a small focus on the Birusa River in Irkutsk oblast (represented by the asterisk in **Figure 2**). Note that the foci of human opisthorchiasis extend further eastwards, with a high prevalence in Krasnoyarsk krai, which is due to high human migration rate and to the marketing of infected fish from Ob River.

It is difficult to make accurate estimates of the number of people infected with *O. felineus*, because as with *O. viverrini* the early stages of infection are asymptomatic in the majority of cases, and the clinical symptomatology in chronic cases is nonspecific and is often diagnosed as a consequence of other diseases. Hence, actual levels of opisthorchiasis prevalence would be considerably higher than currently recorded. It is estimated that the number of people infected with *O. felineus* is 1.5 million.

This is not the case in Southeast Asia where extensive study has shown that *O. viverrini* posses a significant health problem. For example, the pattern of infection in endemic communities is that prevalence and intensity increases with age of the population, thus middle aged people (>20 years) tend to have high prevalence and intensity of infection. Consequently, associated hepatobiliary morbidity also frequently occurs in this age group. The frequency distribution of worm burden in human hosts is not uniform, since the majority of parasites are harbored by a small group of people. In addition, there is evidence of predisposition to heavy infection in which heavily infected individuals have a tendency to return to their

pretreatment infection levels after treatment. These epidemiological characteristics have important implication for control programs.

Role of Aquaculture

Because of the growing fishery export trade from endemic areas of foodborne trematodes to nonendemic areas, both the World Health Organization and Food and Agriculture Organization are aware of the importance of an integrated, multidisciplinary approach to food safety and quality. This should encompass the entire food chain. In the case of aquaculture, the food chain approach required is a responsibility that the fish supplied are safe for human consumption by all involved with the production, processing, trade, and consumption of fish. Development and implementation of good aquaculture practice (GAP), good hygienic practice (GHP), and hazard analysis critical control point (HACCP) are recommended for fish farming in order to reduce snail contamination in fish ponds and hence metacercarial contamination in fish. These measures are possible in intensive, industrial aquaculture practices but for small-scale subsistence fish farming a balance between the operational costs and potential profits should be considered.

A recent investigation in fish farms in Lao PDR supported by the Food and Agriculture Organization demonstrated that some carp species commonly cultured in fish ponds contained *O. viverrini* metacercariae. This preliminary result suggested that apart from captured fish, culture fish can provide an additional source of infection to consumers and thus urgently needs attention.

Source of Infection for Human and Animals

Within the life cycle of *O. viverrini* and *O. felineus* infective metacercariae are found only in specific fish intermediate hosts. Mostly cyprinid species serve as the source of infection to humans and animal hosts. Food preparation methods of these fish are the main issues regarding viability of the metacercariae and hence risk of human infection. In Southeast Asia, particularly in Thailand and Lao PDR, the undercooked fish dishes as source of the liver fluke infection can be grouped into three categories (Figures 4(a)–(c)). Fresh or raw fish dishes without heating is called 'koi pla' and pose a high risk of infection. Of moderate risk is a short fermented dish (1–2 day) known as 'pla som' in which the metacercariae are viable. The last dish is fermented fish known as 'pla ra', which normally requires long-term fermentation, but short term and variable ingredients may provide favorable environments for metacercarial survival. Pla ra is a common ingredient for cooking in Southeast Asia for example in papaya salad ('som tum'). In Lao PDR, the fermented fish is known as 'pla dak.' In other countries such as Cambodia raw fish are prepared as pla hoc that is similar to pla som and these may serve as sources of

Figure 4 (a) Cyprinid fish in Thailand, (b) pla ra (fermented fish), (c) pla som (marinated fish), and (d) koi pla (raw fish preparation).

Figure 5 (a) Salted cyprinid fish in Russia and (b) stroganina (frozen sliced raw fish).

infection. The usual sources of *O. felineus* infection in Russia are dried or salted fish (Figure 5(a)). Other dishes include sliced raw fish ('stroganina,' Figure 5(b)), which is popular among native Siberians, and fish pickled in vinegar.

Diagnosis and Detection

The most widely used methods of opisthorchiasis diagnostics are traditionally based on the detection of *O. viverrini* and *O. felineus* eggs in feces and duodenal contents under the microscope. The eggs of the parasite become detectable during the fourth week after infection. Various fecal examination techniques are employed for diagnoses of liver fluke infection such as the formalin–ether concentration technique, the Kato thick smear and Stoll's dilution egg count technique. Other standard techniques such as direct smear or sedimentation techniques are also used in certain situations according to available facilities. Single examination by these techniques seems to have a high false-negative rate, especially with light infections or those with a history of recent treatment. Repeated stool examinations are necessary to improve the sensitivity of examination. Although repeated examination, yields increased sensitivity, repeated or multiple stool sample collections is difficult in practice, particularly in rural communities. Another complicating factor in accurate detection and diagnosis is that several species of trematodes belonging to the Opisthorchiidae, Heterohyidae, and Lecithodendriidae have eggs and life cycle stages that are similar morphologically.

Since the main factor in liver pathology is changes in duodenocholedochopancreatic organs, diagnosis of opisthorchiasis utilizes clinical and instrumental tools used to diagnose gastroenterological diseases. The most widespread methods are X-ray diagnostics (cholecystocholangiography), ultrasonic examination, and computer tomography. Immunological methods for opisthorchiasis diagnostics are currently available; however, they are nonspecific and lack sensitivity, and they are used as an addition to parasitological methods. Detection of *O. felineus* in fish and snails is also performed by microscopy.

More accurate, yet still infrequently used in clinical diagnostics, are modern molecular diagnostic methods such as polymerase chain reaction (PCR) or specific DNA probe hybridization. They have advantages over conventional methods in higher sensitivity and specificity and they can detect all life cycle stages. There are disadvantages; however, including high costs and the requirement of specialist expertise and facilities. Nonetheless, these diagnostic tools are potentially useful not only for monitoring parasite transmission but also in food inspection and safety.

Prevention, Treatment, and Control

Prevention

The campaign for reduction of raw fish consumption in Thailand to date has proven ineffective and difficult to implement in practice. The difficulty of detecting infected cases that invariably have light infection (egg g^{-1} feces < 1000) and the problem of reinfection are two of the many difficulties facing health workers. However, the millions of people infected and at risk of infection, in addition to the direct and indirect economic losses resulting from liver fluke infection, require the devotion of time and resources to development and implementation of prevention and control measures. Ideally, improvements in hygiene and sanitation practices prevent transmission of eggs from feces, which disrupts the cycle of fluke maturation and transmission. To be successful and long-lasting, prevention programs require broad community acceptance and participation.

The easiest and most promising way of preventing opisthorchiasis is decontaminating fish, as metacercariae are the only source of infection for humans. A multitude of studies have examined the viability of metacercariae under various environmental conditions and it is known that they remain viable when frozen at $-20\,^{\circ}C$ for up to 1 month. Temperatures of $>50\,^{\circ}C$ kill metacercariae in a few minutes, however, to ensure that metaccercariae are killed in frozen pieces of fish they must be cooked for a long time. It is generally recommended to cook fish in boiled water for more than 30 min.

Very popular fish dishes of dried and salted fish are not safe for consumption. Salted fish is generally considered ready in a day or 2. It has been proven that metacercariae remain viable under high salt concentrations for up to 2 weeks. The popular dish in Russia and eastern Europe of slightly salted fish is not safe at all. The same is true for dried fish where greater

than 12 days of drying are required to kill 99% of metacercariae. Given that the weight of fish and the temperature of drying are not constant even at fish plants or after 12-day period, dried fish remain dangerous for consumption.

Hot smoking is a reliable method of fish decontamination. A period of 2–2.5 h at 70–80 °C kills all metacercariae. Alternatively, cold smoking has similar effects to those of drying and salting. The same is true for treating fish with vinegar where it has been shown that 100% of metacercariae remain viable and infectious after 24 h.

Treatment

The acute stage of opisthorchiasis involves desensitizing and detoxication therapies, with the chronic stage usually determined by the affect on the duodenocholedochopancreatic zone. Therefore, treatment involves an integrated therapy following general principles for treating gastroenterological diseases. The drugs of choice are those commonly used in gastroenterology and hepatology, (choleretics, enzymes, and the drugs influencing gastrointestinal tonus and motility) in combination with nutrition therapy. From the 1920s until before the introduction of praziquantel into clinical practice, the standard therapeutic was antimony potassium tartrate, a highly toxic drug requiring long-term administration. The situation changed with praziquantel availability in the mid-1970s. Praziquantel is marketed under many brand names depending on the manufacturer, for example Biltricide and Droncid from Bayer for clinical and veterinary uses, respectively. The doses and course duration depend on the disease stage and age and body weight of patients. Recommended daily doses are 45–70 mg per kg body weight but a single dose of 40 mg kg^{-1} is also used. Treatment programs vary according to local situation and constraints in each country. In Thailand, a control program was implemented via selective treatment approach as opposed to mass treatment. Although the efficacy of praziquantel is relatively high (90–95%) and to date no evidence of drug resistance has been reported, reinfections are a common phenomenon. Therefore, control by chemotherapy alone may not be successful. Chemotherapeutic treatment with praziquantel has proven very effective yielding efficacy of more than 90% in some cases. However, when using higher sensitivity methods such as PCR, the actual treatment efficacy could be lower and this clearly deserves more detailed investigation. This treatment often leads to the resolution of infection and reversal of disease-related abnormalities.We recommend that a multidisciplinary treatment, sanitary improvement and health education approach be implemented. In addition to chemotherapy, health education to avoid eating raw or undercooked fish is essential to reduce and prevent infection. This eating behavior, which is deep rooted, a part of culture and tradition for decades in endemic areas of *O. viverrini*, is not easy to change. Thus, the health education message should be culturally sensitive for an endemic community. For the current population of adults who have been exposed to the infection and are at risk of having hepatobilairy disease and CCA, health education may need to be focused on the importance of reinfection which can further increase the risk of CCA. Another approach for control is targeting the young generation such as school children hence realize the future goal of a parasite-free generation having low or no risk of CCA.

Control

Interrupting the life cycle of the parasite has always been regarded as a promising way of disease control, for example application of molluscicides (drugs that kill snails) to control snail populations. Low concentrations of certain molluscicides (e.g., phenasal and niclosamide) are lethal for infected snails, sublethal for uninfected ones, and presumably nontoxic for other animals. Application of molluscicides was practiced for some time in Russia before the discovery of praziquantel antihelminthic activity. The biggest case against this approach is that it is a huge interference in biocenosis that can have dramatic consequences. Economical factors also negate its usage as the application of molluscicides is practical only for small water bodies since the costs of treating big areas are extremely high. Additionally, it has been shown that snail populations are restored in about 5 years, hence repeated treatments are necessary. Therefore, decontamination of aquatic bodies has been abandoned in Russia.

Control efforts are primarily focused on the reduction and elimination of parasite transmission by ensuring proper food preparation, promoting the development of improved diagnostic techniques, providing chemotherapy, and improving sanitation. A combination of health education, mass treatment, and governmental aid could significantly reduce liver fluke infection. Emphasis on health education should be placed on the younger generation in school as a part of the conventional curriculum.

To aid control measures, the possibility of developing a vaccine against liver fluke is a challenging task. Researchers have been pursuing this approach but it is still at rudimentary stage and none has been generated thus far. The recent development of gene catalogues for both *O. viverrini* and *C. sinensis* should facilitate vaccine development and tests for liver fluke control.

To ensure the success of liver fluke control programs, collaboration with different sectors in society are required, that is, with fisheries and aquaculture institutions and industries, with food production and distribution industries, education sectors and nongovernment organizations. An initiative of the Food and Agriculture Organization (FAO) has stressed the need to assess the relative importance of aquaculture *vis-à-vis* capture fisheries as a source of foodborne trematode infection. The need for food safety assurance and products from aquaculture for both domestic consumption and international trade is needed. The principle of HACCP approach are available for effective control of food risk at the production stage. However, more studies are needed to optimize additional production costs against food safety to meet local conditions and constraints in applying the HACCP approach.

Conclusions and Future Perspectives

Opisthorchis viverrini is a foodborne trematode, which is an important health problem because of the massive numbers of

people infected and its serious morbidities such as hepatobiliary diseases and CCA. Although infections are identified throughout Southeast Asia, the epi-center are northeast Thailand and Lao PDR, where high prevalence coexists but a high incidence of CCA is known in Thailand. Such information in nearby endemic countries, that is, Laos and Cambodia is currently lacking. Despite lower recorded cases of *O. felineus* in certain parts of Europe, recent outbreaks of opisthorchiasis have been documented and further investigation on its current distribution range and intermediate hosts are required.

Metacercariae of *O. viverrini* and *O. felineus* can only be reliably killed by high temperature. They are resistant to drying, high salt concentration and low temperatures (see section 'Prevention, Treatment, and Control'). Even boiled and fried fish is often not safe, as it takes a long time before all metacercariae are killed. A desirable approach for prevention of infection by *O. viverrini* is by the consumption of cooked fish, but the campaign for changes in the eating habits of people appears to have met with little success.

Diagnosis of opisthorchiasis for appropriate treatment is therefore essential to prevent morbidity and disease complication. The standard conventional diagnosis is the parasiotological method of demonstrating eggs in faeces or bile specimens. Several immunological diagnostic methods by antibody or antigen detection are available but careful interpretation of the test result is required. Molecular diagnostic methods have been introduced as alternative methods for diagnosis with the advantage of high specificity and sensitivity. Diagnosis by detection of parasite DNA in fecal specimens is a promising method since it identifies positive tests even in a considerable number of egg-negative specimens and equally sensitive in egg positive cases. The ability to discover positive cases in apparently egg-negative cases as determined by the conventional method, has demonstrated that this PCR-based diagnostic approach is useful for both individual and mass screening diagnosis. It also provides an additional tool for evaluation of the chemotherapy program. With fish aquaculture playing vital role in international aquaculture trade, this PCR-based method serves as a tool for fish inspection for metacercariae, as a significant issue of food safety. The disadvantage of molecular diagnostic methods for detection of the parasite DNA is the requirement of specific molecular instrumentation, thus the development of the detection system into a simplified kit format for routine field and laboratory use is required.

See also: Disciplines Associated with Food Safety: Parasitology. Helminth-Nematode: *Haplorchis*. Helminth-Trematode: *Clonorchis sinensis*; *Heterophyes heterophyes*

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Paragonimus westermani and *Paragonimus* Species

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Glossary

Cercaria (plural: cercariae) One of the larval stages of a trematode. Large numbers of cercariae develop by asexual multiplication in the first intermediate snail hosts. Most have a tail for free-swimming in the water after emerging from the snail. Except for the schistosomes, cercariae usually do not infect humans directly.

Definitive host The host in which the trematode becomes sexually mature.

Eosinophilia Increase in the number of eosinophils: more than 500 μl^{-1} in peripheral blood is considered as eosinophilia. Eosinophilia is not only suggestive of helminth infections but also common in various allergic diseases and autoimmune diseases.

Helminth Eukaryotic multicellular parasites. Medically important helminths are categorized into three principal taxonomic groups: trematodes (flukes), cestodes (tapeworms), and nematodes (roundworms).

Metacercaria (plural: metacercariae) The encysted stage of a trematode in its second intermediate host prior to transfer to the definitive host, usually representing the stage infective to the definitive host.

Paratenic host This is a host in which a parasite can survive for longer periods without maturing. Usually a paratenic host is not essential for the completion of the life cycle of the parasite. In *Paragonimus westermani*, the larvae (excysted metacercariae) can infect and survive in the muscles of wild boars that have eaten crabs. Humans as well as dogs can be infected by ingesting raw wild boar meat.

Pleurisy Pleurisy (pleuritis) is an inflammation of the pleura. In the case of paragonimiasis, pleurisy is often associated with the retention of much exudate fluid (pleural effusion) or air (pneumothorax) in the pleural cavity.

Background

Paragonimiasis is subacute to chronic lung disease caused by the infection of lung flukes of the genus *Paragonimus*. *Paragonimus westermani* is the best known of the lung flukes, but several additional species are now known to develop in human hosts. Infection occurs mainly via consumption of uncooked/undercooked freshwater crabs/crayfish contaminated with metacercariae, an infective larval stage of the fluke. In addition, consumption of raw/undercooked meat of wild boar, which is a paratenic host that can harbor juvenile worms, is an important route of infection especially in Japan. Clinical features of the disease, such as chronic cough, hemoptysis (rusty colored sputum), chest pain, abnormalities by chest radiographic findings, etc., are somewhat similar to those of pulmonary tuberculosis or lung cancer. Even in the paragonimiasis-endemic areas, physicians often do not pay much attention to this disease because of its relatively low mortality and morbidity in comparison with the other two common pulmonary diseases. However, paragonimiasis misdiagnosed as pulmonary tuberculosis or lung cancer can cause enormous socioeconomic loss and create a mental/physical burden for the patient due to unnecessary hospitalization, laboratory examination, surgical operation, and long-term medication. In terms of epidemiology and disease control as well as food safety, identification of *Paragonimus* species is critically important because only a few out of approximately 50 nominal species are infective to humans. Current problems of taxonomy of *Paragonimus* species in relation to their infectivity to humans will be discussed.

Characteristics

Morphology

Adult *Paragonimus* worms are approximately 1 cm in diameter, having oral and ventral suckers, and look like a coffee bean in shape, size, and color while they are alive. They are hermaphroditic but usually live as a pair in a capsule in the lungs of the mammalian definitive hosts (Figure 1). Identification of species using morphological characters can be difficult. The shape, size, and proportion of oral and ventral suckers are, together with the shape of cuticular spines and the size, shape, and positions of the testis and ovary, the main characters used. The size and shape of metacercariae are also commonly used for the identification of the species.

Life Cycle

Like most other digenetic trematodes (flukes), *Paragonimus* species require two intermediate hosts, freshwater snails as the first intermediate host and freshwater crustaceans as the second intermediate host (Figure 2). Adult worms reside in the lungs of the definitive mammalian hosts, as a pair, in the cavity of a granulomatous lesion, called a worm cyst, where they lay eggs. The eggs are coughed up, swallowed down, and voided from the host either in sputum or in feces. In fresh water, a miracidium emerges from each egg and swims to find the first intermediate snail host. In the snail, asexual multiplication through sporocyst and rediae stages yields large numbers of cercariae. The cercariae emerge from the snail hosts and enter a crustacean (freshwater crab or crayfish) second intermediate host, where they encyst and grow to become infective metacercariae. Snail hosts are critically important for the maintenance of the life cycle of *Paragonimus* species, but the various developmental stages in snail hosts are not infective to humans: ingestion of snails does not lead to infection.

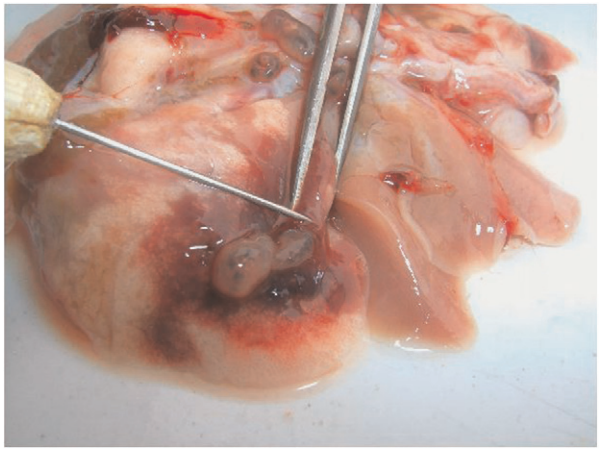

Figure 1 A pair of *P. heterotremus* adult worms squeezed out from a worm cyst in the lung of experimentally infected cat.

Paratenic Host

In Japan, approximately 25 years ago, small-scale outbreaks of paragonimiasis were found among local people living in a mountainous area who dined together on uncooked wild boar meat. Eventually, juvenile *P. westermani* worms were found in the muscles of hunted wild boars. Experimental infections revealed that worms of this species could not mature in wild boars, but could persist as juveniles in this animal, utilizing it as a paratenic host. According to a recent survey, seroprevalence of *P. westermani* infection among wild boars in southern Japan remains as high as 75%. Moreover, approximately 70% or more of human cases of *P. westermani* infection in Japan nowadays are thought to be infected by ingesting raw or undercooked wild boar meat. Recently in Kyushu, Japan, Horii and colleagues revealed that more than 60% of boar-hunting dogs were seropositive against *P. westermani* antigen and active infection was confirmed in many of them by fecal egg examinations. Those seropositive dogs had been allowed free access to, or intentionally fed with, uncooked wild boar meat (Figure 3).

Clinical Manifestations

When metacercariae of *Paragonimus* species are ingested by the definitive mammalian hosts, they excyst in the upper part of the small intestine, penetrate across the intestinal wall, then migrate into the abdominal wall where they grow for a while.

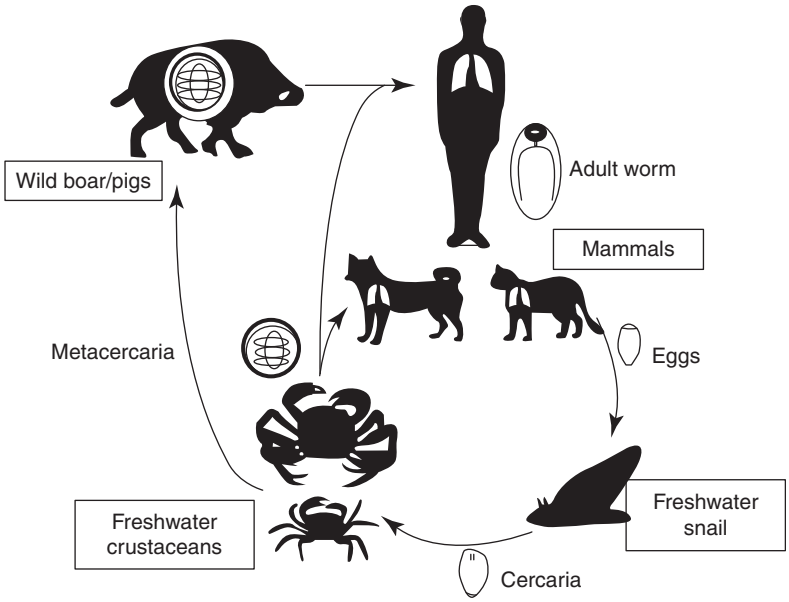

Figure 2 Life cycle of *Paragonimus* species. Reproduced from Nakamura-Uchiyama F, Mukae H, and Nawa Y (2002) Paragonimiasis: A Japanese perspective. *Clinics in Chest Medicine* 23: 409–420.

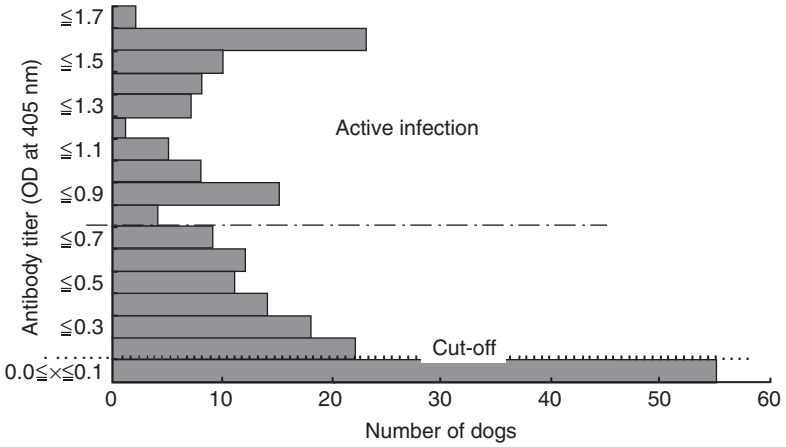

Figure 3 Paragonimiasis seroprevalence of boar-hunting dogs in Kyushu, Japan. The *P. westermani*-specific antibody titers in the sera of 224 dogs were measured by ELISA and the frequency-distribution of ELISA OD values plotted. Out of 224 dogs, 147 (65.6%) were seropositive, when the arbitrary cut-off value (.....) was set at OD = 0.100. Among 147 seropositive dogs, 83 (56.5%) were identified to have active infection because their OD values were more than 0.700 (— - — -). Reproduced from Kirino Y, Nakano N, Doanh PN, Nawa Y, and Horii Y (2009) A seroepidemiological survey for paragonimosis among boar-hunting dogs in central and southern Kyushu, Japan. *Veterinary Parasitology* 161: 335–338.

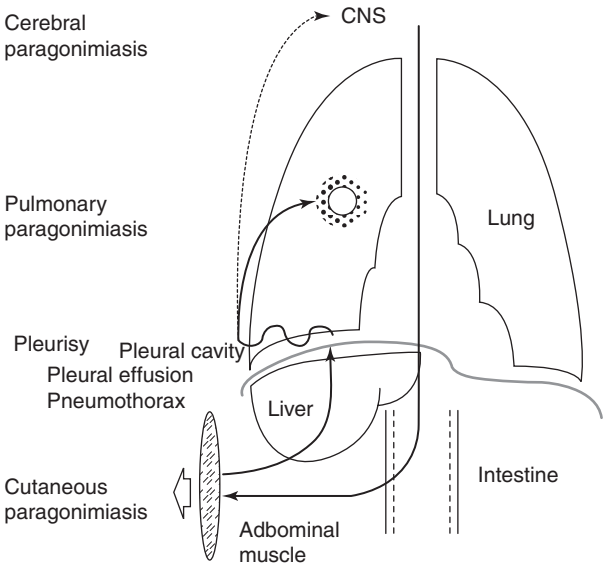

Figure 4 Migration route of *Paragonimus* species in humans. Paragonimasis patients express various symptoms depending on the location of the worms in the body and the stage of worm maturation. Reproduced from Nakamura-Uchiyama F, Mukae H, and Nawa Y (2000) Paragonimiasis: A Japanese perspective. *Clinics in Chest Medicine* 23: 409–420.

Then they come back to the peritoneal cavity, penetrate the diaphragm to reach the pleural cavity where they seek a mate, and then enter the lung parenchyma. Thus, signs and symptoms of the patients should be analyzed depending on the localization of the parasites and their maturation stages (Figure 4).

During the migration period, due to the small size and relatively rapid movement of the worms, patients rarely complain about abdominal symptoms. Acute eosinophilic peritonitis has been reported as a rare form of extra-pulmonary paragonimiasis. While migrating, a larva or juvenile worm may reach the subcutaneous tissue of the abdomen or chest to cause cutaneous paragonimiasis. Experimental infection in laboratory animals revealed that the juvenile worms often migrate through the liver before reaching the pleural cavity. There are a few case reports of eosinophilic liver abscess and hepatic capsulitis due to *Paragonimus* infection.

Once the worms reach the pleural cavity, they induce eosinophilic pleural effusion with/without pneumothorax, which are common features of the early stage of pulmonary paragonimiasis. Patients often complain of fever and chest pain but the degree of those symptoms is rather mild in comparison with those seen in bacterial pleuritis. The worms further migrate from the pleural cavity to the lung parenchyma, where they mate and start to produce eggs. At this stage, nodular lesions associated with scattered minute periovular inflammatory lesions can be seen in chest X-ray examination, computed tomography (CT), or magnetic resonance imaging (MRI). As a consequence of host–parasite interactions, the nodular lesion within the lung turns into a cavitating lesion with a communication into airways. This is now the chronic stage of pulmonary paragonimiasis during which patients show typical signs and symptoms of pulmonary paragonimiasis, namely, chronic cough and rusty colored sputum.

Related to the route of migration of worms, lung lesions are often seen in the lower to middle part of the lung and are frequently connected with the visceral pleura in chest imaging pictures. Because pulmonary tuberculosis lesions are mainly seen in the middle to upper lungs, the localization of lung lesions is suggestive, but not definitive, of the presence of lung flukes.

Juvenile and adult worms may reach the central nervous system (CNS) to cause neuro-paragonimiasis. The major routes of invasion of the CNS are surmised to be direct migration via the soft tissue around the jugular vein, or direct migration from the intervertebral foramina. In the acute stage

of neuro-paragonimiasis, patients are often present with eosinophilic meningoencephalitis. In chronic CNS-paragonimiasis, focal seizure with calcified lesions in the radiogram of brain is a typical form.

Pathology and Pathogenesis

Owing to the complexity of the migratory route of worms from the gut to the lungs, pathological changes in the hosts should be segregated into the tissue migratory phase and the lung phase. During migration, the lesions are mainly acute to subacute eosinophilic inflammation/abscess. As rare cases, eosinophilic peritonitis or eosinophilic liver abscesses have been reported. Before reaching the lung, worms pass through, or reside in, the pleural cavity causing pleurisy associated with eosinophilic pleural effusion, sometimes more than 90% eosinophils. Related to this, high levels of IL-5, a crucial Th-2 cytokine for eosinophilopoiesis and taxis, are detected in the pleural effusion of paragonimiasis patients. Peripheral blood lymphocytes of the patients produce high levels of Th-2 cytokines such as IL-5 and IL-13 on *Paragonimus* antigen stimulation. Peripheral blood eosinophilia of more than 30% is not rare in the early acute pleuropulmonary stage of paragonimiasis. At the extreme, a total white blood cell count of 100 000 mm^{-3} with more than 90% eosinophils was recorded in a child infected with *P. westermani*.

Once adult worms succeed in pairing in the lungs, the initial acute stage of eosinophilic inflammation in the lung parenchyma is followed by gradually progressing fibrotic encapsulation associated with numerous granulomatous lesions around eggs. Although host animals react to encapsulate parasites by immune/inflammatory responses, parasites tend to keep their living space and maintain a communication from the lesion to the respiratory tract of the host to allow eggs to reach the outside environment. For this purpose, parasites produce/release various proteinases in their excretory/secretory products. As a result, a cavitating lesion with a fistula to the airway is formed in the lung parenchyma. Along with the progression of the disease toward the chronic stage, peripheral blood eosinophilia becomes rather reduced and sometimes normalized, rendering recognition of possible parasitic disease in the differential diagnosis more difficult.

Among the common species of *Paragonimus* that can infect humans, *Paragonimus skrjabini* (including *Paragonimus miyazakii*) rarely mature in humans. Instead, they remain as immature worms to cause pleurisy and nodular skin lesions in the anterior chest wall.

Epidemiology of Human Paragonimiasis

Although lung flukes occur widely in tropical and subtropical regions, human cases have been seen only in limited areas of Asia, West Africa, and South and Central America where people have a custom of eating uncooked/undercooked freshwater crustaceans or wild boar meat. According to the World Health Organization in 1995, approximately 20 million people are infected with lung flukes and approximately 300 million people are at risk. The vast majority of the paragonimiasis patients as well as those at risk are in mainland China. Although paragonimiasis used to be endemic nation-wide in Japan, Korea, and Taiwan, the prevalence drastically decreased by the 1980s. Re-emergence of the disease, however, has been reported in Japan. North-eastern India, northern Vietnam, and central to northern Lao PDR were recently added as endemic areas of paragonimiasis.

Among more than 50 named *Paragonimus* species, only a few have been identified as human pathogens. *P. westermani* is the most important species in Asia, followed by *P. skrjabini* (and its Japanese subspecies, *Paragonimus skrjabini miyazakii*) and *Paragonimus heterotremus*, but the infectivity to humans of many other *Paragonimus* species recorded in Asia remains unknown. Human infection with *Paragonimus kellicotti* has been reported in the USA. In Central to South America, *Paragonimus mexicanus* is the species proven to cause human disease. Several other species recorded in this area are now considered as synonyms of *P. mexicanus*. In West and Central Africa, two species, *Paragonimus africanus* and *Paragonimus uterobilateralis*, are known to infect humans.

Paragonimus westermani is widely distributed in the Indian subcontinent (including Sri Lanka), east to Siberia, China, Korea, Japan and Taiwan, and south to the Philippines, Indonesia, and Malaysia. However, its ability to infect humans is variable among different geographical populations. Human cases of *P. westermani* infection have been found in China, Korea, Japan, Taiwan, and the Philippines, but proven/confirmed cases have never been reported from other countries, where this species is endemic in wild animals. Even in experimental infection in laboratory animals, *P. westermani* in Fareast Asian populations can mature in dogs and cats, but those of Southeast and South Asian populations hardly become adults in dogs. In Vientiane province in Lao PDR, *P. westermani* metacercariae were found along with metacercariae of several other *Paragonimus* species in the same crab hosts, but only *P. heterotremus* adults or eggs were detected in human cases in this area. Similar selective infection of *P. heterotremus* in humans was recorded in northern Vietnam where metacercariae of *P. heterotremus*, *Paragonimus vietnamensis*, and *Paragonimus bangkokensis* were concurrently found in the same crab hosts. These experiments of nature clearly indicate that the virulence to humans or other mammalian hosts is variable among *Paragonimus* species or even among the geographical populations of the same species.

Molecular Phylogeny and Biogeography

Of the more than 50 nominal species of *Paragonimus*, most occur in eastern Asia. There are more than 30 nominal species in China alone. Recent molecular phylogenetic analyses revealed that there is often enormous genetic variation within and between species and populations in *Paragonimus*. Molecular work has also demonstrated that morphological variants may not differ from one another in DNA sequences of the markers used. An updated molecular phylogenetic tree based on the ITS2 sequences of some *Paragonimus* species from Asia is shown in Figure 5. This clearly demonstrates the presence of four main groups of species, with *P. vietnamensis* and *Paragonimus macrorchis* standing distinct. Geographic distribution

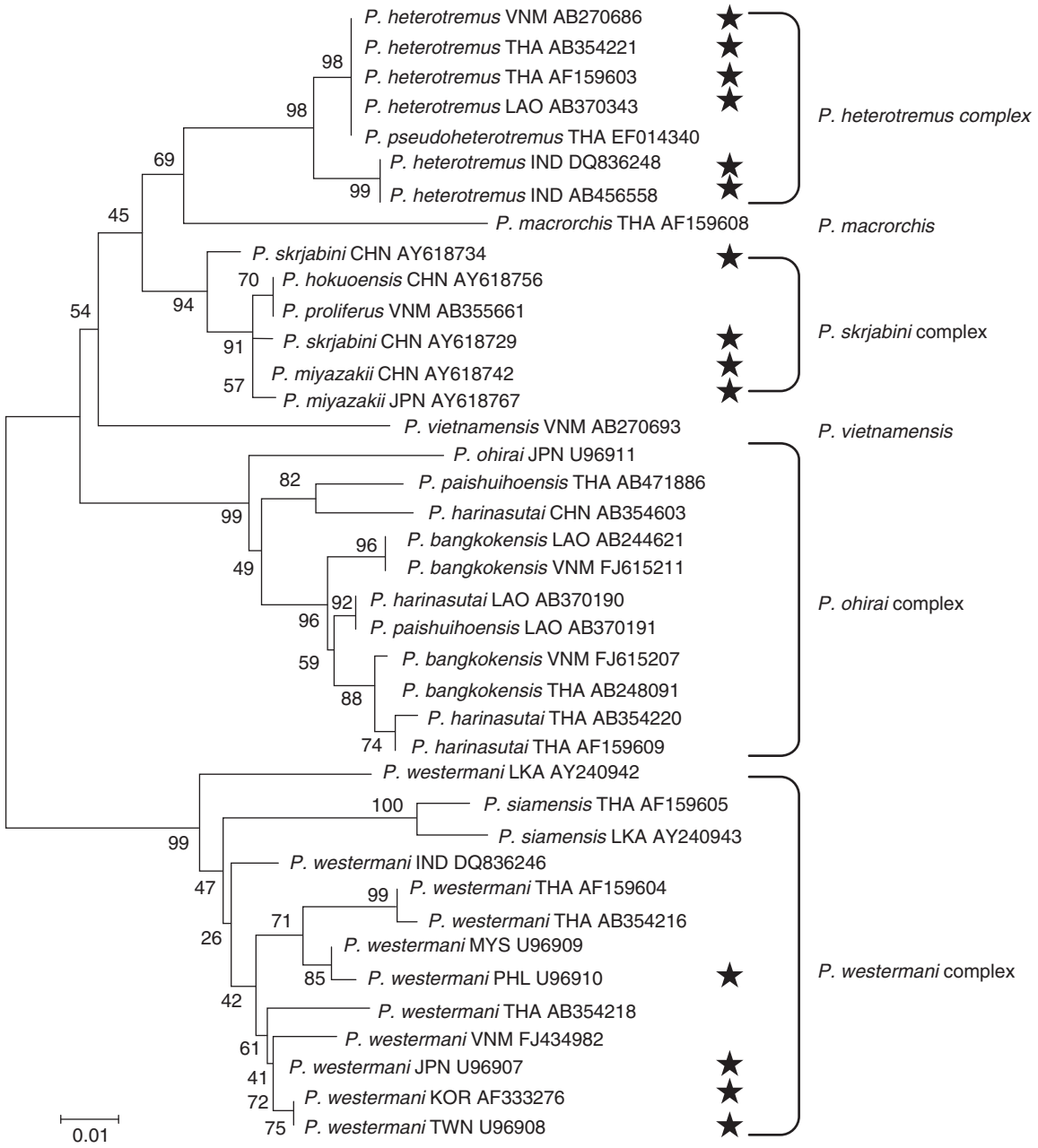

Figure 5 A midpoint-rooted neighbor-joining tree reconstructed from ITS2 sequences of *Paragonimus* species in Asia. The sequence data were obtained from GenBank (http://www.ncbi.nlm.nih.gov/). The three letter country code and the GenBank accession number are given after the species name. CHN, China; IND, India; JPN, Japan; KOR, Korea; LAO, Lao PDR; LKA, Sri Lanka; MYS, Malaysia; PHL, the Philippines; THA, Thailand; TWN, Taiwan; VNM, Vietnam; * sequence from a population known to infect humans. The numbers on the nodes are bootstrap values.

of the four major species complexes is illustrated on the map (Figure 6).

Considerable variation is apparent within *P. westermani* that is consistent to some extent with the differences in infectivity to humans as mentioned above. *Paragonimus siamensis*, although distinct from *P. westermani* in details of cuticular spination, is clustered within the *P. westermani* complex. It may be noted that *P. westermani* found in central Vietnam (FJ434982) and southern Thailand (AB354218) was clustered with Far East populations (Figure 5), but their infectivity to humans is undetermined.

Paragonimus skrjabini miyazakii, a human pathogen in Japan, was proven to be a synonym or sister species of *Paragonimus skrjabini skrjabini*, which is a human pathogen in China. These clustered with *Paragonimus hokuoensis* from China and also with Chinese and Vietnamese populations of *Paragonimus proliferus* to form a species complex. Metacercarial morphology differs greatly among members of this complex. For example, the metacercaria of *P. proliferus* is a gigantic excysted one (2.0–2.4 mm in length) whereas that of *P. hokuoensis* was reported to be within a cyst approximately 350 μm in diameter. Nevertheless, both ITS2 and CO1 sequences derived from both

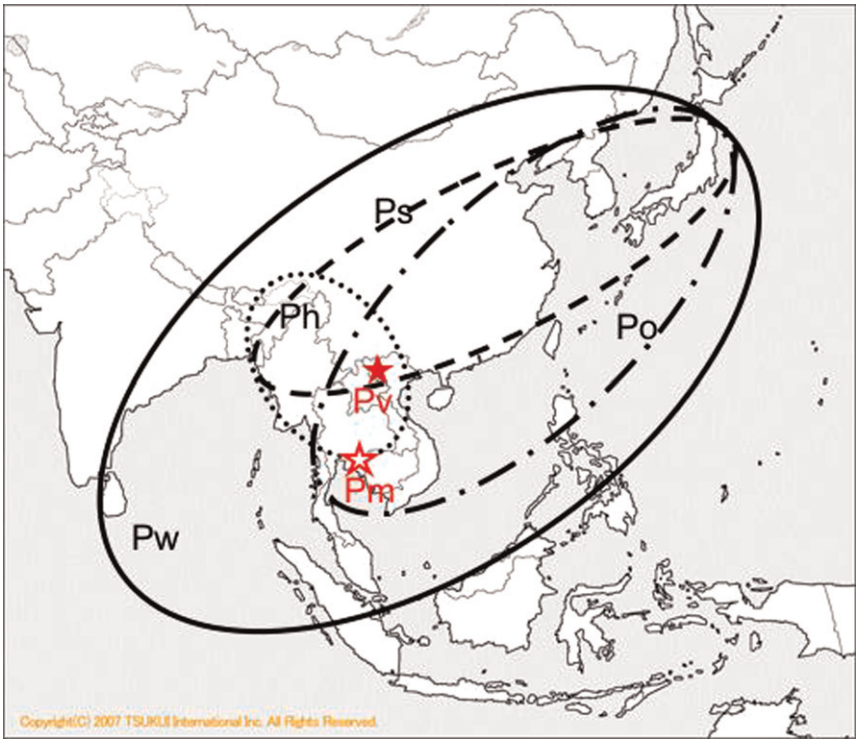

Figure 6 Geographical distribution of *Paragonimus* species complex in Asia: ——— *P. westermani* complex; — - — - *P. ohirai* complex; – – – – – *P. skrjabini* complex; ..... *P. heterotremus* complex; ★: *P. vietnamensis*; ☆: *P. macrorchis*.

kinds of metacercariae were almost completely identical. Furthermore, although the data were not included in Figure 5, several *Paragonimus* species recorded in China, such as *Paragonimus szechuanensis*, *Paragonimus hueitungensis*, *Paragonimus veocularis*, and *Paragonimus fukienensis*, are probable synonyms of *Paragonimus skrjabini* despite considerable variation among them in metacercarial morphology.

Paragonimus heterotremus, the most important human pathogen in Southeast Asia, is clustered with *Paragonimus pseudoheterotremus*, a recently proposed new species, and more distantly with *P. macrorchis*, but the infectivity of the latter two species to humans is not known. The ITS2 sequence of *P. pseudoheterotremus* is almost identical (only one base difference) with that of *P. heterotremus* and the CO1 was very similar to that of *P. heterotremus* from an Indian population (data not shown). *P. pseudoheterotremus* should, thus, be a synonym of *P. heterotremus*.

The *Paragonimus ohirai* complex, named after its oldest named member, also includes *Paragonimus bangkokensis*, *Paragonimus harinasutai*, and *Paragonimus paishuihoensis*. No members of the *P. ohirai* complex (Figure 5) are known to regularly infect humans. This is a large and diverse group containing a number of nominal species and some taxonomic puzzles. *Paragonimus bangkokensis* and *P. harinasutai* were both first found in Thailand and are easily distinguishable by morphological characteristics. Nevertheless, their ITS2 sequences showed extremely high similarities, suggesting they are not distinct species. There is a geographical variation in the sequences of these two nominal species (Figure 5).

Paragonimus vietnamensis was found in northern Vietnam as extremely large (approximately 800 μm in diameter) metacercariae. Molecular phylogenetic analyses revealed that this species is distinct from any other known *Paragonimus* sp. (Figure 5). *Paragonimus sheni* was discovered in Wuyishan, Fujian Province, China, between 1978–1983. Its validity as a distinct new species should await molecular phylogenetic data.

Analytical Methods

In terms of food safety, detection of the infective stage of any parasite is critically important. Metacercariae, the infective larvae of *Paragonimus* species, reside in freshwater crustaceans (crabs, crayfishes). The prevalence and the intensity of metacercarial infection in crabs can be determined by classical parasitological examination. The entire process is shown in a series of photographs (Figure 7). All the necessary equipments is shown in Figure 7(a1), and dissecting microscope is needed for searching the metacercariae and an ordinary light microscope for their morphological identification.

The first step (Figure 7(a)) is to manually remove the hard shell (carapace) of the crab (Figure 7(a2)) and take off the gills (Figure 7(a3)), which are then pressed between two glass plates (Figure 7(a4)) and examined under the microscope.

The second step (Figure 7(b)) is to take the hepatopancreas tissue out (Figure 7(b1)), mix well inside the carapace (Figure 7(b2)), and transfer the mixture into a cup (Figure 7(b3)). Tap water is added to the mixture (Figure 7(b4)), the sediment is allowed to settle for 5 min, and then the supernatant is decanted off. This washing by sedimentation/decantation is repeated until the supernatant fluid

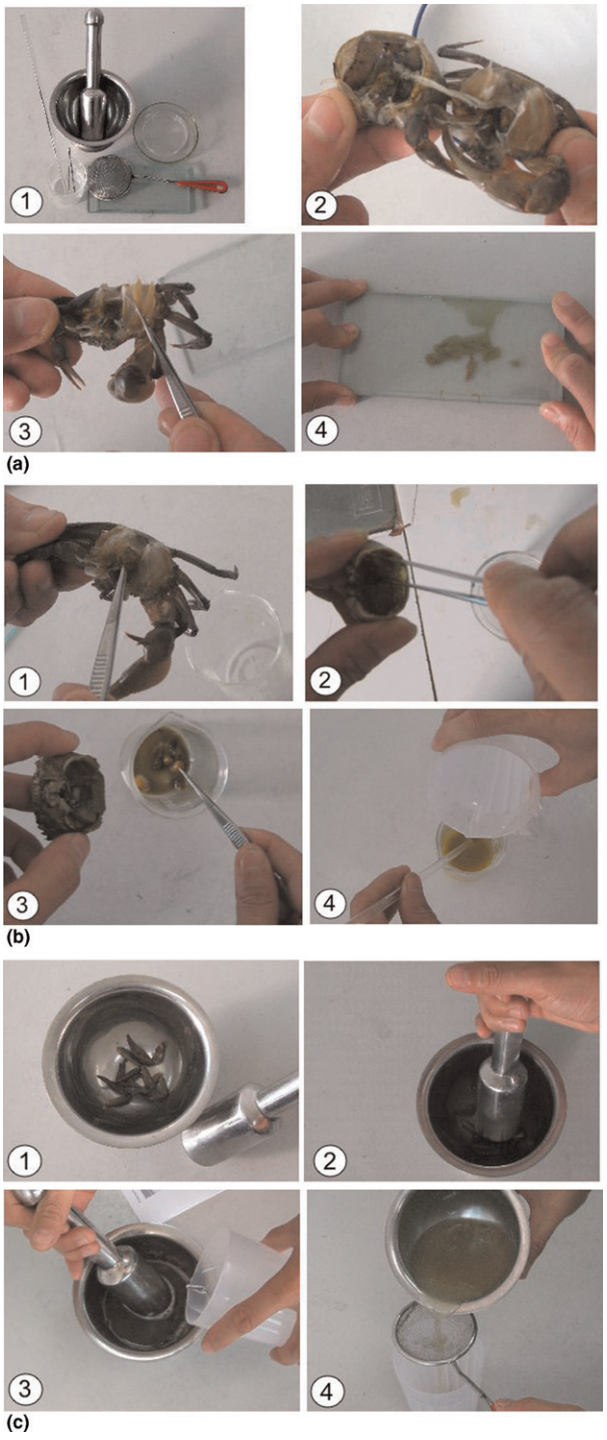

Figure 7 Methods for collecting metacercariae from freshwater crab hosts: (a) examination of the gills; (b) examination of hepatopancreatic tissue; (c) examination of the residual carcass of crabs.

becomes clear. The sediment is transfered to a Petri dish and examined under a microscope for the presence of metacercariae.

Finally (Figure 7(c)) the legs and the body are chopped into fine pieces (Figure 7(c1)) and smashed with a mortar and pestle (Figure 7(c2)). Tap water is added to the mortar and mixed well (Figure 7(c3)). The homogenate is then transferred through a stainless mesh strainer into a cup (Figure 7(c4)). The sedimentation/decantation process is repeated until the supernatant becomes clear, and the sediment is examined under a microscope.

Some of the typical *Paragonimus* metacercariae are shown in Figure 8. Remember that crabs are sometimes infected with metacercariae of more than one species of *Paragonimus* or other trematodes. Morphological and morphometric records are critically important for further identification. Polymerase chain reaction (PCR)/sequencing of some genes like ITS2 and CO1 is extremely useful for confirmation of the identification of species. If sufficient numbers of metacercariae can be obtained, experimental infection of mammalian hosts (dogs, cats, and rodents) is recommended to obtain adult worms for both morphological and genetic identification.

For the examination of the meat of paratenic host animals like wild boar, press thin slices of meat between two glass plates and examine under a microscope. After examination, put the meat slices into artificial gastric juice (pepsin/HCl solution) and incubate at 37 °C overnight. Transfer the digested materials and fluids through the mesh and clear the sediments by sedimentation/decantation; examine the sediment under a microscope.

For the mammalian definitive or paratenic hosts, serological screening by enzyme-linked immunosorbent assay (ELISA) can be used to determine the seroprevalence.

Diagnosis

Diagnosis of paragonimiasis can be made by detecting *Paragonimus* eggs in the sputum or feces by microscopic examination. However, the egg detection rate is not very high, especially in areas of low endemicity like Japan (less than 50%). Even in heavily endemic areas, egg-positive cases are just the tip of the iceberg (Figure 9).

Where egg-positive cases are found, there must also be egg-negative, symptomatic, and seropositive cases as well as egg-negative, asymptomatic, and seropositive cases. Therefore, diagnosis of human paragonimiasis is mainly based on clinical features with the supporting evidence of chest X-ray and immunological tests together with the history of eating intermediate/paratenic hosts. In the classic textbooks, it is often said that nodular/cavitating lesions are the typical radiological findings of *P. westermani* infection, whereas pleurisy is common in *P. skrjabini* (including *P. miyazakii*) infection. However, pleurisy is frequently seen in *P. westermani* infections nowadays in Japan, possibly due to earlier diagnosis and low density infections. Pleurisy was reported to be a good indicator for *P. heterotremus* infection in Lao PDR.

Differential diagnosis for ectopic paragonimiasis is more problematic than in pulmonary cases. Clinical features of cutaneous paragonimiasis are somewhat similar to those caused by other parasites such as *Gnathostoma* or *Sparganum*. The majority of cutaneous parasitoses cases have been diagnosed by postoperative identification of surgically extirpated worms. Because mobile cutaneous lesion(s) with eosinophilia are the suggestive indicators of cutaneous parasitoses, an immunological screening test such as multiple-dot ELISA is helpful to avoid unnecessary surgery. The acute phase of

Figure 8 *Paragonimus* metacercariae of various shape and appearances: (a) *P. heterotremus*; (b) *P. bangkokensis*; (c) *P. westermani*; (d) *P. skrjabini miyazakii*; (e) *P. vietnamensis*; (f) *P. harinasutai*; (g) *P. proliferus*. Scale bar = 200 μm.

cerebrospinal paragonimiasis also needs to be differentiated from other CNS-parasitoses, such as angiostrongyliasis, cysticercosis, gnathostomiasis, etc. In the chronic stage, epileptic seizure is the most common clinical manifestation of cerebral paragonimiasis, and again, differential diagnosis from neurocysticercosis or brain tumor is necessary. Immunoserological tests are helpful, but antibody might not be detected in chronic cases of more than several years duration. Reliable serological tests have been applied for immunodiagnosis of human paragonimiasis; these include ELISA and immunoblotting-based methods. Immunoblotting for the detection of IgG4 antibody to excretory and secretory (ES) products of adult *P. heterotremus* provides a sensitive and specific test. Recently, purified or recombinant proteins of *P. westermani* cysteine proteinases or recombinant egg antigen have been tested as an ELISA antigen with high sensitivity and specificity in serodiagnosis. Nevertheless, none of the immunological diagnosis systems can identify the pathogen to species. A PCR assay for detection of DNA from *Paragonimus* eggs in sputum or in histopathological sections has a potential in clinical epidemiological studies to provide species/geographical population identification.

Treatment

The anthelminthic drug, praziquantel (PZQ), is recommended as the drug of choice for the treatment of *Paragonimus* infection. According to the manufacturer's instruction, 40 mg kg^{-1} body weight should be taken per day, for two days. However, this dose sometimes resulted in incomplete cure for *P. westermani* patients who subsequently required additional doses. A dose of 75 mg kg^{-1} body weight per day for 2–3 days is recommended in Korea and Japan. This regimen gives an

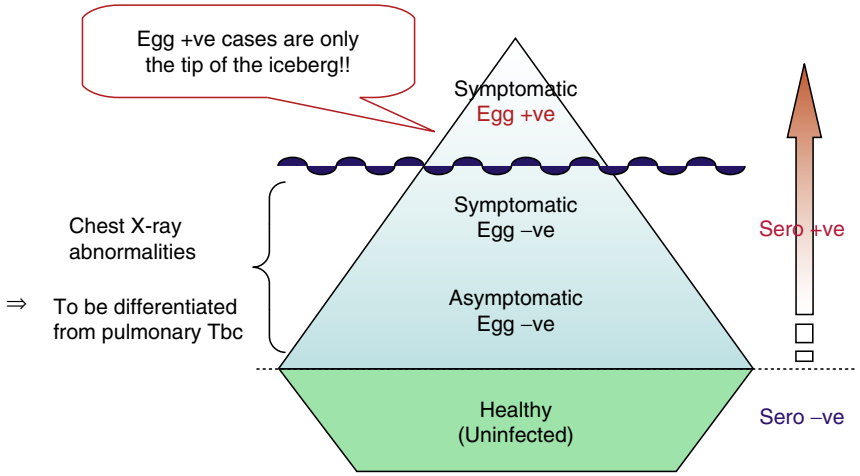

Figure 9 Sputum and fecal egg-positive patients are only the tip of the iceberg! Symptomatic egg-negative or asymptomatic egg-negative cases can be detected only by immunoserological methods Tbc, tuberculosis.

almost 100% cure rate but still a few cases showed resistance. A dose of PZQ 25 mg kg^{-1} of body weight for 1–3 days produces a cure rate of 70–100% for *P. heterotremus* infection in Thailand.

As an alternative to PZQ, field trials of triclabendazole (TBZ), which is a drug of choice for fascioliasis (WHO), was conducted in Cameroon for *P. africanus* infection, and in Ecuador for *P. mexicanus* infection, with reasonably satisfactory results by a single dose of 10 mg kg^{-1} of TBZ . The efficacy of TBZ against *P. skrjabini* was evaluated in experimental infections in dogs and five human cases in China with satisfactory results. However, the efficacy of TBZ against *P. westermani* is not quite satisfactory; in the Philippines, the cure rate of 10 mg kg^{-1} TBZ single dose for *P. westermani* was 18/24 (76%) by sputum egg detection at 90 days after a treatment. Similarly, the cure rate of a single dose of 10 mg kg^{-1} of TBZ for *P. westermani* cases was 3/5 (60%) in Japan. In both countries, the efficacy of 75 mg kg^{-1} day^{-1} PZQ for 3 days treatment for *P. westermani* infections was nearly 100%. The efficacy of TBZ seems to be variable depending on *Paragonimus* species.

Prevention and Control

Theoretically the most effective way to control paragonimiasis is to cure patients or to interrupt the life cycle. However, *Paragonimus* species exploit snail first intermediate hosts and crustacean second intermediate hosts, and a variety of mammalian definitive hosts. Therefore, elimination of the parasite or the interruption of the life cycle would require serious environmental destruction and have significant effects on the biodiversity in the endemic areas. Diagnosis and treatment for mammalian definitive hosts except for humans are practically impossible. The only possible and practical way is to change the habit of consuming raw crabs and crayfishes by education campaigns. Again, this seems to be effective only temporarily, as the re-emergence of paragonimiasis has been occurring in highly developed countries like Japan and the US.

Early diagnosis with effective treatment would be the only practical measure of control. In this regard, repeated education concerning paragonimiasis and other food-borne diseases is critically important and necessary not only for the residents, but also for the clinicians in endemic areas.

Research Needs

1. Identification of natural definitive mammalian hosts to confirm the natural life cycle.
2. Identification of factors determining the infectivity to humans (host specificity).
3. Phylogenetic and biogeographical relationships within and among African, Asian, and American *Paragonimus* spp.
4. Establishment of immunodiagnosis system to discriminate/identify the pathogen at the species level.
5. Development of more effective drugs.

See also: Helminth-Nematode: *Haplorchis*. Helminth-Trematode: *Echinostoma*; *Fasciola hepatica* and *Fasciola gigantica*; *Fasciolopsis buski*; *Heterophyes heterophyes*; *Metagonimus yokogawai*; *Opisthorchis viverrini* and *Opisthorchis felineus*

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Relevant Website

http://dpd.cdc.gov/dpdx/html/Paragonimiasis.htm
CDC's DPDx Laboratory Identification of Parasites of Public Health Concern: Paragonimiasis.

SPIROCHETES

Leptospira

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Glossary

Surveillance In public health and epidemiology, the discipline of continuously gathering, analyzing, and interpreting data about diseases, and disseminating conclusions of the analyses to relevant organizations, to intervene in their patterns in order to control and prevent them.

Zoonosis Any infectious disease that can be transmitted between species (by different ways, by a vector or by their products and food) from animals to humans or from humans to animals (less common).

Introduction

Leptospirosis (also known as Weil's syndrome, canicola fever, canefield fever, nanukayami fever, 7-day fever, rat catcher's yellows, Fort Bragg fever, black jaundice, pretibial fever, and Stuttgart fever) (tenth International Classification of Diseases A27) is caused by infection with bacteria of the genus *Leptospira*, and affects humans as well as other mammals, birds, amphibians, and reptiles. It has been reported in more than 150 mammalian species.

History of Disease

The disease was first described by Adolf Weil in 1886, when he reported an acute infectious disease with enlargement of spleen, jaundice, and nephritis. *Leptospira* was first observed in 1907 from a postmortem renal tissue slice. In 1908, Inada and Ito first identified it as the causative organism and in 1916 noted its presence in rats.

The Pathogen

Though recognized among the world's most common diseases transmitted to people from animals, leptospirosis is nonetheless a relatively rare bacterial infection in humans. The infection is commonly transmitted to humans by allowing water contaminated by animal urine to come in contact with unhealed breaks in the skin or eyes, or with mucous membranes. Rodents can contaminate water as well as food, thus becoming an important source of infection for human beings. Outside of tropical areas, leptospirosis cases have a relatively distinct seasonality with most of them occurring in spring and autumn.

Before 1989, the genus *Leptospira* was divided into two species, *Leptospira interrogans*, comprising all pathogenic strains, and *Leptospira biflexa*, containing the saprophytic strains isolated from the environment. *L. biflexa* was differentiated from *L. interrogans* by the growth of the former at 13 °C and growth in the presence of 8-azaguanine (225 μg ml^{-1}) and by the failure of *L. biflexa* to form spherical cells in 1 M NaCl. Additional to these 2 species 19 other species are described and reported at the National Center for Biotechnology Information (NCBI) taxonomy bank (Table 1).

Both *L. interrogans* and *L. biflexa* are divided into numerous serovars defined by agglutination after cross absorption with homologous antigen. If more than 10% of the homologous titer remains in at least one of the two antisera on repeated testing, two strains are said to belong to different serovars. More than 60 serovars of *L. biflexa* have been recorded, although up to 2012 at the NCBI taxonomy bank only serovars Ancona, Andamana, Canela, Jequitaia, Monteralerio, and Patoc have been recognized. Within the species *L. interrogans* more than 200 serovars have been described and 84 are recognized at the NCBI taxonomy bank for 2012 (Table 2); additional serovars have been isolated but are yet to be validly published. Serovars that are antigenically related have traditionally been grouped into serogroups. Although serogroups have no taxonomic standing, they have proved useful for epidemiological understanding.

There are at least five serovars of importance in the United States of America (USA) and Canada, all of which causes disease in dogs (Icterohaemorrhagiae, Canicola, Pomona, Grippotyphosa, and Bratislava) (Table 2).

There are other (less common) infectious strains (Table 2). Genetically different *Leptospira* organisms may be serologically

Table 1 Species of *Leptospira* – characterized and included in the National Center for Biotechnology Information Taxonomy Browser (2012)

Leptospira alexanderi
Leptospira alstoni
Leptospira biflexa
Leptospira borgpetersenii
Leptospira broomii
Leptospira fainei
Leptospira genomo sp. 1
L. genomo sp. 3
L. genomo sp. 4
L. genomo sp. 5
Leptospira inadai
Leptospira interrogans
Leptospira kirschneri
Leptospira kmetyi
Leptospira licerasiae
Leptospira meyeri
Leptospira noguchii
Leptospira santarosai
Leptospira weilii
Leptospira wolbachii
Leptospira wolffii

Source: NCBI Taxonomy Browser (2012) (http://www.ncbi.nlm.nih.gov/taxonomy).

Table 2 Serovars (84) reported of *Leptospira interrogans* characterized and included in the National Center for Biotechnology Information Taxonomy Browser (2012)

Akiyami	Kirikkale
Anhui	Kremastos
Australis	Kuwait
Autumnalis	Lai
Ballum	Lin
Bangkinang	Linhai
Bangkok	Malaya
Bataviae	Manhao II
Benjamini	Manilae
Biggis	Mankarso
Bim	Medanensis
Bindjei	Medanesis
Birkini	Mini
Bratislava	Monjakov
Broomi	Montevalerio
Budapest	Mooris
Buenos Aires	Muelleri
Bulgarica	Muenchen
Camlo	Mujunkunmi
Canicola	Naam
Carlos	Nanla
Copenhageni	New
Copenhageni/Icterohaemorrhagiae	Paidjan
Djasiman	Panama
Fugis	Perameles
Gem	Pomona
Geyaweera	Portlandvere
Grippotyphosa	Pyrogenes
Gurungi	Rachmati
Hemolytica	Ranaram
Hardjo	Ricardi
Hardjo-bovis	Robinsoni
Hardjo-prajitno	Roumanica
Hawain	Saxkoebing
Hebdomadis	Schueffneri
Ictero	Sejroe
Icterohaemorrhagiae	Sentot
IH CF1	Szwajizak
Jalna	Tarassovi
Javanica	Weerasinghe
Jonsis	Wolffi
Kennewicki	Zanoni

Source: NCBI Taxonomy Browser (2012) (http://www.ncbi.nlm.nih.gov/taxonomy).

identical and *vice versa*. Hence, an argument exists about the basis of strain identification. At present, the traditional serological system seems more useful from a diagnostic and epidemiologic standpoint (which may change with further development and spread of technologies like polymerase chain reaction (PCR)).

Transmission

Leptospirosis is transmitted by the urine of an infected animal and is contagious as long as the organisms are still moist. Although rats, mice, and moles are important primary hosts, a wide range of other mammals including dogs, deer, rabbits, hedgehogs, cows, sheep, raccoons, opossums, skunks, and certain marine mammals are able to carry and transmit the disease as secondary hosts. Dogs may lick the urine of an infected animal, off the grass or soil, or drink from an infected puddle. There have been reports of 'house dogs' contracting leptospirosis apparently from licking the urine of infected mice that entered the house. The type of habitats most likely to carry infective bacteria are muddy riverbanks, ditches, gullies, and muddy livestock rearing areas where there is regular passage of either wild or farm mammals. There is a direct correlation between the amount of rainfall and incidence of leptospirosis, making it seasonal in temperate climates and year round in tropical climates.

Leptospirosis is also transmitted by the semen of infected animals. Slaughterhouse workers may contract the disease through contact with infected blood or body fluids.

Humans become infected through contact with water, food, or soil containing urine from these infected animals (secondary hosts). This may happen by consuming contaminated food or water, or through skin contact. The disease is not known to spread from person to person and cases of bacterial dissemination in convalescence are extremely rare in humans. Leptospirosis is common among water-sport enthusiasts in specific areas as prolonged immersion in water is known to promote the entry of the bacteria. This situation was particularly highlighted in 2000 after an outbreak among 'Eco-Challenge' athletes, in Malaysian Borneo. Surfers and whitewater paddlers are at especially high risk in areas that have been shown to contain the bacteria, and can contract the disease by swallowing contaminated water, splashing contaminated water into their eyes or nose, or exposing open wounds to infected water. Occupations at risk include veterinarians, slaughterhouse workers, farmers, sewer workers, and people working on

derelict buildings. Rowers are also sometimes known to contract the disease. Farmers working closely with cattle, particularly during milking, are mostly at risk. Infection can be acquired during milking through urine splashes or by breathing in an aerosol of urine droplets. The risk of infection is greatest in 'herringbone' parlors where the milkers are below the level of the cows. Infection can also be acquired from discharges after calving or abortion. Milk can be contaminated with *Leptospira*. Leptospirosis can be transmitted via breast milk.

In the past century, many outbreaks related to food contamination were internationally reported. However, most recent outbreaks are associated with occupational and recreational contaminated water exposure. In some countries, however, cans have been contaminated with rats' urine. This highlights the need and relevance of pest management in the warehouses of food industry.

Biological Hazards

Leptospira is considered among the group of viruses and bacteria that cause only mild disease to humans, or are difficult to contract via aerosol in a lab setting, such as hepatitis A, B, and C; influenza A; Lyme disease; *Salmonella*; mumps; measles; scrapie; dengue fever; and human immunodeficiency virus (HIV). This corresponds to biohazard level 2 (mild risk for humans).

Epidemiology

Leptospirosis has a worldwide distribution. This pathology remains one of the most common and dreaded zoonotic infections. Traditionally related to certain socioeconomic or climatic conditions that favor endemicity in animal vectors and human exposure, it is generally confined to the developing parts of the world, particularly in islands of Asia and the Caribbean where incidence can reach figures as high as 432 cases per million population. The incidence is higher in the tropics than temperate regions. This zoonosis is randomly reported from industrialized countries often as an imported disease following international travel to exotic destinations. In both developing and developed countries, leptospirosis is an important public health problem related to poor housing conditions. In recent years, a new trend in human leptospirosis outbreaks has been observed related to recreational activities among the wildlife (a form of tourism that is becoming increasingly popular) and army expeditions, either for training or for combat-related purposes in similar environments. Thus, leptospirosis has become an important infectious agent to take into account when preparing for such expeditions, and this is reflected in efforts to define and utilize successful preventive antibiotic administration policies. Leptospirosis has become an important foodborne zoonosis in travelers.

The disease is seasonal, with peak incidence occurring in summer or fall in temperate regions. Extensive flooding and seasonal rainfall are significant risk factors for exposure to water contaminated with leptospires. A report from Brazil described a relationship between rainfall and human leptospirosis. Much of the resurgent international interest in leptospirosis stems from several large clusters of cases which have occurred in Central and South America following flooding as a result of El Niño-related excess rainfall. However, the occurrence of large outbreaks of leptospirosis following severe floods is not a new phenomenon and not restricted to tropical regions.

Leptospirosis was formerly considered to be primarily an occupational disease associated with agriculture, mining, livestock farming, and military maneuvers.

Eradication of the disease involves not only the attention of veterinarians but also adequate health and surveillance networks in the developing world; awareness of the evolution of the global incidence of human disease is important for acknowledging the relative risks in international travel and highlighting world areas that should move at the epicenter of enhanced disease surveillance and attempts at control from international health organizations.

According to the Office International des Epizooties, data available from the World Animal Health Information System, which is coupled with the World Animal Health Information Database interface (Handistatus), during 2004, 20 countries in the Americas reported human leptospirosis (Barbados, Brazil, British Virgin Islands, Chile, Colombia, Costa Rica, Cuba, Dominican Republic, El Salvador, Guadeloupe (France), Guyana, Jamaica, Mexico, Nicaragua, Panama, Paraguay, Saint Vincent and the Grenadines, USA, Uruguay, and Venezuela). For that year such reports totalize more than 4200 cases (Table 3). This represents a significant burden of disease in this region.

Recent data from serological studies indicated that in some areas of Latin America (e.g., Coffee triangle, Colombia), as many as 33% of cattle can be reported positive. In humans, some studies in Latin America have recently reported figures of 37% (e.g., Madre de Dios department, Peru) or up to 68% (e.g., Córdoba, Colombia), using microscopic agglutination test. In Peruvian Amazon, so far this year (2012), in the Loreto Regional Hospital (HRL) alone, five patients have been treated with severe pulmonary leptospirosis (with pulmonary hemorrhage) who recovered in the intensive care unit; a probable fatal case referred from a private clinic awaiting confirmation of the diagnosis; and a current patient who is in critical condition in the intensive care unit of HRL (22 April 2012). In addition, a man from Nauta with febrile icteric syndrome (confirmed by PCR, died on 22 April 2012) with a diagnosis of severe leptospirosis and renal failure, and two young men with lung injury remained in hospital but they were in stable condition owing to the timely diagnosis and medical attention. In Fiji, Pacific Ocean, in 2012 (from January to April), 279 people have contracted leptospirosis, which is attributed to the January floods.

Clinical Presentation

Leptospirosis has been described as a zoonosis of protean manifestations. In any case, expert consensus is that leptospirosis occurs as two recognizable clinical syndromes: anicteric and icteric leptospirosis.

Anicteric leptospirosis is a self-limited disease similar to a mild flu-like illness. Icteric leptospirosis, also known as Weil's

Table 3 Report of human leptospirosis by countries of the Americas, according the World Animal Health Organization, 2004

Country/territory	*Number of human cases*
Argentina	–
Barbados	28
Belize	0
Bermuda	0
Bolivia	0
Brazil	2394
British Virgin Islands	1
Canada	–
Cayman Islands	0
Chile	25
Colombia	70
Costa Rica	270
Cuba	281
Curaçao (the Netherlands Antilles)	0
Dominica	0
Dominican Republic	124
Ecuador	–
El Salvador	240
Falkland Islands/Malvinas	0
French Guiana	0
Guadeloupe (France)	141
Guatemala	0
Guyana	73
Haiti	+
Jamaica	213
Martinique (France)	+
Mexico	74
Nicaragua	78
Panama	4
Paraguay	12
Peru	–
Saint Kitts and Nevis	
Saint Vincent and the Grenadines	2
Trinidad and Tobago	
United States of America	29
Uruguay	45
Venezuela	98
Total	4202

+, Reported present or known to be present; –, no information available.

disease, is a severe illness characterized by multiorgan involvement or even failure. Two distinct phases of illness are observed in the mild form – the septicemic (acute) and immune (delayed) phases. In icteric leptospirosis, the two phases of illness are often continuous and indistinguishable. At disease onset, clinically predicting the severity of disease is not possible. Subsequent sequelae depend on the serovar involved and the health, nutritional status, and age of the patient, as well as the rapidity of definitive and supportive treatment.

A third syndrome of asymptomatic infection is more controversial, but has been reported and some studies indicated that more than 10–15% of the population in endemic areas would show positive to serological test although not presenting any clinical symptom.

The spectrum of symptoms is extremely broad; the classical syndrome of Weil's disease represents only the most severe presentation. Formerly, it was considered that distinct clinical syndromes were associated with specific serogroups. However, this view was questioned by some authorities, and more intense study over the last 30 years has refuted this hypothesis. An explanation for many of the observed associations may be found in the ecology of animal hosts' maintenance in a geographic region. A region with a richly varied fauna will support a greater variety of serogroups than a region with few animal hosts. In humans, severe leptospirosis is frequently but not invariably caused by serovars of the Icterohaemorrhagiae serogroup. The specific serovars involved depend largely on the geographic location and ecology of local maintenance hosts. The Icterohaemorrhagiae and Canicola serovars are supposed to be present worldwide, but the rest are mostly localized in certain areas of the world. Thus in Europe, serovars Copenhageni and Icterohaemorrhagiae, carried by rats, are usually responsible for infections, whereas in Southeast Asia, serovar Lai is common. In South America, in 2005 serovar Buenos Aires was described and isolated from an aborted dog fetus in Argentina.

The clinical presentation of leptospirosis is biphasic, with the acute or septicemic phase lasting for approximately a week, followed by the immune phase, characterized by antibody production and excretion of leptospires in the urine. Most of the complications of leptospirosis are associated with localization of leptospires within the tissues during the immune phase and thus occur during the second week of the illness.

Prevention

Prevention of leptospirosis can be achieved either by elimination of the environmental risk, by preemptive development of protection through vaccination (in animals) or by prophylactic administration of antibiotics in susceptible populations. Prevention may be also achieved through the use of protective clothing or by changes in animal husbandry, as well as by avoiding food and water contamination with *Leptospira*-infected sources.

As already noted, environmental control of leptospirosis is a futile target because the ecology of the species and serovars continually evolves, partly owing to animal vaccination strategies. Animal vaccination is sufficient to control endemically infected herds. Annual booster vaccination should be carried out in spring in order to boost immunity before summer, which is the time when maximum spread of the infection occurs. However, it is recommended that vaccination should be completed at least 3 weeks before the breeding season begins as vaccination during the breeding season may impair fertility.

Risk factors for the disease include natural disasters such as floods following excessive rain. This is a trend extensively recognized in most endemic areas and can provide a framework for disease, or at least outbreak, prevention. Yet shortcomings of the public health sector in such impoverished endemic areas often preclude such interventions. Similarly, implementation of protective measures to minimize exposure to infected soil and water is subject to public health sector adequacy.

The utility of doxycycline in preventing disease in susceptible populations was excellent in a study done in USA troops stationed in Panama. Administration of doxycycline was also widely used for prophylaxis in subjects exposed during an international outbreak related to recreational activities. In the latter case, however, the doxycycline benefit might actually refer to a percentage of exposed subjects who would not have developed clinical disease anyway. Other efforts to implement doxycycline prophylaxis in endemic settings have not proved as statistically significant. At present, doxycycline administration appears a logical preventive measure on potential exposure to leptospirosis. Moreover, it may serve preventively both for other zoonoses with a common environmental distribution and for other tropical infections that may pose risks for travelers.

In many countries leptospirosis is included in epidemiological surveillance, however, in many other places it is not. This should be enhanced particularly in those developing countries where there is still lack of regular control programs for leptospirosis, foodborne, and zoonotic diseases. Additionally, further development of the prevention strategies is also required to support zoonosis risk reduction impact on disease.

Countries with leptospirosis ought to establish public health programs for its prevention and control, as a part of building a comprehensive initiative for the control of all important infectious diseases. After establishing disease surveillance and laboratory support service, the disease burden must be monitored before and during interventions for control.

Conclusion

Leptospirosis remains as one of the most important hemorrhagic bacterial zoonotic diseases in the World. The last decade has been linked to new epidemiological settings, such as its occurrence in travelers and people practicing recreational activities in aquatic places. Even more, natural disasters, particularly flooding appears to increase disease risk, especially in developing countries, where there is a lack of effective control programs. Contamination of water and food from different sources still make leptospirosis an important foodborne disease particularly in developing countries. Thus, further actions in research and public health control are required to improve its epidemiological burden and significantly reduce its occurrence that as has been shown can lead even to fatal cases.

See also: Food Safety Assurance Systems: Microbiological Testing, Sampling Plans, and Microbiological Criteria. Institutions Involved in Food Safety: Trust in Animals and Food Safety (TAFS) Forum; World Health Organization (WHO). Public Health Measures: Surveillance of Foodborne Diseases

Further Reading

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VIRUSES

Contents

Hantavirus

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Glossary

Cross-immunity One agent causes immunity against other serogroups or serotypes.
Immunoglobulin A protein that acts as an antibody to help the body fight off disease. There are 5 classes: IgG, IgA, IgD, IgM and IgE.
Pathogenesis The natural evolution of a disease process in the body without intervention or treatment.
Platelet Blood cells that are essential to clotting.
Prodrome An early or premonitory sign or symptom of a disorder.
Reservoir A passive host that harbors pathogenic organisms without injury to itself and serves as a source from which other individuals can be infected.
Viremia The presence of viruses in the blood.
Virus A group of infectious agents characterized by their inability to reproduce outside of a living host cell.
Zoonoses Diseases that may occasionally be transmitted from animals to people.

Characteristics

Hantaviruses are named after the Hantaan River in Korea, where the Hantaan virus was shown to be the cause of a deadly disease in soldiers stationed there in 1951. Over the next few years, several other etiologic agents of a similar renal syndrome were discovered across Europe and Asia. At the end of the twentieth century a new pulmonary disease caused by these viruses was discovered in USA.

Hantaviruses are enveloped viruses with a negative-stranded RNA genome composed of three segments (family Bunyaviridae and genus *Hantavirus*). These viruses are distributed worldwide and in nature are hosted by wild mammals, such as persistently infected rodents and shrews, without apparent signs of disease manifestation but secrete virus for prolonged periods. Hantaviruses are transmitted to humans by inhalation of virus-contaminated urine, feces, and/or saliva. It is yet not known if human infection of shrew-borne hantavirus may be possible.

They are associated with two severe diseases:

- Hemorrhagic fever with renal syndrome (HFRS), (5–10% case-fatality rate). It is caused by viruses of the Old World hosted by rodents of two subfamilies, Murinae and Arvicolinae.
- Hantavirus pulmonary syndrome (HPS), or also known as hantavirus cardiopulmonary syndrome, (20–50% case-fatality rate) is caused by viruses confined to the New World and is distributed by Sigmodontinae rodents, thus, due to the distribution of these rodents, HPS is confined to the New World (Table 1).

Treatment is mainly supportive; there are no agents available to eliminate the virus or to avoid a disseminated infection. The drug ribavirin has been effective in HFRS, but not HPS.

The development of vaccines against these diseases has been hampered by the lack of animal models of the disease. None of the Old or New World hantaviruses cause illness or death in any animal model, with the exception of Andes virus (ANDV), which causes a lethal disease in hamsters that is very similar to human HPS. An ideal candidate as a vaccine would be one that protects against the two types of disease.

Clinical Manifestation

The two mainly different clinical forms of the disease are HFRS, which includes diseases such as Korean hemorrhagic

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Table 1 Geographic distribution of major human pathogenic hantaviruses and rodent host associated in the Old and New World

Virus (abbreviation)	*Rodent host associated*		*Associated disease (case-fatality rate)*	*Native region*
	Subfamily	*Common name (species)*		
Hantaan (HTNV)	Murinae	*Apodemus agrarius* (striped field mouse)	Severe FHRS (5–15%)	East Asia
Dobrava (DOBV)		*Apodemus flavicollis* (yellow-necked field mouse)	Severe FHRS severa (5–20%)	Balkans
Seoul (SEOV)		*Rattus norvegicus* (Norway rat) and *Rattus rattus* (black rat)	Moderated FHRS (1%)	Worlwide
Puumala (PUUV)	Arvicolinae	*Clethrionomys glareolus* (bank vole)	Moderated FHRS (1–2%)	Europe
Sin Nombre (SNV)	Sigmodontinae	*Peromyscus maniculatus* (deer mouse)	HPS (20–50%)	North America
Laguna Negra (LNV)		*Calomys laucha*, (small vesper mouse)	HPS (15–25%)	Paraguay, North Argentina
Andes (ANDV)		*Oligoryzomys* sp. (colilargo)	HPS (20–50%)	South America

fever, epidemic hemorrhagic fever, and nephropathia epidemica (fever and renal syndrome) and HPS. However, increasing evidence suggests that the boundaries of these two diseases are no longer perfectly separated, and for this reason it was recently proposed to join the denomination as hantavirus disease (HVD).

Renal Syndrome

The symptoms can be split into five phases:

- The incubation period is 12–16 days (range 5–42).
- The disease begins with a febrile phase characterized by flu-like symptoms: fever, chills, headache, nausea, abdominal and back pain, respiratory problems like dizziness that is accompanied by diarrhea and proteinuria and lasts for 4–7 days.
- There is a leukocytosis and a thrombocytopenia that lead to the formation of petechiae (blood spots) on the buttocks and soft palate, usually called the hypotensive phase. The development of a capillary leakage syndrome leads to a loss of blood pressure at approximately the fifth day. The drop may be small and short lived or large enough to cause shock, and symptoms can lead to tachycardia and hypoxemia. This phase can last for 2 days.
- The disease enters a renal phase, that lasts for 3–7 days and is characterized by the onset of renal failure and proteinuria occurs, affecting the kidneys characterized at first by oligouria then polyuria which can last for a couple of days up to weeks, bleeding of the mucous membranes and edema of the lungs.
- The convalescent phase is when recovery occurs and symptoms begin to improve.

Pulmonary Syndrome

The symptoms can be split into four phases:

- The incubation period is 16–24 days (range 12–35).
- The clinical disease is manifested by a viral prodrome of 1–8 days. The symptoms, which are similar to HFRS, include fever, myalgias, headache, vomiting, and diarrhea; tachycardia and tachypnea generally do not develop until approximately day 6, followed by the precipitous onset of pulmonary edema. Such conditions can lead to a cardiopulmonary phase, where cardiovascular shock can occur. A characteristic of the disease is that it progresses very rapidly, and hospitalization and ventilation of the patient is required.
- Once the cardiopulmonary phase begins, patients can develop a pulmonary capillary leak syndrome leading to respiratory distress symptoms characterized by shortness of breath as the fluid fills the lungs. During this phase, thrombocytopenia is very marked; the decrease in platelets is rapid even when measured 8 h apart. The cardiopulmonary phase persists for up to 1 week, followed by spontaneous diuresis.
- A convalescent phase is characterized by weakness and exertional dyspnea for 3–4 weeks in almost all survivors.

Pathogenesis

Common features of both HFRS and HPS are:

- increased vascular permeability and decreased blood pressure due to endothelial dysfunction,
- syndromes are accompanied by myocardial depression and hypotension or shock,
- diseases appear to be immunopathologic, and inflammatory mediators are important in causing the clinical manifestations, and
- endothelial cells are susceptible to hantavirus infection; however, virus does not cause cytopathic effects, to explain increased endothelium permeability.

In HFRS, the most dramatic damage is seen in the kidneys which can cause fluid overload; the retroperitoneum is a major site of vascular leak and the kidneys suffer tubular necrosis where as in HPS, the lungs, spleen, and gall bladder are most affected. In the case of HPS, pulmonary capillary endothelial cells express high densities of infection; capillary leak is centered in the lungs leading to fulminant noncardiogenic pulmonary edema and may progress quickly to cardiogenic

shock. As in other viral systems, it appears that pathogenic hantaviruses possess mechanisms to antagonize the innate immune system including tumor necrosis factor-α, interleukin 1β, and interferon γ, though this has yet to be clarified. Although antibody-mediated protection is probably the key, cytotoxic T cells may play a role in both protective immunity and pathogenesis of the diseases.

Hantavirus infection in rodents is characterized by an acute phase of peak viremia, viral shedding, and virus replication in target tissues, followed by a persistent phase of reduced, cyclical virus replication despite the presence of high antibody titers.

Epidemiology

The geographic distribution and epidemiology of human cases of the diseases caused by hantaviruses have been considered a consequence of the distribution and natural history of their primary rodent hosts. The precise number of identified hantavirus species is a matter of debate; because the International Committee on Taxonomy of Viruses (ICTV) classification guidelines have not yet demarcated viruses below the species level, and only the main representative viral species pathogenic for humans were summarized in Table 1.

Four hantaviruses are known to cause HFRS:

- Hantaan virus (HTNV), which causes the most severe form of HFRS and is present primarily in Asia.
- Dobrava virus (DOBV), which causes serious HFRS and has been identified in the Balkans.
- Puumala virus (PUUV), a milder form of HFRS which causes a higher proportion of sub clinical infections and is prevalent in Europe.
- Seoul virus (SEOV), which results in a less severe form of HFRS and has a worldwide distribution.

China and Russia have the highest annual incidence of HFRS; most cases are attributable to the SEOV, with the Hantaan virus playing a minor role.

Genetic characterization of the first African hantavirus (Sangassou virus) detected in an African wood mouse (*Hylomyscus simus*) in Guinea was shown to be very closely related to the DOBV clade of hantaviruses, whose members cause the most severe cases of HFRS in Europe. Reports originated from serologic surveys of human populations demonstrated antibodies against this virus although its pathogenicity is unknown.

Since HPS was first recognized it has been identified throughout nearly all American countries. The Sin Nombre (SNV), the cause of the majority of HPS cases in USA and the ANDV of South America, cause the most severe disease, although the number of confirmed annual cases is low (100 cases per country) related to HFRS (approximately 10 000 cases per country).

High rodent population densities and special human activities can result in increased cases of rodent-borne disease. Several rodent species are associated with additional hantaviruses that have not been implicated in human disease. Also, the role of several European and American hantaviruses in transmission of infected nonreservoir rodents (spill-over) is not very clear. Based on a large panel of fragments of the N- and GPC-encoding American strain sequences, phylogenetic studies reported molecular evidence that pathogenic hantavirus in America is represented by different genetic lineages associated in most of the countries with *Oligoryzomys* species according with the geographic distribution.

Person-to-person transmission is rare and has been reported with ANDV in Argentina and Chile, primarily associated with close and prolonged contact, and occurred early in the respiratory phase of the disease. In addition, SEOV was found in several sites in USA, Argentina, and Brazil but was not associated with typical HFRS.

Analytical Methods

Seroprevalence is the first test to do when investigating hantavirus infection, performed principally with an enzyme-linked immunosorbent assay or immunofluorescence assay, to detect immunoglobulin M (IgM) antibodies to hantavirus. The hantavirus-infected patients showed an early and strong IgM, immunoglobulin G, and immunoglobulin A serum antibody response, in most of the cases as early as 1–4 days following the onset of symptoms.

Although native hantavirus antigens are being widely used as diagnostic reagents, their production requires expensive containment laboratory conditions for virus propagation. Recombinant protein technology is less expensive and proteins are generated in larger amounts.

Hantaviruses may be present in clots, lymphocyte fractions, and plasma from patients with acute HPS or HFRS and also in the blood, autopsied tissues, saliva, feces, or urine of infected rodent reservoirs. The difficulty in isolation of hantaviruses has constrained efforts to detect viral genetic material by RT-PCR and sequencing. Real-time RT-PCR offers significant improvements to the quantitation of viral load and in the reduced risk of carry-over contamination into the laboratory.

According to CDC, Biosafety Level 2 (BSL-2) facilities and practices are recommended for laboratory handling of sera from persons potentially infected with hantavirus. Potentially infected tissue samples should be handled in BSL-2 facilities in accordance with Biosafety Level 3 (BSL-3) practices.

Control and Preventive Measures

Most cases of human illness associated with hantaviruses have resulted from prolonged exposure to aerosols from infective saliva or excreta of naturally infected wild rodents, particularly within a closed, poorly ventilated area. Rodent control in and around the home remains the primary strategy for preventing hantavirus infection. Hantaviruses are heat labile and are susceptible to lipid solvents, detergents, and disinfectants like dilute hypochlorite solutions.

The possibilities of infection associated with ingestion of food contaminated with the virus, contact with mucous membranes, or contamination of breaks in the skin barrier have not been clearly evaluated. Hantavirus survival through the digestive tract is unlikely to occur. Very few viruses can survive exposure to saliva because of the potency of antiviral compounds in the saliva like several proteins as secretory

leukocyte protease inhibitor. If it survives, hantavirus must pass through acid condition of the stomach and avoid destruction by enzymes like pepsin, which are designed to disassemble protein or lipid coats of most viruses. But the source of disease instead of being contaminated food could be breathing in air surrounding food, garbage, and food stores contaminated by relatively fresh mouse droppings. *Ex vivo* stability of hantaviruses has been shown to persist much longer in a humid as compared to a dry environment.

The prevention is done primarily by sealing cracks and holes of the food storage area for possible mouse entry with materials that will be resistant to gnawing. The cartilage in rodent's heads allows them to go through a hole as small as 1/4 in. Besides, packaged food must be placed in metal containers, glass bottles, and heavy plastic containers with tight fitting lids, all of them resistant to rodents.

Rodents are the animal species most frequently included in the human diet; in the temperate world, they serve only as a supplement to the regular diet, however in the tropical world, they are widely accepted as a popular source of protein. Consuming rodents in pesticide-treated areas and handling rodents with potential zoonoses like hantavirus are two possible risks. Pesticide materials may be retained in meat. Exposure to zoonoses from ectoparasites, blood, and urine while handling rodents to be used for food is another possible risk that deserves attention.

Research Needs

An integrated investigation at the landscape scale will allow a better understanding of interactions between changes in climate, land use, and human behavior, together with the ecology of animal hosts of hantavirus agents. Further analyses are needed to provide a better knowledge of the serotypes circulating in America because studies on cross-protective immunity among them are hindered by difficulties to propagate virus in cell culture and isolation. Although efforts are continuing to develop safe and effective hantavirus vaccines, viral therapies are not thriving. A finite number of viral agents cause respiratory tract disease in the intensive care unit; some of them like hantavirus are rare but have an immense public health impact. Recognizing these viral etiologies becomes important in treatment, infection control, and public health measures. In many regions, it is still necessary to implement education programs for primary and infectious disease physicians to early suspect hantavirus infections. Wild animals, including rodents, should be taken into account in the national programs for food safety.

See also: Characteristics of Foodborne Hazard and Diseases: International Classification of Diseases; Pathogenesis and Virulence. Disciplines Associated with Food Safety: Food Virology. Food Safety Assurance Systems: Cleaning and Disinfection; Infestation Management in Food Production Premises; Microbiological Testing, Sampling Plans, and Microbiological Criteria. Foodborne Diseases: Foodborne Diseases in Travelers. Institutions Involved in Food Safety: Trust in Animals and Food Safety (TAFS) Forum. Organisms of Concern but not Foodborne or Confirmed Foodborne: Bolivian Hemorrhagic Fever Virus (Machupo Virus); Foot-and-Mouth Disease Virus. Public Health Measures: Challenges of Developing Countries in Management of Food Safety; Challenges of Industrialized Countries in Food Safety Management; Environmental Assessment in Outbreak Investigations; Health Education, Information, and Risk Communication; Monitoring of Contaminants. Safety of Food and Beverages: Cereals and Derived Products; Fruits and Vegetables; Meat and Meat Products; Nuts. Spirochetes: *Leptospira*. Viruses: Lassa Fever Virus

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Centers for Disease Control and Prevention (CDC).

Hepatitis A Virus

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Glossary

Cytopathic effect The cell damage caused by viruses.
Genotype Genetic type of microorganism determined on the basis of the genome sequence using typing techniques.
Hepatitis A A liver disease caused by a picornavirus.
Immunofluorescence A detection technique used for microscopy utilizing target-specific antibodies conjugated to a fluorescent molecule.
Infectivity Ability to produce an infection.
Molecular epidemiology The study of the occurrence and transmission of a disease within populations using molecular methods.
Molecular methods Techniques used for detection or identification of microorganisms at the genome level.
Strain An isolate of the same type of microorganism possessing different properties.
Viral capsid The protein shell that surrounds a virus particle.
Viremia The presence of viruses in the blood of an infected host.

Historical Background

A contagious disease with characteristic symptoms of jaundice, probably viral hepatitis, was first recognized in ancient times. In the twentieth century, the viral etiology of 'infectious hepatitis' was proven by isolation of the agent from stools of infected patients. In due course, hepatitis A has been recognized as an infection that is acquired through ingestion of viruses showing tropism to liver tissue.

Characteristics of Hepatitis A Virus (Taxonomy, Structure of the Virus, Genome Organization, and Virus Strains)

Hepatitis A virus (HAV) is classified into *Hepatovirus* genus within the Picornaviridae family. Mature virions are approximately 27–32 nm in diameter and can only be visualized under an electron microscope (Figure 1). The viral capsid has an icosahedral symmetry and is composed of 32 capsomers or protein subunits. The lack of lipid envelope makes HAV resistant to inactivation by heat, pH changes, and to many disinfectants, which affect enveloped virus infectivity. Cell-adapted HAV survives pH 1 when treated for 2 h at room temperature, and virus infectivity is still retained after incubation at 60 °C for 1 h. Infectivity can be preserved for at least 1 month after drying and storage at 25 °C with 42% humidity or even years at − 20 °C. The longer a virus can survive outside a host, the greater are its chances for transmission. Like other picornaviruses, HAV has a potential to survive in water environments, soil, foodstuffs, and also on surfaces of inanimate objects for extended periods.

HAV has a linear, uncapped, single-stranded 7.5 kb RNA genome consisting of five segments. The 5′-end of viral RNA is linked to the VPg protein followed by the 5′-noncoding region (NCR) and P1–P3 regions. The genome has a short 3′-poly(A) tail acting as the terminator of translation. The genome has only one open reading frame, translated into a single precursor polyprotein, which is post-translationally cleaved to generate the functional viral proteins. The P1 genome segment encodes four structural proteins of the viral capsid (VP1, VP2, VP3, and VP4), whereas the translation of other genome

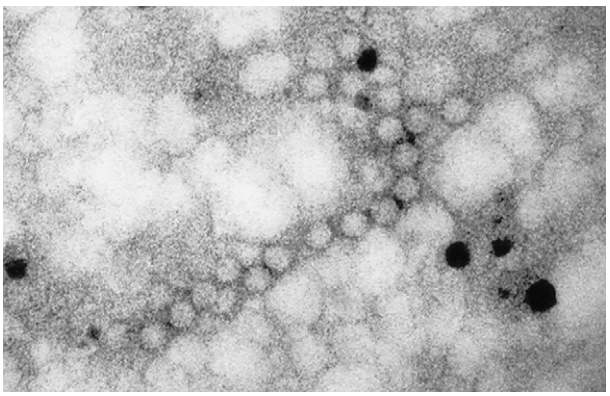

Figure 1 Electron micrograph of HM175 strain of HAV. Reproduced with permission of Albert Bosch and Rosa M. Pintó, Enteric Virus Laboratory, University of Barcelona.

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segments, that is P2 and P3, results in formation of seven nonstructural proteins necessary for RNA synthesis and virion formation. The VP1 and VP3 capsid proteins act as an antigenic epitope on the viral surface which elicits a neutralizing antibody response. The nonstructural proteins of HAV have also been shown to be immunogenic.

Based on molecular differences and homology across the HAV genome, all virus strains are classified into one out of six genotypes. The genotypes I, II, and III have been isolated from humans, whereas genotypes IV, V, and VI are of simian origin. These isolates are genetically distinct from human HAV strains. Genotype I can be further divided into two subgenotypes, IA and IB, differing from each other at approximately 7.5% of base positions. This genotype is the most prevalent worldwide. Despite observing variations on molecular level, all isolates represent only one virus serotype. Consequently, in vaccinated individuals a single vaccine strain can induce the development of protecting antibodies against human variants of HAV, circulating on different continents.

Course of HAV Infection

HAV is infectious only for humans and primates; however, it cannot produce illness in all primate species. Virus transmission occurs mainly through the fecal–oral route either by direct contact with an ill person or by ingestion of contaminated food or drinking water. Disease development after exposure to the urine or nasopharyngeal secretions of infected individuals can occur but is uncommon and not well documented. A parenteral route of virus transmission has also been reported. After ingestion, viruses pass through the gastrointestinal tract to the liver via the hepatic portal vein. When virions reach the liver, they attach to HAVcr-1 receptors present on the hepatocytes, then enter these cells and replicate there. After virus formation, progeny are secreted into liver sinusoids and bile canaliculi, from where they are transported to the intestines to be finally excreted in feces. Liver cell damage is not entirely associated with virus release, but it appears as a result of immune mechanisms in which virus-specific T-cells are involved. Liver hepatocytes are the primary site of virus replication. Nevertheless, viruses have been shown in Kupffer cells and in the cytoplasm of epithelial cells of biliary ducts. The virus is also able to replicate in extrahepatic tissues like epithelial cells of the small intestine and salivary glands. Despite the presence of the virus in saliva, there is no scientific evidence that saliva-transmitted infection has ever occurred.

The mean incubation period of the disease is approximately 30 days with a maximum time of 50 days. A viremia precedes the development of symptoms by 7–14 days. During active virus replication, infected persons remain generally asymptomatic. When symptoms appear, they usually are mild or nonspecific. In adults, virus excretion in feces can occur 2–3 weeks before the onset of jaundice and lasts for at least 6 weeks after onset of symptoms. During that time, virus is shed via feces, which may contain $> 10^6$ infectious viruses per gram. In some cases, virus shedding has been observed 3 months after the onset of clinical symptoms. Generally, the severity of illness is age-dependent with milder symptoms of the disease reported in children than in adults. In young children, subclinical or anicteric infections are common, whereas in adults, hepatitis A is symptomatic with dark urine, pale stools, and jaundice. Symptoms observed in humans during the course of HAV infection are summarized in Table 1. Anti-HAV neutralizing antibodies, mostly IgM, appear in the serum shortly after the onset of symptoms. However, the major role in protection of individuals against reinfection is attributed to IgG antibodies, which confer lifelong immunity. Apart from IgM and IgG antibodies, the presence of fecal and serum anti-HAV IgA has also been evidenced, though their role of secretory immunity in protection against virus infection is limited. Although the disease is self-limiting, in some cases (3–20% of the patients) relapsing or prolonged hepatitis occurs. During relapse, the HAV virus can reemerge and cause jaundice,

Table 1 A summary of the symptoms reported among patients with hepatitis A

Period of infection	*Duration*	*Symptoms*
Incubation period	10–50 days	Lack of symptoms (active virus replication)
Prodromal (preicteric) phase	Several days to more than a week	General symptoms: fever, fatigue, malaise, myalgia, arthralgia Gastrointestinal symptoms: anorexia with disorders of taste and smell, sore throat, nausea, vomiting, diarrhea, constipation, hepatomegaly, splenomegaly, abdominal discomfort Symptoms from respiratory tract: cough Skin symptoms: rash, urticaria, exanthematous skin eruptions
Acute (icteric) phase	3 weeks	Gastrointestinal symptoms: anorexia, nausea, vomiting, hepatomegaly, hepatic tenderness, splenomegaly pale stools Skin symptoms: yellowish discoloration of the mucous membranes (conjunctivae, sclerae), jaundice, pruritus Other symptoms: dark, golden-brown urine, biochemical and hematological changes
Prolonged or relapsing hepatitis	Lasting up to 6 months after onset of illness	Biochemical changes

Source: Adapted with permission from Table 11.1 in Cook N and Rzeżutka A (2006) Hepatitis viruses. In: Motarjemi Y and Adams M (eds.) *Emerging Foodborne Pathogens*, pp. 282–308. Cambridge, UK: Woodhead Publishing Limited, ISBN 1 85573 963 1. www.woodheadpublishing.com

despite the presence of anti-HAV antibodies, with virus being detected in feces and serum. In some cases (approximately 1.5–4.7% of hepatitis patients), atypical manifestations of disease such as acute kidney injury have been reported. There is no evidence of chronic liver disease or persistent infection. A serious complication of the infection is a fulminant hepatitis, observed among young children and adults with underlying chronic liver disease. Fulminant hepatic failure is an extensive necrosis of the liver, resulting in disruption of its normal functions. The estimated mortality rate due to HAV infection is low, estimated at between 0.2% and 0.3%, but in patients over 50 years old, it increases to 2.1%. For the majority of patients (approximately 60%), complete clinical and biochemical recovery takes place within 2 months and within 6 months for the remainder.

Epidemiology

Prevalence of HAV Infections

In some parts of the world like North America, Western Europe, and Australia, the number of HAV outbreaks are declining, which is consistent with a low anti-HAV IgG seroprevalence in the population. Although anti-HAV IgG rates remain high or moderate in South America, Africa, Asia, Middle and Southern Europe, a declining trend in HAV seroprevalence is also observed. Seroprevalence rates are highly correlated with socioeconomic status and access to clean water and sanitation. For instance, in the US, the number of acute HAV cases has declined by 92% over a 12-year period, with 1 case recorded per 100 000 of inhabitants. International travel to countries where HAV infections are frequently reported is the most common risk factor for acquiring the disease. Furthermore, sexual or household contact and consumption of contaminated food or water are also indicated. Annually approximately 100 000 cases of HAV with approximately 500 deaths have been reported in the WHO European Region. The real number of food-borne HAV cases is difficult to estimate, due to prolonged virus incubation period and the lack of suspect food items for laboratory testing when the outbreak occurs. According to the European Food Safety Authority, in 2007, only 46 outbreaks of HAV were linked to consumption of contaminated food. Hepatitis A still remains a global public health concern, with approximately 1.5 million clinical cases reported worldwide annually.

Virus Transmission through Contaminated Food

In most HAV cases reported, a definitive virus source is not recognized and often there is a lack of evidence that food was the sole source of infection. In some outbreaks, the virus source and mode of its transmission was successfully identified through testing of the implicated foodstuff as a follow-up to an epidemiological investigation (Table 2). Detection of HAV outbreaks is complicated by the variable length of incubation period, which is dependent on the dose of the virus ingested and the difficulties in linking the illness with the type of food consumed. Food contamination can occur in different stages within a food supply chain. For instance, a common vehicle of virus transmission is sewage contaminated water, used for farming of shellfish or irrigation of food crops. Furthermore, extensive handling of fresh produce by infected field workers either during production or harvesting, processing, or packaging can result in food contamination. For instance, in an outbreak of hepatitis in New Zealand linked to consumption of blueberries, a traceback investigation of the produce indicated that fruit contamination by HAV occurred in the orchard, and an audit visit performed on the field revealed poor sanitary facilities, which did not facilitate proper hand hygiene among the berry pickers.

In addition, food preparation surfaces contaminated by viruses can also serve as an environmental virus route when produce is in contact with them. Viruses transferred to the surfaces of fruit and vegetables can persist sufficiently long between a contamination event and consumption to constitute a significant risk to consumer health. Most food contamination appears as a result of its handling during processing or at point of service. A good example is an outbreak recorded in Belgium, in which 269 individuals were infected by HAV. Raw beef was identified as the vehicle of virus transmission, and the virus source was a food handler working at the meat distribution plant. He was manually preparing beef meat by cutting and peeling it before its distribution to local butcheries. The food handler had contracted hepatitis A infection (confirmed serologically) and was working with bare hands in the days preceding his symptomatic illness. Blood and serum samples collected from infected persons and from the food handler were virus-positive. Subsequent sequence analysis of VP1/2A junction region of isolated HAV from patients confirmed that the infection was caused by the same virus strain. Although meat samples were not tested for the presence of HAV, there was enough evidence to state that consumption of raw beef was a probable source of infection. In these two outbreaks, the mechanism leading to the food contamination was clear. In both the New Zealand and Belgium cases, viruses were transferred to foodstuffs by infected persons working within the food supply chain. Lack of observance in personal hand hygiene among these workers allowed viruses to enter the food chain.

Another mechanism leading to food contamination can be seen with shellfish, that is, growth in polluted water environments. Shellfish are filter-feeding animals which collect nutrients from the surrounding water by passing them over gills in their body. If shellfish are grown in a sewage-polluted environment, in addition to nutrients, they can also concentrate pathogenic bacteria and viruses. HAV can persist in an infectious state in shellfish for more than 3 weeks following contamination, and viral RNA can be detected after 6 weeks post-contamination. Thus, consumption of shellfish harvested from polluted areas can constitute a risk of infection. The largest ever reported outbreak of HAV resulting from eating of virus-contaminated shellfish took place in China in 1988 and affected almost 310 000 persons. Since then, at least a dozen outbreaks of HAV have been recognized, in which shellfish were confirmed to be the vehicle of virus transmission, as recent example occurred in the US. In this case, the outbreak-related oysters were farmed and harvested from two Louisiana harvest areas in the Gulf of Mexico. The oysters were shipped

Table 2 Examples of food-borne hepatitis A outbreaks

Country	*Year*	*Number of infected persons*	*Implicated food/ food source*	*Virus source*	*Outbreak confirmation*	*HAV strain detected*	*Reference*
Canada	2005	16	Ready-to-eat foods, leafy salads/ restaurant	Food handler	Epidemiological investigation, laboratory testing of blood samples	Genotype IIIA	Heywood *et al.* (2007)[a]
The United States	2006	16	Drinking water/ contaminated spring	Human sewage	Water testing	Subgenotype IA	Tallon *et al.* (2008)[b]
New Zealand	2002	43	Blueberries/ shop	Berry pickers	Epidemiological investigation, testing of food, blood and fecal samples	Not determined	Calder *et al.* (2003)[c]
The United States	1997	242	Frozen strawberries/ school cafeteria	Not determined	Epidemiological investigation, laboratory testing of blood and fecal samples	Not determined	Hutin *et al.* (1999)[d]
Belgium	2004	269	Raw beef/ butchery	Food handler	Epidemiological investigation, laboratory testing of blood and of fecal samples	Genotype IA	Robesyn *et al.* (2009)[e]
Italy	2002	26	Sandwiches/ delicatessen	Food handler	Epidemiological investigation, laboratory testing of fecal samples	Subgenotype IB	Chironna *et al.* (2004)[f]
The United States	2005	39	Oysters/ restaurant	Human waste	Epidemiological investigation, food testing	Genotype IA	Shieh *et al.* (2007)[g]
The United States	2003	805	Green onions/ restaurant	Field worker	Epidemiological investigation, testing of blood samples	Genotype IA Genotype IB	Amon *et al.* (2003)[h]

[a]Heywood P, Cutler J, Burrows K, Komorowski C, Marshall B, and Wang HL (2007) A community outbreak of travel-acquired hepatitis A transmitted by an infected food handler. *Canada Communicable Disease Report= Releve des Maladies Transmissibles au Canada* 33: 16–22.

[b]Tallon LA, Love DC, Moore ZS, and Sobsey MD (2008) Recovery and sequence analysis of hepatitis A virus from springwater implicated in an outbreak of acute viral hepatitis. *Applied and Environmental Microbiology* 74: 6158–6160.

[c]Calder L, Simmons G, Thornley C, *et al.* (2003) An outbreak of hepatitis A associated with consumption of raw blueberries. *Epidemiology and Infection* 131: 745–751.

[d]Hutin YJF, Pool V, Cramer EH, *et al.* (1999) A multistate, foodborne outbreak of hepatitis A. *The New England Journal of Medicine* 340: 595–602.

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[f]Chironna M, Lopalco P, Prato R, Germinario C, Barbuti S, and Quarto M (2004) Outbreak of infection with hepatitis A virus (HAV) associated with a foodhandler and confirmed by sequence analysis reveals a new HAV genotype IB variant. *Journal of Clinical Microbiology* 42: 2825–2828.

[g]Shieh YC, Khudyakov YE, Xia G, *et al.* (2007) Molecular confirmation of oysters as the vector for hepatitis A in a 2005 multistate outbreak. *Journal of Food Protection* 70: 145–150.

[h]Amon JJ, Devasia R, Xia G, *et al.* (2005) Molecular epidemiology of foodborne hepatitis A outbreaks in the United States, 2003. *Journal of Infectious Diseases* 192: 1323–1330.

to several restaurants where they were eaten in a raw state. Only one person among 38 victims admitted eating baked oysters. None of the case patients reported any other risk factors related to infection than food consumption. Oysters harvested from the same areas at the same time as oysters served to the case patients were collected for laboratory testing from a wholesaler who provided shellfish to the restaurants. Viruses were detected by reverse transcription polymerase chain reaction (RT-PCR) in the oyster meat. Hepatitis A RNA sequences derived from oysters were identical to virus sequences obtained from patients' sera. It was confirmed that all cases were due to infection by a single strain classified as

HAV genotype IA. This outbreak originated from a common source, and it is likely that this source contaminated oysters before distribution, because employees involved in shipment had minimal physical contact with the shellfish and none reported hepatitis symptoms. All implicated oysters have been harvested from approved areas remote from any fixed source of sewage discharge. Contamination of the shellfish beds by harvesters was unlikely, as they met all hygienic requirements. The probable sources of contamination were illegal waste disposal from recreational boats within legal harvest areas or illegal harvesting in closed areas.

Virus Transmission through Contaminated Water

Among other risk factors for acquiring HAV than consumption of contaminated foods is exposure to virus-polluted water. Contamination of drinking water sources can occur via improper disposal of untreated or partially treated sewage to the environment. Contamination can also be attributed to the failure in water treatment processes. Although water-borne transmission of HAV is less common compared to other virus types, there have been several HAV outbreaks documented where fecal-contaminated drinking or recreational water has led to infection and disease. For instance, a water-borne HAV transmission was recently described in China, where the outbreak was traced to a school's dining room. Cooked food was ruled out as a source of the virus, and all kitchen staff were negative for anti-HAV antibodies. The putative virus source was well water used for meal preparation and drinking. Some of the ill students admitted to drinking untreated water from the well. Unfortunately, the water source was not tested for the presence of HAV, but bacteriological analysis has revealed *Escherichia coli* contamination. Fecal contamination of the well may have potentially come from two toilets situated in close proximity to the well. No other event or food could explain the source of the disease. Molecular characterization of the HAV VP1/2A junction region showed that all individuals were infected by the same strain representing the IA genotype. Virus isolates revealed 100% homology of the analyzed sequences.

Analytical Methods for HAV Detection

Detection in Clinical Samples

High virus load in the feces of infected persons ($> 10^6$ virions/g) makes the detection of virus particles by electron microscopy possible. Although it allows identification and visualization of the viral agent in the tested sample, it is not routinely used due to its low sensitivity and labor intensity. Clinical diagnosis of HAV infection is based on serological tests, where the presence of anti-HAV IgM and/or IgG antibodies in serum can be confirmed. The concentration of the virus in blood is low, and viremia is transient compared to fecal virus shedding, so if detection of viral antigen is needed, it is performed on fecal samples using molecular tests. Apart from conventional RT-PCR, real-time RT-PCR is also used for direct virus RNA detection in a broad range of clinical and environmental samples. A novel method based on nucleic acid detection is a DNA microarray technique. It uses specific oligonucleotide probes, which can selectively bind to the target virus sequence during microarray hybridization. This method can allow identification of a single nucleotide polymorphism in the analyzed sequences; thus, virus strain, genotype, or subgenotype can be easily determined. This technique offers an alternative to sequencing of amplified PCR products and also has an advantage of being a potentially high-throughput system. A new approach for detection of hepatitis virus antigen in serum samples is integrated automatic electrochemical immunosensor array, enabling simultaneous detection of multiple viral targets in the same sample. The technology is based on immobilized specific virus antibodies on electrochemical sensor arrays. Viral antigen is captured by antibodies from a sample solution, and subsequently the potential change before and after antigen capture is measured. This assay offers simultaneous detection of five different hepatitis virus antigens in a short time as it requires only few minutes to fully analyze the sample. Moreover, it is both sensitive and cost-effective. Currently, this novel method has not been used in routine screening of clinical samples; however, it has an advantage of high-throughput technology, which could be applied in clinical diagnostics.

Infectivity and Cell Culture

Infection of cultured mammalian cells can be used for detection of HAV in clinical or food samples, but for routine diagnostics, it is impractical and time-consuming. Furthermore, wild HAV strains are difficult to adapt to grow in cell cultures, and virus presence in cells should be confirmed by another method, for example, immunofluorescence or molecular testing. Nevertheless, some strains have been successfully adapted to African green monkey kidney cells (Vero, BS-C-1, and BGMK) and to fetal rhesus kidney cell lines (FRhK-4, FRhK-6, and Frp/3). In addition, human hepatoma (PLC/PRF/5), human fibroblasts, and MRC5 human diploid lung cells may also support virus replication. It has been found that progeny virions can either remain associated with infected cells or released into culture medium without visible cell damage. If a cytopathic effect occurs, it is seen 2–14 days after cell inoculation. Successful adaptation of HAV strains to grow in cell culture is based on acquired mutations within the virus genome during growth. Two mutations are essential; one appearing in an internal ribosome entry site of the 5′-NCR and the second in the 2B and 2C region of the virus genome, coding for nonstructural proteins.

Detection in Foods

Methods to detect viruses in foods are composed of two basic parts: (1) sample treatment (preparation) and (2) detection assay. Within each part, variations can exist in application and approach, and this is reflected in methods, which have been developed and published hitherto. Sample treatment involves separating viruses from food substances. It can be performed using PEG precipitation, ultrafiltration, ultracentrifugation, or by using charged membranes or filters. An immunomagnetic separation can also be employed, where viruses are captured by antibodies coated on magnetic beads followed by their

removal from food extracts by magnetic separation. Subsequent to virus concentration, nucleic acids are extracted and purified. Detection assays are generally based on RT-PCR, with selective amplification of specific virus genomic sequences. RT-PCR has the potential for exquisite sensitivity, theoretically being capable of amplifying one target molecule in a single reaction. Different genes within the HAV genome can be targeted by RT-PCR, although the 5′-NCR region has been shown to be the most conserved among all currently isolated HAV strains and is frequently used as target sequence. A limitation of PCR-based analyses is the lack of indication as to viral infectivity. An alternative to RT-PCR is the nucleic acid sequence-based amplification (NASBA) technique, where amplification of RNA occurs through the simultaneous action of three enzymes at one temperature in a single step. The potential detection limits offered by NASBA are similar to those achieved by RT-PCR. Currently, there is no other alternative to molecular methods for virus detection in foods, offering high sensitivity and speed of analysis, and the resolution of the issue of regarding rapid infectivity may need to wait for new technological solutions.

Detection in Water

As with food samples, molecular methods are applied for the detection of viruses in water. However, water samples have to be substantially concentrated as only a small volume of sample can be used for nucleic aid extraction. There are several published methods for HAV concentration from water samples in which viruses are adsorbed to negatively or positively charged filters, glass powder, or glass fiber. In addition, viruses can be removed from water by filtration. Clogging of filters by particulate material can be a major problem. This can be overcome to some extent in tangential flow ultrafiltration systems, where a continuous flow of suspension is passed across the membrane, and liquid passes through pores with sizes approximately 0.01 μm, whereas larger sized particles are swept across them. The system circulates the suspension, and viruses are retained in a diminishing volume of liquid until the retention volume of the system is reached.

Virus Survival in Food

There are several types of produces which are prone to virus contamination, among which leafy green vegetables, berry fruits, and shellfish are of the most concern. Despite the increasing amount of food-borne outbreaks related to consumption of virus-contaminated food, the viral agent has mostly been detected in infected individuals or clinical samples collected from the person handling the food but rarely in the implicated food produce. This may be attributed to the low virus prevalence in food tested or the low sensitivity of the method used. HAV has demonstrated the ability to survive for a few days on lettuce, strawberries, and spinach stored at refrigeration conditions. This indicates that infectious viruses can persist on vegetables for several days under conditions commonly used for storage in households. Also, the potential of HAV to survive in chilled and frozen shellfish was shown in shellfish-related outbreaks. Furthermore, commonly applied food processes such as washing, freezing, and freeze-drying are ineffective in virus inactivation; thus, if produces are contaminated, they may present a potential health risk.

Control and Preventive Measures for HAV Contamination of Foods

Currently, infection in the community caused by HAV can be efficiently controlled through immunization of susceptible individuals. Mass vaccination against HAV is not routinely conducted; however, when it is performed, it usually follows WHO recommendations. Certainly, vaccine use in some countries and improvement of living conditions in others have resulted in a declining rate of HAV prevalence. But despite that, the virus transmission patterns have not changed, with contaminated food and water still recognized as an important risk factors for acquiring the disease.

Various methods are commonly employed to eliminate microbial pathogens from foods. Heating can inactivate HAV but is not appropriate for use with foods such as soft fruit and salad vegetables. Chlorination is in general use in the fresh produce industry, but the conditions employed may not be completely effective against HAV. Gamma irradiation is effective against HAV and other enteric viruses, but at levels which are higher than those generally permitted for use on foodstuffs. HAV can survive freezing, with little if any loss of infectivity, as demonstrated in outbreaks, which have occurred from consumption of frozen berries. High hydrostatic pressures are effective in eliminating infectious HAV.

It is, of course, better to prevent contamination from occurring in the first place than to try to eliminate it after it has occurred. This can be achieved by adoption of appropriate control procedures in food production. Agricultural industries, such as those involved in the production of soft fruit and fresh vegetables, must be adhere to good agricultural practice in order to minimize the risk of transmission of enteric viruses. Water used for irrigation or washing must be of good sanitary quality. Food handlers should be educated about microbial safety guidelines and rules of good hygiene. Any person with symptoms of acute hepatitis (fever, headache, fatigue combined with dark urine and light stools, or jaundice) should be excluded from handling foods or from being present in areas of food production. Where children can be involved in food production, for example, in countries where hepatitis A is highly endemic, it should be kept in mind that they can be infected with HAV without showing any symptoms. Vaccination of food handlers against HAV would be an effective prevention measure, especially when safe and effective inactivated vaccines are commercially available. Unfortunately, they are not used on a large scale within the food industry. This may be partly due to incompletely justified benefits related to the use of vaccine and its high cost. However, vaccination has been used on several occasions to control outbreaks of HAV in communities. Outwith the food industry, vaccination has been recommended for individuals being at risk of acquiring infection.

With shellfish production, there are various government regulations which specify sanitary controls covering several

areas such as the quality of the shellfish-growing waters, processing, and marketing of the food products; although these have been mainly developed with bacterial contamination in mind, they could be revised to take account of viruses. Depuration is often used to reduce microbial contamination but may not be entirely effective in removing viruses.

As the role of viruses as major agents of food-borne disease is becoming more widely recognized, there are consequent international efforts aimed at tackling the problem of contamination of foods by pathogenic viruses. For instance, the Codex Alimentarius Commission Committee on Food Hygiene is currently developing guidelines on the control of viruses in food. The development of virus-specific hazard analysis and critical control point (HACCP) plans would be welcome, and this is underway in European Commission supported research toward integrated monitoring and control of viruses in food supply chains. As a major food-borne pathogen, HAV is specifically included in all these activities, the outcome of which should help to reduce the threat to public health that contamination of foods with this virus causes.

See also: Disciplines Associated with Food Safety: Food Microbiology; Food Virology. Food Safety Assurance Systems: Personal Hygiene and Employee Health. Foodborne Diseases: Overview of Biological Hazards and Foodborne Diseases. Institutions Involved in Food Safety: FAO/WHO Codex Alimentarius Commission (CAC); World Health Organization (WHO). Public Health Measures: Safe Use of Wastewater for Agricultural Production; Surveillance of Foodborne Diseases. Safety of Food and Beverages: Seafood; Water (Bottled Water, Drinking Water) and Ice

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The Centers for Disease Control and Prevention.

www.who.int
The World Health Organization.

Hepatitis E Virus

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Glossary

Asymptomatic Displaying no signs of disease.
Autochthonous Indigenous to an area.
Cell culture Animal cells grown *in vitro*.
Endemic A disease found commonly throughout a particular area.
Seropositivity Having antibodies against a specific agent.
Seroprevalence The proportion of individuals in a population that have antibodies to a specific agent.
Zoonotic Capable of transmission from animals to humans.

Introduction

Hepatitis E is a major cause of waterborne acute hepatitis in developing countries. The disease is endemic in parts of the world with poor sanitation and hygiene, where it occurs in large outbreaks. The first reported outbreak of hepatitis E occurred in Delhi, India, in 1955 and 1956, although it was not recognized as such until the 1990s, when advances in molecular diagnostics and immunodiagnostics made it possible to identify the viral agent. In developed countries, the disease was initially thought to be related only to travel in endemic areas. However, over the past few years a number of cases of locally acquired hepatitis E have been reported in Europe, USA, and Japan and evidence of an animal reservoir have been discovered. Routes of transmission of hepatitis E in developed countries are still unclear, but several cases of foodborne transmission have been reported.

Characteristics of Hepatitis E Virus (HEV)

HEV, previously classified as a member of the Caliciviridae family, is currently, due to differences in genome organization and nucleotide sequence, provisionally classified in a separate genus, *Hepevirus*, of the family Hepeviridae. HEV is a non-enveloped, single-stranded ribonucleic acid (RNA) virus. Virion particles are approximately 27–34 nm diameter, with an icosahedral protein shell or capsid, enclosing a linear 7.5 kb RNA genome. All identified strains of HEV have been classified into four genotypes (1–4), which are distributed by geographic region. In contrast to what is seen in endemic areas in Asia and Africa, where the infecting viruses belong to genotypes 1 and 4, indigenous infections observed in the USA and Europe to date are almost exclusively caused by genotype 3 strains. Despite the presence of four different genotypes only a single serotype is recognized.

The infectious dose of HEV is currently unknown. After entry by the oral route, the virus is passed through the intestinal tract, where it probably replicates. The virus then passes to the liver, and after replication it is released into the bile and blood by mechanisms that are not understood. HEV replication in the liver results in damage to that organ, but the pathogenesis and mechanisms of liver injury during HEV infection are not still explained; however, immune mechanisms are probably involved.

The incubation period of hepatitis E ranges from 15 to 60 days. Virus particles can be found in the bile and feces of an infected person during the late incubation phase, and up to 2 weeks after the onset of clinical disease. The clinical symptoms of the diseases in most cases are very similar to those reported during hepatitis A. During acute HEV infection, the case-fatality rate in the general population is 0.2–1%. The case-fatality rate in developed countries is higher, ranging from 8% to 11%. The most susceptible individuals in developing countries are young adults and pregnant women and the case-fatality rate during pregnancy approaches 15–25%. In developed countries the infection seems to be more prevalent in the middle-aged and elderly men, and is normally lethal in patients with underlying chronic liver disease.

Epidemiology

The most common route of transmission of hepatitis E is consumption of contaminated drinking water, whereas person-to-person transmission is rare. The occurrence of hepatitis E is highest in central and southeast Asia, North and West Africa, and Mexico, areas where fecal contamination of water

is common, and seroprevalence in populations from endemic regions ranges between 3% and 26%. Outbreaks involving several thousand cases have been recorded in countries such as India, Myanmar, and China. In North America and Europe, cases of hepatitis E are uncommon, although seroprevalence ranges from 1% to 5%, suggesting circulation of HEV within the populations there. In industrialized countries, most of the cases are related with travel in endemic areas, but several cases of autochthonous hepatitis E have been reported.

Anti-HEV antibodies have been identified in several animals including chickens, pigs, wild boars, deer, cats, dogs, mongooses, horses, cattle, sheep, and rodents. In some of the animal species positive for anti-HEV antibodies HEV RNA has also been identified. Strains circulating in animals show a high nucleotide identity with human strains identified in the same area, although comparisons are related to short regions of the viral genome only. High levels of HEV infection in pigs are reported from several countries all over the world, although the infection appears to be asymptomatic. HEV strains with very similar RNA sequences have been detected in pigs and humans, which prompts concern over the potential extent of zoonotic transmission of the virus through consumption of contaminated pork products.

Outbreaks of Foodborne Hepatitis E

There have been a few reports of foodborne outbreaks of hepatitis E. However, recent studies have indicated that consumption of contaminated and undercooked meats from infected pigs, wild boars, and deer may be a risk factor for acquisition of this disease. Several studies have suggested that consumption of undercooked pig liver and wild boar meat may have been the cause of some cases of hepatitis E in Japan. Wild boar liver is also often eaten raw in Japan, and this has also been linked to some hepatitis E cases. In Bali, raw pig meat or fresh pig blood is consumed, and seropositivity to HEV is relatively high in the human population. In a case of hepatitis E in the UK, which was caused by an HEV strain very similar to pig strains, the patient had admitted to eating raw pork products, although this was not conclusively the cause of the infection. In USA, 11% of the retail livers tested were positive for HEV RNA and, when inoculated into HEV-free pigs they were able to infect the animals, implying the survival of the virus under storage conditions. The Third National Health and Nutrition Examination Survey in the USA showed that HEV seropositivity was associated with consumption of liver and organ meats. Recently, consumption of shellfish has been indicated as a possible cause of an HEV outbreak on a cruise ship, the shellfish possibly being contaminated by filtrating contaminated water. Consuming uncooked shellfish has also been shown as a possible risk factor of acquiring the infection in other studies.

HEV-positive livers have been shown to still be infectious after incubation at 56 °C for 1 h, but they were unable to infect pigs after being stir-fried at 191 °C (internal temperature of 71 °C) for 5 min or boiled in water for 5 min. This demonstrates that normal cooking procedures can inactivate the virus, but that livers need to be cooked thoroughly to decrease the risk of potential foodborne transmission.

An apparent association with eating raw liver sausage (*figatellu*) typical of Corsica has been reported for eight autochthonous hepatitis E cases diagnosed since 2007 in France. In his Promed contribution this author also remarked that genotype 3 HEV sequences were recovered from seven *figatelli* purchased in supermarkets in southeastern France, finding genetic links between these sequences and those recovered from patients who ate raw *figatellu*.

Detection Methods for HEV in Foods

Detection of HEV in foodstuffs is carried out mainly through qualitative or quantitative polymerase chain reaction (PCR). Extraction methods and detection protocols can vary significantly, and no gold standard has yet been defined for HEV diagnosis. PCR-based methods do not give information on the residual infectiousness of the virus in foodstuffs, and often detection of HEV RNA is not associated with the presence of infectious virus particles. Experimental infections have been performed demonstrating that the virus can remain infectious in foodstuffs. Cell culture replication of HEV has been reported, but to date there is no routine method available for the detection of infectious virus. Development of such a routine method would be very useful for detection of infectious virus, and could be established as the gold standard to test foodstuffs.

Lack of Information

The discovery of HEV strains in pigs related to human strains is potentially significant with regard to the possibility of interspecies transfer and zoonotic infection. Pork products are usually thoroughly cooked at temperatures that should inactivate viruses, but a potential for zoonotic foodborne transfer of HEV may lie in environmental contamination via manure from infected pigs. HEV may be widespread in the general pig population, and if so, it is possible that much of the pig manure, which is stored on farms and subsequently spread onto agricultural land as fertilizer, could contain infectious HEV particles. This could result in exposure of the human population. It may be informative to study prevalence and survival of HEV in the environment and on crops and foods, and also to develop methods to detect interspecies transfer at an early stage.

The incidence of HEV infection in developed countries might be underestimated. The serological data available are as high as 16% for the UK blood donor population and 21% in USA population and are not explained by the relatively low number of autochthonous cases of disease reported. It is likely that the cases of hepatitis E are underreported, considering that they can occur either without any or with nonspecific symptoms. It has been hypothesized that HEV causes clinical symptoms in a dose-dependent manner, and probably individuals in developed countries are exposed only to low doses of the virus. Information still needs to be gathered to clarify the routes of transmission of the nonwaterborne cases of HEV, and the extent of the foodborne transmission in developed countries.

Finally, long nucleotide sequences spanning the entire genome of genotype 3 HEV strains of human and animal origin are needed to understand to what extent swine viruses are in fact representing a real risk of hepatitis transmission to humans via either zoonotic or foodborne transmission pathways.

See also: Disciplines Associated with Food Safety: Food Virology. Food Safety Assurance Systems: Personal Hygiene and Employee Health. Institutions Involved in Food Safety: Trust in Animals and Food Safety (TAFS) Forum. Safety of Food and Beverages: Meat and Meat Products; Water (Bottled Water, Drinking Water) and Ice

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www.medvetnet.org
Med-Vet-Net Association.

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Lassa Fever Virus

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Glossary

Contamination The introduction or occurrence of a contaminant in food or food environment.
Control measure Any action and activity that can be used to prevent a safety hazard or reduce it to an acceptable level.
Disinfection The reduction by means of chemical agents and/or physical methods of the number of microorganisms in the environment to a level that does not compromise food safety or suitability.
Endemic A temporal pattern of disease occurrence in a population with predictable regularity and only minor fluctuations in its frequency over time.
Hemorrhage Instance of sudden bleeding in large amounts.
Infectious A disease capable of being transmitted from person-to-person with or without actual contact.
Mortality rate The number of deaths in a specific period of time.
Pathogen An organism capable of causing disease.
Virus An intracellular parasite that cannot be seen with the aid of light microscope but only with electron microscope.

Background

Lassa fever virus is a highly contagious level IV pathogen which has been implicated in causing the zoonotic disease commonly called Lassa fever or Lassa hemorrhagic fever. Typical clinical symptoms of Lassa fever were first described in Sierra Leone in the early 1950s, unfortunately, little or no attempt was made then to identify the causative agent. However, barely 20 years later, to be precise, in 1969, a similar disease which claimed the life of two missionary nurses occurred in Nigeria. Laboratory investigations of pathological specimens from victims of the Nigerian episode implicated a virus as the causative agent. The name of the isolated virus was coined after the small village of Lassa in the Yedseram River valley in Borno State, North Eastern Nigeria, where the outbreak of the case that led to isolation and subsequent identification of the virus took place. The virus was identified as an arenavirus and classified into the arenaviridae family. Since then, several other outbreaks have been reported in many towns and villages across various states in Nigeria, including the Plateau State capital Jos 1970. Others with outbreaks are Zonkwua from 1974 to 1977, Abo Mbaise and Owerri, a town and Imo State capital, respectively, in 1985; Ekpoma, a town in the Delta State, during 1990–92 and 2012; Lafiya, Nasarawa State capital in 1992 and 2012 and Abakaliki, Ebonyi State capital in 2005, 2007, 2009, 2011, and 2012, Lagos in 2012, Ondo in 2012, and Port Harcourt, River State Capital, in 2012.

In 1970, outbreaks of Lassa fever were reported in Liberia and Sierra Leone. Epidemics of the disease have also been documented in other West African countries including Senegal, Guinea, and Mali. Other African countries such as Central African Republic and Congo Democratic Republic have had their own share of Lassa hemorrhagic fever outbreaks.

Interborder traffic and international travels have resulted in the export of Lassa fever from endemic areas to other parts of the world. The affected countries are the USA, Canada, UK, Israel, Japan, and Netherlands.

The treatment of Lassa hemorrhagic fever with ribavirin was initiated in 1979. Since then, the drug has been found to be highly effective and used extensively to save hundreds of thousands of individuals infected by the virus across the world.

Characteristics

Lassa virus is spherical in shape and is a medium-sized agent that measures between 70 and 150 nm in diameter (Figure 1). It has been classified into the arenaviridae family. A typical mature Lassa virus particle possesses a glycoprotein envelope with T-shaped spikes measuring 7–10 nm on its surface. The envelope encloses a helically coiled nucleocapsid genome which measures between 400 and 1300 nm in length. The genome of the Lassa fever virus is a single-stranded, bisegmented ribonucleic acid (RNA). As a typical arenavirus, it lacks the conventional negative-strand coding arrangement. The interior part of the virus contains electron-dense granule identified as the host cell ribosome from where the name 'arena' meaning sandy was derived.

The genome consists of a small (s) and a large (l) RNA fragment of 3.4 and 7 kilobases, respectively. The small RNA (sRNA) fragment encodes the viral structural proteins like

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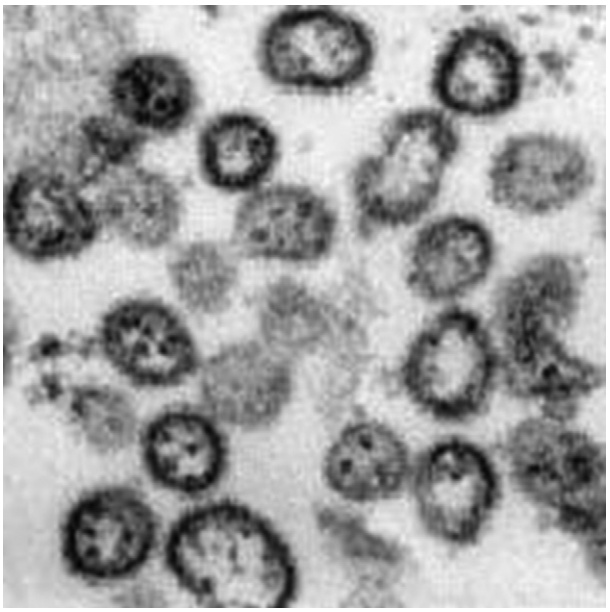

Figure 1 Lassa virus electron micrograph. Image courtesy: C. S. Goldsmith and M. Bowen, Centre for Disease Control and Prevention, Atlanta, USA.

glycoprotein, precursor protein, and the nucleoprotein. The large RNA fragment on its own encodes for nonstructural proteins including viral polymerase and a small zinc-binding (Z) protein. The sequencing of Lassa virus sRNA has enabled the identification and molecular characterization of four Lassa virus strains. These are the Josiah strain from Sierra Leone, two strains from Nigeria, including strain LP and strain Nigeria. The fourth strain designated as AV was exported to Germany by a traveler who had visited Ghana, Côte d'Ivoire, and Burkina Faso. RNA sequencing of the Lassa virus revealed a relatively genetic variation among the circulating strains, however, strain AV appears to be closely related to strain Josiah.

Lassa fever virus can be inactivated by exposure to ultraviolet light, gamma irradiation, or ultrasonic vibration. The effect of these physical factors on the virus depends on the exposure time. Nevertheless, the virus is stable at room temperature and pH range of 5.5–8.5, but heating at 50, 56, 60, and 100 °C inactivates the pathogen within an hour, 30, 15, and 2 min, respectively. Also, the exposure of Lassa virus to acidic and basic solutions above pH 5.4 and 8.6 levels neutralizes its infectivity. Chemical agents like 0.5% sodium hypochlorite, 0.5% phenol, and 10% formalin can kill the virus. This forms the basis of using these agents as disinfectants for cleaning the contaminated surfaces.

Pathogenesis

Rodent rats, particularly the *Mastomy natalensis* sp., commonly found in large numbers in the savannah grasslands and tropical and subtropical forests of West Africa are known to carry Lassa fever virus throughout their life without developing clinical symptoms. Accordingly, the asymptomatic rodent carriers can excrete the virus in their urine, saliva, respiratory secretions, and the exposed blood vessels on anything including uncovered food, food products, meat and its products on contact with any of these.

During the dry period of the year, bush burning usually drives rodents to peoples' homes and grain stores. As the rats search for food, the infected ones among them, can contaminate exposed food, food products, grain, and other materials. Consumption of such food or grains raw, or when not properly cooked causes Lassa hemorrhagic fever.

Eating raw grain, a common practice used for quality testing of stored grains by traders is a possible source of transmission when such products are contaminated.

Hunting and subsequent consumption of rat meat as a source of animal protein for some families is another possible means of contracting the Lassa fever virus. Consequently, the virus has been confirmed to be transmitted in humans through the feco-oral route.

Furthermore, in dirty unhygienic houses infested with rodents, the legs of persons who sleep in such environments are sometimes nibbled by the rats and the infected ones among them can transmit the virus to such persons. Other possible means of contracting Lassa hemorrhagic fever are by inhalation of Lassa virus-contaminated air droplets or sexual intercourse with infected person(s) during the early stage of the infection called the incubation period. In medical practice, use of contaminated medical equipment and needles without adequate sterilization can also transmit the virus.

At the point of entry, the virus undergoes primary replication, before being disseminated through the blood stream, lymph vessels, and other body fluids to multiple organ–system complexes including the reticuloendothelial system for further replication. When the number of virus progeny has approached the infectious dose (population of the virus that can illicit disease), it then causes clinical illness. Infected cells of the intestine, spleen, kidney, liver, myocardium, lungs, and brain are often inflamed, enlarged, ballooned, and the tissues are then infiltrated, resulting in lesions and necrosis.

As a result of this massive destruction of infected cells and tissues, capillary permeability is increased, followed by peripheral vasoconstriction in the presence of disseminated intravascular coagulation that leads to hemorrhagic syndrome. The activities of the virus in the infected cells, tissues, and organs impair their physiological functions which suddenly results in the clinical illness called Lassa fever.

Replication

Replication of Lassa fever virus forms the basis of its pathogenesis. The process starts with adsorption of the virus on widely distributed and highly conserved cell-surface receptor molecules (**Figure 2**). The glycoprotein of the spikes is responsible for the virus–cell interactions. The other steps are penetration, removal of the viral envelope, and liberation of viral RNA into the infected host cell cytoplasm.

Thereafter, duplication of RNA, transcription of mRNA, and translation of proteins take place also in the cytoplasm. During these processes, the cell nucleus provides capped cellular mRNA for priming transcription, whereas the nuclear membrane provides structural support.

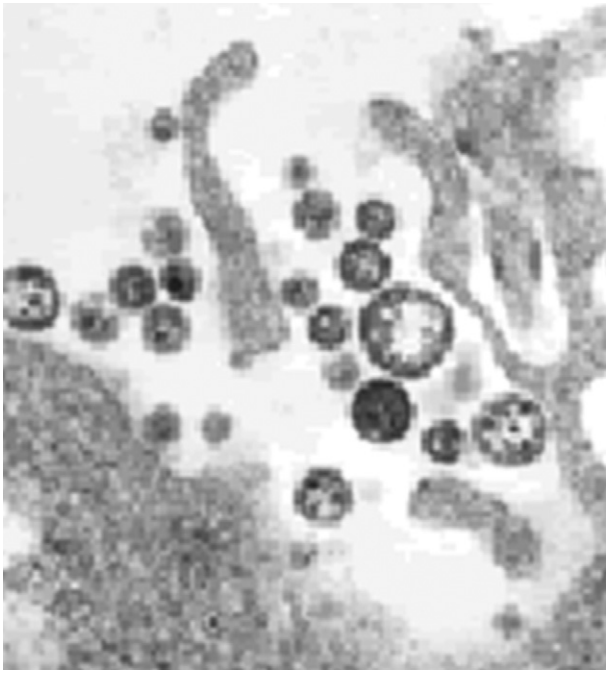

Figure 2 Lassa virus adsorption on cell surface. Image courtesy: C. S. Goldsmith and M. Bowen, Centre for Disease Control and Prevention, Atlanta, USA.

The initiation of replication and transcription starts from the terminus of the template. As the RNA polymerase rails on the template to add new nucleotides that will form the polynucleotide of the new strand, the first two slip back on the template to create nontemplate nucleus, a process peculiar to arenaviruses. After the biosynthesis of macromolecules, the viral progenies are assembled through a process yet to be fully understood. Mature viruses are then released through budding from the plasma membrane of acutely infected cells.

Clinical Manifestation

The incubation period of Lassa hemorrhagic fever is between 7 and 21 days. At onset, the infection is insidious with fever, headache, rigors, myalgia, backache, and malaise. The body temperature may increase to 40 °C, which is usually higher in the evenings than mornings. Being a generalized infection, clinical symptoms of Lassa hemorrhagic fever manifest in every system of the infected individual. The symptoms of respiratory tract involvement include: cough, chest pain, dyspnea, pharyngitis, and pleuritis.

In the oral cavity, ulcerated lesions with white or yellow exudates are present in the soft plates and tonsils. Other symptoms of gastrointestinal tract include difficulty in swallowing (dysphagia), nausea, blood-stained vomit which later turns black, bloody and watery diarrhea that may lead to dehydration, abdominal pain, constipation, as well as hepatitis.

In the cardiovascular system, symptoms like pericarditis, tachycardia, bradycardia, hypertension, hypotension, thrombocytopenia, leukopenia, uremia, depressed lymphocyte counts, and platelet function have been reported. Also lymphoadenopathy; elevated aminotransferase; decrease in prothrombine level; distress of blood circulation; and bleeding through the skin, lungs, gastrointestinal tract, and other mucous membranes have been recorded.

Symptoms of the central nervous system being affected are aseptic meningitis, encephalitis, global encephalopathy with seizures, cerebella ataxia, though not very common, and unilateral or bilateral hearing defect or seizure.

The liver and kidney are often inflamed, enlarged, and are painful on palpation of their respective locations.

The neck is swollen due to inflamed lymph nodes. On the skin are generalized maculopapular rashes. Capillary lesions cause hemorrhage in the stomach, small intestine, kidney, lung, and brain. This can be a prediction of the severe case that leads to death as a result of shock and vascular collapse. During the convalescence period, the virus may no longer be found in the blood samples of some patients.

Diagnosis

Accurate diagnosis of Lassa fever requires a complex approach, taking into consideration the clinical manifestation, epidemiological data, and result of laboratory findings.

The clinical symptoms of Lassa fever can easily be mistaken with other common tropical infections such as severe malaria, typhoid fever, yellow fever, Ebola virus infection, hemorrhagic conjunctivitis, and other viral hemorrhagic fever diseases. As a result, epidemiological parameters have to be taken into account, this including presence of rodent in the area, whether the person has traveled to an endemic place, during what period of the year has there been any contact with somebody with similar illness, if yes, when and how, to mention a few.

Laboratory diagnosis is used for definitive diagnosis. Lassa fever is diagnosed in the laboratories by detecting specific Lassa virus antigens or antibodies in pathological specimens. Specimens for laboratory analysis should be collected as soon as possible from the patient suspected of having the infection. Subsequently, manipulation of suspected Lassa fever virus-infected materials from human and rodent is required to be done in highly specialized biosafety level IV laboratory. Unfortunately, such laboratories are rarely available in poverty-stricken endemic countries, nevertheless, efforts have to be made to send collected specimens to laboratories with adequate facilities for processing and testing highly contagious materials.

The specimens to be collected include patients' blood, urine, pleural fluid, cerebrospinal fluid, throat swab, and in case of death, pathological materials from liver, kidney, spleen, and heart. The same samples should be collected from any available rodents.

In the laboratory, the virus can be isolated using laboratory animals including albino mice, guinea pigs, Vero cell culture, or African green monkey kidney cell culture. Albino mice inoculated intracerebrally die between 3 and 5 days after infection. Lassa fever virus causes conspicuous and total cytopathic effects on confluent monolayer derived from Vero cell culture within 96 h after inoculation. Further, the virus can be seen under electron microscope using specimens obtained from infected persons or infected rodents.

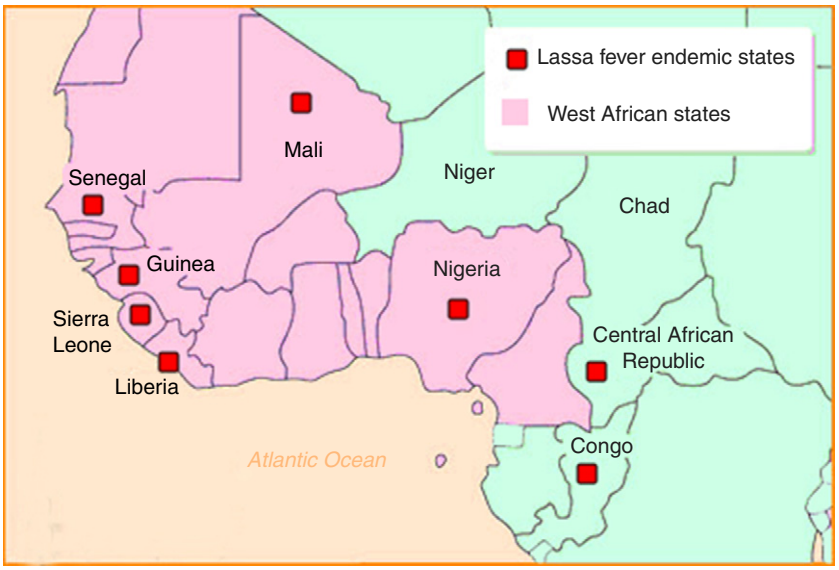

Figure 3 Map of the West African subregion showing Lassa fever endemic countries.

Virus antigens are detectable by enzyme-linked immunosorbent assays (ELISA) using Lassa virus-specific antibodies. The test is easy to handle and rapid, and can be performed with inactivated specimens, which is advantageous in many health institutions where sophisticated equipment is not available.

The indirect fluorescent-antibody (IFA) test has been employed in the laboratory diagnosis of acute Lassa virus infection. The interpretation of IFA results is complicated by the presence of IFA antibodies during both the acute and convalescent stages of infection. The appearance of IFA antibody early in the course of Lassa virus infection is always useful in identifying patients with poor prognosis.

A comparative study of ELISA sensitivity and specificity with a positive reverse transcription-polymerase chain reaction (RT-PCR) test using Lassa virus suspected specimens was carried out. The results proved that the combined ELISA Ag/IgM assay was highly sensitive and specific for the diagnosis of Lassa fever and the antigen detection assay. It therefore offers a unique advantage in providing early diagnosis as well as prognostic information.

Although the RT-PCR assays are higher in sensitivity, their applicability in the West African countries is limited due to the fact that the machines and other facilities are readily available. Further, another valuable diagnostic tool for the detection of Lassa fever virus is the rapid diagnostic immune blot assay but its limitations include low sensitivity and lack of the capacity to provide prognostic information.

Epidemiology

Lassa fever virus was first isolated in Nigeria after an outbreak in a small village called Lassa in Borno State, North Eastern Nigeria. Between 1969 and 2012, several other cases that claimed many lives including those of medical doctors and nurses have been reported in many towns and villages across Nigeria.

In the same vein, series of outbreaks have been recorded in other West African countries including Sierra Leone, Ivory Coast, Liberia, Guinea, Senegal, and Mali. Also the infection has been reported in Central African Republic and Congo Democratic Republic. Documented reports have revealed that between 300 000 to 500 000 cases of Lassa fever resulting in 5000 deaths occur yearly in West African countries (Figure 3).

It has been postulated by the author that the illness may be one of the infectious diseases responsible for many mysterious, uninvestigated, and unreported sudden deaths in many parts of Nigeria and other African countries. Most of the deaths are often believed by many naive individuals to be caused by evil persons (doers) who employ African witchcraft, also called 'African remote control' to kill people, especially those excelling in business and other professions.

Lassa fever has been exported from endemic areas to other parts of the world including USA, Canada, UK, Israel, Japan, and the Netherlands.

The virus is hosted by multimammate rodent rats, specifically the *M. natalensis* sp., which are commonly found in large numbers in the savannah grassland and tropical and subtropical forests of West African. Infected rodents remain carriers of the highly virulent and contagious virus throughout their life without developing clinical symptoms but excrete the virus in their urine, saliva, respiratory secretions, and also through exposed blood vessels. Humans become infected through contact or consumption of food contaminated with infected rodents' excretions or secretions or still eating undercooked or uncooked infected rat meat. Other possible means of contracting the Lassa virus infection are by inhalation of contaminated air droplets containing the virus, use of contaminated medical equipment and needles without adequate sterilization.

Person-to-person transmission of Lassa fever virus can occur through contact with the virus in blood, tissues, secretions, excretions, bruised skin, and sexual intercourse with infected persons. The virus can be isolated in the blood, feces,

urine, throat swab, vomit, semen, and saliva of infected individuals who continuously excrete and secrete the virus for 30 days or more. However, the virus cannot spread through casual contact of intact skin-to-skin.

Outbreak of Lassa hemorrhagic fever can take place at any time of the year. However, in West African subregion, the outbreak is more common during the dry season (from October to early April). During the period, rampant bush-burning practices drive the rodents to people's homes. It is also a time of harvest and as such attracts vector rodents searching for food in grain stores and human habitats. The infected rodent that found itself in the homes can contaminate exposed food, food products, grain, and other materials, as shown in Figure 4. Consumption of such food or grain raw may cause Lassa hemorrhagic fever. Further, eating raw grain, a common practice used for quality testing of stored grain by dealers is also a possible source of transmission. Also, abject poverty among the populace of most West African communities, subjects children between the age of 7 and 15 years and even adults to hunt for rats (a source of animal protein) for family consumption and income generation.

When infected rodents are caught and eaten raw or undercooked, it could cause Lassa fever. Further, a bite from infected rats which share dirty unhygienic houses (common site in endemic environment) with humans can be the source of transmitting the virus to humans.

Lassa virus can always be transported from an endemic area to other Lassa fever-free environments by infected persons during the incubation period or through export of contaminated grains with rodents' excretions and secretions. Still infected persons during periods of viral incubation, who work at food processing industry stand the chance of contaminating food products.

Lassa fever virus infects all age groups and both sex. Individuals at great risk are rural dwellers living in poor sanitation and crowded conditions where the vector rodents *M. natalensis* are usually found. Other persons at risk are close relatives, sympathizers, and care givers who habitually keep close contact with loved ones irrespective of the nature of illness, a common cultural practice in the African setting. Health care workers are not spared if proper barrier nursing and infection control measures are not instituted and maintained.

Figure 4 Rodent rat in food remnants in a typical kitchen. Photo by O. Ogbu and I. Ogbu, Department of Applied Microbiology, Faculty of Biological Sciences, Ebonyi State University, Abakaliki, Nigeria.

Approximately 15–20% of patients hospitalized for Lassa fever die from the illness, whereas, approximately 80% of human infections with Lassa virus are mild or asymptomatic. The prevalence of antibody to Lassa virus varies from 7% in Guinea, 15%, 20%, and more than 20% in Sierra Leone, Liberia, and Nigeria, respectively. Lassa virus causes high mortality in pregnant women and developing fetuses, with mortality rates of 92%, and 75% for fetuses in early pregnancy and third trimester, respectively. Lassa fever has been implicated in 100% mortality in the neonatal period, for full-term babies, whereas mortality rate for gravid women is 7% in the first two trimesters, and 30% in the last trimester, as against average mortality rate of 13% for nonpregnant women. High concentrations of the virus have been found in both fetal tissues and placenta. It has been postulated that maternal T-cells cannot attack Lassa virus in the placenta because of the inability of placental cells to express class I or II major histocompatability complex antigens.

Control and Preventive Measures

In West African towns and villages where there are no facilities for laboratory diagnosis, most Lassa fever infections are treated as malaria. As the patient's condition deteriorates, other supportive medications including fluid replacement, blood transfusion, administration of paracetamol, and broad spectrum antibiotics among other drugs are then initiated. However, very few experienced physicians can empirically start ribavirin therapy while waiting for laboratory results. Early administration of ribavirin is very effective for the treatment of Lassa fever.

Lack of facilities to confirm diagnosis has necessitated that all assays are done in either Europe or the US. By the time the results are sent back to the affected area, irreversible complications might have developed in the first group of patients. Nevertheless, such results are helpful for postprophylactic exposure.

Primary transmission of the Lassa virus to humans can be achieved by avoiding contact with mastomy rodents, especially in disease endemic areas. Food must be kept away from rodents and the premises constantly cleaned to prevent vector rodents from entering homes. Enlightenment campaign against bush burning and rat hunting for eating must be intensified in the endemic areas. Trapping and use of rat poisons are effective in an attempt to reduce rodent populations. Still, storage of grains in modern silos will also help to prevent contamination of stored grain by infected rodents.

Absolute care must be taken while caring for infected patients to avoid further transmission of the disease through person-to-person contact or nosocomial routes. Precautions like isolating infected persons, instituting strict barrier nursing, wearing protective clothing, such as masks, gloves, gowns, and goggles, as well as adequate sterilization of equipment must be maintained until the disease runs its course. Body fluids, excreta, food, and other materials that might have been

contaminated should be handled carefully and disposed properly by burning. All instruments used on the patient, except disposable must be subjected to autoclaving immediately. Absolute care must be taken when collecting and transporting pathological materials to the laboratory for investigations. Correct procedure for the manipulation of materials suspected to contain highly virulent Lassa fever virus have to be observed.

All those who had contact directly with suspected Lassa hemorrhagic fever patients have to be traced, monitored, and specimens from them should be collected for laboratory investigation. Those who test positive have to be isolated and treated as soon as possible with ribavirin.

Overcoming the scientific, political, and economic obstacles in producing a vaccine for human use is very important at this critical period as the outbreak is on the increase. Adequate funding and applications of new vaccine technologies will no doubt stimulate the production of a vaccine for clinical trials.

Conclusion

From this overview, it is unequivocally established that Lassa fever is a very important vectorborne disease that has assumed epidemiological proportions in West Africa where it is highly endemic. The public health implication of the disease cannot be overstated. Apart from possible periodic outbreaks of Lassa fever epidemic within the region, the unprecedented increase in interborder traffic and international travels elevate the chances of introducing the virus to other regions within and outside the African continent.

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Nipah Virus

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Glossary

Date palm sap Sap of the tree *Phoenix sylvestris*. It is collected during winter in Bangladesh by shaving the bark from the top of the tree and directing the sap flow to collection jars overnight. It is either drunk raw or made into molasses.

Ephrin B-2 receptor A transmembrane protein on endothelial cells that Nipah virus G glycoprotein attaches to before entering the cell.

Porcine respiratory encephalitis syndrome The clinical illness associated with Nipah virus infection in pigs characterized by trembling, muscle spasms, leg weakness, uncoordinated gait, and frothy salivation.

Pseudovirus A virus constructed in the laboratory by melding characteristics of natural viruses to permit evaluation of specific viral structures and characteristics.

***Pteropus* bats** Large old world fruit bats, includes 59 recognized species. These bats are important pollinators and seed dispersers, but are also hunted for their meat and in many areas considered as an agricultural pest.

Background

Nipah virus was first identified in a large outbreak in Malaysia from September 1998 through May 1999. On 1 March 1999 Kaw Bing Chua, a medical virologist with the University of Malaya, isolated a novel virus from the cerebrospinal fluid of a patient with fever and mental status changes who lived in Sungai Nipah village. Outbreak investigators ultimately identified 276 human infections and 105 deaths from Nipah virus during this first recognized outbreak.

Characteristics

Nipah virus is a member of the family Paramyxoviradae. It is closely related to Hendra virus, which has caused sporadic infection in horses and persons who come in contact with sick horses. Together, Hendra and Nipah viruses make up the genus *Henipavirus*. Nipah virus is a zoonoses. Its natural reservoir are *Pteropus* bats. These bats are large old world fruit bats. Whenever *Pteropus* bats have been tested, a substantial percentage have antibodies to Nipah virus. The virus has been isolated from *Pteropus* bats in Malaysia, Cambodia, and Thailand. *Pteropus* bats infected with Nipah virus in the laboratory, similar to wild bats that are found to be infected, do not appear clinically ill. Nipah virus is rapidly inactivated by desiccation, but can be recovered after a 60-min incubation in solutions ranging from pH 3 to 11; it can survive for days in bat urine, in fruit juice, or on the surface of a cut mango. Nipah virus can infect a wide range of mammals including pigs, dogs, cats, ferrets, hamsters, African green monkeys, and people.

Clinical Manifestations

In humans, the median incubation period from exposure to virus to onset of illness is 9 days. There are three common acute clinical presentations of human infection with Nipah virus. First, Nipah virus can cause a mild illness with a few days of fever, headache, and rapid complete recovery. Second, most commonly human Nipah virus infection presents as a severe encephalitis with fever, confusion, seizures, and loss of consciousness. Third, human Nipah infection can present as a primary respiratory tract infection with fever, productive cough, and shortness of breath progressing to respiratory failure. The case fatality rate among humans with Nipah virus infection has ranged from 39% in Malaysia where one-half of patients received mechanical ventilatory support to 73% in Bangladesh where mechanical ventilation is largely unavailable. Two-thirds of Nipah patients in Bangladesh presented with cough and developed respiratory difficulty compared with only 14% of patients during the Malaysian outbreak reporting cough on presentation. Eight percent of human Nipah virus infection survivors in Malaysia and 18% in Bangladesh developed recurrent central nervous system infection several months or years after the initial infection. The recurrent encephalitis typically presented with new central nervous system deficits. One-third of survivors of Nipah infection have long-term neurologic deficits. These deficits are more common among patients who suffered symptoms of acute encephalitis.

Virulence Factors

The Nipah virus G surface glycoprotein attaches to the ephrin B-2 and ephrin B-3 receptors and triggers the Nipah virus F

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surface glycoprotein-mediated fusion with the target cell. The ephrin B-2 and B-3 receptors have remarkably similar amino acid sequence homology among animals susceptible to Nipah virus infection. The receptor is commonly expressed throughout the human body including capillaries, arterial endothelial cells, smooth muscle cells, and neurons including neurons of the brainstem. The ubiquity of the ephrin B-2 receptor explains both the wide species susceptibility to Nipah virus as well as its diffuse access to a wide range of organs including lung, brain, and kidney among the infected persons.

Nipah virus strains isolated in Bangladesh are somewhat different from strains isolated during the Malaysian outbreak. Among four Nipah virus isolates from human Nipah virus cases in 2004, the sequences of the nucleoprotein open reading frames of the isolates differed by 0.9% in nucleotide homology, in contrast to the sequences obtained from all of the human cases in Malaysia which were nearly identical to each other. The overall nucleotide homology between a prototypical Malaysian strain of Nipah virus and a strain of Nipah virus from Bangladesh was 91.8% . One hypothesis to explain the higher prevalence of respiratory symptoms among Nipah virus patients in Bangladesh compared with those in Malaysia is that some strains of the virus may possess unidentified virulence factors that selectively favor human pulmonary infection.

Pathogenesis

Nipah infection in humans leads to a generalized vasculitis of small blood vessels characterized by thrombosis, inflammatory cell infiltration, and parenchymal necrosis, with a particular tropism to the brainstem. Nipah virus invades a wide range of neurological cells, though initial infection is likely through endothelial cells. Pathology specimens and magnetic resonance imaging (MRI) investigations suggest widespread neuronal death from infarcts. Other commonly infected organs in humans include the kidney and lung. Nipah virus has been recovered frequently from throat swabs and respiratory secretions of acutely infected patients.

The mechanism of viral latency and recurrent or late onset Nipah encephalitis is incompletely understood, though other paramyxoviruses including measles and Hendra virus also cause recurrent progressive fatal late-onset central nervous system infection. In late-onset Nipah encephalitis, only neuronal cells are infected with virus; the vascular endothelium is not involved.

Epidemiology

Human Nipah virus infections are most commonly recognized in dramatic outbreaks where several previously healthy people living near each other suddenly developed an acute severe illness with high mortality. Human Nipah virus infections have been confirmed in Malaysia, Bangladesh, and Eastern India (Figure 1). A single large outbreak was recognized in Malaysia with no human cases confirmed since 1999. In Bangladesh, recurrent outbreaks and sporadic cases of human Nipah virus infection have been recognized in most years since the first outbreak was confirmed in 2001. Also, sporadic human Nipah infections and the index case in all

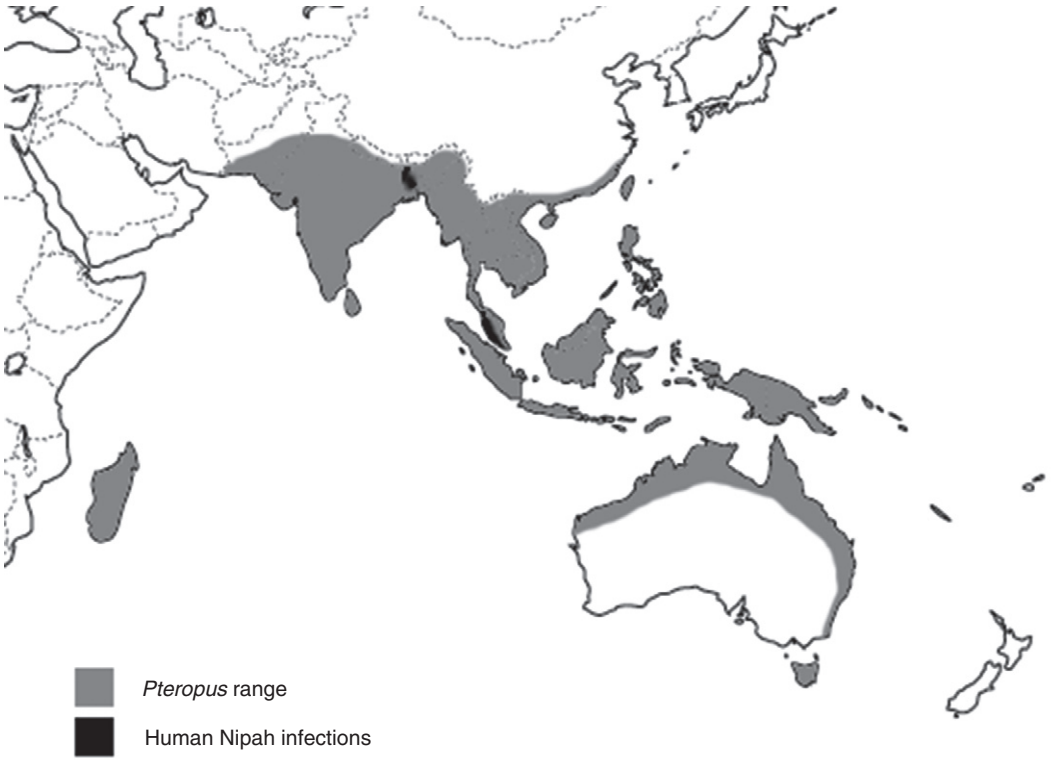

Figure 1 Range of *Pteropus* bats and location of human Nipah infections.

outbreaks had illness onset between December and April. Almost all confirmed human Nipah infections in Bangladesh were among residents of central, western, and northwest Bangladesh. The two outbreaks confirmed in India occurred within 50 km of the Bangladesh border, adjacent to the affected regions of Bangladesh.

Outbreak investigations have identified multiple pathways of transmission of Nipah virus from its bat reservoir to humans. In Malaysia, domestic pigs may have been infected after eating partially consumed fruit contaminated with bat urine or saliva. Compared with people, Nipah causes a more mild illness in pigs, porcine respiratory encephalitis syndrome, with less than 5% mortality. When pigs are infected with Nipah virus, they commonly shed the virus in their saliva, even when they are asymptomatic. The high density of commercially raised pigs in Malaysia allowed the virus to be easily transmitted from pig to pig. The steady introduction of newborn pigs into the piggeries provided a susceptible population that permitted long-term circulation of the virus. Persons who had direct physical contact with pigs including feeding, clipping tails, tagging ears, administering medications, collecting semen from boars and artificially inseminating sows, and handling sick and dead pigs were the primary victims of the Nipah outbreak in Malaysia. Abattoir workers in Singapore who slaughtered Nipah-infected pigs from Malaysia also developed Nipah infection. In Bangladesh, exposure to a herd of pigs was associated with human Nipah infection in a 2003 outbreak in Naogaon. In an outbreak in 2001, illness was associated with contact with sick cattle, and in 2004 one child developed Nipah infection after caring for a sick goat that died following an illness characterized by fever and difficulty in walking.

In Bangladesh, fruit bats commonly feed on date palm sap that is harvested from date palm trees from November through April. Night time infrared photography confirms that *Pteropus giganteus* fruit bats commonly contaminate date palm sap by licking the sap stream during collection. Fresh date palm sap is commonly consumed raw by village residents; it is a popular seasonal delicacy. The only exposure associated with illness in an outbreak in Tangail District in 2005 and in an outbreak in Manikgonj and Rajbari districts in 2008 was drinking raw date palm sap. The time of year when Nipah outbreaks have repeatedly occurred in Bangladesh is the timewhen date palm sap is harvested. The outbreaks have occurred in the regions of the country where date palm sap cultivation is most common.

In Bangladesh and India, extensive person-to-person transmission of Nipah virus infection has been identified in several outbreaks. Most of the care provided to ill patients in Bangladesh is provided by family members and friends, and includes extensive exposure to ill person's saliva including sharing food, sharing a bed, and kissing severely ill patients. People who transmit Nipah virus are more likely to have respiratory symptoms and are more likely to die. Half of recognized cases of human Nipah virus infection in Bangladesh result from person-to-person transmission.

Analytical Methods

Several laboratories including the Centers for Disease Control and the Australian Animal Health Laboratory have developed various enzyme immunoassay (EIA) tests to detect IgG and IgM antibodies to Nipah virus in people and various other animals. Seventy percent of humans who eventually develop antibody to Nipah virus have detectable Nipah virus IgM antibody on their fourth day of illness. Because of the high fatality rate, sole reliance on immunoassay for diagnosis undercounts Nipah infections.

Nipah virus grows readily in cell culture, but because of the high mortality of human infection most countries consider Nipah virus a biosafety level-4 pathogen. Thus, Nipah virus culture and serum neutralization antibody testing requires a high containment facility. Two research groups have developed a recombinant Vesicular Stomatitis Virus which expresses the F and G proteins of Nipah virus. This pseudovirus can be safely used in a biosafety level-II laboratory, has high analytical sensitivity, and provides an alternative more rapid assessment of virus neutralization.

Various polymerase chain reaction (PCR) primers have been developed to identify Nipah virus RNA in the saliva, urine, and cerebrospinal fluid from infected humans as well as in various tissues and fluids from infected animals. Both quantitative real-time PCR and conventional PCR assays have been developed by several research laboratories

Sensitive assay to assess antibodies to Nipah virus G protein using a Luminex microsphere binding assay has been developed for testing Nipah antibodies in a wide range of animals.

Control/Preventive Measures

Limiting fruit bat access to domestic animals and to the human food supply is a first step toward preventing human Nipah virus infection. Separating domestic animals from the immediate environment of trees that produce fruit that are attractive to *Pteropus* bats can reduce risk. Although it is unrealistic to expect animal handlers in regions where Nipah virus circulates to clinically distinguish a domestic animal infected with Nipah virus from much more common infections, encouraging people who handle sick animals to protect themselves from exposure to saliva and to wash their hands with soap after contact with sick animals may reduce the risk of transmission. In Bangladesh, research teams are evaluating programs to encourage the wider use of skirts made from locally available materials that prevent the access of bats to date palm sap intended for raw consumption. The large outbreak in Malaysia ended after widespread deployment of personal protective equipment to people coming in contact with sick pigs, restriction on livestock movements, and culling more than 900 000 pigs. Preventing person-to-person transmission requires developing interventions to reduce saliva exposure among people who care for seriously ill patients. In outbreak investigations of person-to-person Nipah transmission, washing hands with soap after contact with Nipah patients has been associated with a reduced risk of the virus transmission.

Research Needs

As Nipah virus is recently discovered, there are a number of important unanswered research questions in basic science,

diagnostic development, clinical care, epidemiology, and prevention. In the basic sciences, research priorities include understanding the early events of Nipah virus infection and immune evasion, identifying determinants for virulence and host susceptibility and clarifying the mechanism of viral persistence, and reactivation of latent Nipah virus infection. Priorities for diagnostic research include developing a general *Henipavirus* PCR that targets highly conserved genetic targets within the henipavirus and so will detect Nipah virus and other related viruses which may also be human pathogens, and developing recombinant enzyme immuno assay antigens to standardize serological diagnosis.

Research on clinical care should focus on identifying practical interventions for low-income countries that can reduce the very high case fatality rate and rate of neurological sequelae. Priorities for epidemiological research include describing the transmission dynamics of Nipah virus in *Pteropus* bats and the relationship of this transmission to human infections, to identify additional pathways of transmission from bats to humans, and to describe the geographical extent of human Nipah infections and, in the process, to better understand why this pathogen which is widely distributed in bats is observed in humans in more restricted locations.

Priority areas for prevention research include development of a vaccine for domestic animals, development and evaluation of interventions to interrupt transmission from the wildlife reservoir to humans including preventing *Pteropus* bats' access to date palm sap that is destined to be drunk raw, and developing practical approaches and behavior change interventions to reduce the risk of saliva exposure and, therefore, person-to-person transmission for caregivers of severely ill patients.

See also: Food Safety Assurance Systems: Good Animal Husbandry Practice

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Norovirus

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Glossary

Antigenicity The ability of a chemical structure, referred to as an antigen, to bind specifically with T cell receptors or antibodies.

Infectivity The ability of a pathogen to establish an infection.

Introduction

Human norovirus (NoV) infection is popularly known as winter vomiting disease, gastric flu, or stomach flu and the virus itself as the cruise ship virus and vomiting bug. All these nicknames point at some characteristic features of these viruses: an infection is likely to lead to severe vomiting and diarrhea and the outbreaks follow a pattern of winter-seasonality; outbreaks on cruise ships occur regularly and tend to receive a lot of media attention.

NoV are now recognized as one of the most common causes of foodborne gastroenteritis worldwide. Being small nonenveloped particles, NoV are quite stable outside the host and may persist on inanimate surfaces, in water, and on food. The infectious dose is low and transmission of infection between persons is common. These factors contribute to the best known feature of NoV: the tendency to cause outbreaks of gastroenteritis in settings such as hospitals, homes for the elderly, child daycare centers, military establishments, cruise ships, hotels and restaurants. Foodborne transmission is common, but accurate estimates of the exact proportion of human NoV illness that can be attributed to consumption of contaminated food are not available. Currently, the NoV causing foodborne infections are all thought to be of human origin; in other words, a human NoV infection is the result of person-to-person transmission, for which food is one of the intermediate vehicles. In this article we will review essentials of NoV virology and epidemiology with emphasis on aspects that are relevant to their role as foodborne pathogens. For in-depth reviews on specific aspects references have been given.

Historical Information

The acute epidemic diarrhea that is currently known as NoV infection was described by Zahorsky in a paper in 1929, which eloquently described it as the winter vomiting disease, referring to the seasonality and high percentage of patients vomiting. In the decades that followed, outbreaks with similar features were described and a virus was suspected to be the etiological agent because parasite and bacteria-free filtrates transmitted the disease. In 1972, a 27 nm virus-like particle (VLP) was detected by use of immune electron microscopy in stool filtrate obtained from an outbreak of acute gastroenteritis at an elementary school in 1968 in Norwalk (OH, USA). This morphological identification lent the viruses their initial name as small-round-structured viruses (SRSVs). In 1979, another small particle as etiological agent of an outbreak of acute gastroenteritis in an infant home in Sapporo, Japan, was described: the Sapporo virus. The Norwalk agent and Sapporo virus had subtle differences in surface structure when examined by electron microscopy (EM), but more importantly were antigenically distinct. Further characterization was hampered because the viruses could not be grown in the laboratory. In 1982, Kaplan *et al*. formulated what is now known as the Kaplan criteria to identify NoV outbreaks, and in 1983 Pether and Caul published the first paper of NoV as the cause of foodborne outbreak in which the virus was most probably spread via chicken sandwiches prepared by an infectious member of the kitchen staff. Subsequently, Jiang and coworkers used the original Norwalk virus preparations to clone and characterize the Norwalk virus genome. Since then, molecular tools to detect these viruses have developed quickly and polymerase chain reaction (PCR) assays are currently the gold standard for diagnosis. With the beginning of the molecular era, the genome characterization of these viruses was possible, and this was used in virus taxonomy. The Norwalk agent became the prototype of the genus *Norovirus* in the family Caliciviridae, whereas the Sapporo virus turned out to be a distant relative, placed in a separate genus *Sapovirus*.

Nomenclature, Taxonomy, and Classification

Norovirus and *Sapovirus* are the two genera of the family Caliciviridae with humans as the primary host for several strains. The three other genera are *Lagovirus* (known host species: rabbits, brown hares), *Vesivirus* (known host species:

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sea lions, other marine animals, swine, cats, dogs, fish, seals, cattle, and primates), and *Becovirus*, identified in cattle. With the development of sensitive and generic molecular techniques new viruses are also being discovered in different animals and two new genera have been proposed and await independent confirmation. The animal caliciviruses have been associated with a range of clinical syndromes, including stomatitis, upper respiratory tract infections, and systemic diseases with severe hemorrhagic syndromes. NoV are known to infect humans, but have also been detected in pigs, cattle, sheep, mice, cats, lions, and dogs. Sapoviruses infect humans, pigs, and mink. In humans, NoV and sapoviruses cause acute gastroenteritis, and asymptomatic infections, whereas in animals they seem to cause mainly asymptomatic infections and mild gastroenteritis. In immunocompromised mice, the murine NoV can cause encephalitis, vasculitis, pneumonia, and hepatitis indicating a broad tissue tropism.

The diversity within the genus *Norovirus* is great, and a first subdivision of the genus in five genogroups (geno group (G) I–V) is made on the basis of phylogenetic analysis of sequences of the capsid protein. So far, strains belonging to GI have been detected in humans, GII strains in humans and pigs, GIII in cattle, GIV in humans, cats and dogs, and GV has been detected only in mice. The genogroups are further divided in genotypes, again based on phylogenetic analysis of sequences of the capsid protein. Currently, GI includes eight different genotypes whereas GII includes 19 genotypes, three of which have swine as their natural host. Despite this diversity, in recent years few strains, primarily those of genogroup II, genotype 4 (GII.4), have been responsible for a majority of the outbreaks worldwide, although a wider diversity is seen when looking at sporadic cases of gastroenteritis. Because the NoV GII.4 viruses seem to be the main epidemic genotype, in the scientific literature especially this genotype is subdivided into subtypes or variants, for example, NoV GII.4-2004 variant. Full naming of NoV is according to a cryptogram that is organized as follows: host species/genus/species (or genogroup)/strain name/year of occurrence/country of origin. For example Hu/NoV/GI.1/Norwalk/1968/US, Hu/NoV/GII.4/Bristol/1993/UK, Sw/NoV/GII.11/Sw918/1997/JP.

Nomenclature

Until recently, there was no international consensus for the nomenclature of NoV, resulting in some confusion in the literature. Recombinant NoV strains are commonly seen and cause naming difficulties, because they cannot be adequately classified with the current nomenclature system, which is based solely on the capsid gene (ORF2). A recent proposal of a consensus nomenclature scheme including both ORF1 and ORF2 typing is the basis of a publicly available genotyping tool.

NoV Morphology

Caliciviruses are small nonenveloped viruses with a linear single-stranded positive-sense RNA genome of 7.4–8.5 kb. The NoV genome has three open reading frames (ORF) and a 3′ poly A tail. The approximately 5.4 kb ORF1 encodes the six proposed nonstructural proteins, including the viral nucleoside triphosphatase, protease, and RNA-dependent-RNA polymerase. ORF2 is approximately 1.6 kb long and codes for VP1 whereas ORF3 is only approximately 0.6 kb and codes for VP2. The virus particles (virions) consist of a single RNA strand, covalently linked at its 5′ end to one copy of viral protein genome-linked (VPg) and the virus capsid. The capsid is made of a few copies of viral protein 2 (VP2) and 180 copies of a single capsid protein (viral protein 1, VP1) folded into 90 dimers, forming 32 cup-shaped depressions that can be seen in EM pictures of the virus, and has led the family name caliciviruses ('calyx' is cup in Latin). This typical structure is, however, less clear for the NoV that have a more fuzzy appearance. Additionally, the NoV belong to the smaller caliciviruses with a genome of 7.5–7.7 kb (**Figure 1**).

The key antigens of the NoV reside in VP1. This main viral protein contains three domains: a shell domain, constituting the body of the capsid, and two protruding (P) domains. Protruding domain 1 (P1) is the domain between the most exterior/protruding domain P2 and the S domain. Currently, the P2 domain is considered to contain the host cell binding site(s) and key antigens.

NoV Replication

NoV, as all viruses, are obligate intracellular organisms. Because they do not have a metabolism of their own, they are obliged to invade host cells and parasitize the subcellular machinery. Owing to the lack of an *in vitro* propagation system for human NoV, knowledge about the replication strategy of this virus is based mostly on analogies with other single-stranded positive-sense viruses such as members of the Picornaviridae family, to which poliovirus and hepatitis A belong, or viruses from the genus *Vesivirus* within the family Caliciviridae. Only after the discovery of the cultivable murine NoV, MNV-1, in 2003 by Karst and coworkers, it became possible to obtain detailed insights into the NoV replication and processing strategies.

The general steps are clear. The virion interacts with the host cell via a virus-specific receptor and then enters the cell by an as yet poorly understood mechanism. The cellular receptor for NoV binding is unknown, but carbohydrates similar to our ABO blood group are thought to be involved. Subsequently, the capsid is removed or altered and the RNA genome is released into the cytoplasm (uncoating). In the next step, the genome, which is itself a messenger RNA, is translated. ORF1 is translated into a polyprotein that is cleaved in at least six nonstructural proteins by the viral protease. These nonstructural proteins are all involved in the replication of the viral RNA genome. This replication is accomplished by synthesis of a small number of antisense (negative) RNA strands from the genomic RNA and subsequent synthesis of two major positive strand RNA species: a full-length genomic RNA and a subgenomic RNA for the synthesis of the structural proteins VP1 and VP2. Interestingly, for viral genome replication, the nonstructural viral proteins, cellular proteins, and cellular membranes from endoplasmatic reticulum, golgi-complex, and endosomes are needed in a productive replication complex, whereas production of the virus capsid seems to require less stringent regulation and cellular involvement. The

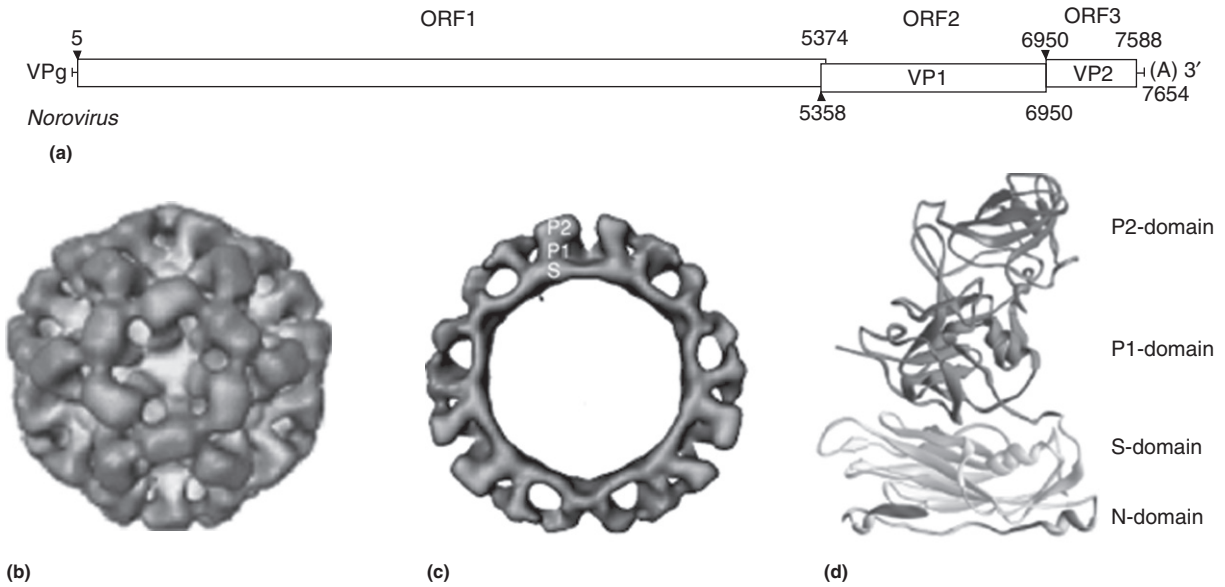

Figure 1 NoV GI genome and virion structure. (a) The genomic organization and reading frame usage (numbers indicate nucleotide position) for Norovirus GI.1 (Hu/NoV/Norwalk/1968/US, GenBank Accession Number M87661). (b) Three-dimensional structure of Norovirus GI.1 recombinant virus-like particles (rVLPs) viewed along the icosahedral threefold axis at approximately 22 Å. An rVLP is made up of 90 dimers of a single protein that form a shell domain from which arch-like capsomeres protrude. (c) A central section of the NoV GI.1 rVLP perpendicular to the icosahedral threefold axis. The arch is composed of the protruding (P) domain (divided into subdomains P1 and P2) and the shell (S) domain. (d) Three-dimensional ribbon representation of an individual subunit from the rNorovirus GI.1 capsomere derived from X-ray crystallography studies of Norovirus GI.1 rVLP at 3.4 Å. Reproduced from Green KY (2007) Caliciviridae: *The Noroviruses*, *Fields Virology*, 5th edn Philadelphia: Lippincott Williams & Wilkins. pp. 950–979.

production of VP1 is efficient in several different expression systems, and when enough copies of this protein are present, self-assembly into VLPs will occur. The next steps in the virus replication cycle are packaging of the viral RNA, maturation of the virus capsid, and subsequent release of infectious viruses.

NoV Pathology

NoV enter the body of their host via the oral route, and after multiplication in the epithelial cells lining the small intestine, leave the body via vomitus (oral) or stool.

The Norwalk virus (GI.1) retain infectivity if stool filtrate is exposed to pH 2.7 for 3 h at room temperature, and no RNA degradation could be measured after 30 min at pH 2, indicating that gastric passage is likely. Based on pathology performed on biopsies of infected symptomatic persons, it is thought that replication occurs in the duodenum and jejunum. Functional and morphological measurements on duodenal biopsies show significantly impaired barrier function during NoV infection: The transepithelial electrical resistance is less than a half that of the control and the passive mannitol leakage is more than doubled. Additionally, there is an increased anion secretion, which can contribute to the diarrhea. Remarkably, no indication for reduced absorption capacity was detected, indicating that the overall function of mature enterocytes was not affected. The functional changes coincide with minor villus blunting, a reduced villus surface area, reduced tight junction protein expression and doubling of the number of apoptotic cells in the villus. Below the epithelium there was a clear infiltration of intraepithelial lymphocytes. Histopathological changes as characterized by villus blunting and infiltration of mononuclear cells were also seen in the jejunum in volunteers during the acute phase of Norwalk virus infection and in the duodenum and jejunum of pigs infected with porcine sapovirus. Concomitant with jejunal pathology, a delay in gastric emptying was observed. If we assume the pathology in the duodenum and jejunum are caused by virus replication, the combination of abnormal gastric function and virus replication so close to the pylorus may explain the projectile vomiting and presence of NoV in the vomitus.

Clinical Manifestation (Disease)

A NoV infection typically results in a mild, short-term, self-limiting illness characterized by (projectile) vomiting and diarrhea, often accompanied by abdominal pain, nausea, and fever. NoV can infect people of all ages and clinical symptoms usually start 12–48 h after exposure and subside for 2–3 days in adults, and approximately 1 week in children. Asymptomatic infections are common. More severe disease has been seen in risk groups such as young infants, elderly, immunocompromised patients, and patients with renal disease and other comorbidities. In hospitalized patients more than 80 years of age, symptoms last longer, and mortality has been observed. Chronic disease has been reported for (severely) immunocompromised patients. Complications are

not common in NoV infections in healthy individuals, but more severe gastrointestinal symptoms and even extraintestinal manifestations have been reported anecdotally. So far, extraintestinal manifestations or viraemia has been rarely reported, but this has not been addressed systematically. Several reports from Japan, Spain, and Italy mention neurological symptoms associated with ongoing NoV infection in children. It remains to be seen if this complication is common around the world.

Asymptomatically infected persons and persons that have gone through a symptomatic phase continue shedding viruses for at least 2 weeks and often longer. Atmar and coworkers showed that for the NoV GI.1 strain (Norwalk virus 8fIIa inoculum) peak levels of shedding from symptomatically infected volunteers occurred after cessation of symptoms, and levels of shedding were as high in symptomatically and asymptomatically infected persons. The median peak amount of virus shedding was approximately 1×10^{10} genomic copies per gram feces, and remained above 1×10^{6} genomic copies per gram feces for up to 2 weeks. Interestingly, in one study (using different quantitative PCR assays) the median viral loads of NoV GI and GII strains in feces was found to be 8.4×10^{5} and 3.0×10^{8} genomic copies per gram feces, respectively: the viral load of NoV GII was more than 100-fold higher than that of GI. This difference is believed to contribute to the higher transmissibility and prevalence of NoV GII strains. The same group also showed that a higher viral load during a NoV GII.4 infection was associated with prolonged diarrhea, something that was not found for NoV GI.1. A straightforward interpretation of these data are hampered because absolute quantification of genomic copies still awaits thorough validation. Furthermore, the detectability and quantification of RNA strands/genomic copies does not necessarily indicate the presence or quantification of infectious viruses.

Shedding of NoV does not only occur via feces, but also via vomitus. The onset of vomiting may be quite abrupt, and has led to contamination of kitchen surfaces and restaurants through aerosols. For several outbreaks the role of vomiting in the spreading of viruses has been described but due to methodological limitations, there are no good data on the level/quantity of infectious NoV in vomitus. Recently, NoV RNA was detected in mouth wash samples from approximately 25% of patients for more than a week after disappearance of symptoms, and even in persons with diarrhea only. The presence of viral RNA in mouth washes might imply that coughing and speaking can contribute to the spread of these viruses but this requires further study.

In addition to the high level of shedding, the infectivity of the NoV is very high. For the NoV GI.1 Norwalk virus it is estimated that the average probability of being infected by a single infectious particle is approximately 0.5: the highest value reported for any pathogen to date. For comparison, this probability is 0.4 for poliovirus, 0.02 for *Campylobacter jejuni*, and 0.000 03 for *Salmonella enterica* (serovar Newport). The probability of becoming ill after exposure to NoV seemed to be dose-dependent and increased to 0.7 at high virus doses ($\sim 10^{8}$ genomic copies). The overall picture is clear: few viruses are needed to cause infection and, with or without symptoms, virus shedding is high.

Most patients with NoV illness do not need treatment. In hospitalized patients, the most common treatment is rehydration. In an experimental replicon system, some effect of antivirals was observed on the level of replication, but this is far from clinical application. Treatment of symptomatic chronic shedders with a range of different approaches has not yet been successful.

NoV Immunology and Genetic Susceptibility

The chance of being infected by NoV after oral exposure to an infectious virus depends on several viral and host factors. The host factors are mainly the immunological status with respect to the virus strain and the genetic susceptibility of the host. In the early volunteer studies it was noted that short-term, strain-specific resistance to disease, not to infection, developed in some volunteers. However, some individuals with high serum-antibody titers could be infected and become sick over 2 years later when exposed to the same virus, whereas individuals without any detectable serum antibodies could not be infected. It is now clear that genetic susceptibility interfered with immunology in these early studies. Just as for *Helicobacter pylori* and *Vibrio cholera* infections, NoV infections depend on the histo-blood group antigens (HBGAs). For NoV, several factors have been shown to correlate with susceptibility to infection by different NoV strains. The first factor is the secretor status, as determined by a functional fucosyl transferase-2 enzyme: secretors are more likely to be infected by NoV than nonsecretors. For example, nonsecretors, constituting approximately 20% of the Europeans, are resistant to infection by NoV GII.4, GII.3, and GI.1, but can be infected by NoV GI.3 and GII.2 strains. The second factor is the Lewis antigens as determined by the fucosyl transferase-3 enzyme. For this factor resistance to infection is far less clear, some strains can infect only Le^{a}, some only Le^{b}. The above implies that the impact of NoV strains on the population may differ greatly, and depends on the ability of a given strain to infect sufficient susceptible individuals for sustained transmission to occur. In the past 10 years, NoV GII.4 have been the most common cause of outbreaks, but these viruses evolve rapidly. Since 2002, when a particularly virulent NoV GII.4 strain emerged, new variants have evolved and spread globally almost every other year. Each of these variants has distinct host binding properties and antigens, making the development of a NoV vaccine quite challenging.

Immunity to NoV is not well understood. Older studies showed that immune responses might be specific and short-term and immunological studies are complicated by the lack of a culture method or a small animal model and differences in host susceptibility for NoV infection. Recent studies do show that on NoV infection antibodies are produced and that these can be measured. Seroprevalence studies have shown that most persons are exposed to a NoV in early life, but disease remains common in people of all ages. This is the first indication of lack of sufficient protective immunity or the true existence of different serotypes. The genetic diversity observed for NoV translates into antigenic diversity. Viruses belonging to different genotypes may induce cross-reactive antibodies, but not those that are directed at the specific host binding, and

thus, influence on the virus infection or disease is doubtful. Studies have shown that even variants within a genotype may have distinct host cell binding properties, due to mutations around the host cell binding domain. This affects antigenicity of the viruses, and thereby escape from herd immunity. This mechanism likely contributes to the success of NoV. However, in mice, cross-reactive T cells may be induced that respond to exposure with viruses from distinct genogroups. These studies show that the immunity to NoV is complex.

Epidemiology

Statistical Data on Prevalence and Incidence of the Disease

NoV are notorious for their ability to cause outbreaks. Outbreaks are very often seen in closed settings with an especially vulnerable population such as hospitals and nursing homes. However, NoV can infect people of all ages and health status, and in several settings attack rates for the patients and staffs are similar. Additionally, cruise ships are also famous for NoV outbreaks , and outbreaks are reported for child daycare centers, schools, concert halls, navy vessels, and scouting camps. Most outbreaks are characterized by person-to-person spread, although introduction of the virus via a point source, such as food or water, could remain unnoticed even if it is common.

Even though outbreaks attract most of the media attention, the number of cases of sporadic infections, or nonreported family outbreaks, is much higher. To estimate the incidence of NoV infection in a population requires costly population-based studies. So far, such studies have been performed only in the US, Australia, the UK, and the Netherlands resulting in burden estimates of viral gastroenteritis varying between 1.3 and 31 infections per 1000 inhabitants. The study in the Netherlands in 1999, estimated the burden of NoV to be 450 disability-adjusted life years (DALYs), with an estimated incidence of 470 000 cases (2.9% of the Dutch population). In another study it was calculated that NoV infections cost the Dutch society 25 million euros in 2004. For comparison, in the same population, the burden of disease for *Salmonella* and *Campylobacter* were estimated to be 8.8 million euros and 19.6 million euros, respectively. We already know the estimates for NoV are likely too low because the incidence of NoV gastroenteritis has increased significantly in recent years, and mortality and hospitalizations due to NoV infections were underestimated.

Geographic Distribution

Reports on NoV outbreaks and studies addressing causes of gastroenteritis in hospitalized children, have demonstrated that NoV is a significant cause of illness around the world. Even though there are large data gaps, particularly in developing countries, NoV are recognized as important pathogens of travelers' diarrhea in various regions of the world. Many of the recent epidemic NoV GII.4 variants have been observed worldwide. Although their impact as cause of outbreaks seems to differ between regions it does show that there are no intrinsic limitations for a global spread of at least some NoV strains.

Reservoirs for NoV, Vehicles for Transmission, and Examples of Implicated Foods

The NoV infecting humans are considered exclusive human pathogens. Even though a huge number of NoV infections and outbreaks have been studied, there is no indication for zoonotic transmission or animal reservoir for human infections. Transmission of NoV is thus per definition person-to-person. What we consider food or waterborne transmission of NoV is actually indirect person-to-person transmission in which food or water is a vehicle for the viruses. This is most clear in food handler transmission. Food handlers can contaminate food with NoV via vomit or via feces. Vomiting as symptom of NoV infections results in widespread dissemination of viruses: viruses contaminate surfaces and fomites in close proximity of the patient by droplets whereas a wider circle and the air is contaminated by aerosols. The viruses are acid resistant and will retain infectivity for at least several days. Contamination via feces occurs through insufficient personal hygiene, especially by persons shedding viruses themselves (e.g., after using toilets), but also by those taking care of infected persons (e.g., changing of diapers) or cleaning toilet areas used by infected persons. Food handlers can also contaminate food by transferring viruses from contaminated surfaces to hands during preparation of ready-to-eat food or by transferring viruses from contaminated food to other ready-to-eat foods. Inanimate surfaces include contaminated utensils, for example, chopping equipment, such as dicers, cutting knives, and serving utensils. Foods reportedly implicated in this route include mostly multiingredient foods such as salads and sandwiches that often require multiple touching for production. Another foodborne route for NoV is via contaminated water, used for drinking or irrigation during cultivation or as production or wash water anywhere in the process. NoV are very stable in water; they will remain infectious for months and virus detection in surface waters is not uncommon. Virus persistence in water is affected mostly by temperature, UV radiation, association and sedimentation with solids, and the microbial flora.

Numerous papers report on NoV infections via drinking water, however reports on NoV in irrigation and process waters are limited for foods other than shellfish. Surface waters that may be used for irrigation or drinking water production can easily be contaminated by human NoV because the virus is shed in high numbers (up to 10^{11} per gram feces) by many people (up to 15% of the population in winter season) and the virus reduction achieved in sewage water treatments plants rarely exceeds 2 log reduction. NoV levels in treated sewage may contain more than 10^3 NoV genomic copies per liter. Interestingly, although in the general population NoV GII infections are much more common than NoV GI infections, in sewage water (influent) NoV GI viruses are regularly detected as often, or even more often, than NoV GII viruses. It is also suggested that removal efficiencies for NoV GI are less than for GII viruses , whereas removal efficiency for bacterial indicators was in general much more.

Peak concentrations of pathogenic viruses occur in source waters used for drinking water production. If seasonal and short-term fluctuations coincide with less efficient or failing treatment, an unacceptable public health risk from exposure to this drinking water may occur. In a recent risk analysis on

the safe use of wastewater in agriculture it was concluded that NoV levels require a 6 log reduction to achieve a WHO accepted risk of infection of 10^{-3} per person per year. In times of water scarcity due to global changes, such as increasing populations and climate change, the use of wastewater for use in irrigation is evaluated but may exceed tolerable risk levels as set by WHO.

Contaminated water can also result in contaminated shellfish. Shellfish are the most common foodborne source of NoV infection, and monitoring systems are established for this product. Often, multiple strains are found, indicating sewage contamination of the growing waters. Because multiple NoV strains are regularly detected in shellfish, there is an added risk of double infections, and consequently a risk of virus recombination, after consumption. Bivalve molluscan shellfish, such as oysters, filter large volumes of water and thereby accumulate and concentrate different types of pathogens. Because bacteria and viruses show differences in terms of accumulation and concentration and are reduced with different efficiencies by depuration from contaminated shellfish, the absence of bacterial contamination does not necessary indicate the absence of viruses. The cells of the digestive tract of at least several oyster species were recently found to present carbohydrate structures that are indistinguishable from the HBGAs that are known to bind several NoV strains. This could mean that oysters can specifically accumulate and concentrate NoV and it might explain the lack of clear effect of depuration on NoV number. NoV GI viruses are detected relatively often in shellfish and shellfish related outbreaks.

Owing to difficulties in detecting viruses in foods, difficulties in linking cases and food in space and time, especially when frozen read-to-eat foods are involved, and the highly efficient person-to-person spreading, the fraction of foodborne outbreaks is hard to establish. However, for Europe it was estimated that ~84% of the outbreaks were reportedly due to person-to-person transmission (including indirect via environmental contamination), and ~16% due to foodborne transmission. From data in a review describing 47 food and waterborne NoV outbreaks from 2000 to 2007, it is estimated that in approximately 40% of the cases a food handler was responsible for the outbreak, followed by fresh produce (28%), bivalve shellfish (17%), and consumed water (15%) (Figure 2).

According to an expert meeting held in 2007, three NoV food commodities were selected as priorities based on scientific proof and epidemiological relevance:

1. NoV in prepared ready-to-eat foods infected by food handlers practicing poor personal hygiene during food preparation and serving.
2. NoV in bivalve mollusks that are consumed raw or lightly cooked: through fecal contamination of waters in which they are growing.

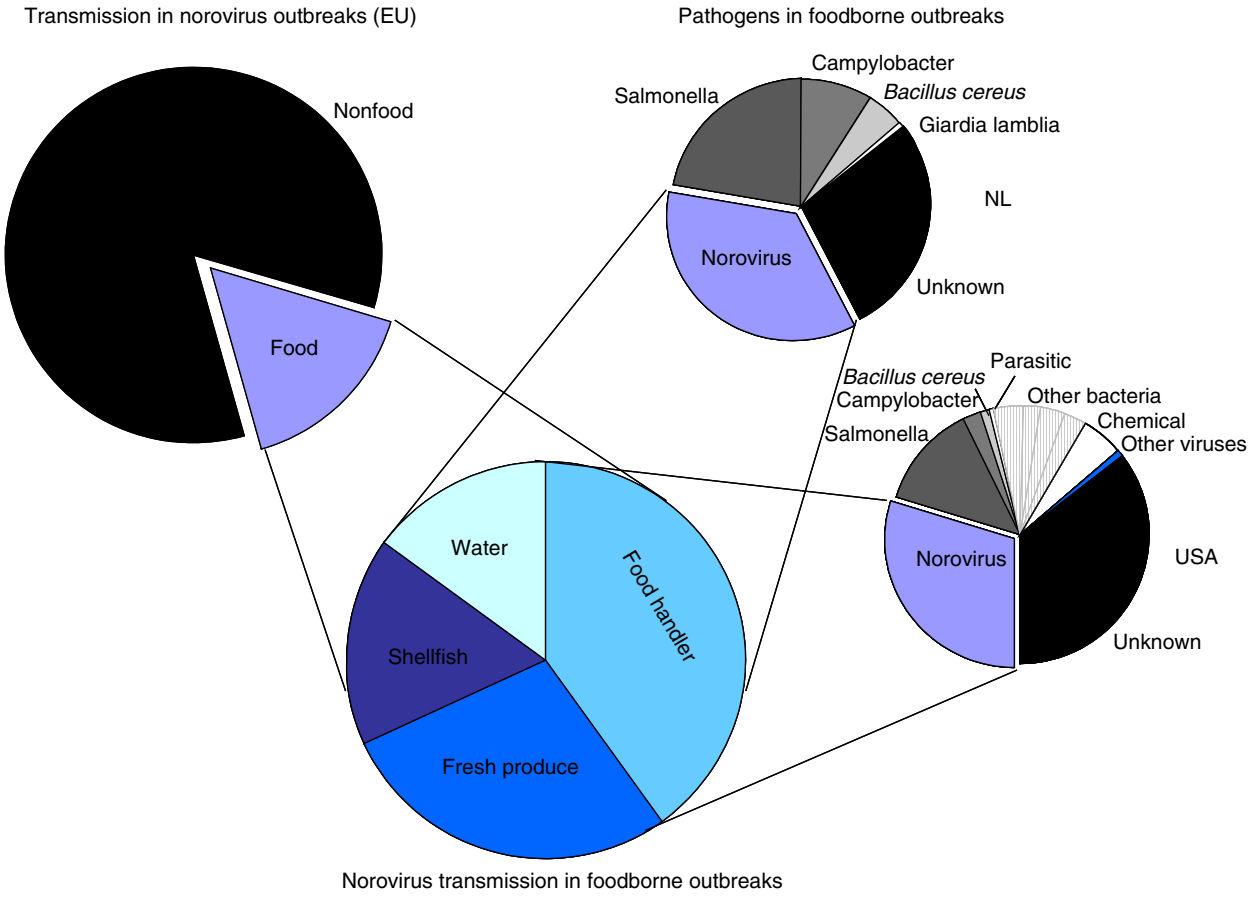

Figure 2 Relative relevance of transmission routes in foodborne NoV outbreaks.

3. NoV in fresh produce: through contaminated water (used for irrigation, or fertilizer application, or wash water) or via contact with contaminated surfaces and contaminated utensils.

Finally, environmental transmission occurs. NoV are very stable outside the human host and may persist in infectious form on surfaces and fomites for weeks and even longer. Environmental transmission has been reported to play a role in NoV outbreaks in hotels, hospitals, food establishments, a concert hall, and on cruise ships. Furthermore, in food establishments associated with NoV outbreaks, widespread environmental contamination has been demonstrated and transfer of viruses from surfaces via fingers and subsequent ingestion is possible.

Methods for NoV Diagnosis and Detection

NoV Diagnosis

Infection with NoV is typically diagnosed by the detection of the virus in patient stool samples, rather than by measuring immune response of the host to the virus. The virus capsids (protein) can be detected by enzyme immune assay (EIA)-like tests and the virus genome by real-time reverse transcriptase-polymerase chain reaction (RT-PCR) assays. Advantages of the RT-PCR method (detection limit of 10–100 viral particles per gram stool) is the high sensitivity and that it can be applied to all kinds of substrates such as feces, vomitus, serum, food matrices, and water. A bottleneck is the high genetic diversity of these viruses. Therefore, generic tests have been developed and no single primer pair can detect all NoV strains. Additionally, generic tests are optimized for the detection of a broad range of viruses, not for the specific and more sensitive detection of one strain. Therefore, false negative tests are relatively common and back-up protocols should be available for additional testing. The sensitivity of EIA-based methods rarely exceeds 70% of that of the RT-PCR assays, indicating that single patient diagnosis by EIA is likely to result in many false negatives. However, most EIA-based tests do show good specificity and they may be used for outbreak diagnosis when at least three samples are tested. When ascertaining possible infection or illness in sporadic cases, validated, and internally controlled PCR-based techniques are preferred. Currently, EIA-based and fast (result within 1 h) point-of-care tests are being developed and validated since early recognition is very important in implementing counter measures to reduce the size of NoV outbreaks. Note that most of the newly developed EIA-based tests seem to have optimized sensitivity for NoV GII.4 viruses, which is a good approach for outbreak diagnosis in general. However, relatively more foodborne outbreaks are caused by NoV GI strains for which sensitivity is often less than the overall 70%. Owing to their higher sensitivity, RT-PCR methods are the method of choice for confirmation of NoV infection in consumers when a food source is suspected epidemiologically.

Because NoV are the most common etiologic agent of foodborne outbreaks of gastroenteritis, diagnostic tests have not been readily available and several clinical and epidemiologic profiles are the characteristic of NoV outbreaks; the Kaplan criteria have been used to distinguish foodborne outbreaks of gastroenteritis caused by NoV from those caused by bacteria. The Kaplan criteria as reevaluated by Turcios and coworkers included: vomiting in $>50\%$ of patients, mean incubation period of 24–48 h, mean duration of illness of 12–60 h, and no bacterial pathogen detected. Using these criteria resulted in a sensitivity and specificity of 68% and 99%, respectively in discriminating the NoV outbreaks form bacterial outbreaks.

NoV Detection in Food and Water

Detection of NoVs in food and water relies mostly on (real time) RT-PCR methods. Owing to the variety of food matrices, there is a challenge in the first stage of the RT-PCR protocol: the extraction of the viral RNA from the matrix. Many studies have focused on the method development to extract viral RNA from shellfish, which has resulted in a variety of methods. Common features of these are dissection of the digestive tract and hepatopancreas and subsequent homogenization, followed by variable (partial) purification and RNA extraction methods. Other matrices such as fresh produce and fruits are often contaminated by an infected food handler or via irrigation or wash water. In such cases viral contamination will be primarily at the surface and protocols are based on elution of the virus particles from the surface of the product, followed by a concentration step (mostly ultra-centrifugation or -filtration) and RNA extraction from the concentrate.

For NoV detection in water a variety of methods are available. Here too, the challenge exists in obtaining concentrates with a detectable level of viral RNA, but a low level of RT-PCR inhibitors. Many protocols varies on a general method which, in short, entails concentration by adsorption to a filter (e.g., a positively charged membrane, glasswool), subsequent elution from the filter and further concentration using a microconcentrator or precipitation step. Immunoaffinity concentration and purification protocols have also been developed for application in water. State-of-the-art detection limits for NoV in water are approximately 10 and 300 genomic copies (RT-PCR units) of NoV G1 and GII, respectively, per 1.5 l water.

Currently, methods for standardized detection of NoV (and hepatitis A virus (HAV)) in soft fruits, leafy greens and bottled water are being validated in the CEN/TAG4 committee of the European Union and Health Canada has listed select validated methods in its Compendium of Analytical Methods for virus detection in foods. Note that all NoV detection methods are based on detection of the presence of NoV RNA (or capsid protein) and that these are not direct measures for infectivity. However, the infectious dose for NoV is extremely low and the presence of NoV RNA does indicate contamination with human feces. At the moment is not clear if there are safe levels of contamination by NoV. As was mentioned before for shellfish, in general there are no indicator organisms (or chemical indicators) for which we know they reliably predict the absence or presence of human NoV in foods, water or environmental samples. Owing to all the difficulties described above, it is unlikely that prospective batch testing of foods to show virus absence will be a reliable and cost-effective strategy.

Prevention of Contamination of Foods

NoV are primarily transmitted directly from person to person. Control of person-to-person transmission and of food handler transmission of NoV relies mostly on strict personal hand hygiene. Proper use of gloves and adequate hand washing, using soap, running water, and disposable towels are keys to prevent contamination of food. Alcohol-based hand sanitizers can be used to supplement the standard hand washing routine. Laboratory data show that the reduction of virus load or infectivity is less, or variable, when alcohol-based hand sanitizers are used instead of washing with water and soap; therefore, the use of only hand alcohol is not recommended.

Foods can also be contaminated by accidents of vomiting in areas where food is being prepared or stored. In a room where a person has vomited all the food items can be contaminated through droplets or via aerosols and these items should be disposed of or consumed only after virucidal treatment. In general, food handlers should not be allowed at the workplace when symptomatically infected with NoV and should only be allowed to return to work after a period of, for example, 48 or 72 h without symptoms. However, because shedding of NoV can continue for 4 or even 8 weeks and asymptomatic infections are common, persons should comply with strict hand hygiene instructions at all times.

Prevention of shellfish contamination relies on the use of clean water for production. Attempts to reduce viral contamination by relaying has proven to be unsuccessful or at best unreliable. The water quality in the growing areas is assessed by continuous monitoring for fecal contamination as measured by quantification of *Escherichia coli*/fecal coliforms/total coliforms. This has proven to be effective in reducing the number of shellfish-consumption related infections due to bacterial and parasitic pathogens. However, viruses may be present in water and shellfish in the absence of *E. coli*/fecal coliforms/total coliforms. To control viral safe production of shellfish, a sanitary survey of the sources and types of fecal contamination (human and animal) in the vicinity of production areas can be helpful. Additionally, methods to detect low levels of NoV in shellfish and growing waters are available and may contribute to a monitoring program with more relevance for viral food safety than mere detection of *E. coli*/fecal coliforms/total coliforms.

Prevention of contamination of fresh produce requires all of the above because contamination can occur by contaminated water or by food handlers applying suboptimal hand hygiene. It is the key to use only clean water (and soil) for the production of food and appropriate sanitary and hand washing facilities should be in close vicinity.

Viral Inactivation in Foods

Virus-infectivity reducing treatments are either treatments that lead to reduction of the virus load or treatments that inactivate the viruses. There are currently no effective, realistic, and validated virus infectivity reducing treatments for food except cooking adequately. The effects of heat treatment on virus infectivity in foods are highly dependent on virus (sub)-type and food matrix. This complicates the assessment of virucidal activity for human NoV. The human NoV cannot be grown *in vitro* yet, information on inactivation rates are based on model viruses such as the feline calicivirus, murine NoV, or HAV, sometimes in combination with reduction in genomic copies of the human NoV. Nonetheless, commonly used cooking procedures for rice, pastas, or potatoes are considered adequate treatments to destroy viral infectivity. Conventional pasteurization (e.g., 63 °C for 30 min, or 70 °C for 2 min) is more effective than high-temperature short-time (71.7 °C for 15–20 s) pasteurization, but NoV are unlikely to be completely inactivated at those treatments. For shellfish, it has been shown that HAV can be inactivated when an internal temperature of 90 °C is reached for at least 90 s. This could also be true for NoV.

The effects of washing on (murine) NoV infectivity are limited. Washing of fresh produce with just water resulted in approximately 1 log reduction of MNV on the produce, addition of 200 mg l^{-1} sodium hypochlorite or 250 mg l^{-1} peroxyacetic acid gave approximately 1 log additional reduction. However, both supplements were successful in preventing cross contamination from spiked fresh produce to clean fresh produce washed in the same washing water. Seven commercial disinfectants were shown to be ineffective at the FDA-permitted concentration when tested for the inactivation of feline calicivirus on strawberries and lettuce. Similar results were found for sodium bicarbonate, chlorine bleach, peroxyacetic acid, and hydrogen peroxide at FDA-approved concentrations.

Because viruses never grow outside their host, preservation methods aimed at preventing or reducing growth of bacteria or fungi will not contribute significantly to viral food safety: freezing, cooling and drying, vacuum packaging, or packaging under protective atmospheres will result in little reduction in viral infectivity. For example, calicivirus infectivity is reduced less than 1 log after five cycles of freezing and thawing and less than 1 log after storage at refrigerator temperatures for 1 week. Outbreak reports show that NoV infectivity will outlast the shelf life of most fruits and vegetables, and NoV infectivity remains at a sufficient level to cause disease after cooled or frozen storage. Furthermore, NoV are very stable at low pH and more than 3 log inactivation may occur only at pH <3, a pH that is often unacceptable for the sensorial quality of foods. Information on virus infectivity after long-term storage, or cooling or freezing in combination with acidification is lacking.

The effects of high hydrostatic pressure (HPP) on human NoV infectivity in foods are unknown, but tests with the cultivable MNV have been performed. Approximately 4 log reduction of MNV was demonstrated in oyster tissues at a treatment 5 min, 400 MPa, at 5 °C. Although only 0.8 log infectivity reduction was found for MNV at homogenization pressures of 300 MPa at 75 °C in PBS (relative to 3 log for MS2). Clearly, virucidal effects depend on the food matrix but HPP may be a measure to reduce viral infectivity.

Studies on the effect of irradiation (gamma or UV) on NoV (calicivirus) infectivity in foods are limited. UV irradiation does reduce virus infectivity but its effectiveness is highly dependent on the presence of the virus on the surface of the food, the food matrix and perhaps even the NoV genotype. It cannot be considered an effective generic measure to reduce

viral infectivity on or in food. UV irradiation can be effective for the inactivation of viruses in water and aerosols.

Virus Infectivity Reducing Treatments for Surfaces

For surface disinfection, many disinfectants recommended for use in food establishments are not effective against the nonenveloped viruses, such as NoV. One effective method is the use of sodium hypochlorite, for which the effectiveness is determined by the availability of free chlorine and the exposure time. The recommended treatment for clean surfaces uses solutions of ≥ 1000 ppm free chlorine for at least 5 min to inactivate HAV, and presumably also NoV.

Feline calicivirus was not efficiently inactivated on environmental surfaces or in suspension by 1% anionic detergents, quaternary ammonium (1:10), hypochlorite solutions with <300 ppm free chlorine, or less than 50% or more than 80% alcohol preparations (ethanol or 1- and 2-propanol). Varying efficacies of 65–75% alcohol are reported, however, short contact times (<1 min) rarely result in effective inactivation. Moreover, the presence of fecal or other organic material reduces the virucidal efficacy of many chemicals tested. MNV was reported to be sensitive to 60% alcohol, alcohol hand rubs, bleach, and povidone iodine-based disinfectant in suspension tests using very low level interfering substances. This was remarkable, because this would suggest a higher sensitivity of MNV than of feline calicivirus for commercial disinfectants and most likely higher sensitivity to hypochlorite of MNV compared to human NoV, because the RNA of human NoV was much better protected during hypochlorite inactivation than the RNA of feline and canine caliciviruses. Overall, human NoV seems to be the most resistant calicivirus tested so far.

Regulatory Measures

Viruses such as NoV have several features that make them behave differently in the food chain than classically recognized foodborne bacteria. It is clear that hazard analysis and critical control point (HACCP) systems and good manufacturing practice (GMP) should be in place and the appropriate guidelines for production of safe foods should be followed. Because it is now recognized that this will not automatically result in virus safe foods, a new CODEX guideline is being drafted. These Guidelines on the Application of General Principles of Food Hygiene to the Control of Viruses in Food aim to minimize the risk of illness arising from the presence of human enteric viruses, more specifically from NoV and HAV, in foods. The guidelines are supplemented with two annexes: Control of hepatitis A virus (HAV) and norovirus (NoV) in bivalve mollusks and Control of hepatitis A virus (HAV) and norovirus (NoV) in fresh produce and could be published as early as 2011.

See also: Disciplines Associated with Food Safety: Food Virology. Food Safety Assurance Systems: Personal Hygiene and Employee Health. Safety of Food and Beverages: Seafood

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ORGANISMS OF CONCERN BUT NOT FOODBORNE OR CONFIRMED FOODBORNE

Contents

Foot-and-Mouth Disease Virus

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Glossary

Acute infection A presentation of a disease with a rapid onset and/or short course of symptoms and disease evolution.

Amino acids These are critical-to-life molecules, which contain an amine group, a carboxylic acid group, and a side chain that varies among different amino acids. They have many functions in metabolism and are the building blocks of proteins.

Carrier An infected individual that carries an infectious agent despite a healthy appearance.

Chronic infection A long-lasting course of a disease in which there is clear presentation of symptoms related to such disease.

Epitope Also known as antigenic determinant, it is the part of an antigen that is recognized by the immune system.

Muller's ratchet effect The process by which the genomes of an asexual population accumulate deleterious mutations in an irreversible manner.

Nucleotide The molecules that join together in chains to make up the structural units of ribonucleic acid and deoxyribonucleic acid.

Peptides or polypeptides Short polymers of amino acids linked by peptide bonds.

Persistent infection The remaining infection after recovery from the disease during which it is still possible to isolate the infectious virus from the individual despite the absence of disease symptoms.

Quasispecies A large group or cloud of related genotypes that exist in an environment of high mutation rate, where a large fraction of offspring are expected to contain one or more mutations relative to the parent.

Background

Foot-and-mouth disease (FMD) was the first viral animal disease identified. The oldest recollection of data describing a disease like FMD was in cattle by Eraclito in ancient Greece (*ca.* 500 BC). The first description of an FMD virus (FMDV) epizootic was recorded in 1514 by Girolamo, in Northern Italy, and in 1546 by Frascastorius, in Venice. In 1764, Michel Sagar individualized the infection identifying it under the name of *Aphthis pecorinis*. But it was not until the experimental studies carried out by Loeffler and Frosch in 1897 that the etiologic agent of FMD was identified and defined as a filterable infectious agent smaller than bacteria. This was the first description of a virus causing an animal disease. In 1922, Vallee and Carre reported the existence of different serotypes, identifying only serotypes A and O. Subsquently, in 1926, serotype C was described by Waldmann and Trautwein; the Asia 1 and South African Territories (SAT) 1, SAT 2, and SAT 3 were described in 1936 by Lawrence. Thus, FMDV exists in seven well-defined serotypes, namely A; O; C; Asia 1; and SAT 1, SAT 2, and SAT 3. However, a large number of subtypes have evolved within each serotype group, making its classification a difficult issue. In fact, the seven original serotypes have been defined based on the lack of cross-reactivity among each virus and the antibodies in sera from the heterologous serotypes. Later, based on the comparisons of VP1 sequence data, it was confirmed that the distribution of FMDV isolates occurs not only in serotype-related groups, but also in genotypes with less

than 15% differences in nucleotide (nt) sequence, which coincide with the geographic origin of the isolates and have been defined as topotypes.

Etiology, and Characteristics of FMDV

The etiologic agent of FMD is a ribovirus of the Picornaviridae family. Within the ribovirus of positive-sense ribonucleic acid (RNA), the family Picornaviridae includes very important pathogens, such as *Enterovirus* (polioviruses and coxsackieviruses), *Cardiovirus*, and several other genera. *Aphthovirus* is one of these 12 genera of the Picornaviridae family comprising the *Equine rhinitis A virus*, the *Bovine rhinitis B virus*, and the FMDV. The diameter of any intact picornaviral particle is approximately 22–23 mμ long and consists of a nonenveloped outer protein coat or capsid with icosahedral symmetry. The protein capsid is composed of 60 capsomeres, each one of them containing a copy of each of the four capsid proteins: VP4, VP2, VP3, and VP1, also known as 1A, 1B, 1C, and 1D, respectively. A single-stranded RNA genome of positive polarity is enclosed in each mature capsid. The FMDV particle is highly labile and rapidly loses infectivity at pH values of less than 6 and greater than 9. However, it is stable at low temperatures, surviving for at least 1 year at 4 °C. Survival times at 37 °C and 56 °C are 10 days and less than 30 min, respectively. In fact, the process of pasteurization of contaminated milk at 72 °C for 15 s does not inactivate FMDV. It must be heated at 100 °C for more than 20 min for virus inactivation. In the environment, the virus can survive in the soil for at least 3 days during winter; in urine, it can persist up to 39 days; and in dry fecal material, it can persist infective up to 14 days in summer and 6 months in winter conditions. For its inactivation, sodium hydroxide 2%, sodium carbonate 4%, and citric acid 0.2% are effective. But it is resistant to iodophores, quaternary ammonium compounds, and hypochlorite and phenolic compounds, especially in the presence of organic matter. FMDV survives in lymph nodes and bone marrow at neutral pH but is destroyed in the muscles due to the acidification process that accompanies the rigor mortis of the carcasses.

Genomic Organization

The approximately 8500-nt long viral RNA consists of a single open reading frame (ORF), flanked by two noncoding regions (NCRs), both predicted to display complex secondary structures, which play a critical role in viral replication and gene expression. A small viral protein (VPg) is covalently linked to the 5′ end of the molecule. A poly-C tract divides the 5′NCR in a small RNA (S) fragment of approximately 400-nt long and a large RNA (L) fragment, which contains the remaining 5′NCR, the unique ORF, and the 3′NCR. Little is known about the function of the Aphthovirus poly-C tract, except that it is also present in only *Cardioviruses*. The picornavirus internal ribosome entry site (IRES) element provides cap-independent translation function. Its length varies among isolates and has been indirectly and inconclusively related with viral virulence. The translation initiation of the FMDV RNA starts at two AUG codons separated by 84 nt, following ribosome recognition of the upstream IRES. The viral ORF encodes a single polyprotein, which is cleaved by viral proteases to yield different viral products. A highly ordered structure is also predicted at the 3′NCR of FMDV genome of approximately 90 nt long, which precedes a long poly-A tail. The viral ORF encodes a single polyprotein, which will be cleaved into a number of intermediate subproducts and 12 final mature proteins. This processing will yield nonstructural proteins (NSPs) known as Lpro, and precursors P2 and P3, plus the capsid or structural proteins (SPs) 1A, 1B, 1C, and 1D, all of them contained in the precursor protein P1. The NSPs are involved in the critical functions of the virus life cycle in infected cells and will result in eight different final products that catalyze the processes of proteolysis, shut-off of host proteins, evasion of host immune system, cytopathic effects, etc.

Viral Structure and Antigenicity

The structure of FMDV capsid has been thoroughly analyzed by X-ray crystallography and was mostly found to contain the combination of four SPs (1A, 1B, 1C, and 1D) in higher structures, as previously described as capsomeres (see Characteristics). One of the important characteristics of the SP is that they contain the most important antigenic structure of the viral particle. Probably, the best known is the antigenic site located in the G–H loop of 1D (also known as VP1), which has been repeatedly related with the generation of neutralizing antibodies. Not all the antigenic sites are linear epitopes, but several conformational or discontinuous epitopes have been described in proteins 1B (VP3) and 1C (VP2).

A second critical function of the capsid proteins is to provide cell recognition factors that make possible the entry of the virus into the host cell. Several studies have determined that the G–H loop of protein 1D contains an arginine–glycine–aspartic acid signal peptide. This motif is critically involved in virus interaction with cell surface integrin receptors. There are also indications of other mechanisms of cell recognition, probably used as alternative routes for infection, such as the capacity to interact with heparan sulfate glycosaminoglycan residues on the cell surface, which is used as a pathway for FMDV variants adapted to cells in culture. The capacity of FMDV to develop and to use multiple pathways for entry into the host cell illustrates the importance of the genetic plasticity of FMDV that results in the evolution of viral antigenicity throughout the action of determined selective pressures. Evolution of FMDV in cell culture can reduce the constraints in an important antigenic site and may allow the virus to explore new antigenic structures.

Variability and Evolution

Many studies have already provided important experimental support to the fact that due to the high mutation rates of replication, RNA viral populations consist of a composite of multiple genetic variants, termed as quasispecies, in which the predominance of special features is the result of the equilibrium between the competitive fitness of each genetic variation in response to the selective pressures acting in each

occasion. This property allows RNA viruses to easily adapt and survive in every different environment. But at the same time, it produces uncontrollable number of spontaneous mutations with detrimental effects that constantly debilitates the population on passages, known as the Muller's ratchet effect. There are enough experimental results to infer that some strains of FMDV may die out if they are restricted to a small contained population of susceptible hosts. However, genetic and antigenic variability of FMDV populations even in the absence of immune pressure are a real challenge for vaccination and control of this disease, and has to be considered for the epidemiology of FMD.

Clinical Manifestation in Animals

FMDV usually produces an acute, systemic vesicular disease. In natural infections, the main route of virus entry is the respiratory tract, and as few as 1–10 infective particles can produce the disease. The initial virus multiplication usually takes place in the pharynx epithelium, producing primary vesicles or 'aphthae.' Within 24–48 h after the epithelium infection, fever and viremia start and the virus enters the blood to spread out and produce secondary vesicles, preferentially in the mouth and feet. Early signs of the disease are fever, prostration, lack of appetite, and lameness. Later, FMD is characterized by the formation of vesicles on the mucosa of the mouth, tongue, lips, cheeks, and palate. Also, vesicles are usually found on the skin between the claws of the feet, coronary band, teats, and udder. Besides farm animals, FMD also affects more than 70 species of wild ruminants. The acute phase of disease lasts approximately for 1 week and declines gradually coinciding with the appearance of a strong humoral response. On infection, FMDV elicits a rapid and broad spectrum of immune mechanisms, including humoral and cellular responses that induce efficient protection against reinfection with homologous and antigenically related viruses. Neutralizing antibodies against B-cell epitopes located on the viral capsid can be detected as early as 4 days after infection and peaks at approximately 10–14 days postinfection. Although FMD does not result in high mortality in adult animals, the disease has debilitating effects, including weight loss, decrease in milk production, and loss of draught power, resulting in a loss in productivity for a considerable amount of time. The recent outbreaks of FMD in a number of FMD-free countries, such as Taiwan in 1997; UK, France, and The Netherlands in 2001; Uruguay and Argentina in 2001; Japan 2002; and others have significantly increased public awareness of its highly disruptive effects from a socioeconomic point of view, as well as its possible use as a terrorist weapon. Despite low mortality rates, FMD severely decreases the livestock production and introduces important trade restrictions on animals and livestock products. In some cases, mortality can be observed among young animals, associated with lesions in the myocardium. In ruminants, an asymptomatic, persistent infection can be established during which the virus can be isolated from the esophagus and throat fluids of the animals. Although the mechanisms that mediate persistence are unclear, both naïve and vaccinated animals can become persistently infected or 'carriers' following acute or chronic, unapparent infection. However, the epidemiological role of these carriers as origin of outbreaks of acute disease is still unknown. Similarly, the genetic determinants for virulence, viral neutralization and host-specificity are yet to be defined.

Under certain circumstances, including the strain and host characteristics, FMD infection can be subclinical, with unapparent symptoms of disease that leads to unnoticed disease, mostly common between sheep and goats. The mechanisms for this kind of resistance to FMDV infection are not well understood and can also occur in animals exposed to the virus after either acute disease or vaccination. Unequivocal evidence of transmission from carrier or unapparent infected animals to susceptible hosts has neither been demonstrated under experimental conditions nor in the field.

Epidemiology and Risks of Transmission to Humans

The disease is enzootic in most parts of the world, with disease-free countries being Australia, New Zealand, North America, and Europe. The global distribution of FMD is clearly associated in areas with lower levels of economic development and it contributes to severe economic problems of many developing countries. The World Health Organization does not consider that FMDV can be transmitted from animals to humans (known as zoonosis). Only few cases, less than a hundred, have been somehow linked to manipulation of heavily infected animals or highly concentrated viral suspensions in laboratories. However, there is no proof of human infection with the animal virus and epidemiological data indicates that potentially contaminated products do not present food safety risks for humans. Long-distance airborne transmission has been documented. FMDV can be mechanically disseminated by animals, farmers, farming equipment, and during animal transport. There is no real proof of FMDV infection in humans; however, records from 1880s show that at least 2 people died of FMD and more than 200 suffered painful symptoms, with 'raw red' mucous membranes and chronic ulcers lined by 'thick puckered edges.' Also, 'the enlarged cervical (neck) glands remained tender, red, and swollen long after the throat symptoms had subsided, resembling, in this respect, scarlet fever; and in some instances, the feet of those who suffered were swollen and painful, simulating rheumatism,' but given the high incidence of the disease in animals across all continents, its occurrence in humans is strikingly rare. The last human case reported in Britain in 1966 was described as mild and self-limiting, mainly causing uncomfortable tingling blisters on the hands, feet, and in the mouth, including the tongue; fever; and sore throat. It has been demonstrated that after contact with FMD-infected livestock, virus can be recovered from nostrils, throat, and saliva; but till date, no implications for human health has been reported, and it is suspected that it might have been mistaken with the HFMD induced by the viruses from the *Enterovirus* group.

FMD is highly contagious and affects artiodactylous, mostly cattle, swine, sheep, and goats. FMD is one of the most communicable of all animal infections; it spreads by direct or indirect contact with infected animals, from their excretions

and secretions, or from contaminated materials and persons. The feeding of uncooked garbage containing infected meat scraps or bones is one of the most common means of spreading. Treatment to limit secondary bacterial pneumonia and mastitis are recommended only where FMDV is endemic, followed by local quarantine and revaccination with the most appropriate vaccine type for the outbreak. Otherwise, in FMD-free countries, the policy of stamping out used to be the choice, the measures taken are killing and disposal of all susceptible livestock on the infected farms and also their immediate contact farms, followed by a thorough disinfection and cleaning of the premises and its equipment, and quarantine with sentinel animals before repopulating the premises. After the 2001 outbreak in UK, in view of the dramatic situation that the stamping-out policy had created, public opinion questioned the need for large-scale slaughter of susceptible animals, particularly the slaughter of healthy animals that were just vaccinated to create a buffer zone around the outbreaks. The culls of healthy animals represent a human tragedy and a traumatic experience for the acting veterinarians, the farmers and their families, and the operators who have to deal with thousands of repeated killings.

Diagnostic Methods

Initial diagnosis is usually made on the basis of clinical symptoms, history of contact between the herd and an infected animal, report of FMD in neighboring farms, visit to market places or fairs, new livestock acquisitions in the farm, and other epidemiological data. In a fully susceptible herd, the clinical signs are frequently severe and pathognomonic. However, in endemic regions, in cattle that have partial natural or vaccine immunity, or in small ruminant's species, clinical signs may be mild and may be missed. Always a differential laboratory diagnostic is needed to rule out FMD from other vesicular diseases, such as swine vesicular disease, vesicular stomatitis, and vesicular exanthema of swine. Currently, FMD is confirmed by reverse transcription polymerase chain reaction (RT-PCR; usually real-time RT-PCR (rRT-PCR)), antigen capture enzyme-linked immunosorbent assay (AgELISA), and virus isolation (VI). Both, the rRT-PCR and conventional RT-PCR take less than 3 h to show results. Meanwhile, ELISA can be obtained in 3–4 h after the sample is received by the laboratory, but a negative result must be confirmed by inoculation of the sample into sensitive cultures (VI) followed by confirmation of the virus by RT-PCR and the virus serotype by AgELISA. These assays can take up to 4 days. RT-PCR methods are much faster and hence, more compatible with the need to rapidly detect disease and initiate an appropriate disease control strategy. Most interesting is the portable onsite diagnosis properties of the rRT-PCR. The assay is specific and as sensitive as VI, and viral RNA can readily be detected in oral and nasal samples, blood, milk, and organs from experimentally infected animals 24–96 h before the onset of clinical signs. In 1966, Cowan and Graves identified a highly immunogenic FMDV-NSP antigen, called the virus infection-associated antigen (VIAA, which was subsequently identified as the viral RNA polymerase), which reacted with the sera from convalescent animals but not with the sera from vaccinated animals and could be used in an agar gel immunodiffusion test to differentiate infected from vaccinated animals (DIVA). However, in later studies, it was seen that sera from vaccinated animals were reactive against VIAA when they had received multiple doses or when the vaccines did not have enough quality and were contaminated with NSPs. The concept of using NSPs as a DIVA test deserved further work to improve the reliability of this diagnostic assay. ELISA-based assays with various NS 3AB, 2C, 3C, and 2B, their respective peptides, the products from recombinant baculovirus, *Escherichia coli*, or synthetically produced peptides to NSPs have been developed and are currently being validated. Whole and clotted blood samples and probang samples may also be sent.

Antibodies to FMDV can be detected in the milk of cattle that have recovered from FMD, using either the liquid-phase blocking ELISA or a specific isotope assay for bovine immunoglobulin G1.

Control/Preventive Measures

FMD control in endemic areas is implemented by regular vaccination, which has resulted in the eradication of the disease in some areas of the world. Conventional vaccines based on chemically inactivated viruses are widely used. They elicit a consistent humoral response, albeit generally weaker than that induced in infected animals. This response correlates with a solid lymph proliferative response and with considerable, although short-lived, protection. However, they do not prevent the persistent infection of protected animals with FMDV and despite the fact that the inactivated virus do no replicate in the vaccinated animals, vaccines might be contaminated with viral NSPs, making the distinction between vaccinated and infected animals impossible. During the past decades, FMD-free countries have started policies of holding strategic reserves of FMD vaccines in the event of an outbreak, storing concentrated antigens indefinitely on liquid nitrogen, which can be rapidly formulated into emergency vaccine in the case of an outbreak (for instance, the North American Vaccine Bank and the European Union Vaccine Bank).

Another concern related with FMD vaccines is the fact that vaccinated animals can become long-term carriers following contact with active FMDV. It does not mean that the vaccine produces a carrier state in the animal, on the contrary, vaccination reduces the amount of FMDV that is released into the environment during an outbreak, but there is always a window of susceptibility between the vaccination and the appearance of protection against infection that will help the establishment of persistent unapparent infections.

Finally, it has to be considered that vaccine production and vaccinated animals have both been frequently associated with emergence of viral escapes and induction of carrier animals. Conventional vaccines against FMD are concentrated with cell culture supernatants from FMDV infected cells that require high technology containment barriers to be handled with reasonable degree of safety against an accidental release. They are the product of the best possible guaranties of inactivation with chemical products and require the use of adjuvants,

$Al(OH)_3$/saponin, and oil-based formulations to enhance the immune response to the FMDV antigen. Most often, more than one serotype is included in the same vaccine formulation allowing protection for a limited spectrum of viral variants. Therefore, it is a strong need of genetically engineered immunogens that provide lower risk of escape and allow DIVA strategy.

All these concerns have inclined the FMD-free countries toward a nonvaccination, stamping-out policy. This nonvaccination policy implies slaughtering of all infected and contact animals, associated to animal movement restrictions, increase of the importation of animals and animal subproducts from the nonaffected areas, and total paralysis in trade and export business. These strict control measures also affect other industries related with products and subproducts, such as products for feeding animals, industries of food complements, leather industry; even tourism is affected due to the restrictions in movements of people across the sanitary barriers. Although effective in maintaining an area free of FMDV, the control of the disease is difficult by several socioeconomic as well as technical factors. The wide range of antigenic diversity of the virus does not help either in making the control an easy task. Our current level of understanding of antigenicity and cross-neutralization factors for FMDV is not enough for the preparation of a universal vaccine. Perhaps most importantly, vaccination with inactivated antigens as is currently done does not allow differentiation between vaccinated and convalescent or FMD-recovered animals. Hence, current control and eradication measures consist of vaccination and stamping-out policies.

FMDV RNA behaves as a messenger; in fact, it is infectious when transfected into susceptible cells. This property of direct translation in the cytoplasm of the infected cell allowed the construction of infectious cDNA clones that can be manipulated and used as a tool for studying functional motives and critical features of the virus, which will help in the development of rational and secure new immunogens that allow DIVA strategy.

See also: Disciplines Associated with Food Safety: Food Virology. Food Safety Assurance Systems: Good Animal Husbandry Practice

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ORGANISMS OF CONCERN BUT NOT FOODBORNE OR CONFIRMED FOODBORNE

Classical Swine Fever Virus

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Glossary

Acute infection A presentation of the disease with a rapid onset and/or short course of symptoms and disease evolution.

Amino acids Critical to life molecules that contain an amine group, a carboxylic acid group, and a side chain that varies between different amino acids. They have many functions in the metabolism and are the building blocks of proteins.

Carrier An infected individual that carries the infectious agents despite a healthy appearance.

Chronic infection Long-lasting course of a disease in which there is a clear presentation of symptoms related with such disease.

Epitope Also known as antigenic determinant, this is the part of an antigen that is recognized by the immune system.

Kbp Kilobase pairs; 1 kilobase contains 1000 pair bases.

Nucleotide Molecules that join together in chains, to make up the structural units of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA).

ORF Open reading frame, the tract of genetic sequence that spans without interruption between a start codon and the stop codon, and that in such a given frame codifies for a unique protein.

Persistent infection Remaining infection after recovery of the disease in which it is still possible to recover infectious virus from the individual despite the absence of disease symptoms.

Positive-strand RNA virus Virus containing positive-sense viral RNA, which is similar to mRNA and thus can be immediately translated by the host cell.

Background and History

Classical swine fever (CSF), otherwise known as hog cholera, pig plague (*Schweinepest*), or *Peste Porcina Clasica*, is a specific viral disease of pigs. It does not affect humans or other vertebrates. According to early reports, CSF was a per acute-to-acute disease with a short incubation period and 100% mortality. The disease was first identified in the early 1830s in Ohio, USA, and has since been widely distributed in the Americas, Europe, and Asia for a long time. It was eradicated from North America in 1978 and has also been eradicated from Australia and most countries in Western Europe, but is endemic in Central and South America, the Caribbean, Asia, and Eastern Europe. With the exception of South Africa, CSF has not been reported from the African continent, although its absence does not correlates with the data that supports freedom from disease. It has also been confirmed in the islands of Madagascar. Other regions believed to be free of CSF include Canada (1962), New Zealand, and Scandinavia.

The Virus

The etiological agent known as the CSF virus (CSFV) is an enveloped small virus of the genus *Pestivirus*. Pestiviruses belong to a large family of positive-strand RNA viruses, the Flaviviridae that includes three genera: *Flavivirus*, *Pestivirus*, and *Hepacivirus*, plus two groups of viruses that have not yet been classified (GBV-A and GBV-C). Like the rest of the Pestivirus, CSFV are spherical, enveloped particles of 40–60 nm in diameter, which contain a single molecule of RNA of positive sense. Co- and posttranslational processing of the polyprotein encoded by the viral RNA gives rise to the following 11–12 final cleavage products or viral proteins: Npro, C, Erns, E1, E2, p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B. Protein C and the glycoproteins Erns, E1, and E2 represent structural components of the virion. E1 and E2 are anchored to the envelope by their carboxy termini, with Erns loosely associated with the envelope. Erns and E2 are present as homodimers linked by disulfide bridges on the surfaces of CSFV virions, whereas E2 is also found dimerized with E1. The genetic basis of CSFV virulence and host range remains poorly understood but recent development of infectious CSFV cDNA clones has enabled genetic approaches for defining mechanisms of viral replication and pathogenesis and identifying viral proteins or protein domains functioning in viral virulence and host range.

The Genome

The CSF genome is a single-stranded RNA molecule of positive sense and approximately 12.3 kb in length. It lacks 5′cap and

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3′ poly-A tract. Instead, 5′ and 3′-non-translated regions (NTRs) flank a unique open reading frame (OFR), which translates in a single polyprotein of approximately 4000 amino acids long. The NTRs harbor important primary and secondary structural elements that have been shown to be of critical importance for efficient replication of the RNA. Additionally, CSF translation initiation is cap independent and mediated by and internal ribosomal entry site that precedes the AUG start codon downstream in the genome. Thus, the positive-strand RNA genome not only functions as messenger RNA allowing immediate translation of the viral proteinsbut also serves as template for RNA replication.

The RNA is translated in a single polyprotein of more than 4000 amino acids, which is processed in the final viral protein products by a combination of cellular and viral proteases. One of the first translated proteins is the protease, known as N^{pro}, responsible for autocleavage of the polyprotein. Other viral serine proteases and host signal peptidase are believed to be responsible for several other cleavages that will lead to the generation of a total of structural (core, Erns, E1, and E2) and nonstructural viral proteins (p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B).

RNA replication is associated with cytoplasmic membranes and occurs between 6 and 24 h post-infection. CSFV mature in intracellular vesicles and are released by exocytosis.

The Target Host Cell

Cells of the monocyte–macrophage lineage are the natural target cells for viral infection and replication. But other cells from the reticuloendothelial system are also affected. On enterring the natural host, the primary site of replication of CSFV is the epithelial tissue that lines the tonsilar crypts; from there, spreads through the lymphatic vessels to the submandibular and pharyngeal lymph nodes that drain the tonsilar region and later to other lymph nodes. CSFV has direct effect on vascular endothelial cells, which is responsible for the characteristic petechial or echymotic hemorrhages in skin, bladder, kidneys, and serous and mucous membranes. Thymic and bone marrow atrophy and destruction of germinal centers in secondary lymphoid organs are the causes that lead to severe leukopenia and immunosuppression in the infected pigs; B-lymphocytes, T-helper cells, and cytotoxic T-cells are all depleted to different degrees. As a result, CSFV-infected pigs are immunocompromised and susceptible to secondary infections that mask the difficult detection of CSF.

Clinical Signs of the Disease

The main natural route of infection is oronasal by direct or indirect contact with infected pigs or by feeding of virus-contaminated feed. In areas with a high density of pigs, spread of virus easily occurs between neighboring pig holdings. Disease transmission via semen of infected boars may also occur. The incubation period in experimentally infected animals is approximately 1 week to 10 days, but under field conditions clinical symptoms may take up to 2–4 weeks or even more if only adult breeding pigs or mild strains of virus are involved. Highly virulent strains can cause clinical symptoms as early as 2 days postinfection. The clinical signs of CSF are extremely variable and depends both on the virulence of the virus strain, the infectious dose, and the immune response of the pig. Highly virulent strains correlate with acute, obvious disease and high mortality and can cause clinical symptoms as early as 2 days postinfection. Nevertheless, even though pigs of all ages are susceptible, adult pigs often develop less severe disease and have a better chance of survival than piglets. Less virulent strains give rise to subacute or chronic infections that may escape detection, while still causing abortions and stillbirths. In these cases, serological surveillance is critical to confirm the absence of disease. Therefore, CSF clinical forms can be distinguished as acute, chronic, and prenatal.

Acute Course of Classical Swine Fever Virus Infection

Some strains of CSF have an hyperacute course of the disease, characterized by a short incubation period with high fever and death 2–5 days after infection. Mortality rises to 90% or 100% of the infected pigs. In acute presentations, the initial signs are nonspecific such as anorexia, lethargy, and fever; the increased fever may lead to redness of the skin, conjunctivitis, swollen lymph nodes, respiratory signs, and constipation followed by diarrhea. The typical hemorrhages of the skin are not always present, but if so they are usually observed on the ears, tail, abdomen, and the inner side of the limbs progressing toward cyanosis near the terminal stage of disease. Neurological signs are frequently seen, such as a staggering hind limb gait, incoordination of movement, and convulsions, progressing to posterior paresis toward the end of the process. Vomiting, transient constipation followed by severe watery, yellow-gray diarrhea are also frequent in this acute and hyperacute forms. Leukopenia and immunosuppression often leads to enteric or respiratory secondary infections that may mask or overlap the most typical signs of CSF and make difficult the identification of the disease to the farmer or veterinarian. Death occurs usually within 1 month. Recovery with production of antibodies does occur, most often in adult breeding animals that do not display severe clinical signs. Antibodies against CSFV are detectable from 2 to 3 weeks postinfection onward.

Postmortem examination shows pathological modifications in lymph nodes, tonsils, spleen, and kidneys. The predominant lesion of acute CSF is hemorrhage. The lymph nodes become swollen, edematous, and red marmoreal. Hemorrhages of the kidney may vary in size from hardly visible petechiae to ecchymotic hemorrhages. Similar hemorrhages can also be observed in the urinary bladder, larynx, epiglottis, and heart, and sometimes widespread over the serosae of the abdomen and chest. Necrotic foci may also be present on the palatine tonsils. Splenomegaly and spleen infarction occurs sometimes and can be considered pathognomonic of CSFV and African swine fever virus (ASFV) infections. Lesions associated with encephalitis, microgliosis, and focal necrosis in central nervous system can be observed. Lesions due to secondary infections may also be seen, which may mislead the pathologist, such as infarctions and hemorrhages are seen in the lung, pericardial, and peritoneal accumulation of serosous fluids, as well as catarrhal to fibrinous bronchopneumonia and pleuritis as a result of secondary infections.

Chronic Course of Classical Swine Fever Virus Infection

The chronic course of infection starts as any acute infection, with anorexia, depression, intermittent fever, chronic enteritis, and wasting, but is followed by a stage of recovery, characterized by the general improvement of the clinical status of the animal. Later on, a third stage of relapse and deep sickness, often accompanied by death, follows. The typical hemorrhages of the skin are missing, although multifocal and necrotic lesions can occur. Animals can survive more than 3 months before their eventual death. Constipation and diarrhea are alternated, in periods that last for weeks or even months. Poor growth and bad condition are typical (runted pigs).

Pathological changes are similar but less severe than those in acute and hyperacute forms, especially concerning the lack of hemorrhages on organs and serosae. In animals showing chronic diarrhea, necrotic lesion in the ileum, the ileocecal valve, and the rectum are common. Secondary bacterial infections are more common, and cecum and colon can be found ulcerated.

Congenital Course of Infection and Late Onset of Disease

Depending on the virulence of the strain of virus infecting the pregnant sow and the stage of gestation, the outcome of the CSFV infection varies. High virulence strains of CSFV usually results in abortions or early death of the piglets, but infection with moderate and low virulence strains are difficult to detect. In these cases, the disease is subclinical for the sow with a transient fever, slight anorexia, and poor reproductive performance. CSFV is able to pass across the placenta of pregnant animals and infect the fetuses; thus when the infection occurs during early pregnancy may result in abortions and stillbirths, mummification, and malformations. In this case, all that is noticed is a reduction of the fertility index of the herd. When the infection of sows occurs during the first 90 days of pregnancy, healthy-looking, persistently infected piglets are born. These piglets do not show antibodies against CSFV, have persistent viremia, and can excrete large amounts of virus during their short but clinically normal life. Usually after 2–11 months from birth, anorexia, depression, conjunctivitis, dermatitis, diarrhea, and paresis lead them to death.

Prevention and Control

CSF live attenuated vaccines have been in use for a long number of years for controlling and eradicating the disease. The better known are the Chinese (C), the Japanese GPE, the Thiverval, and the Mexican PAV strains. These vaccines have shown to be effective in protecting against clinical signs, virus replication, and virus shedding. Therefore, their inclusion in measures to control CSF can reduce costs and limit spread of CSF outbreaks. The only problem of using vaccination as a control measure is that it is not possible to distinguish between vaccinated and naturally infected serologically positive animals. A general and fruitful effort in all laboratories interested in CSF has been, and still is, the development of new immunogens that allow distinguishing between infected and vaccinated animals. Currently, at least two subunit vaccines based on the use of the protein E2 glycoprotein as the only CSF antigen has been developed. Also, an infectious clone of bovine viral diarrhea virus (BVDV) has been used to prepare deletion of genetic mutants that protect against challenge with virulent strains, but do not produce either clinical signs when inoculated or viral shedding or persistent infection of the inoculated and/or challenged pigs. Natural infection induces strong immunity, but 10–12 days after infection there are circulating neutralizing antibodies, and survivor pigs develop long-lasting humoral immunity.

Regulatory measures, restriction trade policies, and periodic clinical inspections in critical points of commerce and production of pork products are significant tools for prevention. Additionally, CSF prevention has a strong component that can only be achieved through effective communication between veterinary authorities, veterinary practitioners, and pig farmers; effective disease reporting and animal identification system; a strict import control of live pigs, fresh, and cured meat; prohibition of feeding pigs with waste food; and biological and serological surveillance. Finally, the importance of serious rules of on-farm biosecurity cannot be overestimated. The ban on commercial slaughter and sale of pigs meant that all of these pigs had to be destroyed in such a way that they do not enter the food chain, creating a massive problem of disposal. The conclusion is that although the control strategy applied was effective and the costs were justified when compared to that of control failure, they could have been greatly reduced if good biosecurity had been implemented during the outbreak.

To achieve eradication of CSF after an outbreak, stamping out of infected and in-contact pig herds with destruction of the carcasses is traditionally considered to be the best option. Protection areas of infected, surveillance, and control around each outbreak, with restrictions on pig movements should be established as soon as possible. Then, an epidemiological investigation for tracing the source and spread of infection should be carried out. And an emergency vaccination program around the outbreak can also be used to limit disease spread and reduce the number of slaughtered animals. Because vaccine-produced antibodies are indistinguishable from antibodies originated after infection with the virus, all vaccinated animals should be eliminated later on. However, following the control and resolution of the outbreak, the vaccinated animals can be used in commercial slaughter. After an outbreak has been eradicated, it is necessary to put in place a monitoring and surveillance system to demonstrate, at an acceptable level of confidence, that the eradication was successful and the area or country is free of CSF infection. Such surveillance systems need to be maintained for a sufficient period to be sure that eradication has been accomplished, and on-going systems designed to ensure early detection of CSF.

Public Health

Humans are not susceptible to CSF infection. CSF persists infectious in uncooked pork and processed pork that has not been heated to high temperatures for long periods, including salted, smoked, and fresh chilled pork (months and even years depending on the conditions of preservation); however, no cases of disease or seroconversion have ever been reported in CSF endemic countries. On the contrary, feeding pigs uncooked

swill that may contain pork products is extremely dangerous, illegal introduction of meats from CSF endemic countries, vehicles used to transport pigs between high-risk or endemic and free of CSF areas are all extremely dangerous practices because it has been demonstrated that CSF can be transmitted that way. In summary, CSFV is relatively stable in moist excretions of infected pigs, pig carcasses and fresh pig meat, and some pig meat products but is readily inactivated by detergents, lipid solvents, proteases, and common disinfectants.

Diagnosis

CSF is often difficult to recognize because of the wide range of clinical signs and lesions reported according to the amount and virulence of the virus and the age, breed, and immune capability of the pig. Besides, most of the general symptoms can be confused with other diseases, in particular not only ASF, but also porcine reproductive and respiratory syndrome (PRRS) and bacterial septicemia or pneumonia. Acute CSF needs to be differentiated from erysipelas, PRSS, cumarin poisoning, purpura hemorragica, postweaning multisystemic wasting syndrome, porcine dermatitis and nephropathy syndrome, *Salmonella* or *Pasteurella* infections, or any enteric or respiratory syndromes with fever, which do not respond to antibiotic treatment. When present, hemorrhages on the skin and ears are quite easy to detect and lead to the suspicion of acute ASF or CSF, because few other diseases cause similar lesions, but in chronic cases, the multifocal and necrotic skin lesions can be confused with those seen in porcine dermatitis and nephropathy syndrome and erysipelas. Reduced fertility and abortions can be caused by CSFV as well as parvovirus infection, PRRS, Leptospirosis, and Aujeszky's disease. Materials aborted due to CSF infection cannot be distinguished grossly from abortions due to other diseases. In case of an infectious disease of the reproductive tract, investigation for CSF must be immediately carried out in case that the holding in question can be considered at risk for CSF (e.g., due to location of the holding in an area where CSF occurs in feral pigs) and, in any case if more common infectious diseases of the reproductive tract have been excluded.

Therefore, laboratory diagnosis and confirmation of the infectious agent is of paramount importance for CSF. Despite all, the close genetic and immunogenic relationships between all pestiviruses (BVDV and border disease virus) make difficult even the laboratory discrimination of CSFV. Samples of choice for detection of CSFV are tonsillar tissue, lymphoid tissues (lymph nodes and spleen), and the distal ileum. A recent experimental study demonstrated that the nictitating membrane (third eyelid) provides a useful source of virus in pigs that have undergone autolysis, because this organ is much less affected by autolysis than the internal organs. Whole blood samples, nasal swabs, tonsillar scrapings, and tonsillar swabs may be taken from live pigs. Because the disease may run a chronic course, with animals in different stages of postinfection, samples should be taken from as many animals as possible.

Isolation of the virus remains an essential element of the laboratory tools to diagnose CSF, although this is made difficult by the fact that it is a noncytolytic virus and indirect methods are needed to detect it in cell cultures. Pig kidney cell cultures followed by immunofluorescence or immunoperoxidase staining are performed using anti-CSFV polyclonal or monoclonal antibodies as conjugates. It can also be confirmed using reverse transcriptase polymerase chain reaction (PCR) and/or sequencing the nucleic acid product obtained from the infected cell culture. Enzyme-linked immunosorbent assay (ELISA) technology is also used to detect viral antigen in blood or organ samples and offers a rapid way of screening large numbers of samples, but is less sensitive than the PCR. Direct immunofluorescence detection of virus in histological cuts is widely used. A variety of polyclonal and monoclonal antibodies are available for detection of CSFV antigens in frozen tissues, impression smears, and bone marrow aspirates. The assay based on avidin–biotin complex monoclonal antibody is highly sensitive and successful for working with frozen tissue sections.

For detecting antibodies in sera of suspicious or surveillance animals, the indirect immunofluorescence test, virus neutralization test are available. ELISA is also used to detect antibodies in sera, but only some of the tests available distinguish between antibodies produced in response to CSFV and those elicited by related viruses.

Finally, the use of sequencing and molecular genetic studies of the CSFV virus provides important insights on the relationships of viruses isolated from different outbreaks. This analysis can help to point out the possible origin of outbreaks, and demonstrates whether in a particular area all the outbreaks of CSF are likely to be due to a single or multiple introduction of the disease.

Epidemiology

The potential of this disease to spread over long distances and cause outbreaks in areas previously free is widely demonstrated. CSFV transmission takes place through direct contact of susceptible swine with excretions, secretions, semen, blood, saliva, or other contaminated substances from infected animals. Acutely infected pigs that are shedding large amounts of virus in their saliva, as well as lesser amounts in urine, feces, ocular, and nasal secretions, are a potent source of infection for other pigs. Pigs start to shed virus for a few days before clinical signs develop, and continue to do so until antibodies develop, which usually happens approximately 11 days after infection. Congenitally infected piglets may be persistently viremic and may shed the virus for months. These piglets may play a crucial role in spreading the disease and in the maintenance of virus persistence within a population as they constantly shed virus until death. Indirect contact through contaminated vehicles, clothes, instruments, needles, pig traders, and farm visitors has also been described as the source of virus. Aerosol transmission over distances of less than 500 m has been demonstrated experimentally, but is not considered important except possibly under unusual circumstances. Investigations have indicated that persistently infected wild boars, either due to transplacental or postnatal infection, are unlikely to play a role in maintaining the virus. Spread from infected wild boar to domestic pigs and vice versa has taken place on several occasions in the past in some areas of Europe. Spread from wild boars to domestic pigs occurs when free-ranging domestic pigs come into direct

contact with infected boars or scavenge the remains of infected boars that have died or been killed by hunters. Experimental infection has demonstrated that the warthog (*Phacochoerus aethiopicus*) and the bush pig (*Potamochoerus larvatus*) are susceptible to infection and can spread disease to in-contact animals of the same species but the degree of its importance in the epidemiology of CSF is still unknown.

See also: Organisms of Concern but not Foodborne or Confirmed Foodborne: African Swine Fever Virus

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ORGANISMS OF CONCERN BUT NOT FOODBORNE OR CONFIRMED FOODBORNE

Bolivian Hemorrhagic Fever Virus (Machupo Virus)

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Glossary

Anthroponosis An infectious disease in which the etiological agent is carried by humans and is transferred to other humans and animals.
Foodborne disease Any disease of an infectious or toxic nature caused by or thought to be caused by consumption of contaminated food, including drink.
Organism life cycle A period involving all different generations of a species succeeding each other through means of reproduction, whether through asexual reproduction or sexual reproduction (i.e., a period from one generation of organisms to the same identical point in the next).
Surveillance In public health and epidemiology, it is the discipline of continuously gathering, analyzing, and interpreting data about diseases, and disseminating conclusions of the analyses to relevant organizations, to intervene in their patterns in order to control and prevent them.
Taxonomy The science of classification; in microbiology the arrangement of microorganisms into a classification.
Zoonosis Any infectious disease that can be transmitted between species (by different ways, by a vector or by their products and food) from animals to humans or from humans to animals (less common).

Introduction

Bolivian hemorrhagic fever (BHF) or Machupo hemorrhagic fever (ICD-10 A96.1) was first identified in 1959 as a sporadic hemorrhagic illness in rural areas of Beni department, Bolivia (Figure 1). BHF is a potentially severe febrile illness caused by Machupo virus (a member of the family Arenaviridae). Clusters of BHF patients were noted the same year and by 1962 BHF was recognized as a new epidemic infectious disease. In 1963, the etiological agent, the Machupo virus was first isolated from patients with acute hemorrhagic fever in San Joaquin, Bolivia.

The Pathogen

The etiological agent is a single-stranded ribonucleic acid (RNA) negative-strand virus (Arenaviridae–arenavirus of the New World). The Arenaviridae are a family of viruses whose members are generally associated with rodent-transmitted disease in humans (a zoonotic disease). They are classified as New World arenaviruses and Old World arenaviruses (Table 1). Each virus is usually associated with a particular rodent host species in which it is maintained. Arenavirus infections are relatively common in humans in some areas of the world and can cause severe illnesses. Other family members for the New World include Guanarito virus (etiological agent of the Venezuelan hemorrhagic fever), Junin virus (etiological agent of the Argentine hemorrhagic fever (AHF)) and Sabia virus (etiological agent of the Brazilian hemorrhagic fever) (Table 1).

Ecologic investigations established the rodent *Calomys callosus*, which is indigenous to the disease-endemic region of northern Bolivia (Figure 1), as the reservoir for Machupo virus. This rodent can enter homes in this region, thereby leading to contamination of dwellings, food, and water.

Chapare virus, shown in Table 1, also cause hemorrhagic fever in Beni department, Bolivia (Figure 1). Real time-polymerase chain reaction analysis of the Chapare virus isolates of 2003 and 2004 cases and subsequent analysis of the complete virus S- and L-RNA segment sequences identified the virus as a member of the New World clade B arenaviruses (Table 1), which includes all the pathogenic South American arenaviruses. The virus was shown to be most closely related to Sabia virus, and distinct from Machupo virus, which is the etiological agent of BHF.

Machupo virus infection in *C. callosus* results in asymptomatic infection with shedding of virus in saliva, urine, and feces; 35–50% of experimentally infected *C. callosus* are chronically viremic and shed virus in their bodily excretions or secretions. Although the infectious dose of Machupo virus in humans is not well known, exposed persons may become infected by inhaling virus shed in aerosolized secretions or excretions of infected rodents, by eating food contaminated with rodent excreta (then making it a foodborne disease), or by direct contact of excreta with abraded skin or oropharyngeal mucous membranes. Reports of person-to-person

Figure 1 Map of Latin America showing Bolivia (in yellow), the Beni department (in red) with its provinces (box) and indicating the countries with borders to this country.

transmission are uncommon; however, hospital contact with a patient resulted in person-to-person spread of Machupo virus to nursing and pathology laboratory staff (nosocomial transmission).

Transmission have been more often reported during spring and summer (April–July–September, the so-called dry season, in the upper savanna region of eastern Bolivia, Beni) (Figure 1), and particularly among agricultural workers.

Biological Hazards

Machupo virus, as many other hemorrhagic fever viruses (such as AHFs, dengue hemorrhagic fever, Marburg virus, Ebola virus, hantaviruses, Lassa fever, and Crimean–Congo hemorrhagic fever) is considered among the group of viruses and bacteria that cause severe to fatal disease in humans, and for which vaccines or other treatments are not available. This corresponds to biohazard level 4 (highest risk for humans).

Epidemiology

Close to 2000 cases of BHF have been reported since 1959 until May 2012. Most of those cases occurred during the first years of the history of BHF (1100 between 1959 and 1963). However, the disease is endemic and currently active. The last case (which was fatal) was reported on 20 April 2012. In 2012 three cases (all of them fatal) have been reported in Bolivia, all of them in Beni department (Table 2). As can be seen from the summary of cases, fatal cases and locations where these cases occurred, BHF has a high case-fatality rate, which can be approximately 22.73% (IC95% 20.60–24.48%) (Table 2), although in some outbreaks the rate can be as high as 100%. Besides these cases, more cases probably have occurred, but as a very restricted geographical disease, few additional information is available with international agencies (e.g., *Office International des Epizooties* or World Health Organization).

As can be seen from Table 2, the disease is endemic in the provinces of Mamoré, Iténez, and Yacuma, in the Beni department, Bolivia (Figure 1).

Outside Bolivia, in Panama imported cases of two North American researchers who were working in Beni have been reported. In Brazil, although no cases have been reported, in 1977, studies conducted in rodents (*C. callosus*) reported a high proportion of them with splenomegaly in an area close to the Iténez province, Beni department, Bolivia (Figure 1).

Given the epidemiology of this disease, international interest on research is limited. There are still many aspects of disease to be better defined. In Index Medicus database, using PubMed (http://www.pubmed.com/) there are less than 30 articles directly related to BHF (medical subject heading: 'bolivian hemorrhagic fever [ti]') (1965–2009).

Food contamination

Rodent excretions are the main source of contamination for food that can be ingested by susceptible humans and become infected. Rodents in rural areas can enter homes, contaminating dwellings and food and water. Raw food in endemic areas can contaminated with Machupo virus and become a source and risk for foodborne infection.

Table 1 Viruses included in the Arenaviridae family, showing the New World and Old World arenaviruses characterized and included in the NCBI Taxonomy Browser (showing those classified up to 2012) (http://www.ncbi.nlm.nih.gov/taxonomy)

New World arenaviruses	*Old World arenaviruses*
Allpahuayo virus	Ippy virus
Amapari virus	Lassa virus
Bear Canyon virus	Lymphocytic choriomeningitis virus
Chapare virus	Mobala virus
Cupixi virus	Mopeia virus
Flexal virus	
Guanarito virus	
Junin virus	
Latino virus	
Machupo virus	
Oliveros virus	
Parana virus	
Pichinde virus	
Pirital virus	
Sabia virus	
Tacaribe virus	
Tamiami virus	
Whitewater Arroyo virus	

Clinical Presentation

BHF is clinically similar to AHF, however, neurological signs are more common in AHF, whereas hemorrhagic diatheses are more common in BHF. After an incubation period of 5–19 days, initial symptoms include headache, fever, arthralgia, and myalgia. In the later stages of this illness, patients may develop hemorrhagic manifestations including petechiae, subconjunctival hemorrhage, epistaxis, hematemesis, leukopenia, thrombocytopenia, melena, adenopathy, relative bradycardia, pulmonary edema, and hematuria, as well as neurological signs including tremor, seizures, and coma, and internal hemorrhage may occur. Pulmonary edema is the most common cause of death.

During the BHF epidemics of the 1960s, convalescent-phase immune plasma from survivors of BHF was administered to selected patients infected with Machupo virus. However, there is, at present, a paucity of survivors of BHF who can donate immune plasma and no active program for collection and storage of BHF-immune plasma.

Diagnosis of BHF

Diagnostic tests available for BHF include viral culture (using VERO cells) from biological samples of the suspected cases (blood, urine, and pharynx) and serology (enzyme-linked

Table 2 Cases reported through ProMEDmail (http://www.promedmail.org), historical records and surveillance of the Bolivian Ministry of Health (http://www.sns.gob.bo/), 1959–2012

Year	*Number of cases*	*Fatal cases*	*%CFR*[a]	*Location*
1959–63	1100	260	23.6	Mamoré and Iténez provinces, Beni
1963–64	650	122	18.8	San Joaquin, Mamoré province, Beni
1963	2	–	–	Panama (imported from Beni)
1968	6	6	100.0	Magdalena, Iténez province, Beni
1969	9	–	–	Magdalena, Iténez province, Beni
1971	6	5	83.3	Cochabamba department (southern border of Beni), nosocomial, index case came from Beni, one case was health care occupational
1971	4	–	–	Yacuma province, Beni
1974–75	4	2	50.0	El Recuerdo, Mamoré province, Beni
1976–92	–	–	–	Apparently no cases reported
1993	1	1	100.0	San Ramon, Mamoré province, Beni
1994	10	7	70.0	Magdalena, Iténez province, Beni
1996	3	–	–	Beni deparment
1999	5	–	–	Santa Cruz department (southeast border of Beni)
1999	3	–	–	Tarija department (without borders with Beni, border with Argentina)
2004	2	2	100.0	Huacareje and Magdalena, Iténez province, Beni
2007	20	3	15.0	Magdalena, Iténez province, Beni
2010	1	1	100.0	Beni deparment
2011	3	1	33.3	Beni deparment
2012	1	1	100.0	Beni deparment
2012	1	1	100.0	San Ramon, Mamoré province, Beni
2012	1	1	100.0	Penas Verdes, Mamore province, Beni
Total	1832	413	22.5	

[a]%CFR: case fatality rate.

immunosorbent assay, ELISA) to detect antibodies anti-Machupo virus. Limitations to its diagnosis include that owing to its pathogenicity, Machupo virus requires biosafety level 4 conditions, the highest level.

Management, Prevention, and Control

Although no effective treatment is available for BHF, intravenous ribavirin has been used with some favorable results in the management of disease. However, more extensive clinical studies to assess the usefulness of ribavirin for treating BHF are needed.

The standard management of BHF includes strict isolation, use of specific immune plasma, and ribavirin at a dose of 2.0 g intravenous (i.v.), 1.0 g i.v. Q6 h $\times$ 4 days, and 0.5 g i.v. Q8 h $\times$ 6 days.

The main strategy to prevent this disease is to control rodent populations. In San Joaquin, Mamoré province, Beni department (**Figure 1**), Bolivia, a study has shown the importance of using rodent traps and rodenticides that destroyed more than 3000 specimens of *C. callosus* in 60 days. As rodents can contaminate food and water, rodent control is fundamental to avoid foodborne disease transmission.

A vaccine developed for the genetically related Junin virus causing AHF has shown evidence of cross-reactivity to Machupo virus and may, therefore, be an effective prophylactic measure for people at high risk of infection. Postinfection (and providing that the person survives the infection), those who have contracted BHF are usually immune to further infection from the disease.

Conclusions

BHF is a highly fatal disease, also known as black typhus or Ordog fever, that still needs and deserves further research in many aspects. More published research on BHF has been focused on epidemiological, viral, and molecular diagnoses; however, understanding of its importance and impact as a zoonotic foodborne disease is still very limited.

See also: Food Safety Assurance Systems: Microbiological Testing, Sampling Plans, and Microbiological Criteria. Institutions Involved in Food Safety: Trust in Animals and Food Safety (TAFS) Forum; World Health Organization (WHO); World Organisation for Animal Health (OIE). Public Health Measures: Surveillance of Foodborne Diseases

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ORGANISMS OF CONCERN BUT NOT FOODBORNE OR CONFIRMED FOODBORNE

African Swine Fever Virus

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Glossary

Acute infection A presentation of the disease with a rapid onset and/or short course of symptoms and disease evolution.

Carrier An infected individual who carries the infectious agents despite a healthy appearance.

Chronic infection Long-lasting course of a disease in which there is clear presentation of symptoms related with such disease.

Persistent infection Infection that remains after recovery from the disease where it is still possible to recover infectious virus from the individual despite the absence of symptoms.

Background and History

African swine fever (ASF) is also known under the following names: *Peste Porcine Africaine, Peste Porcina Africana, Pestis Africana Suum, maladie de Montgomery,* warthog disease, *Afrikaanse Varkpes,* and *Afrikanische Schweinepest.*

ASF virus (ASFV) was first described as a disease in 1909 when it infected domestic pigs of European origin in Kenya. It is thought that ASF has existed for many years as an inapparent infection in warthogs, bush pigs, and giant forest hogs in endemic areas of Africa. For a long time it was confined to Africa, but in 1957 it was discovered in Portugal. This first incursion in the European continent was eradicated in 1958. Outbreaks occurring along the Spanish border in France in 1964, 1967, and 1974 were also eradicated by slaughtering infected and exposed animals. Since the mid-1960s, outbreaks have occurred in Italy, Spain, Malta, Sardinia, Belgium, and the Netherlands. In the western hemisphere, ASF first appeared in Cuba in 1971, and later in Brazil, the Dominican Republic, Haiti, and again, Cuba. Finally, during the 1990s, the disease was eradicated by depopulation everywhere except in Africa, with the only exception being the island of Sardinia in the Mediterranean Sea. The most recent outbreak of ASF outside Africa started in early 2007 in Georgia, and has since spread to the countries of Armenia, Azerbaijan, Iran, and Russia. Major outbreaks of ASF in Africa are regularly reported to the World Organisation for Animal Health (OIE).

The Virus

The virus responsible of this disease (ASFV) is the only known animal virus with a deoxyribonucleic acid (DNA) genome that is transmitted by arthropods. Ticks of the *Ornithodoros* genus become infected by feeding on infected warthogs and bush pigs, and transmit to other hosts. Direct transmission of ASF has been proved to occur between domestic pigs, while indirect transmission through ticks is the most common way of transmission in wild swine species. ASF manifests itself in several forms, ranging from highly virulent forms that kill most pigs to subclinical forms that result only in seroconversion. Currently, there is no vaccine and the control of the disease is made by animal quarantine and slaughter.

ASFV is a large, enveloped, double-stranded DNA virus that replicates in the cytoplasm of the infected cell. Is the only member of the Asfarviridae family despite the fact that it shares many similarities with other DNA families like Poxviridae and Iridoviridae. In terms of replication strategy and genome structure, the ASFV genome shows enough genetic divergence relative to the other large DNA families as to be considered apart.

ASFV particles have a complex multi-layered structure that includes a nucleoprotein core or nucleoid that contains the viral genome, surrounded by a core shell or matrix which contains several different proteins that is itself surrounded by two lipid bilayers, also known as the inner membrane or internal envelope, which likely derives from the cellular endoplasmic reticulum. Surrounding the matrix is the capsid, assembled by capsomers of structural proteins that provides the icosahedral structure to the virion. Finally, although not required for virus infection, there is sometimes an external membrane as a consequence of the budding through the plasma membrane. The viral particle is large and contains enzymes and factors needed for viral replication in the cytoplasm of the infected cells.

The Genome

Contained inside the virion, there is a single molecule of linear, double-stranded (ds) DNA that is cross-linked at the

ends, which is between 170- and 192-kbp long. The DNA contains one left and one right terminal genomic regions characterized for carrying: terminal inverted repeats, terminal cross-links, and multigene families; additionally, there is a long central region with conserved and variable sequences and direct repeats interspersed with unique sequences. There are between 160 and 175 open reading frames (ORFs), that encode for more than 150 different proteins, including among others, homologs of cellular ubiquitin, inhibitor of apoptosis, myeloid differentiation primary response antigen MyD116, lectine- and CD2-like proteins, and components of a base-excision repair pathway.

Recent research on the molecular aspects of ASFV, making use of recombinant viruses that contain complete deletions of the ORF for a given gene, has allowed identification of some of the functions of ASFV genes. There are a number of genomic regions involved in virulence, tropism, cell response regulation, and host-range that have been identified. However, despite the short evidence of those genes functionality, they alone are usually sufficient to completely knock down the virulence of the virus, indicating that more than one viral determinant or genomic region might be involved in the same functions. The targeted deletion of genes which causes a reduction in virulence, altered tropism or reduced viability, and replication ability in cell cultures, though not always, correlate with the total loss of virulence in domestic pigs. This illustrates the complexity of the pathological manifestation of the virus in natural hosts. The information that these techniques have provided to the understanding of ASFV is highly valuable and has provided a solid basis for the future development of an effective vaccine, probably through the sequential deletion of targeted genes.

The Target Host Cell

In common with other viral hemorrhagic fevers, the main target cells for replication are those of monocyte–macrophage lineage. The ability to replicate and kill macrophages appears to be a critical factor of virulence in ASFV. Infection induces a progressive down regulation of host protein synthesis at early times postinfection. Virus replication takes place in perinuclear factory areas, and assembly of the icosahedral capsid occurs on modified membranes from the endoplasmic reticulum, where immature capsids and mature viral particles can be detected after 12–24 h of infection (hours post infection (hpi)). The infection of pigs results in lymphocyte, macrophage, and megakaryocyte apoptosis, which heavily impacts the structure and functionality of lymph nodes, spleen, and thymus, which is reflected in lymphoid cell depletion and immunodeficiency.

Clinical Signs of the Disease

Sudden deaths with few lesions are characteristic of the peracute form and may be the first sign of an infection in a herd. The acute form of the disease is characterized by a short period of incubation, between 5 and 15 days, starting with high fever; anorexia; congestion and cyanosis of the skin (particularly on the ears and flanks); shivering and incoordination; diarrhea, which is initially mucoid and later may become bloody; and death within the 2–9-days period of showing symptoms. At necropsy, there are hemorrhages in lymph nodes, spleen (very enlarged), kidneys, and respiratory and gastrointestinal tracts with severe interlobular lung edema. Pregnant sows abort; in some cases, abortions may be the first signs of an outbreak. The disease spreads through the herd over several days or sometimes more slowly over several weeks and many pigs die.

In subacute presentations, the disease lasts 3–4 weeks with remittent fever, pneumonia, dyspnea, *caquexia*, and swelling of the joints. Some outbreaks of the disease in places where the virus is endemic in the domestic pig population are milder, run a longer course and spread more slowly through a herd, with fewer deaths. Some affected pigs become very thin and stop growing and develop signs of pneumonia, skin ulcers, and swollen joints. In this form of the disease, the death rate is generally lower in adult swine, but may still be very high in very young animals. In subacute disease, fever, thrombocytopenia, and leukopenia may be transient, and affected pigs usually die or recover within 3–4 weeks. Sometimes, animals infected with isolates of low virulence may seroconvert without symptoms, abort, or develop chronic ASF. Persistent infection occurs in warthogs and in domestic pigs surviving the acute or subacute viral infection, and anecdotic reports of sudden reactivation and death with ASF symptoms have been recollected. Under experimental conditions, viral DNA was detected in monocytes more than 500-days post infection by polymerase chain reaction (PCR). However, the infectious virus has never been isolated in samples from those animals.

Prevention and Control

ASF is reportable to the OIE. There is no vaccine available and only homologous (rarely heterologous) protection has been described in infection-surviving individuals. Pigs surviving infection with attenuated or low-virulent strains produce antibodies that protect it from fatal infection with the homologous virus, but the neutralizing value of such immune response is still a matter of discussion for some ASF experts.

Prevention relies on strict regulations on the import of animals and animal products from countries where ASF is known to occur. These regulations are enforced through point-of-entry inspections and the compulsory boiling of waste animal products under licensed supervision before feeding to pigs. In Africa, prevention includes measures to keep warthogs and materials contaminated by warthogs away from the herd. The application of a slaughter policy when the disease is diagnosed is mandatory.

Eradication is based on the slaughter of infected and in-contact animals, and disposal of carcasses, often by burying, rendering, or burning. Rapid diagnosis and the prevention of disease spread to feral or wild pigs are very important. Strict quarantines must be imposed. ASFV can survive for long periods on fomites and in the environment, thus sanitation and disinfection are important in preventing further spread. Disinfectants specifically approved for ASF such as sodium

hypochlorite and some iodine and quaternary ammonium compounds are effective. Potential tick vectors should be controlled with acaricides. In addition, biting insects that may be able to transmit the virus mechanically should be controlled.

Public Health

Humans are not susceptible to ASFV. ASFV remains infectious for 3–6 months in uncooked pig products such as sausage, filet, and dry hams. In 1983, ASF spread from Sardinia to the mainland in boar meat.

Diagnosis

Suspect ASF in the field is based on clinical signs and necropsy findings in affected pigs, and a high death rate in affected herds. However, it will be extremely difficult to identify the disease caused by moderate or low virulence strains, as the clinical signs are variable and very often obscured by other common pathogens acting simultaneously. The clinical symptoms of ASF are very similar to classical swine fever (CSF) and the two diseases normally have to be distinguished by laboratory diagnosis. However, before collecting or sending samples from animals with a suspected foreign animal disease like ASF, the proper authorities should be contacted. Samples should only be sent under secure conditions and to authorized laboratories to prevent the spread of the disease.

At necropsy, bright red hemorrhages in the lymph nodes, kidneys, heart, and linings of the body cavities are common findings. There may also be excess hemorrhagic fluid in the body cavities and gelatinous fluid in the lungs. The spleen may be enlarged, darkened, and crumble on slight pressure.

The sample of choice from live animals for laboratory diagnosis of ASFV are whole blood and serum. From dead animals tonsils, lymph nodes, spleen, kidneys, and distal ileum are best recommended. ASFV is not found in aborted fetuses; in cases of abortion, a blood sample should be collected from the dam.

It is essential to isolate and identify the virus, which may be isolated in primary cultures of pig bone marrow or peripheral blood leukocytes from the blood or lung. Infected cells hemadsorb pig red cells will adhere to them, in what is known as the hemadsorption or 'rosette' test. Most isolates of ASFV induce hemadsorption of pig erythrocytes to the surface of infected cells. However, nonhemadsorbing isolates can be missed with this test. ASFV antigens can also be found in tissue smears or cryostat sections, as well as in the buffy coat, with the fluorescent antibody test. The OIE does not consider this test alone to be sufficient for diagnosis, although it is useful in conjunction with other assays. Nucleic acids can be detected with a PCR assay or by the hybridization of nucleic acid probes to tissue sections. PCR is particularly useful in putrefied samples that cannot be used for virus isolation and antigen detection. A rapid, real-time PCR technique using tonsil scraping samples has recently been published. This test can detect the virus a few days before the onset of symptoms. Antibodies to ASFV persist for long periods after infection and serum antibody titers may be tested in a number of ways. These tests include the enzyme-linked immunosorbent assay (ELISA), immunoblotting, indirect fluorescent antibody, and counter immunoelectrophoresis (immunoelectro-osmophoresis) tests. The ELISA is prescribed for international trade. Finally, in doubtful cases, samples can be injected into experimental pigs, although this test is no longer recommended by the OIE due to humane considerations and the complexity of the test.

The differential diagnosis includes CSF (hog cholera), acute porcine reproductive and respiratory syndrome, porcine dermatitis and nephropathy syndrome, erysipelas, salmonellosis, eperythrozoonosis, actinobacillosis, Glasser's disease (*Haemophilus parasuis* infection), Aujeszky's disease (also known as porcine pseudorabies), thrombocytopenic purpura, warfarin poisoning other generalized septicemic or hemorrhagic conditions, and heavy metal toxicity.

Epidemiology

Outbreaks occur from time to time in domestic pig herds in Africa and even in the large control areas in South Africa where a slaughter policy exists. Herds usually become infected by eating warthog flesh and blood or from tick infestations or from other infected domestic pigs. The main threat to pig herds outside Africa is the introduction of infected pork products in waste food from aeroplanes and ships arriving from the southern half of Africa. This is unlikely to occur in the most developed countries because of the strict rules governing the disposal of such waste, but the rules may not be so strict in some other countries.

The ASFV is highly resistant to environmental conditions. It can survive for a year-and-a-half in blood stored at 4 °C, 11 days in feces at room temperature, and at least a month in contaminated pig pens. The virus will also remain infectious for 150 days in boned meat stored at 39 °F, 140 days in salted dried hams, and several years in frozen carcasses.

ASF can be transmitted by direct contact with infected animals, indirect contact with fomites, and tick vectors. Transmission during direct contact is usually by oronasal spread. Aerosol transmission is thought to be unimportant, as it only seems to occur over short distances when pigs are in close contact. ASFV can be found in all tissues and body fluids, but particularly high levels are found in the blood. Massive environmental contamination may result if blood is shed during necropsies or pig fights, or if a pig develops bloody diarrhea. The virus can also spread on fomites, including vehicles, feed, and equipment. There is evidence that some pigs may become carriers. ASF is also spread through the bite of infected soft ticks *Ornithodoros* spp. In tick populations, transstadial, transovarial, and sexual transmission occur. In Africa, ASFV is thought to cycle between newborn warthogs and the soft ticks (*O. moubata*) that live in their burrows. Individual ticks can apparently remain infected for life, and infected soft tick colonies can maintain this virus for years. *Ornithodoros erraticus* became infected with ASFV when the virus was enzootic in Spain and Portugal, and additional *Ornithodoros* spp have been infected in the laboratory. Other blood-sucking insects such as mosquitoes and biting flies may

also be able to transmit the virus mechanically. Stable flies (*Stomoxys calcitrans*) can carry high levels of the virus for 2 days. Under experimental conditions, these flies could transmit ASFV 24 h after feeding on infected pigs.

See also: Organisms of Concern but not Foodborne or Confirmed Foodborne: Classical Swine Fever Virus

Further Reading

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ORGANISMS OF CONCERN BUT NOT FOODBORNE OR CONFIRMED FOODBORNE

Spoilage Microorganisms

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Glossary

Aerobic Bacterial growth in the presence of oxygen.
Anaerobic Bacterial growth in the presence of little or no oxygen.
Mesophile An organism that grows at moderate temperatures (20–40 °C).
Modified atmosphere packaging Packaging of food in a gaseous atmosphere in which the proportions of carbon dioxide, nitrogen, and oxygen are different from air.
Psychrotroph An organism that grows at low temperatures (0–7 °C).
Vacuum packaging Packaging of food in the absence of air.

Introduction

Why is an article dedicated to spoilage microorganisms being included in the *Encyclopedia of Food Safety*? Are spoiled foods necessarily a food safety hazard?

Some spoiled foods present no food safety hazard at all. They are simply objectionable and/or offensive from a sensory perspective. These products contain odor, flavor, and/or textural characteristics that cause most humans to reject them. Other spoiled food products, however, can present a food safety hazard.

Evaluation of spoilage is a culturally subjective decision making process. Some food products may be considered acceptable in one part of the world, whereas in another part these products would be rejected. Spoilage assessment is also based on personal preference. Some humans prefer to eat certain food products, whereas others find the same offensive.

Some nonpathogenic spoilage microorganisms generate chemicals that cause foodborne illness. Histamine- and other biogenic amine-producing bacteria can be predominant spoilage microorganisms in certain fish species when storage conditions are favorable for their proliferation. *Morganella morganii, Enterobacter aerogenes, Hafnia alvei, Raoultella planticola/ornithinolytica,* and *Photobacterium damselae* are capable of producing high histamine levels in time/temperature-abused scombrotoxin-producing fish. Consumption of these fish may result in scombrotoxin (histamine) fish poisoning in humans. Articles elsewhere in this encyclopedia contain in-depth discussions addressing this subject matter.

Food spoilage molds including *Aspergillus* spp., *Fusarium* spp., *Penicillium* spp., *Alternaria* spp., etc., may also produce harmful toxins. The food safety implications of these molds and their toxins are addressed in other articles of this encyclopedia.

Food Spoilage – Overview

Food spoilage results when microbiological, chemical, or physical changes occur, rendering the food product unacceptable to the consumer. Microbiological food spoilage is caused by the growth of microorganisms which produce enzymes that lead to objectionable by-products in the food. This is the most prominent type of food spoilage encountered worldwide. Chemical food spoilage occurs when different components in the food react with each other or with some added component which alter the food's sensory characteristics. Examples of this include: oxidation; enzymatic browning; and nonenzymatic browning. Physical food spoilage results when moist foods are excessively dehydrated or dried foods absorb excessive moisture. This article has been dedicated to spoilage microorganisms and shall, therefore, focus on microbiological food spoilage.

Microbiological food spoilage is dependent on both the intrinsic characteristics of the food product and the extrinsic characteristics of handling and the storage environment.

Intrinsic characteristics include:

1. moisture content (A_w);
2. pH;
3. oxidation–reduction potential (E_h);
4. available nutrients;
5. physical barriers;
6. natural antimicrobial substances.

Extrinsic characteristics include:

1. storage temperature;
2. gaseous environment;
3. relative humidity;
4. numbers and types of microorganisms present.

When the above factors are considered in totality, it is obvious that microbiological food spoilage can progress in a

myriad of ways. Discussing all potential food commodities and storage possibilities would be quite onerous and therefore this article will focus on certain representative fresh food commodity groups and how spoilage progresses for each. The intrinsic and extrinsic characteristics that most prominently contribute to microbiological spoilage of each commodity will be considered. In addition, interactions between pathogenic and spoilage microorganisms that affect the quality and safety of each commodity group will be discussed. The following commodity groups have been included in this article:

1. meat and poultry;
2. seafood;
3. raw and pasteurized milk;
4. minimally processed vegetables.

Meat and Poultry

Fresh meat and poultry products are excellent substrates for microbial growth and spoilage. They have a high moisture content (A_w 0.98–0.99) and a normal pH of 5.4–6.6. An ample supply of nutrients is available to sustain microbial growth, including: carbohydrates (glucose, glycogen, and lactic acid) and nitrogen sources (free amino acids and dipeptides). Physical barriers (i.e., hide and skin) are removed during processing, providing access to the muscle tissue for microorganisms. Natural antimicrobial substances are minimal and have little or no effect on microbial growth.

The muscle tissue of healthy animals is essentially sterile. However, during slaughter and processing, this tissue becomes contaminated with microorganisms that originate from animal surfaces, the gastrointestinal tract, lymph nodes, and the processing environment. After slaughter, microbial counts on muscle surfaces typically range from 10^2 to 10^4 cm^{-2}, depending on the sanitary practices and conditions in the processing plant. The types of microorganisms commonly associated with fresh meat and poultry include mesophilic and psychrotrophic bacteria, yeasts, and molds. Spoilage by the mesophilic and psychrotrophic bacteria will be discussed further because these organisms are most frequently responsible for meat and poultry spoilage.

The initial microflora of fresh meat and poultry generally consists of *Staphylococcus*, *Micrococcus*, coryneforms, *Flavobacterium*, *Pseudomonas*, *Moraxella*, *Acinetobacter*, *Alcaligenes*, and Enterobacteriaceae. *Pseudomonas*, *Moraxella*, and *Acinetobacter* will become the dominant spoilage flora under refrigerated, aerobic storage conditions. These organisms are psychrotrophic and thus grow well at typical refrigeration temperatures ranging from 0 to 7 °C. The mesophilic organisms will not grow at these temperatures and are outcompeted by the psychrotrophs. When fresh meat and poultry products are stored under refrigerated vacuum packaging (VP) or modified atmosphere packaging (MAP), the dominant spoilage microorganisms will be lactic acid bacteria (i.e., *Carnobacterium*, *Lactobacillus*, *Leuconostoc*, and *Pediococcus*) and *Brochothrix thermosphacta*. These organisms grow well at refrigerated temperatures under oxygen-limited conditions and will dominate over mesophiles and aerobic psychrotrophs.

Temperature abuse of fresh meat and poultry products may occur at any point in the distribution chain. When aerobically stored meat and poultry are temperature abused, both mesophilic and psychrotrophic bacteria will be present as part of the spoilage microflora. Under moderate abuse conditions (10–20 °C), *Pseudomonas*, *Moraxella*, and *Acinetobacter* will be dominant with lower levels of Enterobacteriaceae, *Micrococcus*, and *B. thermosphacta*. Alternatively, when severe abuse is encountered (20–30 °C), Enterobacteriaceae and Staphylococci may comprise a larger proportion of the microflora. When meat and poultry products stored under oxygen-limited conditions experience moderate temperature abuse, the microflora is commonly dominated by Enterobacteriaceae with lower levels of lactic acid bacteria, whereas severe temperature abuse results in a microflora consisting principally of *Clostridium* with lower levels of lactic acid bacteria and Enterobacteriaceae.

Seafood

Similar to meat and poultry, fresh seafood products are excellent substrates for microbial growth and spoilage. They have a high moisture content (A_w 0.98–0.99) and a pH ranging from 5.4 for red meat species (e.g., tuna) to > 7.0 for some crustaceans. In contrast to meat and poultry, the nutrient supply in most seafood is very low in carbohydrates with the exception of some mollusks, which may contain glycogen levels as high as 5%. However, seafood products contain high levels of nonprotein nitrogen (NPN) including free amino acids, trimethylamine oxide, and urea. These NPN compounds serve as the primary substrate for microbial growth. Physical barriers (i.e., skin and shells) may be removed during butchering or processing, giving microorganisms access to the muscle tissue. Some seafood, however, is stored whole, which requires microorganisms to invade the muscle tissue before spoilage. Microbial growth on edible seafood tissues is not commonly affected by natural antimicrobial substances.

The internal muscle tissue of healthy seafood species is essentially free of microorganisms. However, during butchering and processing, this tissue becomes contaminated with microorganisms, which originate mostly from the harvest waters that seafood species inhabit. The primary microbial reservoirs for postharvest seafood are the gastrointestinal tract, gills, and skin, as well as the harvest and processing environment. After harvest, microbial counts on muscle surfaces typically range from 10^2 to 10^6 cm^{-2}, depending on the handling conditions during harvest and processing. The types of microorganisms isolated from seafood are determined by the harvest environment (i.e., cold or warm waters, salinity, etc.), harvest method (i.e., trawl, longline, traps, etc.), and postharvest handling. The microflora commonly found include both mesophilic and psychrotrophic bacteria. Spoilage of seafood products by these microorganisms will be discussed below.

The initial microflora of freshly harvested seafood from temperate waters typically consists of *Acinetobacter*, *Aeromonas*, *Alcaligenes*, coryneforms, *Flavobacterium*, *Micrococcus*, *Moraxella*, *Pseudomonas*, *Shewanella*, *Vibrio*, and Enterobacteriaceae. The initial microflora of seafood from warm tropical waters is

similar, but with higher levels of Gram-positive cocci, *Bacillus*, and *Vibrio*. In aerobic refrigerated or iced storage conditions, the dominant spoilage flora will be *Pseudomonas* and *Shewanella putrefaciens*, followed by lower levels of *Acinetobacter* and *Moraxella*. These psychrotrophic organisms grow well at refrigeration temperatures and have faster generation times than other psychrotrophs and mesophiles. When seafood products are stored under refrigerated, VP, or MAP conditions, the dominant spoilage microorganisms will be *Photobacterium phosphoreum* and lactic acid bacteria including the genera *Carnobacterium*, *Lactobacillus*, *Lactococcus*, and *Leuconostoc*. *Shewanella putrefaciens* may also play a role in seafood packed in oxygen-limited conditions. These organisms grow well at refrigerated temperatures under oxygen-limited conditions and will dominate over mesophiles and aerobic psychrotrophs.

Fresh seafood products are highly perishable and tend to spoil rapidly when they are temperature abused. The spoilage microflora of temperature-abused seafood stored under aerobic conditions will comprise both psychrotrophic and mesophilic bacteria. *Pseudomonas* and *S. putrefaciens* may still be present, but members of the family Vibrionaceae become predominant, especially at severe abuse temperatures. In addition, some members of the family Enterobacteriaceae may be detected at lower levels. Seafood products that experience moderate temperature abuse while being stored under VP and MAP tend to be dominated by lactic acid bacteria, primarily *Lactobacillus* and *P. phosphoreum*. Research addressing the spoilage microflora of severely temperature abused VP and MAP seafood is very limited. However, mesophilic lactic acid bacteria, Enterobacteriaceae, Vibrionaceae, and *Clostridium* are likely to be present. The lack of research in this subject area is likely due to the increased potential for toxin production by *Clostridium botulinum* under these storage conditions. Effects of spoilage bacteria on growth and toxin production by *C. botulinum* are discussed in the Interactions between Spoilage and Pathogenic Microorganisms section.

Raw and Pasteurized Milk

Similar to meat, poultry, and seafood, raw and pasteurized milk also readily support the growth of pathogenic and spoilage bacteria. Their high moisture content (A_w 0.99) and pH (6.3–6.6) are ideal for numerous Gram-positive and Gram-negative organisms and carbohydrate (lactose), nitrogen (protein and NPN), and lipid sources are plentiful. In contrast to many other foods, milk contains natural antimicrobial substances that need to be considered with respect to microbial spoilage. These include conglutinin, lactoferrin, lysozyme, and the lactoperoxidase system. Conglutinin inhibits Gram-negative bacteria and lysozyme inhibits Gram-positive bacteria. Lactoferrin binds iron, making it unavailable for microbial utilization. The lactoperoxidase system produces hypothiocyanate, which is inhibitory to Gram-positive and Gram-negative bacteria. Although these inhibitory substances do exist in milk, their concentrations and antimicrobial activity are not sufficient to prevent spoilage.

The microflora of raw milk may originate from the environment, inside the udder, udder and teat surfaces, dairy farm workers, and milking and processing equipment. Bacterial counts range from 10^4 to 10^7 ml^{-1}, depending on handling and storage conditions. As would be expected, it is a diverse flora comprised mainly of Gram-positive and Gram-negative bacteria. Sources of the microflora in pasteurized milk are heat-resistant bacteria, postpasteurization contamination by processing equipment, and the processing environment. Bacterial counts are usually at the 10^3 ml^{-1} level but can be higher if the raw milk used for pasteurization has elevated bacterial counts.

The initial microflora of raw milk may contain *Aeromonas*, *Alcaligenes*, *Bacillus*, *Clostridium*, coryneforms, Enterobacteriaceae, *Flavobacterium*, *Microbacterium*, *Micrococcus*, *Paenibacillus*, *Pseudomonas*, lactic acid bacteria, *Staphylococcus*, and *Streptococcus*. The organisms that are most commonly associated with spoilage of refrigerated raw milk are the psychrotrophs – *Pseudomonas*, *Flavobacterium*, and *Alcaligenes*. *Pseudomonas fluorescens* and other pseudomonads are of particular concern because they produce heat-stable lypolytic and proteolytic enzymes that can degrade pasteurized milk, even in the absence of the living organisms. Temperature-abused raw milk is typically spoiled by mesophilic lactic acid bacteria including *Lactococcus lactis* that outcompete the Gram-negative psychrotrophs.

Pasteurization is a heat process intended to destroy vegetative cells of bacterial pathogens and to reduce the levels of spoilage microorganisms. Immediately after processing, pasteurized milk has an initial microflora comprised primarily of thermoduric bacteria that survive the pasteurization process. These organisms include *Micrococcus*, *Streptococcus*, *Bacillus*, *Paenibacillus*, and *Clostridium*. The psychrotrophic spore formers, *Bacillus* and *Paenibacillus*, are often associated with spoilage of pasteurized milk held at refrigerated temperatures. When postpasteurization contamination occurs, the Gram-negative psychrotrophs, *Pseudomonas*, *Flavobacterium*, and *Alcaligenes* are typically the cause of refrigerated pasteurized milk spoilage. Spoilage of temperature-abused pasteurized milk will likely be caused by *Pseudomonas*, *Flavobacterium*, and *Alcaligenes* after postpasteurization contamination or thermoduric survivors of the pasteurization process when postpasteurization contamination does not occur.

Minimally Processed Vegetables

Fresh minimally processed vegetables are different from other commodity groups being discussed in this article because of their much lower protein content. However, they do have an adequate supply of free amino acids and carbohydrates to support a good growth of pathogenic and spoilage bacteria. Vegetables generally have a pH ranging from 5.0 to 7.0 and an A_w ranging from 0.95 to 0.99, depending on species. Their outer surfaces (i.e., peels and skins) give some protection against bacterial invasion, but inner tissues may harbor bacteria without negative impacts to the living plant. Cruciferous vegetables may release isothiocyanates when they are cut, shredded, or bruised. These chemical compounds possess some antimicrobial activity. However, for the vast majority of fresh vegetables, antimicrobial substances have minimal impact on the spoilage microflora.

The surfaces of fresh vegetables typically have a microflora that originates from water, soil, air, insects, fauna, and

handling practices during harvest. This microflora is highly variable and may change depending on any number of environmental or agricultural factors. The primary flora is comprised of bacteria, but yeasts and mold are also present. Although bacterial counts on freshly harvested vegetables usually range from 10^4 to 10^7 g^{-1}, counts as high as 10^9 g^{-1} have been reported. This microflora consists of Gram-positive and Gram-negative bacteria, which may be psychrotrophic or mesophilic. Minimal processing (e.g., cutting, shredding, and peeling) of fresh vegetables results in release of cellular fluids, which provide water and nutrients that promote growth of spoilage bacteria.

The initial microflora of fresh vegetables consists of *Aeromonas, Bacillus, Clostridium*, coryneforms, *Enterobacter, Erwinia, Flavobacterium, Pantoea, Pseudomonas*, lactic acid bacteria, and *Xanthomonas*. The bacteria most commonly associated with spoilage of refrigerated aerobically stored vegetables are members of the genus *Pseudomonas*, including *Pseudomonas marginalis, P. fluorescens*, and *Pseudomonas viridiflava*. These fluorescent pseudomonads cause a form of soft rot in vegetables, which results from the activity of the enzyme pectate lyase. They are also able to grow well at temperatures of 4 °C and below. If refrigerated minimally processed vegetables are stored in VP or MAP conditions, the spoilage microflora tends to be dominated by lactic acid bacteria, particularly *Leuconostoc mesenteroides*. Refrigeration and reduced oxygen storage conditions give these organisms a competitive advantage over mesophiles and aerobic psychrotrophs.

Microbial spoilage of fresh minimally processed vegetables is greatly influenced by temperature and atmosphere of storage. When these products are stored aerobically and abused at moderate temperatures (10–20 °C), the spoilage flora will most likely consist of fluorescent pseudomonads. Storage at severe abuse temperatures (20–30 °C) results in a microflora that may be dominated by members of the genus *Erwinia*, including *Erwinia carotovora* and *Erwinia chrysanthemi*. *Erwinia* spp. are mesophilic facultative anaerobic bacteria that, like the fluorescent pseudomonads, cause soft rot of fresh vegetables. However, lactic acid bacteria have also been shown to dominate the spoilage flora of fresh minimally processed vegetables stored aerobically at 30 °C. The microflora of temperature-abused fresh vegetables stored under VP and MAP conditions also tend to be dominated by *Erwinia* spp. and lactic acid bacteria.

Microbial Pathogens as Spoilage Microorganisms

Throughout this article, intrinsic characteristics of foods and extrinsic characteristics of the storage environment have been discussed in view of their relationship to the growth of spoilage microorganisms and the resulting progression of food spoilage. For the most part, the spoilage organisms discussed have been harmless bacteria that pose no threat to human health. However, in certain situations, the bacteria responsible for food spoilage may, in fact, be pathogenic. *Bacillus* spp. are frequently present in raw and pasteurized milk and have routinely been identified as milk spoilage organisms. The pathogenic species, *Bacillus cereus*, has been linked to spoilage and human illness in fluid milk products. *Clostridium* spp. and members of the family Enterobacteriaceae can constitute a significant proportion of the spoilage flora in temperature-abused fresh meat and poultry products. *Clostridium perfringens* can comprise a large portion of the spoilage flora under anaerobic storage conditions. In addition, Enterobacteriaceae, including pathogenic *Salmonella* spp. and *Escherichia coli*, can dominate the spoilage microflora under aerobic and anaerobic storage conditions. *Vibrio* spp. are commonly present on seafood products harvested from warm water climates. These organisms tend to dominate the microflora of seafood products that are abused at temperatures more than 20 °C. In particular, the human pathogen *Vibrio parahaemolyticus* has been shown to be the predominant spoilage organism in shrimp held at temperatures of 24 °C or greater.

Interactions between Spoilage and Pathogenic Microorganisms

Microorganisms present in foods (or any other environment) interact with each other. The primary interaction from a food safety perspective is competition. It has been shown that some bacteria proliferate in foods at the expense of others. This is sometimes the case with the interactions between spoilage and pathogenic bacteria. When the spoilage microflora (or a portion of it) is able to inhibit, suppress, or eliminate a microbial pathogen in a food product, that food product has less chance to cause consumer illness. This type of microbial interaction will be discussed in the remaining part of this section.

How do spoilage microorganisms inhibit pathogens? Spoilage microorganisms can inhibit pathogens by numerous mechanisms. These include nutrient limitation, competition for space, production of toxic substances (bacteriocins, acids, antibiotics, etc.), iron sequestration, and nonspecific inhibition. Nutrient limitation occurs when spoilage organisms consume the nutrients available in foods and leave little or none for the pathogens. In competition for space, the spoilage organisms attach to the available surfaces, leaving no room for other organisms to attach. Toxic substances produced in sufficient quantities by spoilage organisms may negatively affect pathogen growth and/or survival. Iron sequestration, resulting from siderophore production, binds to iron making it unavailable for use by pathogenic microorganisms. This is essentially a specialized form of nutrient limitation. Nonspecific inhibition occurs when the presence of background microflora and/or spoilage organisms in foods results in a reduction in the numbers of pathogens, but the mechanism of action is unknown or can only be speculated. Of course, a combination of the inhibitory mechanisms discussed in this paragraph may also be employed.

Foodborne bacterial pathogens that can be inhibited by spoilage microorganisms include *Aeromonas sobria*, *B. cereus*, *Campylobacter jejuni*, *C. botulinum*, *E. coli* O157:H7, *Listeria monocytogenes*, *Salmonella* spp., *Staphylococcus aureus*, and *Yersinia enterocolitica*. The most extensively studied spoilage microorganisms that exhibit the ability to inhibit bacterial pathogens are *Pseudomonas* spp. and lactic acid bacteria. Other naturally occurring bacteria, yeasts, and molds have also been shown to exert inhibitory activity toward pathogens. Some *Pseudomonas* spp. have also been shown to inhibit other

spoilage microorganisms including other pseudomonads and *S. putrefaciens*.

Pseudomonas

Pseudomonas spp. primarily inhibit bacterial pathogens by nutrient limitation and iron sequestration in food stored under aerobic conditions. This has been demonstrated in several studies involving microbiological media, meat, poultry, milk, fish, and fresh vegetables. *Bacillus cereus*, *L. monocytogenes*, *Salmonella infantis*, *S. aureus*, and *Y. enterocolitica* levels were reduced in a minced meat medium when they were inoculated with *Pseudomonas fragi* and *P. fluorescens* after 3 days of aerobic incubation at 6 °C. *Pseudomonas aeruginosa* has been shown to inhibit growth of *S. aureus* in milk. *Pseudomonas fluorescens* produces positive, negative, and no effects on growth of *L. monocytogenes* in microbiological culture media, depending on the pH, salt content of the media, and the incubation temperature. *Pseudomonas fluorescens* isolated from raw pork and chicken has displayed inhibitory activity against *C. jejuni*, *E. coli* O157:H7, *Salmonella enteritidis*, *Y. enterocolitica*, *L. monocytogenes*, and *S. aureus*. Naturally occurring *P. fluorescens* and *P. aeruginosa*, isolated from fresh vegetables, have demonstrated antimicrobial activity toward *E. coli* O157:H7, *L. monocytogenes*, *Salmonella* Montevideo, and *S. aureus*. *Pseudomonas* strains isolated from fish inhibited the pathogens *A. sobria*, *E. coli*, *L. monocytogenes*, and *S. aureus* as well as the spoilage bacteria *P. fluorescens* and *S. putrefaciens*. All inhibitory activities reported for *Pseudomonas* spp. were attributed to iron sequestration via siderophore production, nutrient limitation, or other unreported mechanisms.

Lactic Acid Bacteria

Lactic acid bacteria including the genera *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, and *Pediococcus* may inhibit microbial pathogens by producing organic acids (primarily lactic acid), bacteriocins, hydrogen peroxide, and/or diacetyl. Nutrient limitation may also play an inhibitory role for some lactic acid bacteria. As in the case of *Pseudomonas* spp., this has been demonstrated in numerous studies incorporating microbiological media, meat, poultry, milk, fish, and vegetables. Similar to the discussion presented in the *Pseudomonas* section *B. cereus*, *L. monocytogenes*, *S. infantis*, *S. aureus*, and *Y. enterocolitica* levels were reduced in a minced meat medium when they were inoculated with *Lactobacillus brevis* and *Lactobacillus plantarum* after 3 days of incubation at 6 °C. However, only the lactic acid bacteria *Pediococcus damnosus* has displayed inhibitory activity against *Salmonella*. *Carnobacterium piscicola* has been shown to have inhibitory activity against *L. monocytogenes* in culture media. Besides the incubation temperature, the degree of antimicrobial activity depends on the pH and salt content of the media. *Carnobacterium piscicola* has also been shown to control growth of *L. monocytogenes* in crabmeat, creamed corn, frankfurters, and milk. Growth of this pathogen can be controlled more effectively when the food products are incubated at 5 °C rather than at 19 °C. Three unidentified naturally occurring Gram-positive bacteria isolated from fresh bell peppers displayed inhibitory activity against *E. coli* O157:H7, *L. monocytogenes*, *Salmonella* Montevideo, and *S. aureus*. Bacteriocin production by these isolates was postulated, suggesting that they may have been lactic acid bacteria. *Lactobacillus fuchuensis*, *Lactococcus piscium*, *Leuconostoc gelidum*, and *Carnobacterium alterfunditum* isolated from various seafood products showed antimicrobial activity against numerous bacterial pathogens and spoilage bacteria including *E. coli*, *L. monocytogenes*, *S. enterica*, *S. aureus*, *P. phosphoreum*, *Pseudomonas* Group I, and *S. putrefaciens*. Some of these lactic acid bacteria isolates demonstrated bacteriocin-type activity, whereas others did not. Inhibitory activity of the nonbacteriocin-producing isolates was tentatively attributed to nutrient limitation. The three most common inhibitory activities reported for lactic acid bacteria in the literature are pH reduction, bacteriocin production, and nutrient limitation.

Inhibition of foodborne microbial pathogens by lactic acid bacteria is an important consideration when foods are stored under VP or MAP. As mentioned in the preceding sections, lactic acid bacteria commonly dominate the spoilage microflora in foods stored in this manner, especially under refrigerated conditions. This is due to their psychrotrophic and anaerobic growth capabilities. Lactic acid bacteria are also able to grow when carbon dioxide is present in the gaseous atmosphere of stored foods, which provides them a competitive advantage. *Lactobacillus fuchuensis*, *L. piscium*, *L. gelidum*, and *C. alterfunditum*, mentioned in the previous paragraph of this section, were originally isolated from MAP salmon, MAP sea bream, and MAP roughhead grenadier, and inhibitory activity experiments were conducted under anaerobic conditions. This demonstrates that these lactic acid bacteria have antibacterial activity against *E. coli*, *L. monocytogenes*, *S. enterica*, and *S. aureus* in oxygen-limited storage conditions. The background microflora of ground beef has been shown to control the growth of *E. coli* O157:H7 stored under both aerobic and anaerobic conditions. This background microflora was comprised primarily of lactic acid bacteria and the dominant isolate was *Lactobacillus sakei*. *Lactobacillus sakei* has also been shown to inhibit growth of *E. coli* O157:H7 and *L. monocytogenes* in VP cooked meat and *L. monocytogenes* in VP cooked ham. *Enterococcus mundtii* has shown inhibitory activity toward *L. monocytogenes* in a vegetable-based culture media but not in fresh mungbean sprouts stored under oxygen-limited conditions. *Lactococcus lactis* and *L. sakei* isolated from VP chilled meat inhibited the growth of *L. monocytogenes* and *C. jejuni* in meat-based agar stored under anaerobic conditions.

Clostridium botulinum: Time to Toxin Formation Versus Organoleptic Spoilage Detection

Clostridium botulinum is a bacterial pathogen of major concern in foods packaged under reduced oxygen conditions. These anaerobic spore-forming bacteria are commonly found in nature and may grow and produce toxin in hermetically sealed, VP, or MAP foods. Some strains including *C. botulinum* Type E and nonproteolytic types B and F are able to grow and produce toxin at refrigerated temperatures. One strain of *C. botulinum* Type E has been shown to produce toxin

at temperatures as low as 3.3 °C. *Clostridium botulinum* Type E has routinely been isolated from fish and seafood products and has caused a number of illness outbreaks associated with seafood consumption. This has resulted in an area of research addressing time to toxin formation versus organoleptic spoilage detection for VP and MAP refrigerated fresh fish. The primary goal of this type of research is to determine under what storage conditions will these products display spoilage characteristics (e.g., off odors) before the toxin is detected. Thus, this answers the question – will the natural microflora present spoil the fish before a food safety hazard arises?

When VP and MAP cod, whiting, and flounder were inoculated with *C. botulinum* Type E spores and stored at temperatures ranging from 4 to 26 °C, the flounder spoiled before toxin was produced at low temperatures but did not spoil before the toxin was produced at 26 °C. Whiting and cod did not spoil before the toxin was detected at any incubation temperature or gaseous atmosphere tested. Air-stored, VP, and MAP catfish fillets inoculated with *C. botulinum* Type E spores and stored at temperatures ranging from 4 to 16 °C spoiled before toxin was detected in all atmospheres at 4 °C. However, in all atmospheres with storage at 16 °C, and in VP with storage at 8 °C, toxin was detected on the same day as spoilage. In flounder fillets inoculated with a mixed culture of *C. botulinum* Type E and nonproteolytic Types B and F, sensory spoilage was detected before toxin production for samples stored in oxygen permeable film at 4 and 10 °C. However, at the same storage temperatures, sensory spoilage was detected after toxin production in VP samples. These studies show that strict temperature control is extremely important when raw fish is stored under VP and MAP conditions. It should be noted that in VP and MAP stored fish, a large portion of the spoilage microflora is likely to be lactic acid bacteria.

Clostridium botulinum also is a food safety concern when minimally processed produce is stored in MAP. Research has shown that nonproteolytic *C. botulinum* Types B and E can produce toxin at temperatures as low as 5 °C in fresh MAP vegetables, whereas proteolytic *C. botulinum* Types A and B can produce toxin at 15 °C and higher. Most samples displayed organoleptic spoilage characteristics before toxin was detected. However, butternut squash and onion were not considered to be spoiled before toxin was produced. Fresh mushrooms stored in MAP at 20 °C have also been shown to support toxin production by *C. botulinum*. The mushrooms did not appear to be spoiled at the time toxin was detected (3–4 days). Similar results have been found for fresh cabbage stored in MAP.

In general, organoleptic spoilage cannot be relied on to predict the presence of botulinum toxin in VP and MAP foods, especially those that use refrigerated storage as the only means to control growth and toxin production. Additional control strategies should be considered for refrigerated foods packaged in oxygen-limited conditions. These include reduced moisture content, acidification, food additives, pasteurization, or some combination of 'hurdles' that reliably prevent growth and toxin production.

See also: Food Additives: Natural Preservatives; Preservatives. Food Technologies: Aseptic Packaging; Biopreservation; Chilling; Cleaning and Disinfection Technologies (Clean-In-Place, Clean-Out-of-Place); Drying; Fermentation; Food Irradiation; Freezing; High Pressure Processing; Microwave Heating; Packaging; Pasteurization; Pulsed Ultraviolet Radiation Processing; Sterilization. Mycotoxins: Aflatoxins; Deoxynivalenol and Other Trichothecenes; Fumonisins; Ochratoxin A; Patulin; Zearalenone. Other Significant Hazards: Food-Related Choking. Processing Contaminants: Biogenic Amines

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NATURAL TOXICANTS

Contents

Alkaloids

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Glossary

Extraction A means of extracting specific compounds (such as alkaloids) from food by treatment (usually shaking) with a solvent.

Gas chromatography (GC) A means of separating volatile compounds by passing in a stream of gas through a very narrow heated glass tube coated with an absorptive phase.

High-performance liquid chromatography (HPLC) A means of separating nonvolatile compounds by passing in a solvent under pressure through a column packed with an absorptive phase.

Mass spectrometry (MS) An instrumental means of detecting, identifying, and measuring compounds by producing and separating ions. Used as a detector for gas chromatography (GC–MS) or for high-performance liquid chromatography (LC–MS).

Solid phase extraction (SPE) A means of extracting specific compounds (such as alkaloids) from a solvent extract of food on to a solid medium as a means of separation from food. Alternatively, unwanted compounds can be removed from the extract.

Alkaloids

Alkaloids are chemical substances having one or more nitrogen atoms, usually contained in a heterocyclic ring system, and they function as amines. Most are naturally produced by plants, and many have a pharmacological activity in the animals and humans that consume them, affecting the nervous system and other essential processes. Some insects are immune to the toxic effects and use the alkaloids for their own defense by making their own bodies toxic to predators. Chemical structures of the major alkaloids discussed in this article are presented in Figure 1.

Plant alkaloids are secondary metabolites in almost all cases, that is, their production is not essential for the plant survival. In fact, their purpose in most plants is poorly understood, but in some cases, is known or assumed to be to deter animal and insect predators. Alkaloids have a bitter taste, and often accumulate in the fresh young growing parts of plants that are most likely to be eaten by predators.

Food Safety Issues

Toxic alkaloids can be present in food for various reasons. They are in some cases found as natural components of some important food plants such as potatoes, and in other cases they can contaminate food that does not itself contain alkaloids. Certain fungi that grow on food crops produce toxic alkaloids either during the growth of the plant or during storage. The most important example of this is the fungus ergot, which infects mainly rye, wheat, and barley, and has achieved notoriety through major poisoning outbreaks.

Toxic weeds or their seeds frequently contaminate cereal grains or animal feed, and can cause human poisoning directly or through animal products such as milk and eggs. Furthermore,, many toxic plants are consumed intentionally for their bioactive effect, where some real or perceived health benefit or effect on the nervous system is desired. These herbal food supplements or medicines are defined as foods in some countries, and as medicines in others.

Alkaloid Excretion in Breast Milk

The transfer of alkaloids from food or feed into the milk of humans is of considerable concern, because newborn babies are very sensitive to toxins, and may derive all of their food from breast milk. Alkaloid transfer into the milk of cows, sheep, and goats can also lead to human poisoning. The rate and degree of alkaloid transfer into breast milk are affected by

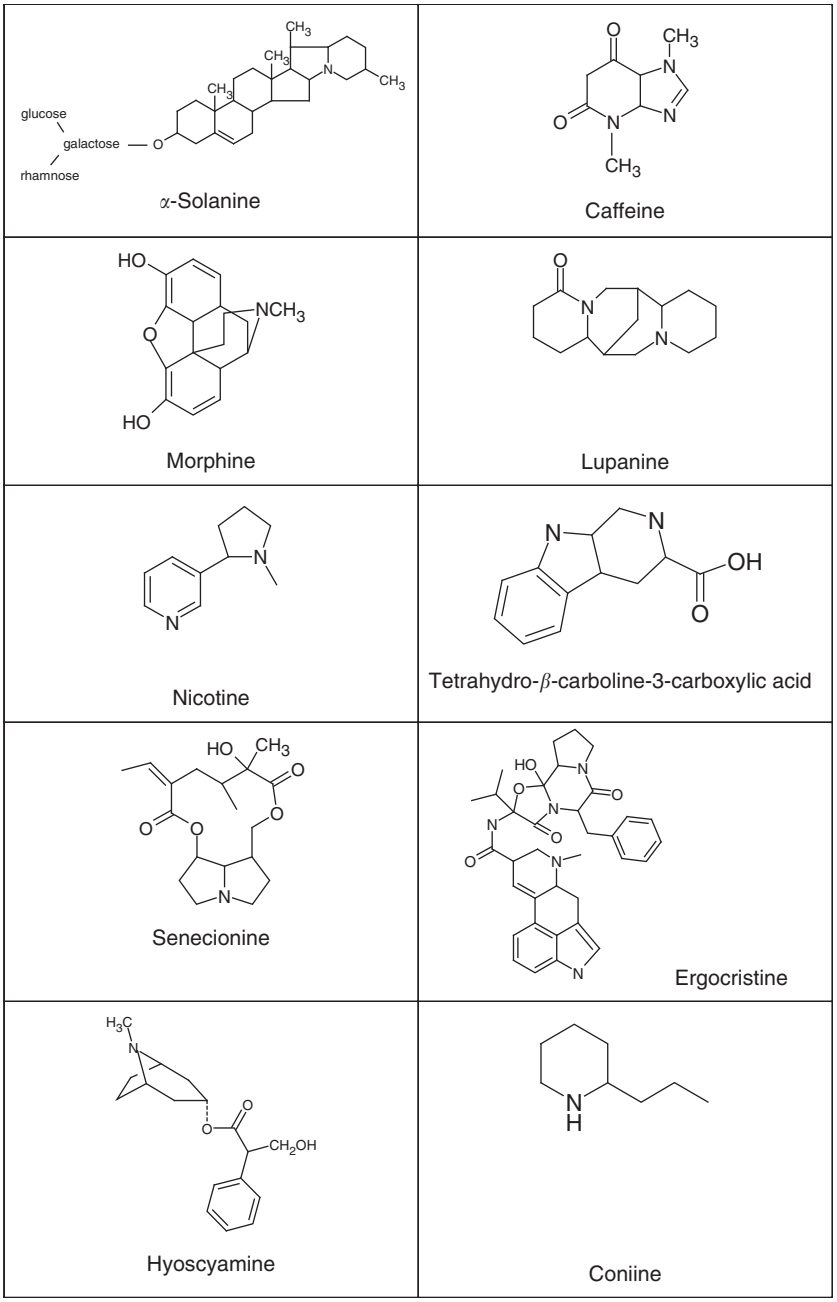

Figure 1 Chemical structures of some common alkaloids.

the solubility of the alkaloid and the fat content of the milk. So, relatively fat-soluble alkaloids will accumulate more in high fat milks.

Some alkaloids can be found at relatively high levels in milk whereas others, notably solanine and ergot alkaloids, remain absent regardless of high ingestion levels. Medicinal morphine has been shown to enter human breast milk and be transferred to babies, but food sources of opiates are unlikely to produce detectable levels in milk.

Experimental doses of caffeine are rapidly absorbed into the blood and pass into breast milk, with ingested doses of approximately 100 mg caffeine producing breast milk concentrations of 2–4 μg ml^{-1}.

The quinolizidine alkaloid anagyrine has been implicated in a human poisoning incident involving the consumption of milk from goats that had grazed on lupins.

Nicotine and cotinine from smoking mothers contaminates milk and can be detected in infants' urine, and so ingestion of these alkaloids in food will presumably also present some risk to babies.

In the paragraphs below the major alkaloids presenting a health risk in food through natural occurrence or contamination

are described. In the analysis of alkaloids in plants and in food, several general approaches apply to almost all situations, thus an overview of these techniques is provided first, with deviations specific to particular alkaloids described in the main text.

Analysis

The determination of alkaloids in food usually takes the form of extracting the compounds with a suitable solvent followed by a detection step that provides either qualitative or quantitative information. Measurement methods vary in complexity from relatively simple techniques that typically produce a color reaction, indicating their presence, to complex instrumental analyse that can identify and quantify individual compounds.

In almost all cases the alkaloids have to be separated from a food matrix, that is, a complex mixture of insoluble fiber and protein, lipids, and water-soluble components. The basic nature of alkaloids aids their extraction considerably. They are mostly soluble in relatively polar organic solvents such as methanol, diethyl ether, or chloroform, but are also soluble in water at acid pH. Typically, alkaloids are extracted into organic solvents and then partitioned into dilute aqueous hydrochloric or sulfuric acid. Alternatively, they may be extracted directly with the acid. The acid solution is then shaken with a nonmiscible organic solvent, such as chloroform, which dissolves and removes much of the coextracted material. The organic layer is discarded and the aqueous extract made strongly alkaline by, for example, the addition of strong ammonia solution. The basic conditions greatly reduce the alkaloids' solubility in the aqueous phase and they can then themselves be extracted into an immiscible organic solvent. This partitioning between aqueous and organic phases can be repeated to produce an extract suitable for alkaloid detection.

Recently, small disposable solid phase extraction (SPE) columns containing a cationic exchange phase have been used for trapping alkaloids from food extracts, and this procedure is likely to dominate future applications.

Alkaloids may be detected in extracts by their reaction with reagents that produce colored products. The reagents include phosphomolybdic acid, iodoplantinate, potassium bismuth iodide (Dragendorff's reagent), and dimethylaminobenzaldehyde (Ehrlich's reagent). The reagents can be used to detect alkaloids on thin-layer chromatography plates, or the colored product may be quantified using spectrometric techniques to give the total alkaloid content. The method has the advantage that another related alkaloid can often be used where specific standards are not available, whereas the major disadvantage is that alkaloid components cannot be quantified individually.

For quantitative analysis of single alkaloids in a mixture, they are usually separated by chromatographic techniques. Most alkaloids are sufficiently volatile to be analyzed by gas chromatography (GC). In some cases, blocking of the polar hydroxyl groups (derivatization) is used. The alkaloids are detected by general purpose detectors such as the flame ionization detector, a nitrogen-specific detector, or more often a mass spectrometer (GC–MS).

Nonvolatile compounds can be separated by high-performance liquid chromatography (HPLC) using normal or reverse phase conditions. Alkaloids can be detected by various means including UV absorbance, refractive index, and mass spectrometry (LC–MS), the choice depending on both the analyte and the solvent used. The most comprehensive information and the best confirmatory evidence are provided by LC–MS.

Alternative techniques include immunoassays that have potential application to inexpensive field-based dipstick tests, and the more expensive nuclear magnetic resonance (NMR) methods that help elucidate chemical structures.

Alkaloids Present Naturally in Food

Solanum Alkaloids

Occurrence

The potato plant (*Solanum tuberosum*) and the aubergine (*Solanum melongena*) are related to some very toxic plants – deadly nightshade, henbane, and tobacco – and themselves contain toxic alkaloids that cause sporadic outbreaks of poisoning in humans. Solanine is present in most of the 1700 species in the genus *Solanum*. Potatoes are a staple part of the diet for very many people, with a worldwide annual production of approximately 350 million tons, and all contain detectable alkaloid glycosides. Therefore, their safety is of considerable concern. Toxic alkaloids were first isolated from potato in 1820 but were not characterized until the 1950s. The major alkaloids in edible *Solanum* species are α-chachonine and α-solanine, which are bound to sugar molecules as glycosides. The alkaloid part of the molecule has a steroidal structure and is attached to a branched trisaccharide or tetrasaccharide.

The glycoalkaloid content of potatoes may increase during storage, particularly when exposed to light (although the greening is independent of toxicity), or as a result of damage during handling (Figure 2). Potatoes can contain up to 350 mg kg^{-1} glycoalkaloids in the fresh tubers with much more being present in the green parts of the plant, including the sprouts. The levels are higher in organically grown plants. The levels are also higher in wild species of potato used in breeding new varieties, and therefore analysis of the progeny

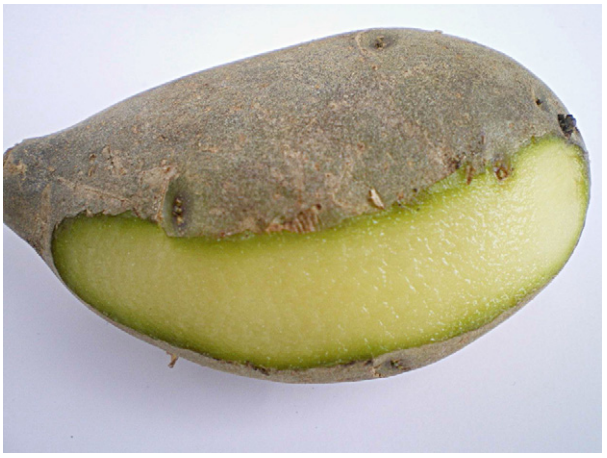

Figure 2 A potato showing evidence of exposure to light.

resulting from breeding trials is required. The glycoalkaloid content is much higher in the potato skin than in the main flesh and can be removed by peeling of the outer 3–4 mm. The alkaloids persist to some extent when potatoes are processed, with potato chips containing up to 100 mg kg^{-1}. Some countries have set limits (typically 200 mg kg^{-1}) for levels of glycoalkaloids in potatoes.

All parts of the aubergine (*S. melongea*, eggplant) contain solanine, however some cooking processes remove the toxin. Potatoes, aubergines, and several other vegetables (tomatoes, cabbage, sweet potatoes, sweet peppers, and chilli peppers), and some fruits (physalis and mulberries), also contain polyhydroxylated nortropane alkaloids called calystegines. Tomatoes contain the related tetrasaccharide glycoalkaloids dehydrotomatine and α-tomatine, but there is no strong evidence of their toxicity.

Toxicity

Solanum glycosides inhibit the cholinesterase enzymes *in vitro*, although clinical cholinergic symptoms are not observed. The toxins cause nerve damage, leading to hallucinations, delirium, and eventually coma. Ingestion of potatoes with high glycoalkaloid content can cause stomach pains, weakness, nausea, vomiting, and breathing difficulties between 2 and 24 h after ingestion, which may persist for several days. Moderately contaminated potatoes (140 mg kg^{-1}) have a bitter taste and higher levels induce a burning sensation. Several outbreaks of potato-related illness have been reported, from which the toxic alkaloid dose has been estimated to be 2–5 mg kg^{-1} body weight.

The glycoalkaloids appear to be more toxic to humans than to other animals. It is believed that they, or their metabolites, suppress fertility, increase infant mortality, and induce neurological defects, including spina bifida. The related potato alkaloids solanidine and salasodine have been shown experimentally to cause teratogenic defects such as craniofacial malformations.

Calystegines are more usually associated with some toxic nonfood plants, but they are potent glycosidase inhibitors, and are therefore of concern, although the degree of human toxicity has not yet been established.

Control

Solanine is not removed by boiling, but it can be destroyed by frying. Solanine poisoning is uncommon as cooks and the public are aware of the problem and tend to avoid green potatoes, in any case, consumption of up to 5 g of green potato per kg body weight per day does not appear to cause acute illness.

Control of solanum glycoside toxins in potatoes is based on careful selection and breeding of seed potato varieties, and attention to handling and processing with a view to reduce mechanical damage and exposure to light. Some chemical treatments, such as the use of sprouting inhibitors, can reduce the formation of the toxins.

Analysis

HPLC with UV detection (HPLC-UV) is, today, the preferred analytical method for glycoside toxins. The presence of the hydrophilic sugar in the molecule means that the toxins are very soluble in water and, therefore, aqueous extraction is used.

Xanthine Alkaloids

Occurrence

The xanthine alkaloids include caffeine, theobromine, and theophylline, and are well-known components of tea (*Camellia sinensis*), coffee (*Coffea arabica*), cola ingredients (*Cola* spp.), and cocoa (*Theobroma cacao*). These are obviously consumed in considerable quantity around the world. Caffeine is the most important xanthine alkaloid. It is a mildly stimulant drug found in tea, coffee, cocoa, and the kola nut and is usually associated with the alkaloids theophylline and theobromine, which are mild cardiac stimulants. Coffee seeds (beans) show considerable variations in caffeine concentration, as do the various brews prepared from the roasted and ground bean. A typical serving of coffee contains between 40 and 100 mg of caffeine. Coffee contains trace amounts of theophylline, but no theobromine. The tea plant contains more caffeine than coffee, however, the brewing process results in the caffeine content of the beverage being rather lower than that of coffee.

The kola nut species *Cola acuminata*, *Cola nitida*, and *Cola anomala* contain theobromine and caffeine. Caffeine is present in *Cola* seeds at levels of approximately 10 000 mg kg^{-1} of the fresh seed. The alkaloids are absent from the related cola plant *Garcinia kola*. Kola nuts are an ingredient of cola drinks, which typically contain approximately 10–50 mg of caffeine per serving, but energy drinks can contain considerably more, often 80–100 mg of caffeine per serving. Guarana is another ingredient of energy drinks that contains caffeine, theobromine, and theophylline. Chocolate (cocoa) contains mostly theobromine and also caffeine and theophylline in low concentrations.

Toxicity

Caffeine is a stimulant of the central nervous system, causing minor enhancement of alertness. This effect contributes to the popularity of beverages prepared from plants such as tea and coffee, and of its use as an ingredient in soft drinks and energy drinks.

Caffeine is metabolized in the liver by the cytochrome P450 oxidase enzyme system which produces the dimethylxanthines, paraxanthine (the major product), theobromine, and theophylline. Paraxanthine increases lipolysis, the process by which fats are split into glycerol and fatty acids. Theobromine has a vasodilatory effect, increasing the oxygen and nutrient flow to the brain and muscles. Theophylline relaxes smooth muscle and increases heart rate and efficiency. The mechanism whereby caffeine causes alertness is unclear, but it might be related to adenosine which is believed to control sleepiness.

Xanthines act as nonselective antagonists of adenosine receptors. Adenosine protects the brain in times of stress by suppressing neural activity and increasing blood flow. Caffeine counteracts this by binding to adenosine receptors without activating them, thus reducing cerebral blood flow. Excesses of caffeine can lead to insomnia, headaches, nervousness,

irritability, anxiety, palpitations, and twitching. The LD_{50} of caffeine in humans is estimated to be approximately 150–200 mg kg^{-1} of body mass, roughly 80–100 cups of coffee for an average adult taken within a limited time frame that is dependent on half-life. Heavy caffeine consumption is believed to increase the risk of miscarriage.

Control

The consumption of caffeine and its content in foods and beverages is not regulated, but government agencies often impose labeling requirements. For example, European Commission regulations call for clear labeling on any beverages containing more than 150 mg l^{-1} of caffeine to state that there is 'high caffeine content' in the product. Because of the risk of miscarriage, the UK Food Standards Agency has recommended that pregnant women should limit their caffeine intake to less than 200 mg of caffeine a day – the equivalent of two cups of instant coffee or a half to two cups of fresh coffee.

Analysis

Caffeine has been determined by gravimetric and spectrophotometric methods, but these are often tedious and unreliable. GC can be used but HPLC-UV is now preferred. The xanthines are typically detected using a photodiode array detector.

Opium Alkaloids

Occurrence

Poppy seeds obtained from the opium poppy (*Papaver somniferens*) are used all over the world for the decoration of baked foods such as bread rolls. The seeds contain a range of morphine alkaloids, but in much lower levels than the parts of the plant used for opium production. The seeds typically contain 5–60 mg kg^{-1} of morphine and 5–50 mg kg^{-1} of codine. These alkaloids can regularly be detected in baked foods that have been decorated with poppy seeds.

In 2005, the opiate content of poppy seeds surveyed in Germany was found to be high enough to cause adverse health effects. Seventy six percent of 110 samples exceeded the guidance level of 4 mg morphine per kg poppy seeds proposed by the Federal Institute for Risk Assessment, but almost 30% contained more than 20 mg kg^{-1}, although these levels would be reduced by processing, especially baking, which has a marked effect on morphine levels. The main interest in morphine is in the analysis of body fluids where it is necessary to distinguish between morphine due to drug abuse and that from legitimate foods.

Toxicity

Morphine appears to mimic the body's endorphins that are responsible for analgesia (reducing pain), causing sleepiness, and feelings of pleasure. Morphine is a phenanthrene opioid receptor agonist which binds to and activates opioid receptors in the central nervous system. Morphine is substantially broken down in the liver, with most being rapidly excreted in the urine. It is metabolized primarily into glucuronides.

Poppy seeds only rarely provide doses of morphine and other opiates that are significant in terms of human health. Ingestion of the seeds is of more importance in terms of verifying opiate abuse indicated by screening tests and distinguishing between innocuous ingestion via food and illegal intake.

Control

Maximum values for morphine in poppy seeds have been set in some countries, for example, a maximum value of 30 mg kg^{-1} in raw poppy seeds sold in Hungary.

Analysis

Opiates are easily determined by GC and GC–MS.

Lupin Alkaloids

Occurrence

Lupin seed is a good potential source of protein as the dry seed contains approximately 40% of protein with a reasonably good amino acid profile (Figure 3). Flour from lupin has been used in the manufacture of products including biscuits and pasta. It has been proposed as a component of infant formula and bread and as a replacement for soya flour. Protein concentrates isolated from lupin have lower levels of alkaloid and improved technical and functional properties, making them more attractive as novel food ingredients.

Lupins and related plants of the Fabaceae family contain alkaloids known as quinolizidines. These secondary metabolites are known to have a defense function against predatory herbivores and microorganisms. The major quinolizidine alkaloid is lupanine, but a number of minor alkaloids are present according to the species, including albine, hydroxylupanine, sparteine, anagyrine, lupinine, and angustifoline.

Lupins that have low alkaloid content are referred to as sweet lupins as opposed to the bitter species or varieties. The most commonly used sweet lupins are *Lupinus albus* (white lupin) and *Lupinus angustifolius* (blue lupin). The mean total alkaloid content of sweet lupin seed is approximately 130–150 mg kg^{-1}, but bitter plants contain approximately four times that amount, with a similar alkaloid pattern. The alkaloid content is affected by geography and climate.

Figure 3 Seeds of lupin (*Lupinus albus*).

Toxicity

Lupin alkaloids are acutely toxic to humans. Quinolizidine alkaloid poisoning causes trembling, shaking, excitation, and convulsion. Lupanine and other lupin alkaloids show a moderate toxicity in vertebrates, whereas α-pyridone alkaloids such as cytisine and anagyrine are strong poisons. It is likely that the molecular target of quinolizidine alkaloid poisoning is the acetylcholine receptor. Lupanine, angustifoline, and 13-hydroxylupanine do not show any mutagenic activity. The lethal dose of lupanine has been determined to be approximately 100 mg kg^{-1} of lupin seeds. It is substantially destroyed by cooking, but if the cooking procedure is insufficient, 10 g of seeds may liberate more than 100 mg of lupanine. Anagyrine causes malformations (crooked calf disease) in young sheep and calves, when their mothers feed on lupins or broom. It has been shown that goats that consume lupin seeds pass anagyrine in the milk, and there has been a single reported case of a child born with limb deformities after maternal ingestion of goat's milk.

Control

Sweet lupin flour may be used to enhance the protein content of foods if the alkaloid content is suitably low. Some countries (Great Britain, France, Australia, and New Zealand) have imposed limits on the quinolizidine alkaloid content of seed or flour, typically at 200 mg kg^{-1}. It is likely that the alkaloid content could be reduced by breeding techniques, but the use of isolates of the protein fraction has been shown to be the safest approach. In most foods prepared from lupin flour, the alkaloid content is slightly changed by the processing, but protein isolates contain only lupanine, at levels below the limit of 200 mg kg^{-1} where the lupin protein isolates used exceeds approximately 15%.

Analysis

Lupin alkaloids can be analyzed by gas chromatography–flame ionization detector (GC–FID) and GC–MS, and also by HPLC.

Nicotine

Occurrence

Nicotine (1-methyl-2-pyrrolidinyl)pyridine, is the major alkaloid of the tobacco plant (*Nicotiana tabacum*). It is present at 2–10% levels in tobacco, and at considerably lower concentrations in other Solanaceae plants including tomatoes, aubergines, peppers, and potatoes. Nicotine has been used as a convenient and inexpensive insecticide and can therefore contaminate food crops. Recently, nicotine has been found in dried wild mushrooms (ceps) from China, which might have absorbed the drug from tobacco leaves used to hold the mushrooms during drying.

Very low levels of nicotine, less than 0.01 mg kg^{-1}, have been reported in fresh potatoes, tomatoes, aubergines, and sweet peppers. There are conflicting data regarding relative levels in the flesh and peel of potatoes. Exposure to nicotine through the diet is much lower than that from even passive smoking, and absorption from ingestion is also much lower by this route. Processing and cooking have little effect on nicotine levels in potatoes.

Toxicity

Low doses of nicotine, for example, from cigarette smoke causes hypertension and respiratory stimulation. There is, however, unlikely to be any detectable physiological effect from ingestion of levels present naturally in food.

Control

The use of nicotine as a pesticide is well regulated. Following the mushroom contamination incident, the European Food Safety Authority (EFSA) proposed the introduction of temporary maximum residue levels (MRLs) of 0.036 mg kg^{-1} nicotine for fresh mushrooms and 1.17 mg kg^{-1} for dried mushrooms (2.3 mg kg^{-1} for ceps).

Analysis

A variety of methods have been used for the analysis of nicotine, but ones based on GC–FID and GC–MS are most popular.

Carboline Alkaloids

Occurrence

Toxic carboline alkaloids are produced during the high temperature cooking of high protein foods. They are neurotoxins and can form mutagenicity promoting derivatives. They have several varied properties and actions including adverse effects on the nervous system and cytotoxicity, but being antimicrobial, antiviral, and antioxidant they also have some beneficial activity.

Carbolines are heterocyclic compounds with an indole ring system joined to a pyridol ring in which the position of the nitrogen molecule can vary. Where the nitrogen is in the 2-position carbon atom, the compounds are called β-carbolines, whereas α- and γ-carbolines have the nitrogen in other positions. In tetrahydrocarbolines, the pyridol ring is saturated.

Tetrahydrocarbolines are formed in food processing by reaction of indolalkyamines such as tryptamine, serotonin, or tryptophan with carbonyl compounds, principally aldehydes. They undergo oxidation to form dihydrocarbolines and ultimately β-carbolines. The highest concentrations of carbolines are found in soy sauce (150–2000 mg kg^{-1} β-carboline) and in coffee (0–12 mg kg^{-1} norharman).

Tetrahydro-β-carboline-3-carboxylic acid (THCA) and methyl-tetrahydro-β-carboline-3-carboxylic acid (MTCA) are isomeric carbolines found in some commercial fruits. Citrus fruits (orange, lemon, grapefruit, and mandarin) have been found to contain 0.15 to more than 8 mg kg^{-1} MTCA with lower levels in other fruits (banana, pear, grape, tomato, and apple). Levels of THCA are lower, usually less than 0.05 mg kg^{-1}. MTCA increases in both pears and bananas as the fruit ripens and softens during storage. Tetrahydro-β-carbolines have also been found in chocolate and cocoa with total levels typically being 1–5 mg kg^{-1}.

Toxicity

The compounds of greatest concern because of their mutagenicity and carcinogenicity are α- and γ-carbolines. *N*-methylcarbolines bioactivated in the liver may act as neurotoxins, and β-carbolines influence the action of neurotransmitters. The tetrahydro-β-carbolines can also act as precursors of *N*-nitrosamines, which are potent carcinogens.

Control

There are no specific approaches to control the intake of carboline alkaloids, however, consumers are occasionally advised not to overcook meat on account of the range of carcinogenic or toxic compounds that are believed to be formed by excessive heating.

Analysis

Carboline alkaloids may be determined by GC–MS or LC–MS, or by HPLC with fluorescence detectors.

Alkaloids that Contaminate Food

Pyrrolizidine Alkaloids

Occurrence

Pyrrolizidine alkaloids are probably the major alkaloidal food contaminants in terms of wide distribution and the degree of economic and health damage caused. They are found worldwide in a very large number of plants. The pyrrolizidine alkaloid content of seeds is often very high (up to 5% dry weight) and the effects can be acute or cumulative. Pyrrolizidine alkaloids not only cause liver damage in the form of veno-occlusive disease, but also have numerous other damaging effects including lung disease and an elevated risk of liver cancer.

Plants containing pyrrolizidines that have been used as food or food supplements include comfrey (*Symphytum*) and a number of vegetables consumed in Japan, including *Petasites, Symphytum*, and *Tussilago*. They are also found in the seed of borage *(Borago officinalis)* and species of *Echium*. These seeds are used in producing specialty oils on account of their beneficial fatty acids, but pyrrolizidine alkaloids are not particularly soluble in oil and reports of its contamination are very limited. However, the major human health problems with pyrrolizidine alkaloids are associated with contamination of food that does not naturally contain the alkaloids.

Bees that feed on plants containing pyrrolizidine alkaloids transfer the toxins into honey and into foods and supplements produced from pollen (Figure 4). This has become a real problem where the plants grow profusely, such as *Echium* in Australia, and guidance has been issued to reduce consumer exposure to contaminated honey. There have also been limited studies indicating that pyrrolizidine alkaloids may be transferred into animal milk and poultry eggs.

Figure 4 *Echium* surrounding beehives in Argentina. Reproduced with permission of Quality Services International, Bremen, Germany.

Toxicity

Pyrrolizidine alkaloids are converted in the liver to highly reactive pyrroles which stimulate proliferation of cells in the liver causing hepatic veno-occlusive disease, hepatic venous thrombosis, ascites, jaundice, and an elevated risk of liver cancer. Acute poisoning causes necrosis of the liver, damage to the lungs, heart, kidney, stomach, reproductive system, and brain, and probably a higher incidence of liver cancer. Pyrrolizidine alkaloids are teratogenic and are transmitted through breast milk.

Most poisonings result from contamination of food grain with seeds of pyrrolizidine alkaloid-containing plants. Pyrrolizidine alkaloids have been responsible for some very serious contamination incidents. In 1975, people in four Indian villages were poisoned by *Crotalaria*-contamination of grain, as a result of which 28 of 67 affected people died from liver disease. Seeds of *Heliotropium*-contaminated wheat in Afghanistan in 1976, causing liver disease in almost 1500 villagers who consumed it. In 1992, *Heliotropium* growing in wheat contaminated the flour, which was consumed because of famine in the area, and this resulted in approximately 4000 casualties. In a more recent outbreak in Afghanistan (2008), more than 150 people were poisoned, and 20 died after consuming pyrrolizidine alkaloid-contaminated wheat flour and goat's milk.

Control

Methods for the control of pyrrolizidine alkaloid contamination are based partly on legislation and advice, and partly on control of the plants responsible.

Most of the plants that produce pyrrolizidine alkaloids have very successfully invaded large area of land in Europe, Asia, Australia, and the USA. Despite the fact that they have

few natural predators, their control by biological means (usually with toxin-resistant insects) is today preferred because of the undesired environmental impact of herbicides.

Acting on government advice, the herbal medicine industries in the UK and in the USA have voluntarily removed preparations containing comfrey root from the market. The German Federal Health Bureau has introduced a limit of pyrrolizidine alkaloid intake from herbal medicines to levels providing less than 1 μg day^{-1}, or to 0.1 μg day^{-1} when used for more than 6 weeks. These limits could be exceeded by consumption of contaminated eggs and honey. The Food Standards Australia New Zealand organization has set a provisional exposure level of 1 μg pyrrolizidine alkaloid per kg body weight per day and has advised consumers not to eat honey from *Echium plantagineum* every day.

Analysis

Pyrrolizidine alkaloids have been analyzed by GC and color reactions, but today LC–MS methods are preferred, especially for food samples. Metabolites of pyrrolizidine alkaloids can be detected in body fluids by this technique.

Ergot Alkaloids

Occurrence

Ergot is the broad term used to describe the infection and toxins produced by the fungus *Claviceps purpurea*, which infects cereal grains. Ergot is most common in rye and triticale, a hybrid of rye and wheat, and it occasionally infects wheat and barley, but rarely oats. Grasses can also be infected and pass spores on to cereal crops. The quantity and pattern of ergot alkaloids vary between fungal strains and the host plant. The fungus produces dark growths known as sclerotia on the grain, which survive milling and baking processes to contaminate bread and other products (Figure 5).

The ergot sclerotia are poisonous, containing alkaloids including ergotamine, ergocornine, ergocryptine, ergocristine, ergoclavine, ergonovine, and ergometrine. These are amide and peptide derivatives of lysergic acid; and tremorgenic indolediterpene alkaloids. Ergot alkaloids containing C9-C10 double bond epimerize to form mixtures of left-hand rotation isomers based on lysergic acid and known by the suffix -ine (e.g., ergotamine) and right-hand rotation isomers based on isolysergic acid known by the suffix -inines (e.g., ergotaminine). In nature, -inines always accompany -ines.

Toxicity

Ergot alkaloid poisoning causes many symptoms including fatigue, burning sensations, muscle spasms, convulsions, and numbness of extremities. In extreme cases, gangrene ensues, leading to loss of extremities, hallucinations, and abortion. Large-scale human poisoning from ergot has been recorded recently in France, India, and Ethiopia.

The ergot isomers differ in toxicity with -ines being biologically active and -inines inactive, thus it is important that analytical methods distinguish between the two.

Figure 5 Ergot (*Claviceps purpurea*) on wheat. Reproduced with permission from D Waylett.

Control

Control of ergot is based on the planting of seed that is free of spores, and from which sclerotia have been removed by seed cleaning equipment or by brine flotation, plowing in of infected seed, and removal of grasses that can carry the fungus. When present in the field, *Claviceps* can be killed by fungicides. Burning of wheat stubble will also reduce the number of viable sclerotia. Cereal cultivars vary in their susceptibility to ergot with those having short flowering periods being less susceptible.

Despite effective cleaning procedures, ergot alkaloids have been detected in surveys of Canadian and European cereals and cereal products at high levels of more than 7000 mg kg^{-1}.

Legislation

Legislative limits have not been set for ergot alkaloids in food as the ergot problem primarily affects animal feed. Ergot alkaloids are usually measured in terms of the weight of sclerotia in a grain sample, with grain having more than 0.05% ergot by weight typically being declared ergoty and unsafe for human consumption. The setting of limits has been hampered by the difficulty in determining the alkaloids and separation of the toxic and nontoxic isomers.

Analysis

The analysis of ergot alkaloids has best been achieved by LC–MS where modern methods can separate the isomeric pairs and give a more accurate picture of exposure. Care is required to minimize the exposure of the samples and extracts to light and heat, in order to avoid epimerization.

Argemone Alkaloids

Occurrence

The seed oil of the plant *Argemone mexicana* contains the toxic quaternary benzophenanthridine alkaloids sanguinarine (approximately 90%) and dehydrosanguinarine (approximately 5%) with lesser quantities of cheletrythrine and coptisine, and small quantities of berberine and protopine, which are isoquinoline alkaloids. The seeds contain 30–35% of oil which itself contains 0.13% alkaloids.

Argemone seed occasionally contaminates cereals, but more frequently its oil contaminates edible oils, especially mustard oil from *Brassica nigra*. Perhaps more commonly in some countries, argemone seed oil is used to adulterate mustard oil.

Toxicity

Consumption of mustard oil contaminated with argemone oil leads to epidemic dropsy. Its effects are dilatation, proliferation, and increased permeability of capillaries, leading to edema of the lower body and limbs, with oxidative stress and death of red blood cells causing anemia and, ultimately, congestive heart failure. The visible symptoms are diarrhea and vomiting, erythema (redness of the skin), coughing, and shortness of breath. Toxicity may be observed following short-term poisoning or cumulative low-dose exposure. Despite these issues, sanguinarine has potential anticancer activity and antimicrobial activity and has been tested as a feed additive for poultry.

Control

Control of argemone poisoning takes two forms. First, yellow-seeded mustard is cultivated, in which the argemone seeds are visible, in mixtures, and the darker oil also shows up. This is encouraged by programs of education for farmers, who are also taught to identify the argemone plant. Second, in countries such as India, official control measures are enforced, including maximum permissible upper limits of argemone oil in edible oils, bans on the sale of unpacked mustard oil, and certification of mustard oils.

Analysis

HPLC-based methods are preferred for all argemone alkaloids, with diode array or mass spectrometric detection.

Mushroom Alkaloids

Mushrooms commonly cultivated or collected for human consumption do not appear to contain toxic alkaloids. The total alkaloid content of the common edible mushroom *Boletus edulis* has been reported as relatively high, but the individual alkaloids have not been identified.

Occurrence

Several mushroom-poisoning incidents are reported every year. The majority are associated with misidentification of toxic species as safe ones by people collecting wild specimens for food. Most serious poisonings are attributable to the fungal genus *Amanita*. Some members of this genus are safe to eat, but others are very poisonous, in particular the Fly Agaric, *Amanita muscaria*, and the Death Cap, *Amanita phalloides*.

Toxicity

The toxins in these fungi are the alkaloids ibotenic acid and related compounds muscarine, muscaridine, and muscimol. Levels of ibotenic acid are typically 300 mg kg^{-1}. It is decarboxylated to the more psychoactive compound muscimol, which produces symptoms on ingestion of 10–15 mg.

Ingestion causes paralysis of the respiratory center and cardiac muscle. There are numerous symptoms, including profuse salivation, sweating, severe colic, vomiting, and purging, often followed by coma and death.

Control

Control of mushroom poisoning can only be provided by education and advice. The most common consumers of these toxins are collectors of wild mushrooms who are aware of the risks and are likely to have identification guides, of which many are available. The major problem appears to be misidentification, especially where the juvenile stages of safe and toxic mushrooms are very similar in appearance.

Minor Alkaloids

Some minor alkaloids rarely contaminate food but have the potential to do so. Tropane alkaloids are a large group of compounds found in some of the Solanaceae, but not in food crops. They have a two-ringed structure characterized by a pyrrolidine and a piperidine ring that share a nitrogen atom and two carbons atoms. The most important tropane alkaloids are (−)-hyoscyamine and (−)-scopolamine (also known as hyoscine). Tropane alkaloids are present at high levels in the Datura species *Datura stramonium* (thorn apple, Jimson weed), *Datura ferox*, and *Datura innoxia*. The highest levels of the alkaloids are found in the seeds, which often contaminate soya beans and linseeds used in the manufacture of animal feeds. Food poisoning by *Datura* occurs occasionally when people mistake the plant for an edible one, for example, the root of *Datura* resembles that of edible burdock.

Tropanes block the muscarine acetylcholine receptors of the central nervous system, preventing the binding of acetylcholine. Poisoning symptoms include tachycardia and mental confusion. High doses (approximately 100 mg in adults) cause coma and death and sometimes lower doses (less than 10 mg) can be fatal.

The hemlock plant (*Conium maculatum*) contains a small number of toxic alkaloids including coniine and coniceine. Hemlock does not normally poison humans as a result of food contamination, but it has been confused with several umbelliferous wild food plants including wild carrot (*Daucus carota*), wild parsnip (*Pastinaca sativa*), and fennel (*Foeniculum vulgare*). It has also been claimed that birds, notably quails, that feed on seeds become toxic and can poison people consuming them.

Summary

Toxic plant exposure is a common cause of poisoning, and although many are caused by alkaloids, the more frequent incidents do not arise through food contamination. Alkaloids

naturally present in important foods such as potatoes can be detected quite readily, and the incidence of their occurrence will increase as analytical methods improve. However, advances in quality control and general awareness can ensure that for virtually all consumers, the dose ingested is well below that likely to cause ill effects.

A more serious threat is accidental or intentional contamination with alkaloids of high toxicity. Large-scale accidental poisonings are still likely to occur occasionally in the poorer countries where the toxic plants are more common, the means of their control are absent, alternative foods are not available, and consumer awareness is poor. Today, there is the modern threat of a moderate-scale intentional contamination of food supplies with poisons, of which the alkaloids are likely agents as they are relatively easier to obtain from natural sources. The answer to this problem lies partly in advances in detection techniques, which are fortunately proceeding at a rapid rate.

See also: Analytical Methods: Overview of Methods of Analysis for Chemical Hazards. Public Health Measures: Challenges of Developing Countries in Management of Food Safety; Food Defense: Prevention of Sabotage and Bioterrorism

Further Reading

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NATURAL TOXICANTS

Naturally Occurring Toxins of Plant Origin

C Crews and D Clarke, The Food and Environment Research Agency, York, UK

Glossary

Biosensors These are detectors that incorporate a biological agent such as an enzyme.
Chromatographic methods They are a means of separating chemical compounds in a sometimes complex mixture, usually by high-performance liquid chromatography (HPLC) or gas chromatography (GC).
Colorimetric detection It is a detection based on the absorbance of light of a particular wavelength, usually after producing a colored derivative of the analyte.
Gas chromatography (GC) It is a means of separating volatile organic compounds before detection. The separation is based on the different affinities of compounds for a solid stationary phase and mobile gas phase pumped through a column being heated slowly.
Gas chromatography–mass spectrometry (GC–MS) A combination of GC with a mass spectrometer as a means of identifying and measuring the compounds eluting from a column.
Glycoside It is an active compound bound to a carbohydrate (sugar) molecule.
High-performance liquid chromatography (HPLC) It is a means of separating nonvolatile organic compounds before detection. The separation is based on different affinities of compounds for a solid stationary phase and mobile liquid phase (solvent) pumped through a column.
Liquid chromatography–mass spectrometry (LC–MS) It is a combination of HPLC with a mass spectrometer as a means of identifying and measuring the compounds eluting from a column.
Rapid test kits These are usually portable kits that can be used to measure toxins outside a laboratory, often based on immunoassays.
Reverse-phase high-performance liquid chromatography It is a system of HPLC where the mobile liquid phase is more polar than the solid stationary phase. This is the most common type of HPLC.
Ultraviolet (UV) detection A means of detecting compounds eluting from an HPLC column by detecting their absorbance of UV light of certain wavelengths.

Introduction

Plants regularly cause poisonings on account of certain chemicals they contain. Plant toxins are generally secondary metabolites, that is, they are not essential for the plant to grow but have another function, usually in the plant's defense from animal and insect predators.

Poisonous plants affect nearly all living creatures from insects to humans. Their effects on humans vary from causing individual poisoning to mass outbreaks and from having relatively minor to rare fatal consequences. The means of human exposure varies from direct physical contact and intentional consumption through to accidental ingestion both directly and through contamination of plant- and animal-based foods. Ingestion of toxic plants is almost always deliberate and not accidental. Plants are eaten for food or because they are believed to have health benefits or desirable psychoactive properties. In other cases they may be consumed as an act of suicide.

Some toxin-containing plants are occasionally eaten on account of mistaken identity. Some common bulbs such as daffodils and tulip which are mildly toxic can be mistaken for onions. The fruits of toxic plants such as the very poisonous deadly nightshade appear similar to edible fruits such as blueberries, and the very toxic poison hemlock and hemlock water dropwort resemble wild versions of carrot, parsnip, and chervil.

Food preparation procedures are important in some cases. Lupin seeds need treatment and at home several raw beans need substantial cooking.

Natural herbal remedies have become very popular and can cause poisoning on account of poor knowledge of the plants' properties, misidentification, or lack of awareness of the potential dangers. Today regulation and licensing are addressing these issues in the developed world.

Poisoning can be caused by accidental overdose, overconsumption, or disbelief of the toxicity. Poisons can be transferred from plants to human foods in animal products such as milk, bird's eggs, and honey produced by bees foraging on toxic plants. Toxins can be present in some or all parts of plants including the roots, leaves, fruits, and seeds; and the toxin can be effective when taken internally or through skin contact. Poisonous plants are consumed as found or processed by drying or cooking. These activities affect the toxicity of most

poisonous plants. Many toxins can be deactivated by cooking, but others are concentrated by drying or by extraction into a tea-like beverage.

Some people, such as children and the infirm, are more at risk due to less effective immune systems and form the most important risk group. Very young children are at the highest risk from plant toxins as they regularly put almost anything of suitable size within reach into their mouths, and might find berries and leaves attractive. Poisons affect children more rapidly and severely because their body weight is much lower, thereby increasing the concentration of the toxins.

Some highly toxic plants including water hemlock (*Cicuta* sp.), poison hemlock (*Conium maculatum*), and hemlock water dropwort (*Oenanthe crocata*) contain highly dangerous toxins (cicutoxin, coniine, and oenanthotoxin, respectively). They are not food items but closely resemble edible foods and herbs such as parsnip and dill (**Figure 1**).

Cyanogenic Glycosides

Cyanogenic glycosides are compounds that are chemically bound to sugars and release cyanide in the form of hydrogen cyanide (HCN, prussic acid) under certain conditions, notably in an acid environment or through enzyme action. Many plant species can produce cyanide and over 50 cyanogenic glycosides are known.

Occurrence

In most parts of the world, plants containing cyanogenic glycosides are relatively uncommon dietary items. The major food plants that do contain cyanogenic glycosides are cassava (*Manihot esculenta*), sorghum (*Sorghum* spp.), and corn (*Zea mays*).

The concentration of cyanogenic glycosides in plants varies with many factors such as the organ, plant maturity, time of the year, and growing conditions. Most parts of the plants contain cyanogenic glycosides, with the highest concentrations in the young growth and seeds. The flesh of the ripe fruits remains edible.

The cyanide part of the molecule is synthesized from nitrate as a part of normal plant metabolism, but in competition with amino acid production. The glycosides are present in the vacuoles of the cells and are released when the cell walls are broken by chewing, grinding, or cooking. These processes also release the enzymes that cleave the cyanide–sugar bond, hence releasing the free toxin.

Cassava is a major foodstuff for many people in Africa and South America and is responsible for most cyanide poisoning

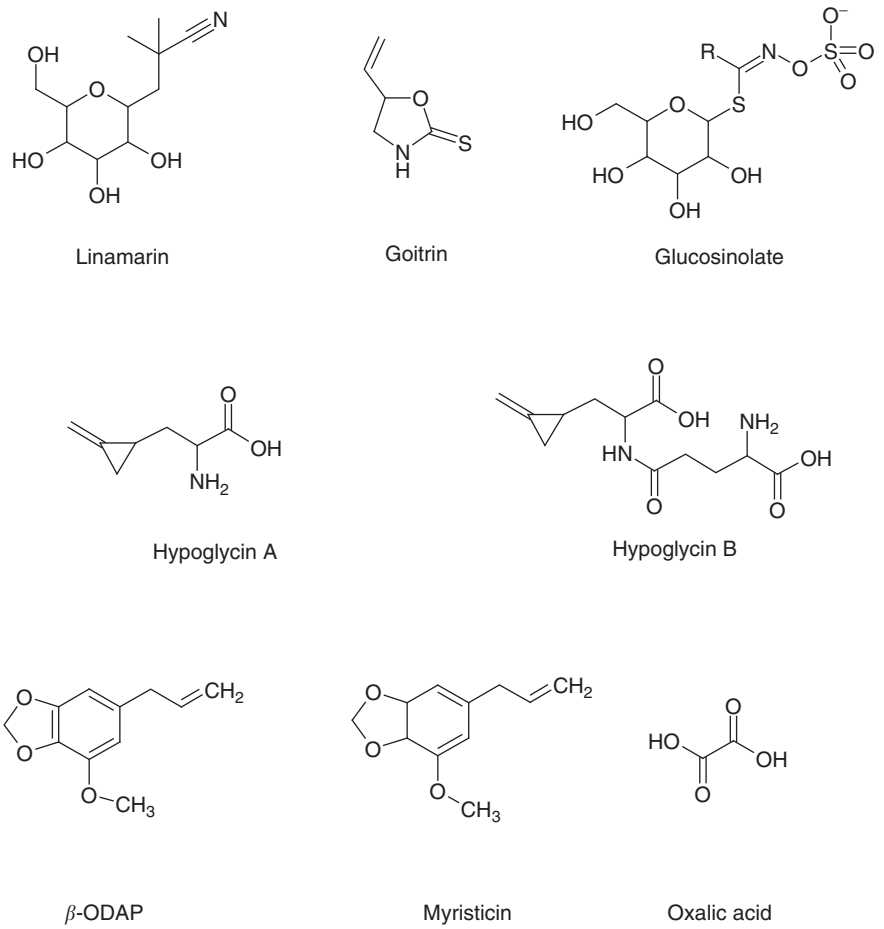

Figure 1 The structure of some important plant toxins.

Figure 2 Cassava root.

in humans. It contains cyanogenic glucosides, linamarin, and lotaustralin that are hydrolyzed by the endogenous enzyme linamarase when the plant is eaten or processed. The root of the cassava plant is eaten (Figure 2).

There have been two large epidemics of cassava poisoning in recent times, in the Democratic Republic of Congo from 1936–37 and in Mozambique in 1981, with many smaller outbreaks in other African countries.

Other food plants well known to be poisonous are several *Prunus* sp. comprising edible fruits such as apricot, cherry, peach, plum, and sloe and *Malus* sp. (apples and crab apples). The edible *Prunus* sp. are usually deciduous trees that are widely cultivated in temperate zones. The toxic glycosides are fortunately contained in the seed kernel, which is usually not consumed intentionally in a large quantity. Flax and white clover contain the glycosides linamarin and lotaustralin, sorghum contains dhurrin, and lima beans contain linamarin.

The cyanogenic glycoside found at high levels in bitter almond (*Prunus amygdalus*) is known as amygdalin or laetrile. In the 1970s, laetrile was popular as a cancer cure but was soon withdrawn on account of its toxicity and lack of evidence of its supposed beneficial properties.

Toxicity

In the body, cyanide released from the hydrolysis of cyanogenic glycosides deactivates cytochrome C oxidase in the mitochondria, disrupting electron transport and stopping aerobic respiration. As a consequence, oxygen is not extracted from the blood and death follows quickly.

Clinical Manifestations

The cyanide ion is readily absorbed from the intestinal and respiratory tracts and has a strong affinity for binding with trivalent iron of the cytochrome oxidase molecule, inhibiting its enzymatic action and preventing cellular respiration. The characteristic cherry red venous blood seen in acute cyanide poisoning results from the failure of the oxygen-saturated hemoglobin to release its oxygen to the tissues because the enzyme cytochrome oxidase is inhibited by the cyanide.

The body can detoxify cyanide quite quickly, so very small doses have no obvious effect. Small quantities of cyanide are detoxified by cellular enzymes and thiosulfates in many tissues to form relatively harmless thiocyanate, which is excreted in the urine. When large quantities of cyanide are rapidly absorbed and the body's detoxification mechanisms are overwhelmed, cyanide poisoning occurs. In most species, the lethal dose of HCN is in the range of 2–2.5 mg kg^{-1} bodyweight.

Low doses of cyanide are goitrogenic in humans and animals. Chronic exposure to low levels of cyanide causes goiter and tropical ataxic neuropathy, a disorder that damages the nerves and makes movements uncoordinated and eventual lameness occurs. Enlargement of the thyroid gland is caused by the formation of thiocyanate, which inhibits the intrathyroidal transfer of iodine and elevates the concentration of thyroid-stimulating hormone.

Moderate poisoning causes headache, tightness in throat and chest, and muscle weakness. Acute poisoning causes more severe effects, notably an irreversible paralytic disorder called Konzo, and sometimes death. Large doses (200–500 ppm) taken at once are often fatal. Konzo and tropical ataxic neuropathy often occur in local epidemics, especially during famines when proper cassava preparation procedures are neglected. Cyanide can also poison workers processing cassava when the HCN fumes are inhaled in enclosed spaces.

Diagnosis and Treatment

The appearance of toxic effects is usually delayed because the glycosides have to reach the gastrointestinal tract before hydrolysis releases cyanide. Tissues lose cyanide very rapidly, so samples must be analyzed quickly or frozen as soon as collected. Cyanide poisoning can be identified by measuring HCN in body fluids by the methods described above.

Treatment for cyanide poisoning consists of binding cyanide in a nontoxic form to reactive compounds. For this purpose intravenous injections of sodium nitrite and sodium thiosulfate are used. Sodium nitrite converts hemoglobin to methemoglobin that reacts with cyanide to form cyanomethemoglobin. This reactivates the cytochrome oxidase system. Sodium thiosulfate combines with cyanide to form sodium thiocyanate, which can be excreted rapidly.

Amyl nitrite is given by inhalation to stimulate respiration. Antidote kits are available containing sodium thiosulfate or hydroxocobalamin and amyl nitrite.

Mitigation

Selective breeding of certain varieties of plant species with naturally low glycoside content has resulted in varieties that are low in cyanogenic glycoside, and has increased their food value for humans and animals. The development of sweet almond varieties with low cyanogenic glycoside content has facilitated the human consumption of almond seeds.

Sorghum varieties low in cyanide have also been developed. Reduction of the enzyme levels can reduce the cassava's toxicity. The genes responsible for linamarin synthesis can be blocked by genetic engineering that can almost completely remove the cyanogenic glycoside content of the roots.

Cassava can be detoxified by chopping and grinding in running water to wash away the glycosides, but long-term consumption of cassava that has been poorly prepared in diets is quite common, resulting in the disease tropical ataxic neuropathy.

Fresh cassava roots rot rapidly and cannot be stored, so detoxification is sometimes neglected. Cassava processing procedures vary depending on the food products being prepared and on local cultural practices. In Africa, cassava is usually boiled or fermented by bacteria and molds in aerobic or fermentation methods. Fermentation and the associated crushing disintegrates the tissues, increasing the rate of glycoside hydrolysis and reducing cyanide by 70–95%. Drying, however, decreases the cyanide content of cassava by only 10–30% and boiling alone does not remove cyanide effectively.

Analysis

In a simple field test, paper strips containing salts of picric acid are used, picrate turns from yellow to red in an atmosphere containing HCN. The measurement of HCN released by chemical or enzyme treatment can also be made using rapid test kits and biosensors based on immobilized cyanidase, usually with colorimetric detection. Cyanogenic glycosides can be analyzed in the intact form by chromatographic methods such as capillary electrophoresis (CE), gas chromatography (GC), and high-performance liquid chromatography (HPLC) or HPLC/mass spectrometry (MS), sometimes with post-column enzymatic cleavage.

Saponins

Saponins are steroidal and triterpene compounds bound to sugar molecules. They produce soap-like foaming when shaken with water, having both a hydrophilic part (glycoside) and lipophilic part (triterpene). Some saponins act as antifeedants, protecting the plant from microbes, fungi, and animals. They may also have beneficial medicinal and dietary effects in humans.

Occurrence

Saponins are mostly associated with the plant ackee (*Blighia sapida*), the fruits of which are a popular food item in Jamaica. *Blighia sapida* is a tall tree found originally in Africa and introduced into the West Indies at the end of the seventeenth century. It also grows in Central America and southern parts of Florida. The fruits are pear-shaped, approximately 10-cm long and weigh approximately 100 g. When ripe, the reddish pericarp opens to reveal the shiny black seeds. The fruits usually contain three seeds, but in 30–50% of the fruits one or two of the seeds are very undeveloped. At the base of the seeds is a fleshy seed mantle (aril) which is cleaned of fibrous material, boiled in water, and eaten. The fruit is usually cooked by baking, or boiling in milk or water to produce a soup (Figure 3).

Figure 3 Ackee fruit showing the seeds and mantle.

Toxicity

The unripe fruits of ackee are poisonous, even after cooking. Consumption of the unripe fruits or of water in which the ackee fruit has been cooked may cause rupturing of the red blood cells (erythrocytes), technically known as hemolysis, leading to epidemics of hypoglycemia (lowered blood glucose). The plant mainly poisons young children who might be unaware of its effects. In Jamaica the condition is well known as 'Jamaican vomiting sickness.' Approximately 270 cases of poisoning were reported in Jamaica between 1980 and 1991.

The ackee fruit toxins are amino acids hypoglycin A and dipeptide hypoglycin B and the γ-glutamyl peptide of hypoglycin A. Hypoglycin A is 2-amino-4,5-methanohex-5-enoic acid or L-(methylenecyclopropyl)-alanine, and is the most toxic compound in the ackee fruit. It is found in the unripe seeds at a level of approximately 0.95% by weight and in the aril at approximately 0.7% by weight. The flesh of the fruit contains less than 0.04% hypoglycin A. Levels in the seed and aril decrease on ripening of the fruit.

Clinical Manifestations

Symptoms of poisoning often occur rapidly after consumption, beginning with intense thirst, severe and persistent vomiting without diarrhea, thus leading to dizziness, weakness and tiredness with tingling sensations (paresthesia), and twitching of the limbs. In severe cases consciousness is lost and coma and death can follow within a few days. The physiological effects are extreme hypoglycemia (low blood sugar) and hypokalemia (low blood potassium).

Hypoglycin A is metabolized to methylenecyclopropylacetic acid in the body, and it is this compound that is directly responsible for the ackee's toxicity. Methylenecyclopropylacetic acid inhibits the metabolism of fatty acids and affects acylCoA dehydrogenase. This leads to fatty acids accumulating in the blood serum. Glucose synthesis is prevented, and

glucose and hepatic glycogen are therefore depleted, leading to hypoglycemia.

Diagnosis and Treatment

Hypoglycemia can be detected by clinical tests, and further testing of blood electrolytes, kidney, and liver functions are advised, along with computed tomography scanning of the brain. The fatty acid profile shows an overabundant proportion of medium-chain fatty acids. Treatment consists of gastric lavage and attempted adsorption of the toxin on to activated charcoal, and actions to help breathing and correct electrolyte imbalance. Intravenous injections of glucose can correct hypoglycemia.

Mitigation

The fruits are banned in the US.

Analysis

Hypoglycin A can be determined using normal methods for amino acids analysis, such as using automated amino acid analyzers, but problems may be caused by coelution with the beneficial amino acids on chromatographic systems. Another approach is to use reverse-phase high-performance liquid chromatography with ultraviolet (UV) detection, after derivatization of the amino acid with *o*-phthalaldehyde, *o*-phenylisothiocyanate, or phenylthiocarbamate.

Glucosinolates

Glucosinolates are sulfur-containing oximes and glycosides associated with brassicas. They have both toxic and beneficial effects on the body, impairing thyroid function in both humans and animals at high doses, but at subtoxic doses, they act as chemoprotective agents against chemically induced carcinogens by blocking the initiation of tumors in a variety of rodent tissues, such as the liver, colon, mammary gland, pancreas, etc.

Occurrence

There are upwards of 200 individual glucosinolates. These are found in common vegetable foods of *Brassica* species, including cabbage, broccoli, cauliflower, and rapeseed. Spicy condiments such as mustard, horseradish, and wasabi owe their piquant nature to high concentrations of glucosinolates.

Toxicity

When the tissues of brassica plants are macerated or cooked, the glycosides are hydrolyzed to release volatile isothiocyanates or nitriles and thiocyanates. The major toxicity is associated with oxazolidine-2-thiones, such as goitrin (5-vinyloxazolidine-2-thione), which impair thyroid function by inhibiting the formation of thyroxine by binding iodine and suppressing thyroxine secretion from the thyroid.

The oil of the brassica rapeseed is often assumed to have cardiovascular toxicity on account of its high level of erucic acid. Despite evidence for this being rather poor, special varieties of low erucic acid rapeseed are now cultivated for edible oil production.

Nitriles released from glucosinolates depress growth and cause damage to the liver and kidneys in severe cases.

Clinical Manifestations

Exposure to toxic levels is revealed as goiter, liver damage, and stunted growth. However in many cases a swelling in the neck is the only symptom observed.

Diagnosis and Treatment

Diagnosis is by detection of thyroid abnormalities in the absence of physiological causes and a history of brassica ingestion. Treatment beyond withdrawal of the glucosinolate source is usually unnecessary

Mitigation

Ingestion of toxic levels of glucosinolates is uncommon and action to prevent exposure is unnecessary.

Analysis

Glucosinolates and derived isothiocyanates in vegetables are normally determined by LC–MS or GC–MS after extraction from plants with polar solvents such as mixtures of water and methanol.

Phytohemagglutinins (Kidney Bean Lectins)

Lectins are proteins that bind to soluble carbohydrates that are parts of glycoproteins or glycolipids. They cause agglutination (clumping together) of certain animal cells, and their function in plants is uncertain.

Occurrence

Red kidney beans (*Phaseolus vulgaris*) and several other beans contain the lectin phytohemagglutinin. This is a lectin that binds to oligosaccharides on the erythrocyte membrane, affecting protein transport across cell membranes and inducing mitosis. It agglutinates mammalian red blood cells causing nausea, vomiting, and diarrhea. It also affects transport systems across cell membranes, and cell permeability to proteins. Red kidney bean poisoning is known as Kinkoti bean poisoning (Figure 4).

Toxicity

Phytohemagglutinin is found in many types of beans, but red kidney beans contain more lectins than other species, typically three times the level found in white beans and ten times that found in broad beans. The toxin content of beans is measured

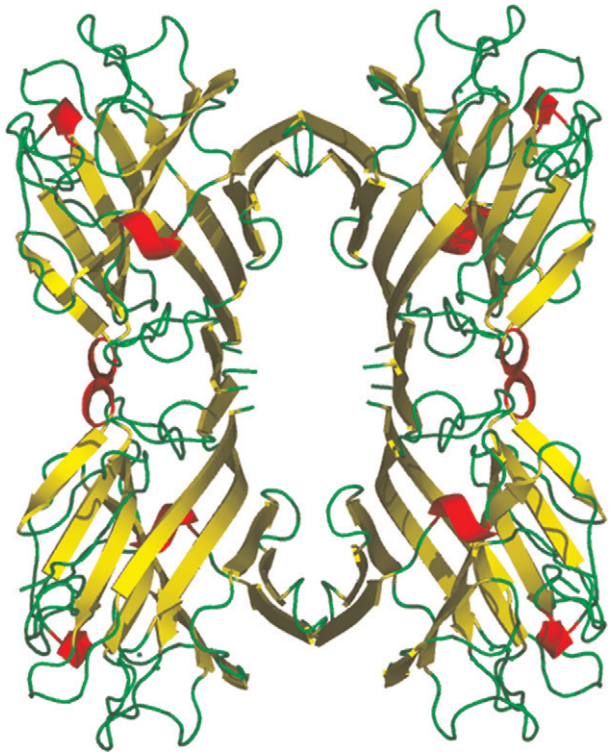

Figure 4 The structure of a phytohemagglutinin.

in hemagglutinating unit (hau), of which the raw kidney beans contain 20 000–70 000 hau. Cooking reduces the hemagglutinin activity levels to 200–400 hau.

Clinical Manifestations

Poisoning can be caused by ingestion of very few beans. Poisoning incidents are not uncommon.

Diagnosis and Treatment

Diagnosis probably often attributes the symptoms to more common causes such as bacterial food poisoning.

Symptoms are apparent between 1 and 3 h of consumption of improperly prepared beans. They begin with extreme nausea, followed by severe gastroenteritis, vomiting, and diarrhea. People affected usually recover very quickly, typically 3–4 h after the onset of symptoms.

Mitigation

Phytohemagglutinin can be deactivated by cooking the beans at 100 °C for 10 min, although much longer times are required to fully cook the beans. It is recommended that the dried beans are first soaked in water for a minimum of 5 h before cooking and then the soaking water is discarded. The cooking procedure is very important and the use of 'slow cookers' or other procedures operating at a relatively low temperature may not destroy the glycoprotein lectin; and warming might increase the toxicity of these foods.

Figure 5 The castor oil plant.

Analysis

Analysis of the toxins is indirect, based on hemagglutination of red blood cells.

Ricin

The castor oil plant (*Ricinus communis*) contains a highly poisonous proteinaceous toxin called ricin. A similar toxin called abrin is found in the plant jequirity (*Abrus precatorius*).

Occurrence and Exposure

The castor plant is a familiar tall annual or perennial with distinctive red stalks and large palmate green–red leaves and fruit pods and a distinctive spiky seed capsule. The attractive seeds, known as castor beans, are very hard, shiny, smooth, marbled, and approximately 17×8 mm in size, with a small protuberance. They contain ricin, which is an extremely toxic water-soluble protein. The seeds contain the highest amounts of ricin and some lesser toxins. Ricin seeds have a hard impermeable seed coat and are generally harmless when swallowed whole, but when chewed or otherwise broken the toxin can be released. Ricin poisoning can occur when broken seeds contaminate cereals, notably soya beans; intact seeds may pass through the digestive tract without releasing the toxin. The lethal dose in adults is considered to be 4–8 seeds (Figure 5).

Castor oil has qualities that gives it numerous industrial uses. In medicine it has famously a strong laxative action but it contains no toxin.

Abrus seeds are very distinctive, being glossy bright red in color and having a single black spot. The seeds are not knowingly eaten by adults but may be attractive to children.

Toxicity

Ricin binds to a specific adenine molecule of the 28S ribosomal ribonucleic acid (RNA) preventing protein synthesis. Poisoning symptoms are gastrointestinal hemorrhage; necrosis of liver, spleen, and kidneys; and a much increased raised white blood cell count.

Clinical Manifestations

If ingested, symptoms may be delayed by up to 36 h but commonly begin within 2–4 h. These include a burning sensation in mouth and throat, abdominal pain, purging, and bloody diarrhea. Within several days there is severe dehydration, drop in blood pressure, and decrease in urine. Unless treated, death can be expected to occur within 3–5 days.

Diagnosis and Treatment

There is no specific treatment beyond supporting care.

Mitigation

Exposure is best reduced by providing education regarding the toxic nature of the plant.

Analysis

Ricin can be detected by the application of tests to measure hemagglutionation. It is difficult to measure on account of its size and proteinaceous nature. It can however be quantified by HPLC with UV detection or by LC–MS applied to tryptic digests.

Ricin is accompanied by ricinine, a small alkaloid that can be determined by GC–MS or LC–MS as a marker of the likely presence of ricin.

Neurotoxic Amino Acids

Legumes of the species *Lathyrus* which grow in Europe, Asia, and Africa contain neurotoxic amino acids which cause large-scale disease epidemics.

Occurrence

The most familiar toxic Lathyrus species is *Lathyrus sativus* (Indian pea) which contains *β*-*N*-oxalylamino-L-alanine acid (*β*-ODAP), *β*-*N*-oxalyl-L-*α*,*β*-diaminopropionic acid, *β*-aminopropionitrile, and dimethylaminopropionitrile. *Lathyrus sativus* is cultivated in large quantities in several countries. The plant can be grown in soils and conditions that do not support safer primary crops. The peas are usually milled to produce flour, which is most often eaten when more preferred foods are unavailable, such as during famines. The concentration of *β*-ODAP in the seed can vary widely, from 0.2 mg to more than 1 mg g^{-1}.

Toxicity

The toxins cause paralysis of the lower body and wasting disease (neurolathyrism) if eaten over a long time. Consumption of the peas has caused major poisoning incidents, most recently in India in 1958 when over 25 000 people were poisoned in one district. *β*-ODAP is a nerve stimulating toxin acting as an agonist of certain nervous system glutamate receptors. It is found together with a nonneurotoxic isomer, *α*-ODAP. *β*-amino-propionitrile inhibits the enzyme lysyl oxidase, affecting the maturation of collagen.

Clinical Manifestations

The disease is manifested after several months of consumption as neurolathyrism. Symptoms of muscle spasms and leg muscle weakness develop, and paralysis ensues.

Diagnosis and Treatment

Diagnosis of lathyrism is based on evidence of consumption and paralysis with no other apparent cause. Lathyrism is treated mainly by withdrawal from the consumption of the peas.

Analysis

β-ODAP can be measured by LC after reaction with amine derivatization reagents such as *p*-nitrobenzyloxycarbonyl chloride. Isomerization of *β*-ODAP to *α*-ODAP should be inhibited where possible when a measure of the toxicity of a plant is required.

Mitigation

The neurotoxic amino acids can be removed from the seeds by soaking in water before cooking.

Oxalic Acid

Rhubarb (*Rheum rhabarbarum* L.) is a common and ,when in season, widely consumed plant in Europe and Asia. It is widely known that the leaves of the plant are poisonous, due to the content of oxalic acid.

Occurrence

Rhubarb is well known and widely found on many continents. The herb sorrel (*Rumex acetosa*) also contains oxalic acid and can cause similar kidney damage.

Toxicity

Serious poisonings are associated with consumption of the leaves of the plant, with the stems (petioles), which are normally the only parts consumed, being much less toxic. Children are poisoned more frequently than adults.

Rhubarb contains oxalic acid and dihydroxyanthracene derivatives. Oxalic acid is nephrotoxic and has a corrosive action, its lethal dose is approximately 10–25 g depending on age. In blood, oxalate binds to calcium to form an insoluble calcium oxalate, which can result in severe hypocalcemia and involuntary muscle contraction. Rhubarb leaves typically contain approximately 0.5% oxalic acid and much higher quantities of the nontoxic malic acid.

Cooking rhubarb with alkaline solutions containing sodium or potassium salts can increase their toxicity by forming soluble salts of oxalic acid.

Clinical Manifestations

Rhubarb poisoning causes abdominal pain, diarrhea, and profuse persistent vomiting, followed by internal bleeding, convulsions, and coma.

Diagnosis and Treatment

The symptoms of rhubarb leaf poisoning show clinical signs such as low production of urine (oliguria), excretion of acetone in the urine (acetonuria), and excessive albumin in the urine (albuminuria). In rare fatalities there are signs of damage to the kidneys.

Mitigation

Exposure can be reduced by warnings of the toxic effects of consuming the leaves and exercising moderation in the consumption of the stems.

Analysis

Treatment consists of gastric lavage with alkali and administration of a laxative (calcium sulfate) rich in calcium which forms an insoluble salt with oxalic acid.

Oxalic acid can be measured in rhubarb by HPLC, CE, or enzymatic methods based on the oxidation of the acid, derivatization, and colorimetric determination.

Minor Plant Toxins

Consumption of several other plants can have an adverse effect on certain individuals but the incidences are so few that they do not warrant the attention given to major plant toxins. Two examples of these are given below.

Avocado

The edible fruits (pears) of the avocado tree (*Persea americana*) are oblong and green with a knobbled green skin and edible greenish-yellow flesh surrounding a single large stone. Avocado is cultivated in many tropical and semitropical areas including Central America, Brazil, the US, and Israel. Avocado has a high content of tyramine which causes hypertension when consumed by patients taking monoamine oxidase inhibitor drugs. Another substance in avocado pears, 1-(acetyloxy)-2-hydroxy-12,15-heneicosadien-4-one (named persin) is known to damage the mammary gland of some animals, and some people are allergic to the fruit.

Nutmeg

The nut of the nutmeg tree (*Myristica fragrans*) contains the compounds myristicin and elemicin that have possible neurotoxic effects and may have psychoactive properties at high dose. The psychoactive effects are rather vague but include euphoria and nausea, and visual hallucinogenic effects are claimed; consequently, most poisoning results from deliberate ingestion of quantities far in excess of culinary portions. The effects last in excess of 24 h.

Conclusions

Our knowledge of plant toxins has improved considerably with the growth in medical and analytical science. The number of toxic plants consumed has probably increased slightly with improvements in the global distribution of foods and the increased awareness of and attraction to foreign diets. Contamination of food with toxic plants has possibly increased slowly with a reversion to 'natural' crop production methods, and the expansion of the geographical spread of plants with global warming. Population growth and climate change have contributed to the most serious plant toxicity problems, with drought and famine driving people to eat plants that are known to have toxicity. Better communications can provide aid, and developments in medicine and plant breeding can mitigate some problems, but the problem of plant toxins is likely to remain for the foreseeable future.

Further Reading

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Mushrooms and Toadstools

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Glossary

Fungal toxin A chemical entity produced by fungi that may cause illness or death when eaten.
Inedible toadstool A fungi that when consumed produces a non-lethal gastric upset, which is not usually assigned to an individual toxin.
Mushrooms The edible fruiting bodies of fungi that are large enough to be picked by hand.
Rhabdomyolysis A rupture of muscles due to toxins or compression. Release of myoglobin leads to renal failure.
Toadstools The fruiting bodies of fungi that are classified as inedible or poisonous.

Introduction

Mushrooms are defined quite simply as the edible fruiting bodies of fungi, which are large enough to be picked by hand. Conversely, toadstools are the inedible, or poisonous, equivalents that are often mistaken for mushrooms.

Approximately 14 000 of the described fungi species from the estimated total of 1.5 million in the world produce fruiting bodies that are large enough to be considered as mushrooms or toadstools. The world market for the mushroom industry in 2005 was valued at more than US$45 billion. During the 1979 production year, button mushroom, *Agaricus bisporus*, accounted for more than 70% of the world's supply of edible mushrooms. The number of individuals engaging in foraging for wild mushrooms will always be unclear; the only solid data are the rates of poisonings.

General Safety

Recently, there has been a rapid change in the pattern of mushroom production in that many species, which were previously considered wild, are now grown commercially for food. This carries no inherent risk in itself as the feedstock cultures are collected from recognized local edible species in the wild and grown under controlled conditions until reproducible and economically viable production processes are established. This includes regular inspection to ensure that the mushrooms in growth are true to type and not outcompeted by any alternative or related species. The result of this is that even the smaller local supermarkets will routinely offer several different species of fresh mushrooms for sale that were previously considered as wild species.

The single biggest food safety aspect associated with mushrooms is basic food hygiene practice rather than chemical intoxication. Wild mushrooms are often contaminated with significant quantities of harmful bacteria. Insects can bore into the mushroom cap to lay their eggs and the burrowing tracks of the resultant larvae introduce bacteria deep into the cap, which cannot be removed by simply peeling and washing. Any unsound mushrooms, such as those with obvious damage and insect larvae, should not be collected and eaten.

The more widely recognized danger of a number of toadstool species is the chemical toxins that are contained within and their often lethal effects. These require oral ingestion of only a modest quantity to cause harm, but they can be handled quiet safety (and so no early warning signal is received) as there is little or no dermal absorption unless the toadstool is waterlogged and the juices are squeezed out onto bare skin. The dangers of eating poisonous mushrooms cannot be overstated. The greatest cause of poisoning is mistaken identity, where a forager assumes they can identify an edible species and collects a common look-alike poisonous species. It is essential to learn foraging skills alongside a trained and experienced professional, normally through a recognized fungal society, where training can be provided in identification of all species of mushroom, irrespective of classification as edible or inedible in locally published field guidebooks. Until a target mushroom and all of its look-alikes can be identified in juvenile, adult, and overripe forms, it is simply not safe to forage unsupervised with the intent to consume wild fungi.

The toxic properties of some mushrooms have been known throughout recorded history. Consumption of fly agaric (*Amanita muscaria*; **Figure 1**) for the ceremonial purpose of ritual intoxication predates written history. *Amanita* poisoning is ascribed to be the cause of the 'poisoning' of at least three Roman emperors and a Pope. More recently, in November 2012, four residents in a California senior-care facility died after consuming mushroom soup prepared by a caretaker, from fungi growing in the grounds of the facility. California alone recorded 1700 cases of mushroom-related illness, of the national total of 5902, with two fatalities during 2009–10.

Figure 1 The death cap mushroom *A. phalloides* (upper) and the fly agaric mushroom *A. muscaria* (lower).

A further potential risk to public safety are wild mushroom dishes in local restaurants. Although many of the species are now produced by safe commercial methods, the presence of a true wild mushroom risotto on a menu implies foraging has taken place. Wise diners would be advised to inquire as to such arrangements, and a manager should welcome the opportunity to reassure that a qualified professional collects and identifies any truly wild produce. A chef in Australia recently caused his own death and that of another diner with a dish prepared with 'mushrooms' he had collected himself.

Identification of fungi is then at the heart of mushroom safety. The fungi of the British Isles are considered the most studied in the world, which, with lichens included, is some 12 000 species. A selection of field guides are listed in the Further Reading section, and relevant local books should be sought, but even the best guides cover only 1000–2400 local species. A new method of fungi identification is the use of a computer-based system named MycoKey. Although recognized as a work in progress, version 4.0 now adequately covers more than 1000 genera that occur in Northern Europe and provides a visual identification system of measuring, drawing, and coloring the fungi to be named and requesting further details to differentiate between look-alikes until the fungus is identified to the genus level and it is clearer what detailed reference texts should be consulted.

Toxicity

Only a few percent of fungi are truly poisonous (2%) and a similarly low percentage are considered as choice edible species (4–5%). It is the species containing toxins and resulting in death that are of the highest degree of public concern. The more poisonous fungi often have appropriately evocative names such as Satan's bolete, yellow sickener, the deadly fiber cap, beechwood sickener, funeral bell, fools mushroom, and false morel.

As the number of individual chemicals identified in fungi that can be considered as harmful is too large to cover in any detail, this work is restricted in scope to those toxins for which there is clear evidence of lethal effect and toxicological data are available documenting the toxin as a lethal dose-50 (LD_{50}), the quantity of toxin required to kill 50% of those humans or animal surrogates who are exposed (Table 1). Individual sections are then given to the most toxic, those with LD_{50} values lower than 10 mg kg^{-1} bodyweight, where a dose of 0.6 g will then have a 1-in-2 chance (50%) of causing death to a 60 kg person, and which poses a real and significant hazard.

Table 1 Summary of mushroom toxins, their source, toxicity, and effects

Toxin class	*Toxin*	*Fungi species*	*LD_{50} mg kg^{-1}*	*Poisoning*
Acromelic acid	Acromelic acid	*Clitocybe acromelalga*		Acroduria – neuroexcitation
Cyanides	Hydrogen cyanide	*Lepista nuda*	3	Cyanosis
Cyclopeptides	Amatoxins	*Amanita phalloides*	0.2–0.5	Hepatic and renal failure
	Virotoxins	*Amanita verna*	2.5	Hepatic and renal failure
Cyclopropanes	Coprinine	*Coprinus atramentarius*	–	Antabuse syndrome
	Prop-2-ene carboxylic acid	*Russula subnigricans*	2.5	Rhabdomyolysis
Hallucinogens	Psilocybin	*Psilocybe semilanceata*	–	Hallucinations
	Bufotenine	*Amanita citrina*	200	Hallucinations
Hydrazine	Gyromitrin	*Gyromitra esculenta*	10–30	Convulsions and GI disorders
Isoxazoles	Muscimol	*Amanita muscaria*	7.5–10	Mycoatropinic and delirium
	Ibotenic acid	*Amanita pantherina*	45	Mycoatropinic and delirium
	Muscarine	*Clitocybe dealbata*	150	
	Tricholomic acid	*Tricholoma muscarium*		
Necatorin	Necatorin	*Lactarius necator*		
Norleucines	Aminohydroxyhexynoic acid	*Trogia venenata*	71	Sudden unexplained death
	Stizolobic acid	*A. pantherina*		Hallucination, spasms, and sleep
Nucleosides	Clitidine	*C. acromelalga*	50	Pain and red coloration of toes
Orellanines	Orellanine	*Cortinarius orellanus*	15–20	Renal failure
Terphenyls	Ustalic acid	*Tricholoma ustale*	Kills mice	Diarrhea, tremors, and death
Trichothecenes	Roridin	*Podostroma cornu-damae*	1	
	Verrucarin	*P. cornu-damae*	0.7	
	Satratoxin	*P. cornu-damae*	0.5	
Triterpenes	Hebevinosides	*Hebeloma vinosphyllum*	66	Paralysis
	Crustulinols	*Hebeloma crustuliniforme*	100	GI disorders and renal failure
	Fasciculols	*Naematoloma fasciculare*	50–168	
Sesquiterpenes	Illudin S	*Lampteromyces japonicus*	5	GI disorders
Lectins	Bolesatine	*Boletus satanas*	3.3	Hemolytic poisoning
	Rubescenslysine	*Amanita rubescens*	0.15–0.3	Hemolytic poisoning
	Phallolysin	*A. phalloides*	0.2–0.7	Hemolytic poisoning

Abbreviation: GI, gastrointestinal.

The actual risk is related to the range and quantities of toxins normally present in any given toadstool. As these are normal metabolic products rather than residual contaminants, the required levels of grams of toxin per kilogram of fresh toadstool are often surpassed in those fungi regarded as poisonous. As an illustration, a total of 500 mg kg^{-1} of amatoxins (LD_{50} 0.2–0.5 mg kg^{-1}) can be found in the death cap (*Amanita phalloides*; **Figure 1**) and this then requires only a 50 g portion of the fresh toadstool to be considered lethally dangerous. The named toxic classes and individual chemicals, a representative fungal source, toxicity, and medical symptoms are summarized in **Table 1**. In reality, some 95% of deaths from 'mushroom' poisoning are due to a single cause – the amatoxins, which are present in species found around the world. Research into medical treatments and supporting analytical chemistry has been heavily weighted toward this single genus–toxin combination as a direct result of the extremely low amount required to be toxic and the likelihood of misidentifying examples of the *Amanita* genus such as 'death caps' and 'destroying angels' with edible *Amanita* and *Volvariella* look-alikes. Nevertheless, new classes of toxins are still being identified after activity guided isolations of 'mushrooms' believed to have caused human deaths (**Figure 2**).

Many mushrooms that are neither choice edible nor poisonous species will often produce undesirable gastrointestinal symptoms that are unrelated to bacteria food poisoning. These symptoms often do not manifest in every consumer, which complicates disease etiology. Individual species in the genera – *Paxillus*, *Agaricus*, *Entoloma*, *Boletus*, *Hebeloma*, *Tricholoma*, *Russula*, *Lactarius*, *Ramaria*, *Chlorophyllum*, *Scleroderma*, and *Laetiporus* – are known to induce nausea, vomiting, abdominal pain, and diarrhea within hours of ingestion. Such symptoms, in the absence of true toxins, can be treated by rehydration but often require hospitalization.

The occurrence, toxicity, clinical manifestations, diagnosis, and treatment for some of the major toxin classes are summarized in **Table 1**. These are provided as illustrative only, and

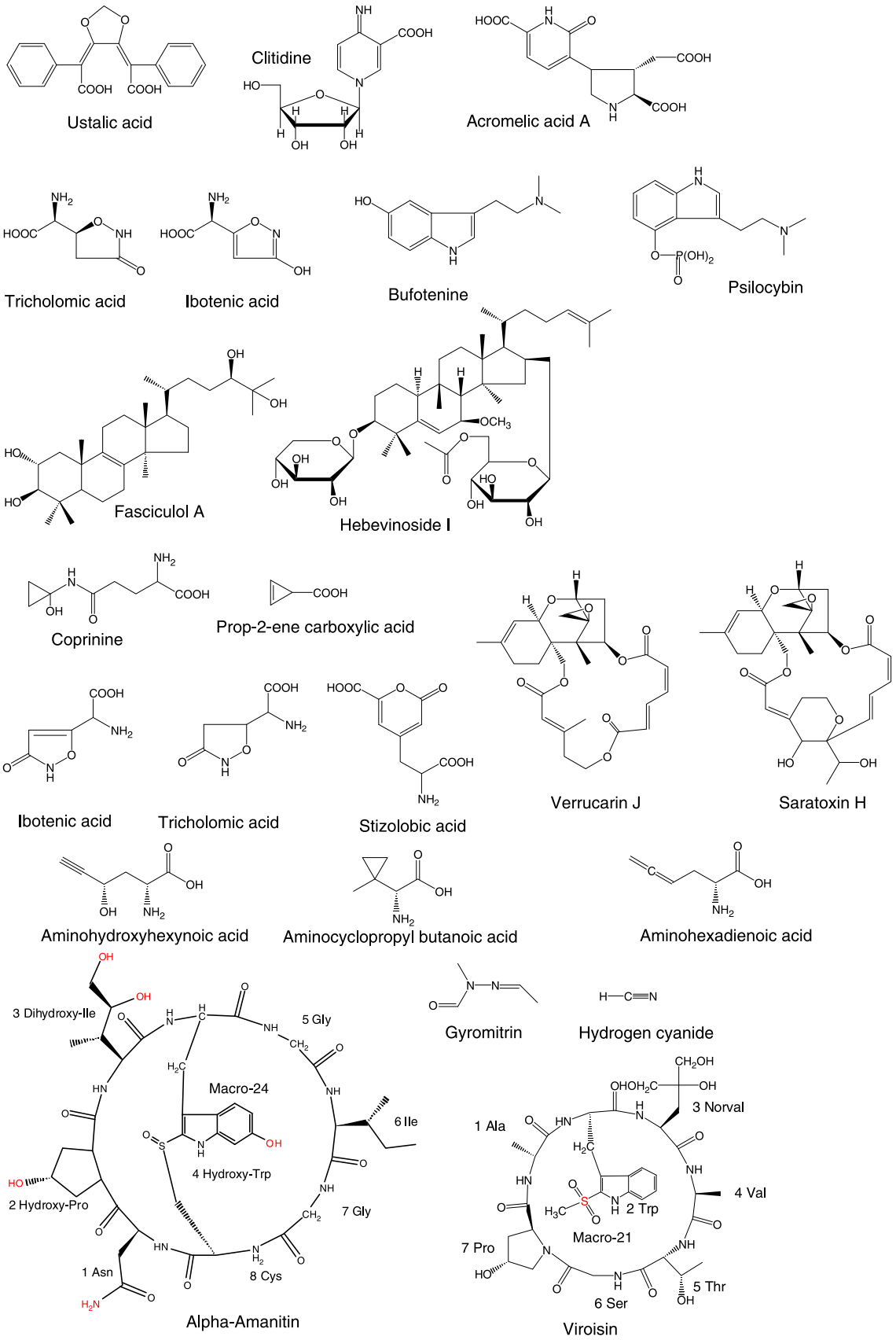

Figure 2 Structures of representative fungal toxins.

of course, expert medical advice and assistance should always be sought in the case of suspected poisoning.

Amatoxin Poisoning

Occurrence

Amatoxins are found in certain fungi in the unrelated genera *Amanita*, *Galerina*, *Lepiota*, and *Conocybe*. Of particular concern are the death cap *A. phalloides*, the European destroying angel (*Amanita virosa*), and the American destroying angel (*Amanita bisporigera* and *Amanita verna*). The sac-like volva from which the fruiting body arises is a distinguishing feature of the genus *Amanita*. A modern overview of the diagnosis and treatment of amatoxins mycosis has been published.

Toxicity

The cytotoxic agents present in these species are amatoxins and virotoxins. These toxins target the liver and inhibit transcription from deoxyribonucleic acid to messenger ribonucleic acid and impair protein synthesis, which leads to cell death.

Clinical Manifestations

The symptoms of severe gastrointestinal distress (stomach cramps, vomiting, and diarrhea) are delayed at least 6–12 h following ingestion. This phase lasts for 1–2 days followed by an apparent remission stage in which the patient feels better as the stomach is emptied. In the third phase, the patient may fall into a coma or die as a result of irreversible liver and kidney failure. The central nervous system deteriorates, and dehydration and exhaustion may impair kidney function, which is further complicated by renal damage.

Diagnosis and Treatment

There is no known antidote. Amatoxin poisoning is particularly dangerous because symptoms are delayed after the mushroom is eaten. By the time the patient feels sick, it is too late for emesis or gastric lavage to be of any use. The stomach can be emptied shortly after ingestion if no more than 2 h have passed. Treatment is on a case-by-case basis and involves careful monitoring of the patient's liver enzyme levels and general condition with supportive care. Charcoal filtration of the blood may be performed in some cases. Even patients who appear to have total remission often subsequently develop chronic active hepatitis. Liver transplantation may also be considered.

Orellanine: *Cortinarius* Poisoning

Occurrence

Orellanine is found in toadstools in the genus *Cortinarius*. This is the largest known fungi genus with 2000–3000 species. The individual species are extremely hard to distinguish and identify visually with any degree of accurately. Most of the lethal species such as *Cortinarius orellanus* and *Cortinarius speciosissimus* are a cinnamon brown color. The distinguishing feature of the genus is the presence of a cobwebby veil (the cortina).

Toxicity

Orellanine is a potent nephrotoxin. A metabolite of orellanine is believed to inhibit protein synthesis in the kidneys.

Clinical Manifestations

Cortinarius poisoning is characterized by an extremely long delay. A minimum of 3 days, or as long as 10 days to 3 weeks, may pass between eating the mushroom and the onset of symptoms. Symptoms include vomiting, diarrhea, loss of appetite, headache, a feeling of coldness, and eventual kidney failure, which may lead to death. Signs of renal impairment are headache, fatigue, thirst, chills, and abdominal, lumbar, and muscle pain.

Diagnosis and Treatment

Cortinarius poisoning is particularly dangerous because of the delay in the occurrence of symptoms. As most foragers simply avoid this genus, it is fortunately quite rare for the mushroom to be associated with the illness. It can be treated as kidney failure, by hemodialysis or peritoneal dialysis. Some patients recover spontaneously; others may require dialysis or kidney transplant.

Monomethylhydrazine Poisoning

Occurrence

Precursor toxins are found in toadstools of the genus *Gyromitra*, particularly in the false morel *Gyromitra esculenta*.

Toxicity

Monomethylhydrazine poisoning is among the most confusing of mushroom poisoning syndromes. The amount of the gyromitrin toxin can vary greatly from toadstool to toadstool, and susceptibility can vary greatly from person to person. The precursor gyromitrin toxin decomposes in the stomach to monomethylhydrazine, which reduces pyridoxine in the central nervous system. The method of preparing the mushrooms makes an immense difference to the toxicity as the toxin is volatile and is destroyed by heating. The 'safest' way to prepare *Gyromitra* species is to parboil the mushrooms (being careful not to inhale any of the vapors, which could contain the volatile toxin), discard the cooking water, and then fry the mushrooms in a clean pan. This removes the majority of the monomethylhydrazine.

Clinical Manifestations

A latent period of 6–8 h is followed by a feeling of fullness in the stomach, then vomiting and watery diarrhea, which may persist for up to 2 days. Headache, lassitude, cramps, and intense pain in the regions of the liver and stomach may be

followed by jaundice. Red blood cells may be broken down. Poisoning can be fatal.

Diagnosis and Treatment

Emesis may help if employed within 2 h after ingestion, but will be ineffective if symptoms have already occurred. Fluid replacement may be necessary if the patient is dehydrated. Patients should be hospitalized so laboratory tests can be performed to detect signs of hemolysis or liver or kidney failure. Glucose infusion prevents hypoglycemia. An infusion of pyridoxine at 25 mg kg^{-1} is generally given in cases affecting the central nervous system.

Antabuse Syndrome

Occurrence

It is caused by the fungi *Coprinus atramentarius*, *Clitocybe clavipes*, other *Coprinus* spp.

Toxicity

Coprinus species produce the compound coprine. This is not itself a poison but interferes with the alcohol detoxification process by inhibiting one of the enzymes (alcohol dehydrogenase) that processes alcohol. Alcohol is then broken down only partially to acetaldehyde. The symptoms of coprine poisoning are due to the build-up of acetaldehyde in the blood. Incidentally, the alcohol antabuse treatment disulfiram operates in the same manner, hence the syndrome being named 'antabuse.'

Clinical Manifestations

Symptoms may occur shortly after the consumption of both the fungus and an alcoholic beverage up to 48 h after the fungi is eaten. Symptoms include a flushing of the face and neck, a metallic taste in the mouth, tingling of the extremities, rapid heartbeat, and a feeling of swelling in the face and hands. The initial symptoms may be followed by nausea and vomiting. Occasionally visual disturbances, vertigo, weakness, and confusion occur.

Diagnosis and Treatment

The symptoms will subside on their own in time, although the patient may be convinced that he or she has been seriously poisoned. Coprine poisoning is unpleasant but will run its course in a couple of hours without treatment. *Coprinus atramentarius* is edible and safe if cooked and if no alcohol is ingested within 2–3 days of eating the fungi.

Tryptamine Hallucinogens

Occurrence

Psilocybin and related tryptamine substances are found in *Psilocybe*, *Stropharia*, *Panaeolus*, and in some species of *Conocybe* and *Gymnopilus*.

Toxicity

These tryptamines are hallucinogenic, and are not toxic themselves, but the hallucinogens often occur with other classes of dangerous toxin. Poisonings often occur when the fungi are used as recreational drugs. These hallucinogens produce pharmacological effects similar to lysergic acid diethylamide (LSD) by stimulation of central serotonin receptors and blocking of peripheral receptors.

Clinical Manifestations

A change of mood usually occurs 20–60 min after ingestion of the mushroom. The patient may experience an altered sense of space and time, fear, excitement, hilarity, hallucinations, vertigo, loss of coordination, anxiety, agitation, headache, nausea, dilation of pupils, seizures, rapid heart rate, or rapid breathing.

Diagnosis and Treatment

Poisoning is rarely serious in adults; moving a hallucinating patient to the hospital when in a disordered state may increase the sense of fear and confusion. Reassurance and time are usually sufficient treatment.

Muscimol and Ibotenic Acid

Occurrence

Primarily found in *A. muscaria* (Figure 1) and *Amanita pantherina*, but similar toxins may occur in *Amanita cothurnata*, *Amanita frostiana*, and *Amanita gemmata*.

Toxicity

Ibotenic acid is metabolized to the toxin muscimol, which causes the symptoms of this poisoning syndrome. The syndrome is produced by the body's efforts to process ibotenic acid. Muscimol is thought to bind to receptors in the brain, causing disordered neurotransmission. Some other *Amanita* spp. are less well studied and have rarely been eaten. Loss of muscular control may be pronounced. Poisonings, including fatal outcomes, are more likely to occur with *A. pantherina*, which contains higher levels of toxin than *A. muscaria*.

Clinical Manifestations

Drowsiness is experienced 30–60 min after ingestion, followed by a state resembling alcoholic intoxication. Following this, a hyperactive state of confusion, muscular spasms, delirium, and visual hallucinations occurs, lasting as long as 4 h. Vomiting usually does not take place. Drowsiness and deep sleep follow.

Diagnosis and Treatment

Recovery is usually quite rapid without treatment, though a fatality rate of 1–5% has been reported.

Muscarine

Occurrence

Muscarine is found in certain members of the genera *Inocybe* and *Clitocybe*. *Inocybe* spp. are often called 'fiber caps.'

Toxicity

The syndrome is not usually particularly dangerous, but is decidedly unpleasant. There are no recorded deaths due to poisoning by this toxin. The toxin attaches to acetyl cholinesterase receptors in the parasympathetic nerves and affects the organs supplied by these nerves.

Clinical Manifestations

The symptoms include perspiration, salivation, lacrymation, urination, defecation, gastritis, and emesis, which occur 30–120 min following ingestion.

Diagnosis and Treatment

Atropine is a specific antidote for muscarine poisoning. Atropine is toxic and should be administered only by a qualified physician. This is the only mushroom poison for which a specific antidote is known.

Unsaturated Norleucine Amino Acids

Occurrence

An undescribed toadstool species *Trogia venenata* has caused 260 deaths over 30 years, known as Yunnan sudden unexplained death (SUD) syndrome. Amino-hydroxy-hexynoic acid, an unsaturated acetylenic acid, has recently been identified as the active poison.

Toxicity

The cause of SUD was tracked epidemiologically to toadstools and a new species *Trogia venenta* was thereby identified and named as the cause. Two new amino acids 2*R*-amino-4*S*-hydroxy-5-hexynoic acid and 2*R*-amino-4*S*-5-hexynoic acid and a known toxin γ-guanidinobutyric acid were implicated. The new toxins have an LD_{50}s of 71 and 84 mg kg^{-1}, respectively. As observations of SUD suggested that cardiac muscle was affected, creatine kinase levels in serum were studied, but they were found not to be elevated as in rhabdomyolysis from cycloprop-2-ene carboxylic acid poisoning.

Clinical Manifestations

SUD may cause profound hypoglycamia within hours. The lack of serum glucose and depletion of adenosine triphosphate lead to neural cell death and a rapid fatal outcome.

Diagnosis and Treatment

Diagnosis is by postmortem determination of 2*R*-amino-4*S*-hydroxy-5-hexynoic acid in the blood of SUD victims. As this is a new syndrome, no treatment is as yet recommended.

Analytical Methods

The presence of toadstool toxins can by definition be established by universal bioassays using rodents to test the lethality of either the intact toadstool or specific fractions of its extract.

Traditionally toadstool toxins have been extremely difficult to measure precisely or accurately. The most powerful tool for identification and determination has hitherto been gas chromatography coupled with mass spectrometry. This technique, however, requires the molecule to be volatile. The few toxins that are volatile are too small for the best use of mass spectrometry and require derivatization to larger structures. Those toxins that are nonvolatile are too polar to chromatograph well and are derivatized to reduce polarity.

The most studied class of toxin has been amatoxins, in which the oligopeptide structures were more amenable to prevailing chromatography and detection techniques. Enzyme-linked immunosorbent assays and simple color-change tests are also used. Amatoxin poisoning is still routinely diagnosed by an acid-catalyzed condensation reaction between the amatoxin indole functional group and lignan-like components in lignan paper. The resultant complex gives a diagnostic blue-colored spot, but this perhaps simple conjugate has not yet been characterized.

Quantitative information on the identity and concentration of toadstool toxins can now be best achieved by modern liquid chromatography and mass spectrometry (LC–MS) techniques. Development of methods to identify and quantify the toxins will allow more effective diagnosis and treatment of poisonings. When working with unknown toxins, this must still be linked to a bioassay to prove the cause and effect.

Modern LC–MS methods are well positioned to help resolve the identification challenges. Development of a universal method to identify and quantify the toxins would allow more effective diagnosis and treatment of poisonings. However, given the diversity of these toxins and their mechanisms of toxicity, the development of such a universal method is not likely in the near future.

There are several more documented poisoning syndromes under study, and it can be expected that further toxins and their diseases will continue to be defined. These include Paxillus syndrome from long-term ingestion of *Paxillus involutus*, encephalopathy after ingestion of *Hapalopilus rutilans* experienced in Germany, and *Pleurocybella porrigens* poisonings in Japan. It is noted that many of the toxins that have resisted identification will be of a more complex chemical structure. In the *P. porrigens* example, a heat-stable high-molecular-weight toxin releases the sugar *N*-glycolylneuraminic acid. In *Amanita smithiana* the novel toxin may be an amino sugar, a conjugate of a norleucine hexadienoic acid and an as yet undefined sugar.

It was noted during the initial work on LSD in the 1960s that further indoles of a similar or greater psychoactive effect than LSD were present in *Boletus manicus*, a mushroom known for its visual and auditory hallucinogenic effects from the Western Highlands of Papua New Guinea. The field of hallucinogenic fungal compounds is an aspect of food safety not receiving great attention.

Conclusions

Many new fungal poisoning syndromes have been described since the early 1990s. This greatly increases the number of classes of potential toxin chemicals in fungi that require consideration when collecting wild fungi, or attempting to diagnose and treat a suspected poisoning.

See also: Foodborne Diseases: Overview of Chemical, Physical, and Other Significant Hazards. Mycotoxins: Mycotoxins – General. Risk Analysis: Risk Assessment: Chemical Hazards; Risk Management: Application to Chemical Hazards

Further Reading

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Relevant Websites

www.mycokey.com
MycoKey™: Jens H Petersen & Thomas Laessoe.

www.mykoweb.com
MykoWeb: Michael Wood.

www.rogersmushrooms.com
Rogers Plants Ltd.

NATURAL TOXICANTS

Tetrodotoxin

K Campbell and S Haughey, Queen's University Belfast, Belfast, UK

Glossary

Biosensor An analytical tool that detects an analyte through the conversion of a biological response into an electrical signal with a physicochemical detector.

Neurotoxin A toxic component that targets specifically nerve cells or neurons primarily by interacting with membrane proteins such as ion channels.

Regulation A principle, rule, or law designed to control or govern conduct.

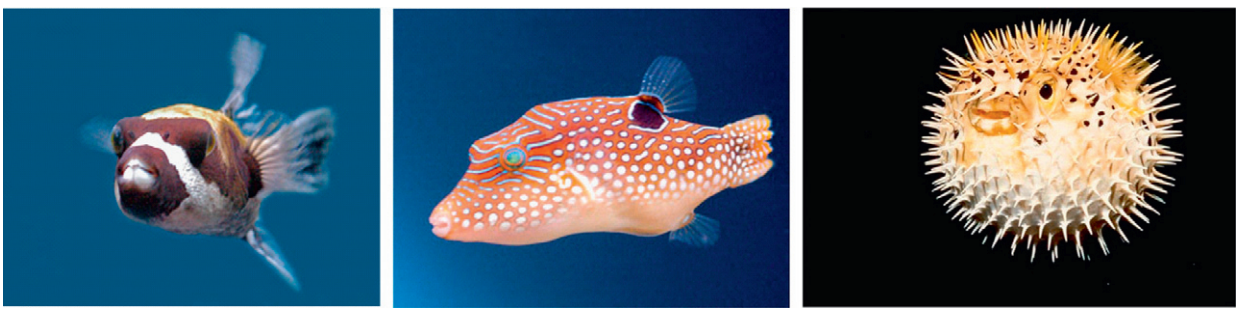
Tetrodotoxin Potent neurotoxin produced by bacteria.

Toxicity The degree by which something is poisonous to an organism.

History and Background

Tetrodotoxin (CAS number 4368-28-9) is a powerful, low molecular weight (~319 Dalton (Da)), naturally occurring neurotoxin. It is named from the Teleost fish order Tetrodontiformes from which the toxin was first isolated and described. This fish species received their name because they have four very strong teeth that almost fuse together to form a beak-like structure, which they use to chew and crack shells open to get food. Tetrodontid fish, which include puffer fish or *fugu*, are long established as being toxic (Figure 1).

There is evidence from the early Egyptians (fifth dynasty, *ca*. 2500 BC), Chinese herbal medical writings (*ca*. 200 BC), the logs of Captain James Cook (1774), and other historical texts (e.g., Kaempfer's *History of Japan*) that there was an awareness and knowledge of the toxicity associated with these fish. The first report of formal research into the pharmacology of tetrodotoxin was by Charles Remy in 1883 who described the symptoms of tetrodotoxin poisoning and documented the high concentrations of tetrodotoxin present in the gonads of puffers. Tetrodotoxin was formally named in 1909 by Dr Yoshizmi Tahara who isolated and prepared a crude extract from puffer fish. Pure crystalline tetrodotoxin was not isolated until 1950 when Yokoo isolated tetrodotoxin from the ovaries of *Fugu rubripes* and described it as spheroidine after a genus of puffer fish. The nomenclature of tetrodotoxin was corroborated in 1952 when Tsuda and Kawamura isolated an identical toxin using chromatographic methods. Since the 1960s, the chemistry, pharmacology, and synthesis of tetrodotoxin have been the subject of a voluminous body of work and reviews. The complete low molecular weight (319 Da) structure of tetrodotoxin was first described in 1964 at the Natural Products Symposium of the International Union of Pure and Applied Chemistry by a total of four different laboratory groups including Tsuda, Goto, Woodward, and Mosher. It is important to note that while three of these groups had been working on toxin isolated from puffer fish, the Mosher group was reporting on compound, they named tarichatoxin, isolated from eggs of the newt *Taricha torosa* (Figure 2).



(a) Dog faced puffer fish (b) Jewel puffer fish (c) Porcupine puffer fish

Figure 1 Examples of puffer fish.

Figure 2 California newt (*Taricha torosa*).

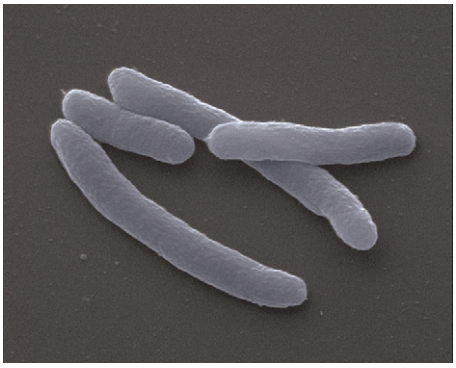
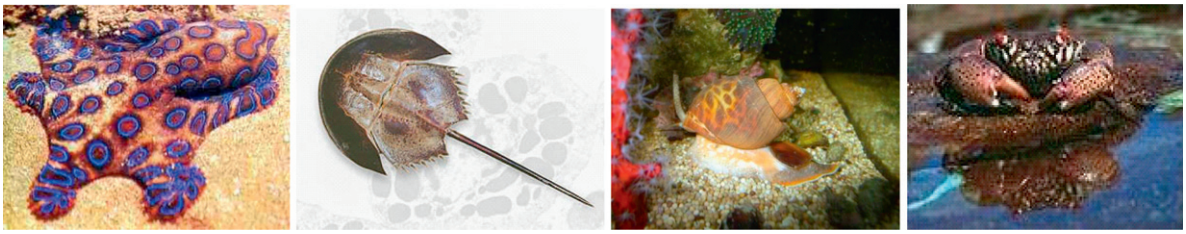


Figure 4 *Shewanella alga.*



Hapaloclaena maculosa *Carcinoscorpius rotundicauda* *Nassarius species* Xanthid crab

Figure 3 Examples of other species known to contain tetrodotoxin.

Occurence of Tetrodotoxin

Tetrodotoxin now has been found in a wide genre of species. Other marine organisms have been found to store tetrodotoxin and include the Australian blue-ringed octopus (*Hapaloclaena maculosa*, which uses tetrodotoxin as a toxin for capturing prey), parrot fish, triggerfish, goby, angelfish, boxfish (*Ostracion* spp.), tobies, porcupine fish, molas or ocean sunfish, globefish, seastars, starfish (*Astropecten scoparius)*, xanthid crabs (*Eriphia* spp.), a horseshoe crab (*Carcinoscorpius rotundicauda)*, two Philippine crabs (*Zosimus aeneus* and *Atergatis floridus*), a number of marine snails, flatworms, sea squirts, several nemerteans (ribbonworms), and several species of *Chaetognatha* (arrow worms), which use tetrodotoxin as a venom for prey, molluscs (*Nassarius* spp. and the Japanese trumpet shell Boshubora), and marine algae (*Jania* spp.). Terrestrial organisms include the Harlequin frogs (*Atelopus* spp.), Costa Rican frog (*Atelopus chiriquiensis*), three species of California newt (*Taricha* spp.), and members of the Salamandridae (salamanders). The number of species found to contain tetrodotoxin continues to grow (Figure 3).

It is unlikely that these tetrodotoxin-bearers possess a common gene that codes for tetrodotoxin production. The ecologic environments of tetrodotoxin-bearing animals seem to have no common factor other than being closely related to an aquatic system. Bacteria, omnipresent organisms that commonly inhabit the aquatic system, are implicated as the primary source of tetrodotoxin. The bacteria believed to be involved are *Shewanella alga* (Figure 4), *Vibrio* species, *Alteromonas* species, and *Pseudomonas* species and their proposed mechanism of tetrodotoxin accumulation in marine animals was described by Noguchi and Arakawa using the flow chart (Figure 5).

Therefore, the tetrodotoxin of puffer fish is not endogenous (produced by the puffer fish itself), but exogenous (taken from outside and accumulated) via the food chain. It has been suggested that the puffer fish accrue tetrodotoxin as a biological defense agent. There appears to be a symbiotic association between tetrodotoxin-producing bacteria and higher organisms, which offers distinct advantages to both partners. The bacteria have a host as a safe place to live, eat, and reproduce whereas the host uses the toxin for predation or defense or both. The normal visual defense mechanism for slow-swimming and clumsy pufferfishes is their ability to inflate to several times their normal size by swallowing air when threatened, and tetrodotoxin may be an inadvertent weapon. Generally, they are left alone by predators.

Synthesis of Tetrodotoxin

In 1972, the first total synthesis of D,L-tetrodotoxin was described by Yoshito Kishi and co-workers at Nagoya University, Nagoya, Japan. In 2003, Isobe and co-workers at Nagoya University and Du Bois and co-workers at Stanford University, USA, reported the asymmetric total synthesis of tetrodotoxin by two different synthetic approaches. Isobe's synthesis was based on a Diels–Alder approach and Du Bois's synthesis involved C–H bond activation.

Structure and Mode of Action

Tetrodotoxin is known as a guanidinium toxin as it consists of a positively charged guanidinium group made up of three nitrogen atoms incorporated as part of a pyrimidine ring with additional fused ring systems.

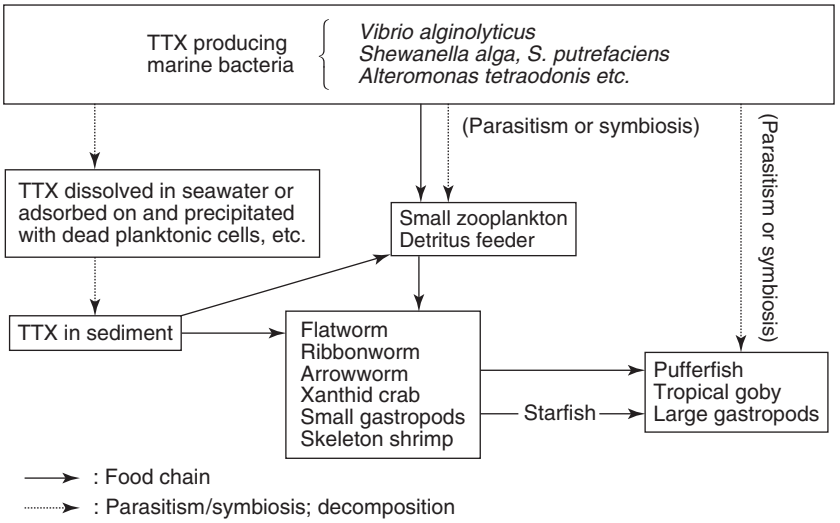
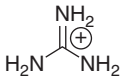


Figure 5 Flow diagram of mechanism of tetrodotoxin accumulation. Reproduced from Noguchi T and Arakawa O (2008) Tetrodotoxin – Distribution and accumulation in aquatic organisms, and cases of human intoxication. *Marine Drugs* 6: 220–242.



Guanidinium group

The ring systems, of which there are five in total, contain hydroxyl groups. Modifications of the parent compound can occur at five sites designated R_1–R_5 and the main modification at these sites are primarily hydrogen to hydroxyl substitutions. The chemical structure of tetrodotoxin and its derivatives are highlighted (Figure 6).

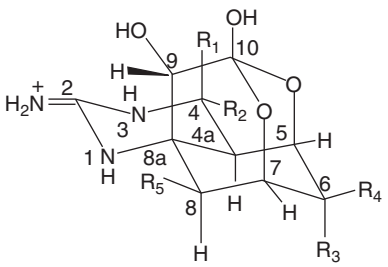
Tetrodotoxin is an extremely potent neurotoxin, specifically blocking voltage-gated sodium channels on the surface of nerve membranes. The flow of sodium ions into nerve cells is a necessary step in the conduction of nerve impulses in excitable nerve fibers and along axons. Normal axon cells have high concentrations of K^+ ions and low concentrations of Na^+ ions and have a negative potential. Stimulation of the axon results in an action potential, which arises from a flow of Na^+ ions into the cell and the generation of a positive membrane potential. Propagation of this depolarization along the nerve terminal presages all other events. The Na^+ ions flow through the cellular membrane employing the sodium-ion channel, a channel that is selective for sodium ions over potassium ions by an order of magnitude. The sodium channel itself is made up of a single peptide chain with four repeating units with each unit consisting of six trans-membrane helices. The trans-membrane pore is formed when the four units fold into a cluster with the center of the cluster being the pore.

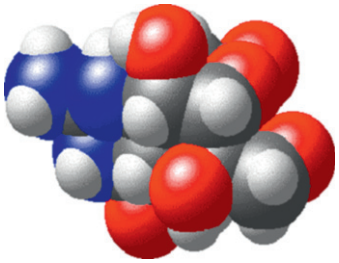
Tetrodotoxin acts by competing with the hydrated sodium cation for the sodium channel. It is proposed that the binding occurs through the positively charged guanidinium group of the tetrodotoxin molecule and negatively charged carboxylate groups on side chains in the mouth of the channel. The guanidinium group fits into the external orifice of sodium channels, but the rest of the molecule is too large to penetrate the channels, acting like a cork in a bottle. This results in plugging the sodium channels from outside preventing the flow of sodium ions into the channel, effectively shutting down sodium movement; thus, the conduction of nerve impulses along nerve fibers and axons ceases. Tetrodotoxin is quite specific in blocking the sodium-ion channel (and, therefore, the flow of sodium ions) while having no effect on potassium ions. Binding to the channel is relatively tight ($Kd = 10^{-10}$ nM). The hydrated sodium ion binds reversibly on a nanosecond time-scale, whereas tetrodotoxin is bound for tens of seconds. The different tetrodotoxin derivatives display different binding affinities to the channel based on the changes in their chemical structure (Figure 7).

The Japanese tiger puffer fish, *F. rubripes*, has been the subject of intensive genetic sequencing studies. A single point mutation in the amino acid sequence of the sodium-ion channel in this species causes it to be immune from being bound and blockaded by tetrodotoxin. Changing the amino acid sequence of any protein alters its structure, and, therefore, its function. Such spontaneous mutations occur frequently in animal populations. Most such changes are neutral or disadvantageous to an organism's survival, but occasionally one confers a selective advantage. In the case of *F. rubripes*, one tiny change enabled the fish to incorporate tetrodotoxin-producing bacteria into its tissues and use the toxin to its own advantage.

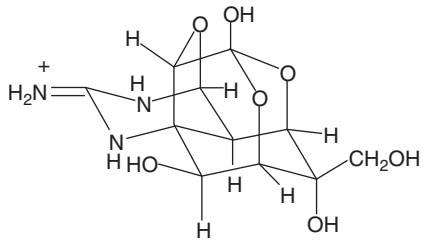
Toxicity

The intravenous and oral median lethal doses for mice are reported as 7.3 and 334 μg kg^{-1}, respectively, in mice. On the presumption that these doses are comparable for humans, 0.5 and 25 mg of tetrodotoxin would be expected to kill a 75 kg

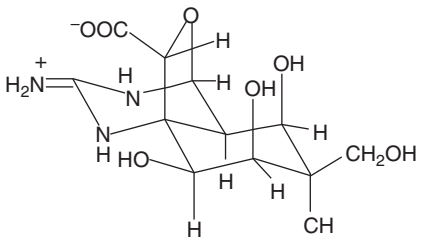




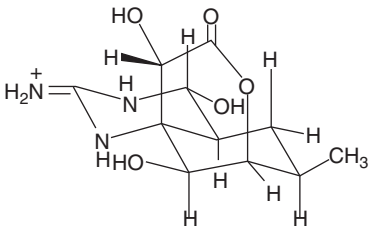
	R_1	R_2	R_3	R_4	R_5
TTX	H	OH	OH	11-CH_2OH	OH
4-*epi*TTX	OH	H	OH	11-CH_2OH	OH
6-*epi*TTX	H	OH	11-CH_2OH	OH	OH
11-deoxyTTX	H	OH	OH	11-CH_3	OH
11-oxoTTX	H	OH	OH	11-CHO	OH
TTX-11-carboxylic acid	H	OH	OH	11-COO^-	OH
11-norTTX-6,6-diol	H	OH	OH	OH	OH
11-norTTX-6(*S*)-ol	H	OH	OH	H	OH
11-norTTX-6(*R*)-ol	H	OH	H	OH	OH
chiriquitoxin	H	OH	OH	11-CH(OH)CH(NH_3^+)COO^- (12 *R*, 13 *S*)	OH
TTX-8-*O*-hemisuccinate	H	OH	OH	11-CH_2OH	$OOC(CH_2)_2COO^-$ (1′ 2′ 3′ 4′)



4,9-anhydroTTX



tetrodonic acid



5,6,11-trideoxyTTX

Figure 6 Structure of tetrodotoxin and analogues. Reproduced from Yotsu-Yamashita M, Sugimoto A, Takai A, and Yasumoto T (1999) Effects of specific modifications of several hydroxyls of tetrodotoxin on its affinity to rat brain membrane. *Journal of Pharmacology and Experimental Therapeutics* 289(3): 1688–1696.

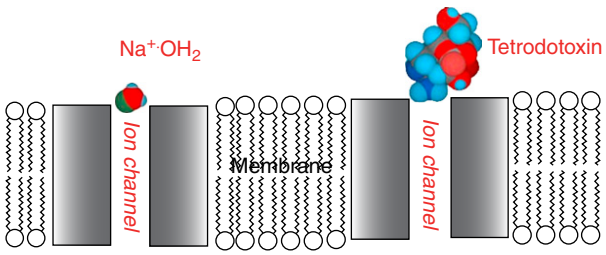


Figure 7 Illustration of tetrodotoxin binding to the sodium ion channel.

person by injection and through consumption. Commonly, reports of a human mortal dose of tetrodotoxin are 1–2 mg with the victim eventually dying from respiratory paralysis.

Tetrodotoxin is 10–100 times as lethal as black widow spider venom (depending on the species) when administered to mice, and more than 10 000 times deadlier than cyanide. It has a similar toxicity as saxitoxin, which causes paralytic shellfish poisoning and also blocks the sodium channel – both are found in the tissues of puffer fish. A recently discovered naturally occurring congener of tetrodotoxin has proven to be four to five times as potent as tetrodotoxin. Except for a few bacterial protein toxins, only palytoxin, a bizarre molecule isolated from certain zoanthideans (small, colonial, marine organisms resembling sea anemones) of the genus *Palythoa*, and maitotoxin, found in certain fishes associated with ciguatera poisoning, are known to be significantly more toxic than tetrodotoxin. Palytoxin and maitotoxin have potencies nearly 100 times that of tetrodotoxin and saxitoxin, and all four toxins are unusual in being nonproteins.

Clinical Manifestations

The first symptoms occur between 15 min and several hours postingestion of tetrodotoxin-containing food. Almost all toxicity is caused by the ingestion of fugu, but other species of animals have been shown to produce tetrodotoxin (e.g., California newt, parrot fish, and blue-ringed octopus). A death from ingestion of tetrodotoxin from a California newt has been documented.

A recent report on toxicity found that initial symptoms may occur up to 20 h after ingestion. Initial symptoms include lip and tongue paresthesias, followed by facial and extremity paresthesias and numbness. Salivation, nausea, vomiting, and diarrhea with abdominal pain develop early. Motor dysfunction with weakness, hypoventilation (may be from dysfunction of central and peripheral nervous systems), and speech difficulties then develop. A rapid ascending paralysis occurs over 4–24 h. Extremity paralysis precedes bulbar paralysis, which is followed by respiratory muscle paralysis. Deep tendon reflexes are preserved early in the course of paralysis. Finally, cardiac dysfunction with hypotension and dysrhythmias (bradycardia), central nervous system (CNS) dysfunction (e.g., coma), and seizures develop. Patients with severe toxicity may have deep coma, fixed nonreactive pupils, apnea, and loss of all brain stem reflexes. Death can occur within 4–6 h. Typically, death occurs from respiratory muscle paralysis and respiratory failure.

Prognosis

Mortality rates are difficult to establish though estimates as high as 200 cases per year with mortality approaching 50% have been reported in spite of good supportive care. Symptoms may last several days even in nonlethal ingestions, but prognosis is reported to be good if the patient survives the first 24 h. Tetrodotoxin is absorbed with activated charcoal. The treatment is symptomatic and supportive with special attention to airway management and cardiac support. Only a few cases have been reported in the US, and outbreaks in countries outside the Indo-Pacific area are rare.

Hazard Characterization

Since 1958 in Tokyo, it is required that specially trained fish cutters or chefs have a license to process and prepare puffer fish. It can take up to 11 years to become a fully competent fugu chef commencing with a 3-year apprenticeship in separating edible and inedible parts. Fugu may be prepared only in kitchens used solely for this purpose. Remarkably, even the waste from fugu preparation must be disposed off in hermetically sealed containers handled by specially trained companies as homeless people may scavenge the bins for food. However, there is no centralized regulation and it is difficult to say for sure who is licensed and who is not with only 19 of Japan's 47 prefectures requiring chefs to pass an exam to obtain a fugu license (Figure 8).

The Food and Drug Administration (FDA) advises consumers in the USA to eat puffer fish (fugu, bok, blowfish, globefish, swellfish, balloonfish, or sea squab) only from two recommended sources. The sources are imported puffer fish that have been processed and prepared by specially trained and certified fish cutters in the city of Shimonoseki, Japan, and puffer fish caught in the mid-Atlantic coastal waters of the US, typically between Virginia and New York. Puffer fish are imported into the US two to three times per year for special occasions, by only one approved New York importer, Wako International, under an FDA/Japanese Government agreement. This is the only acceptable source of imported puffer fish.

Figure 8 Licensed chef in Japan preparing fugu from puffer fish.

A number of the reported poisoning incidences, however, arise from recreational fisherman whereby the fish is mis- or unidentified as being potentially poisonous and consumed by families in rural fishing areas.

Risk Management

Japan is currently the only country to have a regulatory level established for tetrodotoxin in puffer fish at 2000 $\mu g\,kg^{-1}$ of fish. In the USA, it is illegal to import puffer fish but problems do arise due to mislabeling of imported products. The unpublished action limit in other countries for tetrodotoxin is the same as that for paralytic shellfish poisoning toxins, saxitoxin, at 800 $\mu g\,kg^{-1}$ of fish. Below these levels, the fish is deemed fit or safe for consumption. A total of 22 species of puffer fish, all belonging to the Tetraodontidae family, are currently registered as tetrodotoxin poisoning. The levels found in puffer fish vary depending not only on the species but also in the different organs with the dispersal appearing to be species-specific. In marine species, the liver and ovary commonly have the highest toxicity, followed by intestines and skin. Muscles and testis are normally nontoxic or weakly toxic and are regarded as edible by the Japanese Ministry of Health, Labor, and Welfare. The liver generally displays very high toxicity during the year except in the spawning season, at which time the ovary becomes highly toxic through the buildup of tetrodotoxin transferred from the liver. One theory is that tetrodotoxin in the eggs spawned from the ovary could play a role in protecting the eggs from predators. Similarly, when toxic puffer fish are threatened, their bodies swell to two or three times their usual size and tetrodotoxin is postulated to

be excreted from their skin to fend off their attacker. Levels of 160 000 μg kg^{-1} have been reported in the liver.

To reduce poisoning incidents, particularly in Asia, recreational fishermen and consumers should be instructed through food safety campaigns about which puffer fish is edible or inedible. It is believed that this may help official authorities to regulate the capture and consumption of toxic puffer fish species in China in order to avoid the risk of lethal poisoning.

Methods of Analysis

In Japan, puffer fish intended for human consumption are tested using the mouse bioassay. However, the mouse bioassay shows low precision and also requires a continuous supply of mice, which is unethical. Tetrodotoxin can also be tested by high-performance liquid chromatography (HPLC), thin-layer chromatography (TLC), capillary zone electrophoresis, gas chromatography–mass spectrometry (GC–MS), and liquid chromatography–mass spectrometry (LC–MS). These methods are accurate, but may be extremely slow as they require extensive sample clean up extraction steps and, therefore, cannot allow rapid screening when large numbers of sample analysis are required and the instrumentation can be expensive.

Biosensors have been highlighted as alternatives to current Regulatory Methods for marine biotoxins including (1) the use of an antitetrodotoxin specific antibody as the biological reporter and amperometric detection with a screen-printed electrode in an indirect competition assay; (2) differential pulse voltametry with screen-printed electrode in the form of a competition immunoassay; (3) a tissue biosensor has been developed with frog bladder membranes, which have a high concentration of Na^+ channels and, therefore, a Na^+ specific electrode measures the transport of Na^+ through the membrane and its dose-dependent inhibition by tetrodotoxin; (4) tetrodotoxin biosensor based on inhibition of cell function has been designed using murine spinal cord neuronal networks cultured on microelectrode arrays where the biological response is monitored as extracellular potentials; and (5) several enzyme-linked immunosorbent assays have been developed as well for the detection of this toxin, some of them preparing the ground for the development of the electrochemical antibody-based biosensors. More recent methods for tetrodotoxin detection have been employed using surface plasmon resonance and fluidic force discrimination that achieve low levels of sensitivity.

See also: Safety of Food and Beverages: Seafood

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MYCOTOXINS

Contents

Mycotoxins – General

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Glossary

Mycotoxicosis A disease caused by a mycotoxin.

Secondary metabolite A chemical compound produced by an organism during growth, but not part of primary metabolism.

Mycotoxins and History

Mycotoxins are fungal metabolites, which when ingested, inhaled, or absorbed through the skin, can cause sickness or death in humans or domestic animals, including birds. By common agreement, toxins ingested when fungi are eaten as food (mushrooms) are excluded, as are compounds that show toxicity only to lower animals, such as insects or plants.

Mycotoxins have been responsible for major epidemics in humans and animals throughout history. The most important epidemics have been ergotism, due to growth of the fungus *Claviceps purpurea* in rye grains, which killed and maimed hundreds of thousands of people in Europe in the past millennium; alimentary toxic aleukia, caused by T-2 toxin produced by *Fusarium sporotrichioides* in grain, which was responsible for the death of at least 100 000 Russian people between 1942 and 1948; and stachybotryotoxicosis, caused by growth of *Stachybotrys chartarum* in hay, which killed tens of thousands of horses in the USSR in the 1930s. With the possible exception of ergotism, these diseases are causing little problem at the present time.

Despite a few excellent studies of disease due to molds in food or feed in the first half of the twentieth century, understanding of the significance of mycotoxins in human and animal health came only more recently. The term mycotoxicosis was first used in 1952 for the diseases resulting from the growth of fungi in foods and feeds. The event that ushered in the modern era of mycotoxin investigation was the discovery of aflatoxins due to the growth of *Aspergillus parasiticus* in a peanut meal, which killed 100 000 young turkeys in the UK in 1960. Laboratory experiments and the investigation of field outbreaks of the disease rapidly led to the discovery that many commonly occurring fungi – both spoilage fungi (the molds) and plant pathogens – are able to produce a staggering array of toxic secondary metabolites, many with chemical structures of types never before encountered in natural product chemistry.

Chemical Characterization

Mycotoxins are secondary metabolites, i.e., they appear to have no role in the normal metabolism concerned with growth of the fungus and they are usually, though not exclusively, produced as the fungus matures. They are frequently bizarre molecules, with structures ranging from single heterocyclic rings with molecular weights of scarcely 50, to groups of irregularly arranged 6- or 8-membered rings with total molecular weights greater than 500 (Figure 1). Some, such as patulin, appear to be relatively isolated compounds, whereas others, most notably the trichothecenes, exist as a family of 100 or more compounds, all fungal metabolites. No particular group of atoms or moiety can be considered to define 'mycotoxin,' as the active groups vary widely among classes of toxins.

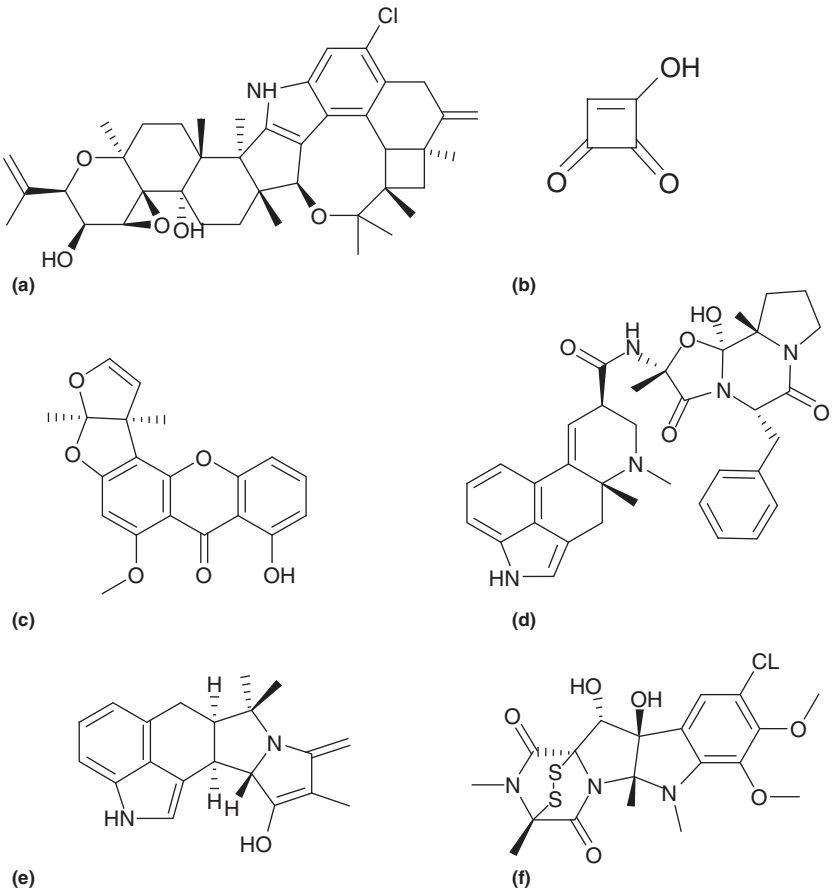

Figure 1 Chemical structures of some mycotoxins. (a) Penitrem A; (b) moniliformin; (c) sterigmatocystin; (d) ergotamine; (e) cyclopiazonic acid; (f) sporidesmin.

Hazard Identification and Characterization

Mycotoxins exhibit four basic kinds of toxicity: acute, chronic, mutagenic, and teratogenic. The most commonly described effect of acute mycotoxin poisoning is deterioration of liver or kidney function, which in extreme cases may lead to death. However, some mycotoxins act primarily by interfering with protein synthesis, and produce effects ranging from skin sensitivity or necrosis to extreme immunodeficiency, which can lead to death from infectious agents. Others are neurotoxins, which in low doses may cause sustained trembling in animals, but at only slightly higher levels, can cause permanent brain damage or death.

Long term effects of low levels of mycotoxin ingestion are also varied. The prime chronic effect of many mycotoxins is the induction of cancer, especially of the liver. Some toxins affect DNA replication, and hence, can produce mutagenic or teratogenic effects.

Unlike bacterial toxins, most mycotoxins are not proteinaceous in character and, being relatively small molecules, are not usually detectable by the immune systems of humans or animals. A major potential danger of mycotoxins in the human diet, therefore, resides in our inability to detect them biologically i.e., in the absence of antibodies.

The symptoms of mycotoxin poisoning are almost as diverse as the chemical structures of the compounds themselves. Some compounds may elicit few symptoms until death results, whereas others may induce severe effects including skin necrosis, leukopenia, and immunosuppression. Doses producing chronic disease are usually far below those responsible for acute effects, and hence long term effects, such as cancer or tumor induction, are undetected at the time of ingestion, and indeed may remain so until the disease is quite advanced. Epidemiological studies on mycotoxins are difficult to conduct and interpret because of uncertainties in the duration and level of exposure. A complication is that mycotoxins in animal feed may in some cases reach detectable levels in edible tissues, such as meat, eggs, and milk. Consequently, the possible presence of mycotoxins in animal tissues may be of importance for hazard identification.

Fungal Sources

Many mycotoxigenic fungi are of widespread occurrence, and indeed in some cases appear to have a strong ecological link with major crops used for human food supplies. The natural fungal flora existing in conjunction with food production is dominated by three genera: *Aspergillus*, *Fusarium*, and *Penicillium*. *Fusarium* species are destructive pathogens on cereal crops and other commodities, and produce mycotoxins before, or immediately after, harvest. Certain species of

Aspergillus and *Penicillium* are also plant pathogens or commensals, but these genera are more commonly associated with commodities during drying and storage. The fungi that produce the major mycotoxins are dealt with in detail under the particular toxins with which they are associated.

After the discovery of aflatoxins, over a period of 20 or more years, searches for these and other toxic compounds led to postulates of relationships of a particular mycotoxin with a wide range of fungi. Many such reports were inaccurate. Only comparatively recently have the major associations between fungi and mycotoxins been firmly established. Most mycotoxins are produced by a single fungus, or a group of closely related species. Only a few mycotoxins are produced by species from more than a single genus.

Categorizing Mycotoxins

Mycotoxigenic compounds can be divided into three broad risk management categories on the basis of both their toxicity and their occurrence in foods and feeds:

1. Major mycotoxins: compounds that have definitely been established to cause sickness in humans or domestic animals, and result in economic problems associated with their presence in food commodities;
2. Minor mycotoxins: compounds with demonstrated toxicity, known to occur naturally in toxic concentrations, and which from time to time may cause sickness or economic loss on a limited scale; and
3. Mycotoxins of less importance: compounds with demonstrated toxicity, but no known disease syndrome, usually due to uncommon occurrence in foods or feeds.

Major Mycotoxins

Five mycotoxins are considered to be of major importance in human or animal health today: aflatoxins, ochratoxin A, fumonisins, deoxynivalenol (and the related trichothecene nivalenol), and zearalenone. Aflatoxins are produced by *Aspergillus* species primarily in cereals and nuts; ochratoxin A is produced by both *Aspergillus* and *Penicillium* species in a range of foods – grapes and products including wines and dried vine fruits, cereals in cool temperate climates, and coffee, cocoa, and chocolate. The remaining major toxins are primarily produced by *Fusarium* species in cereals. These compounds are dealt with in detail in separate articles, together with patulin, considered by some authorities to be important, though no human or domestic animal health issues have ever been ascribed to this compound.

Minor Mycotoxins

A variety of compounds can be considered to be mycotoxins of lesser importance, either because health effects are less pronounced, or because they occur in a limited geographical area. These can be divided between those that occur in pasture or fodder crops, and those produced by *Aspergillus* and *Penicillium* species, which mostly occur postharvest.

Field Toxins

Fungi from the genus *Claviceps* grow on grasses, including cultivated cereals, throughout temperate zones. *Claviceps* species infect the flowers of susceptible hosts, and replace the ovaries with a specialized mass of fungal tissue, a sclerotium, generally known as an ergot. The ergots produced by the most important species, *Claviceps purpurea*, are dark purple to black and are found mostly in rye, but can also occur in barley, oats, and wheat. Ergots of *C. purpurea* contain toxic alkaloids, of which ergotamine (Figure 1d) is the best known. Ergotism, the disease caused by consumption of ergots from rye, may cause convulsions or gangrene. The convulsive symptoms are often accompanied by hallucinations, whereas gangrene, caused by constriction of peripheral blood vessels accompanied by a burning sensation, may result in loss of limbs. This disease syndrome was well known in the Middle Ages, when it was commonly known as 'St Anthony's Fire.' Ergotism has declined in importance with the decrease in use of rye and with increased knowledge of the problem, but outbreaks have occurred in Africa in recent years.

Facial eczema is a disease primarily of sheep in New Zealand, but also known from Australia and South Africa. It is caused by sporidesmin (Figure 1f), produced by *Pithomyces chartarum* as it sporulates on pasture in late summer and autumn. Sporidesmin causes obstruction of the bile duct, liver damage, and photosensitization. The disease known as perennial ryegrass staggers also occurs in New Zealand. *Acremonium lolii*, an endophytic fungus in ryegrass (*Lolium* spp.) interacts with the plant to produce lolitrem, a potent tremorgenic toxin, which causes trembling, staggers, and lack of muscle coordination in sheep, cattle, and horses grazing affected pasture. Death may result in severe cases.

Lupinosis is important in Western Australia, where sheep and cattle are often grazed on lupin stubble after harvest. This disease has also been reported from South Africa and Germany. It is caused by phomopsin A, produced in lupins by the pathogenic fungus *Phomopsis leptostromiformis*. Lupinosis in animals is characterized by jaundice and the development of yellow, fatty livers. Death may occur within a few days. Phomopsin is also produced in lupin seed, and is of concern as lupin seed is increasingly being sold for human consumption.

An important field mycotoxicosis in southern Africa is diplodiomycosis, due to grazing cattle on maize plants that become infected by *Diplodia maydis* after grain harvest. This disease is a neurotoxicosis, characterized in cattle by ataxia, a peculiar walk, incoordination, and often paralysis and death. The chemical cause of diplodiomycosis remains obscure.

In Eastern Europe, stachybotryotoxicosis is a well known disease of domestic animals. It was responsible for the deaths of tens of thousands of horses in the Ukraine in the 1930s, and also affected farm workers when the problem was severe. Symptoms varied, but in horses the most common was necrosis of membranes in the mouth, the result of direct contact with straw infected by *Stachybotrys chartarum*. A general

toxicosis followed, consistent with modern knowledge that the cause was a group of macrocyclic trichothecenes. The same fungus and toxins are one postulated cause of pulmonary distress in US children.

Certain *Alternaria* species grow before harvest in small grain cereals, especially wheat and sorghum. One species, *Alternaria alternata*, produces tenuazonic acid, a well known cause of ill thrift in chickens.

Aspergillus Toxins

Apart from the aflatoxins and ochratoxin A, *Aspergillus* species produce a variety of other mycotoxins. Cyclopiazonic acid (Figure 1e) is produced by several species including *Aspergillus flavus*, with or without simultaneous aflatoxin production. Classical 'turkey X' disease has the characteristics of toxicity due to both aflatoxins and cyclopiazonic acid.

It has recently been shown that *Aspergillus niger* frequently produces fumonisins, until now known exclusively from specific *Fusarium* species. As *A. niger* is of common occurrence in fresh fruits, vegetables, cereals, and nuts, fumonisins are much more widespread than previously believed. The public health significance of fumonisin production by *A. niger* has not yet been evaluated.

Penicillium Toxins

Penicillium species produce a wide variety of toxic compounds, which occasionally cause concern. Penitrem A (Figure 1a) is a potent neurotoxin, responsible for several outbreaks of neurological disorders in horses and sheep, including sustained trembling and brain damage. A number of cases in dogs have also been well documented, where dogs that have eaten moldy food, especially discarded hamburger buns, have exhibited sustained trembling. Fortunately penitrem A appears to have an emetic effect in humans, limiting its toxicity.

As well as *Aspergillus* species, cyclopiazonic acid is produced by several *Penicillium* species, the most important being *Penicillium commune*, a common cheese spoilage mold. As it is water soluble, cyclopiazonic acid appears to have limited mammalian toxicity, but is one cause of ill thrift in birds. A second common water soluble toxin, citrinin, produced primarily by the very common saprophyte *Penicillium citrinum*, has also been implicated in poor growth performance by domestic birds.

Mycotoxins of Less Importance

A wide range of other fungal metabolites have been shown to be toxic to animals in the laboratory or less commonly in the field. Compounds not listed above can be considered to be unimportant in practice, in most cases because the causal fungi rarely grow to a significant extent in foods or feeds.

Less Important *Penicillium* Toxins

Rubratoxins A and B are highly toxic, and were studied quite intensively 20 years ago. However, they are produced by only three known isolates of *Penicillium*, which do not have a recognized name. Rubratoxin was implicated in one poisoning outbreak involving two people, who had eaten homemade rhubarb pie.

Verruculogen is a potent neurotoxin produced by some isolates of *Penicillium simplicissimum* and *Penicillium paxilli*. The rarity of these species in foods and feeds make their practical importance low. The same applies to janthitrems, also neurotoxins produced by *Penicillium janthinellum*, and to viomellein and xanthomegnin, kidney toxins produced by *Penicillium viridicatum* and some *Aspergillus* species. *Penicillium viridicatum* was erroneously believed to be a major source of ochratoxin A until the 1980s.

Citreoviridin falls into a different category. This potent toxin, produced by *Penicillium citreonigrum*, was the likely cause of acute cardiac beri beri, a disease of young healthy Japanese people 100 years ago. As the result of pioneering toxicological work by Sakaki before 1900, Japan banned the sale of yellow rice in 1915, and the disease disappeared. *Penicillium citreonigrum* appears to be a rare species now. However, a recent disease outbreak in northern Brazil suspected to be caused by toxic rice has been attributed to this species. Citreoviridin is also produced by *Eupenicillium ochrosalmoneum* when it infects maize kernels, but the significance of this finding is unknown.

Like *P. citreonigrum*, *Penicillium islandicum* was believed to be common in rice in the nineteenth and early twentieth centuries. It produces four toxic compounds, the skyrins, erythroskyrin and leuteoskyrin, and the cyclic peptides islanditoxin, and cyclochlorotine. It is now an uncommonly isolated fungus.

Less Important *Aspergillus* Toxins

Sterigmatocystin (Figure 1c), produced by *Aspergillus versicolor* is a toxic precursor to the aflatoxins. Its practical importance appears to be low, because it is highly insoluble and it is not clear that ingestion is followed by absorption in either human or animal guts. Also, *A. versicolor* is a slowly growing invader of dried foods, and rarely grows on the large scale necessary for significant toxin production.

Gliotoxin, produced by *Aspergillus fumigatus*, assists this highly pathogenic fungus to invade lung tissue. As *A. fumigatus* primarily occurs in decaying vegetation, it is of low importance as a source of foodborne disease. *Aspergillus clavatus* is of common occurrence in malt houses, growing on malting barley. It is a cause of 'malt workers' lung,' due to the production of patulin as well as a number of other mycotoxins of marginal importance, including kojic acid, cytochalasin E, and tryptoquivalones.

Less Important *Fusarium* Toxins

Like *Penicillium*, *Fusarium* species make a wide range of metabolites with demonstrable toxicity, but which rarely, if ever, are produced at toxic levels in foods or feeds. Moniliformin (Figure 1b) is produced by at least 20 *Fusarium* species and can be expected to be of widespread occurrence in grain crops. Butenolide is produced by several *Fusarium* species, and is

moderately toxic to mice. Fusarochromanone, produced by *Fusarium equiseti*, can cause bone deformities in chickens. Sambutoxin, a metabolite of *Fusarium oxysporum* and *Fusarium sambucinum*, was found to cause hemorrhaging of the digestive tract in rats. None of these toxins has been reliably associated with any disease of humans or animals.

Chemical Analysis

Methodology for analysis of the major mycotoxins is highly developed, and is dealt with under the particular toxins. Specific assays exist for some of the less important toxins mentioned above, but assays are rarely performed outside specialist laboratories with access to standard compounds. Over the past 20 years, numerous papers have described multimycotoxin assays for 50 or even more toxins. However, it is rare for any particular batch of suspect food or feed to have been infected by more than a single mycotoxigenic species, so determining the likely fungus before attempting mycotoxin assays is still the preferred approach to pinpointing the cause of an unknown toxicity. Traditionally, identification of mycotoxigenic fungi is carried out by growth in culture and identification using standard texts. Molecular methods are coming into use at this time, and their utility will increase as more species are molecularly characterized.

Occurrence and Control of Mycotoxins in Foods

Before harvest, mycotoxins are produced in a particular crop only by specific fungi that are pathogens on that crop, or by commensals that have an affinity with that crop. Fitting this category are the production of aflatoxin in peanuts and maize by *Aspergillus flavus*, deoxynivalenol, and related trichothecenes in wheat and sometimes other small grains by *Fusarium graminearum*, and fumonisins in maize by *Fusarium verticillioides*. The major mycotoxin problems in the world's food supply are the result of this type of association. Control of this type of mycotoxin problem has proved to be very difficult. Biocontrol by competitive exclusion has been effective in controlling aflatoxins in some crops and circumstances.

In a slightly different category, *Aspergillus carbonarius* is able to invade grapes before harvest, not because of a specific affinity, but because grapes are frequently damaged at or before harvest. Infection by pathogenic fungi, splitting due to rain, or mechanical damage permit entry of *A. carbonarius*, that thrives in the acid, high sugar environment. Control requires management of these viticultural problems.

By the twenty first century, it is believed that the common species that are responsible for particular toxins are all known, so that the particular crops that needs monitoring, and the climatic conditions that permit fungal growth are well understood.

The other mycotoxins of importance in foods occur after harvest, before crops are fully dry. Here the specificity for formation of a particular mycotoxin appears to be a combination of fungal ecology and climate. Ochratoxin A formation in small grains, only in cool climates, and in coffee, only in warm climates, fits this category – as the result of growth by quite different fungal species. Control here relies on good agricultural practice – dry crops quickly and keeps them dry.

If foods are inadequately dried, badly stored, or incorrectly transported, at too high a moisture content or humidity, or subject to temperature fluctuation, contamination by a wide range of mycotoxins is possible. This problem occurs all too often in tropical countries, but also is a well recognized risk of long distance transport, especially across the tropics. Good agricultural practice, i.e., drying food commodities as soon as possible and keeping them dry, is the only sure defense against mycotoxin formation in stored commodities.

See also: Foodborne Diseases: Overview of Chemical, Physical, and Other Significant Hazards. Mycotoxins: Aflatoxins; Deoxynivalenol and Other Trichothecenes; Fumonisins; Ochratoxin A; Patulin; Zearalenone

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MYCOTOXINS

Aflatoxins

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Glossary

Commensal A fungus which grows in plant tissue without causing discernible damage to the plant, i.e., is not pathogenic.
Conidium (pl. conidia) Microscopic asexual spores produced by molds (filamentous fungi with small fruiting bodies); the main means of propagating many species.
Metabolite Chemical compound produced by a fungus during growth.
Metula (pl. metulae) Microscopic structures sometimes found supporting phialides in *Aspergillus* species.
Phialide (pl. phialides) Microscopic structures bearing conidia in some asexual genera of fungi, such as *Aspergillus*.
Sclerotium (pl. scletoria) Small (less than 1 mm diameter) often hard bodies produced by some filamentous fungi; a resting stage that may or may not lead to the formation of sexual spores (ascospores).

Chemical Characterization

Aflatoxins are highly substituted coumarins, with a fused dihydrofurofuran moiety. There are four naturally occurring aflatoxins of importance. Aflatoxins B_1 (CAS 1162-65-8) and B_2 (dihydroaflatoxin B_1; CAS 7720-81-7), so named because of their blue fluorescence under UV light, are characterized by fusion of a cyclopentenone ring to the lactone ring of the coumarin. Aflatoxins G_1 (CAS 1165-39-5) and G_2 (dihydroaflatoxin G_1; CAS 7241-98-7), which fluoresce greenish yellow, include an additional lactone ring (Figure 1).

When aflatoxin B_1 and aflatoxin G_1 are ingested by lactating animals, small proportions (1–2%) are excreted in milk as aflatoxin M_1 (4-hydroxyaflatoxin B_1; CAS 6795-23-9) and M_2 (4-hydroxyaflatoxin G_1; CAS 6885-57-0), hydroxylated derivatives of the parent compounds.

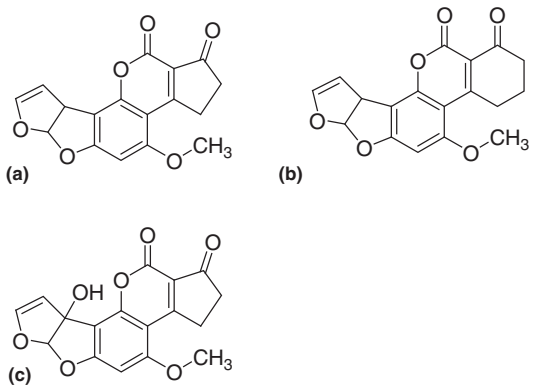

Figure 1 Structures of aflatoxins: (a) aflatoxin B_1; (b) aflatoxin G_1; (c) aflatoxin M_1.

Fungal Sources, Physiology, and Ecology

Aflatoxins are produced in foods, primarily by *Aspergillus flavus* and the closely related species *Aspergillus parasiticus*. On standard identification media, Czapek yeast extract agar and malt extract agar, both species produce rapidly growing colonies characterized by green conidia (asexual spores), under the microscope7 borne on typical *Aspergillus* fruiting structures (Figure 2). The two species differ in small details: *A. flavus* usually bears conidia (asexual spores) on metulae and phialides, whereas in *A. parasiticus* spores are borne on phialides alone. Conidia of *A. parasiticus* exhibit rougher walls than those of *A. flavus*. Isolates of *A. flavus* produce only B aflatoxins, whereas those of *A. parasiticus* produce both B and G aflatoxins.

At least eight other *Aspergillus* species make aflatoxins. Only two of these are of possible importance in foods: *Aspergillus nomius* and *Aspergillus minisclerotigenes*. Both resemble *A. flavus* in culture, but *A. nomius* produces bullet-shaped sclerotia, as distinct from the spherical sclerotia produced by many *A. flavus* isolates, whereas *A. minisclerotigenes* produces small spherical sclerotia. Unlike *A. flavus*, both of these species produce both B and G aflatoxins. Other species are rare, and none is of consequence in foods or feeds.

Detection of these species from foods or soils is facilitated by the use of *A. flavus* and parasiticus agar (AFPA). After 42–48 h incubation on AFPA at 30 °C, colonies of *A. flavus* and *A. parasiticus* exhibit a brilliant orange–yellow reverse coloration.

Physiology

A. flavus will grow from a minimum of 10–12 °C to a maximum of 43–48 °C, and optimally at approximately 33 °C. It

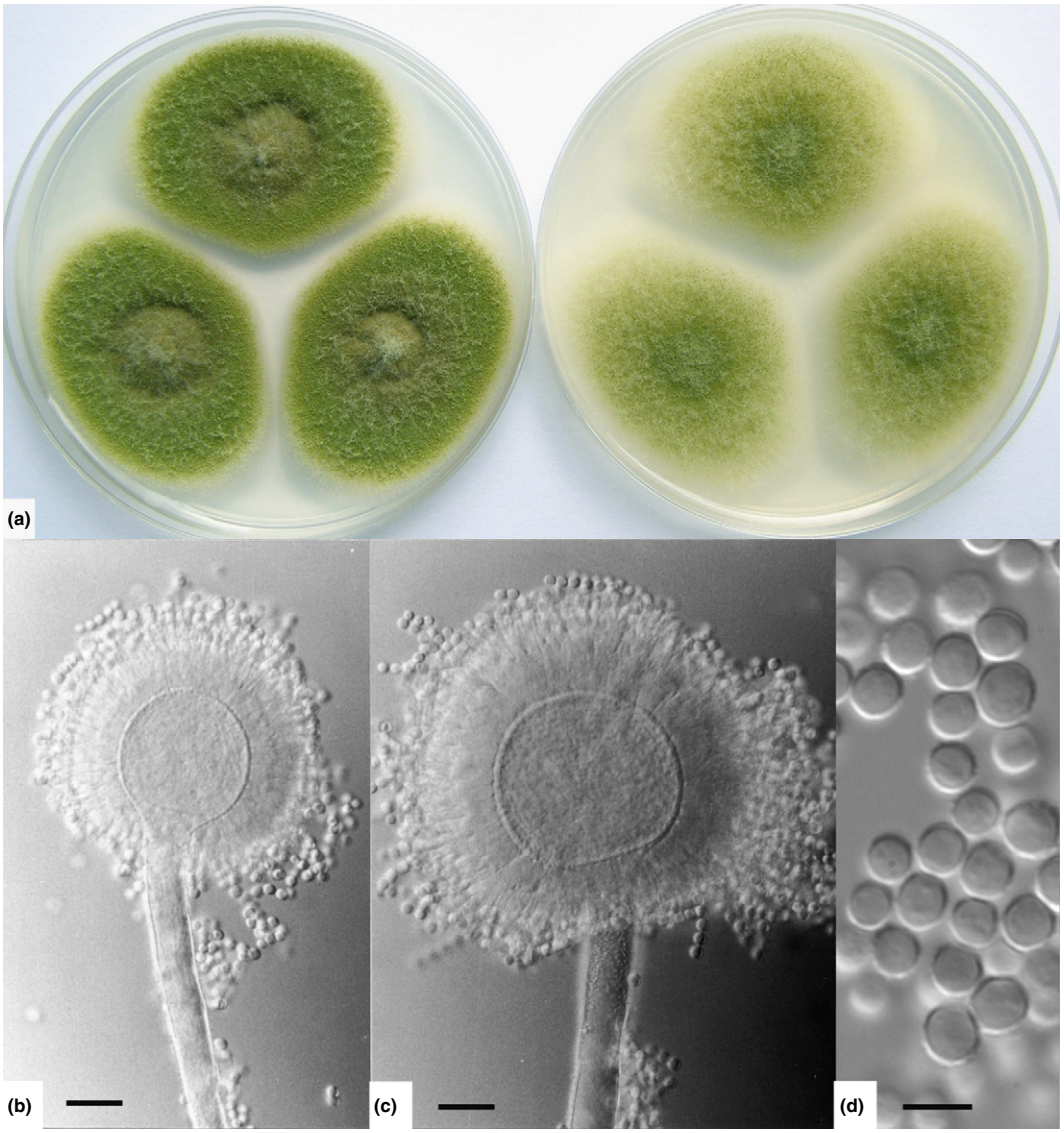

Figure 2 *Aspergillus flavus*: (a) colonies on Czapek yeast extract agar and malt extract agar, 7 days, 25 °C; (b, c) fruiting structures, bar = 10 μm; (d) conidia, bar = 5 μm.

has been shown to grow down to a minimum of 0.82 water activity at 25 °C, 0.81 at 30 °C, and 0.80 at 37 °C. This species will grow in a pH range of, at least, 2.1–11.2 at 25, 30, and 37 °C, with optimal growth near pH 7.5.

The most reliable figures for heat resistance of *A. flavus* conidia indicate a D_{45} value of more than 160 h, a D_{50} of 16 h, a D_{52} of 40–45 min, and a D_{60} of 1 min, at neutral pH and high water activity, with z values ranging from 3.3 to 4.1 °C.

Growth of *A. parasiticus* has been reported over the range 12–42 °C, with an optimum at 32 °C. The effects of water activity and pH on growth are very similar to those described for *A. flavus*. Conidia of *A. parasiticus* have a low heat resistance: D_{55} values of up to 9 min at pH 7 in phosphate buffer have been reported. Values were much higher if water activity was reduced.

Genetics

The aflatoxin biosynthetic pathway in *A. flavus* has been studied extensively and is now quite well understood. The pathway is a single unit on a chromosome within linkage group VII, and includes more than 20 enzymes and gene products. Many of these have now been isolated and characterized. Two regulatory genes, *AflR* and *AflS*, are known. The gene products of *AflR* regulate the biosynthetic pathway at the transcription level, whereas the function of *AflS* appears to be to regulate *AflR* activity.

Ecology

The quest for knowledge about potential aflatoxin problems means that *A. flavus* has been sought in every conceivable kind

of foodstuff. *A. flavus* has become the most widely reported foodborne fungus, reflecting its economic importance and relative ease of recognition as much as its ubiquity. It is especially abundant in the tropics, and it has a particular affinity for nuts and oilseeds as substrates. It grows as a nondestructive pathogen, or commensal, in the tissues of peanuts and cotton plants, and perhaps maize as well, and that is reflected in the widespread occurrence of aflatoxins in those crops. It does not appear to have that advantage in other crops, so that, in commodities apart from nuts and oilseeds, spoilage or unacceptable levels of aflatoxins should not occur in the absence of gross mishandling. Figs are an exception, because of the unique way in which the fungus is able to enter the fruit before harvest.

It is interesting that *A. flavus* is of universal occurrence in tropical and subtropical countries, whereas the closely related *A. parasiticus* is uncommon, indeed almost unknown, in South East Asia. In addition, *A. parasiticus* is not usually found in maize or cotton, but is often dominant in peanuts, even where both species are found together in soils where these crops are being grown.

History

Aflatoxins were discovered in 1960, as the result of an outbreak of a mysterious disease that became known as 'turkey X' disease which killed 100 000 turkey poults in England. It was soon established that peanut meal from Brazil was the cause, and it was subsequently shown that the toxin was produced by the common spoilage fungus *A. flavus.* In the same year, it was discovered that trout fed cottonseed meal while being reared in hatcheries in California frequently had liver tumors: indeed this problem was first reported in 1934. Within 2 years, the toxins had been identified, named aflatoxins after their fungal source, and assays developed using the new technique called thin layer chromatography. By 1970, it had been established that aflatoxins were widespread in tropical and warm temperate commodities, especially peanuts, maize, and cottonseed, and that aflatoxins were potent carcinogens in animals. Indeed aflatoxin B_1 was found to be the most potent liver carcinogen known. During the 1970s, outbreaks of human illness and death showed that aflatoxins can be acutely toxic, and carcinogenicity to humans was postulated. However, in the 1980s, it became clear that the hepatitis B virus caused liver cancer, and the validity of aflatoxin as a cause was questioned. During the 1990s, it was established that both hepatitis B and aflatoxin caused liver cancer, and were synergistic. Equations were developed relating aflatoxin intake to the likelihood of developing liver cancer, in the presence and absence of the hepatitis B virus. In the past decade, it has become apparent that aflatoxins are likely to play an even wider role in human health.

Hazard Identification and Characterization

Aflatoxins have been known to cause acute and chronic toxicity for many years. However, these compounds now appear to have even more diverse effects on human and animal health than believed previously. Aflatoxins have a likely involvement in five toxic effects: acute toxicity, liver carcinogenicity, growth retardation in children, immunosuppression, and liver cirrhosis.

Acute Toxicity – Aflatoxicosis

An outbreak of hepatitis due to aflatoxin ingestion from maize occurred in India in 1974: almost 400 cases were identified, and 106 deaths were reported. Clinical features were jaundice preceded by fever, vomiting and anorexia, with ascites and edema in the lower limbs in extreme cases. Levels in analyzed foods were often extremely high, up to 15 mg kg^{-1}. Given that adults normally consumed more than 300 g of maize per day, estimated daily ingestion was 2–6 mg of aflatoxins. Two outbreaks of aflatoxicosis have been documented from Kenya, in 1981 and 2004. In the latter outbreak, 317 cases and 125 deaths were reported. A smaller outbreak occurred in Malaysia in 1988, where 13 children died from acute hepatic encephalopathy from consumption of commercially prepared noodles contaminated with aflatoxin.

With the exception of the Malaysian incident, aflatoxicosis only occurs when drought or famine causes exceptionally high levels of aflatoxins in the diet, and forces the eating of substandard food. Much more insidious, and more frequent, is liver cancer development from consuming much lower levels of aflatoxin over longer periods of time.

Liver Carcinogenicity

The International Agency for Research on Cancer recognizes aflatoxin B_1 and naturally occurring mixtures of aflatoxins as Class 1 carcinogens, i.e., they are recognized as carcinogenic to humans. Although earlier studies (1970–1990) drew this conclusion, studies in the past 20 years have identified that hepatitis B virus also causes human liver cancer. The two agents provide different histopathology, so it has become evident that both agents are causal and synergistic. In its risk assessment of aflatoxins, the Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) has derived two potency factors for cancer formation by aflatoxins: for aflatoxin alone, 0.01 cases per 100 000 people per annum per nanogram per kilogram body weight per day, and for individuals carrying hepatitis B infection, 0.30 cases. Thus the two agents together are approximately 30 times as potent as aflatoxin alone.

In the liver, aflatoxin B_1 is converted by cytochrome P450 enzymes to the 8–9 epoxide. This is capable of binding to liver proteins, leading to liver failure, and potentially to aflatoxicosis. This epoxide is also able to bind to DNA, a precursor step to the development of liver cancer.

Stunting in Children

A number of studies have now provided evidence that aflatoxin exposure before birth and in early childhood is associated with stunted growth – defined by WHO as height for age being more than two standard deviations below the average

height for age in a given population. It is apparent from the high numbers of people believed to be consuming uncontrolled levels of aflatoxin, that stunting is an important disease burden, only recently recognized.

Immunosuppression

Aflatoxins have been shown to suppress the cell-mediated immune response in both cell lines and domestic animals. Effects include the impairment of delayed-type hypersensitivity, decreases in the phagocytic activity of macrophages, increased susceptibility to infection, and reduced response to vaccines. Few studies have been reported in humans, but it is apparent that if the effects in humans mirror those in animals even approximately, then the immunosuppressive effects of aflatoxins probably have very wide implications on human health.

Liver Cirrhosis

Direct evidence that aflatoxins are involved in liver cirrhosis is limited, but as death from cirrhosis of the liver is an important cause of mortality, any enhancement by aflatoxin or other factors in causing cirrhosis will have important consequences.

Exposure Assessment

Accurate exposure assessment of aflatoxins in food supplies has proved to be very difficult and is only now being addressed adequately. People in developed economies, with effective regulatory controls, are seldom exposed to levels of aflatoxins that might lead to disease consequences. However, many people in less developed economies are likely to be exposed to deleterious levels. A number of reasons exist. Peanuts are a drought-tolerant crop, and are widely grown in tropical countries, often under conditions of drought stress that promote aflatoxin production. Peanuts are a very valuable source of income to tropical economies. However, because of stringent regulations in developed importing countries, particularly Europe, the best quality nuts are usually exported, whereas poorer batches are consumed locally. If sorting by color is carried out, the nuts of inferior quality are seldom destroyed, but sold to poorer people. Peanuts and maize grown by subsistence farmers in many countries are rarely assayed for aflatoxins, and due to drought or insect damage are frequently of poor quality and contain excessive aflatoxin levels.

Risk Characterization

In general terms, the risk from aflatoxin in developed countries is low. Commodities, especially peanuts, maize, and cottonseed, are closely monitored and sorted to ensure the safety of foods and animal feeds. Foods for meat-producing ruminant animals can contain quite high levels of aflatoxins, up to at least 300 $\mu g\,kg^{-1}$, without effect on the animal or leaving aflatoxin residues in the meat, as ruminant microorganisms detoxify aflatoxins. However, feeds for dairy cows must contain very low levels of aflatoxins to limit the risk of aflatoxin M_1 in milk. Poultry and fish are sensitive to aflatoxins, so feeds must contain only low levels. As a consequence, residues in poultry meat, eggs, or aquaculture products are normally very low.

In many tropical and subtropical areas of the world, peanuts and maize are grown under suboptimal conditions of moisture and insect control, frequently leading to excessive aflatoxin levels. Concentrations of 100–1000 $\mu g\,kg^{-1}$ are not uncommon. Control measures, such as good agricultural practice or sorting of defective grains or kernels, are often neglected. A recent authoritative review has estimated that up to 5 billion people worldwide are at risk from exposure to uncontrolled levels of aflatoxins in their diets.

Chemical Analysis

Analysis of aflatoxin levels in the main commodities in which these compounds occur has become standardized. AOAC International (The Scientific Association Dedicated to Excellence in Analytical Methods) provides such standard methods, used throughout the world. These are briefly summarized below.

Sampling

Aflatoxins are usually very unevenly distributed in commodities, especially peanuts, where it is common for no more than 1 nut in 1000 to be contaminated, but contamination levels in individual nuts are often high, up to 1 $mg\,kg^{-1}$. For this reason, sampling is often the largest source of error in aflatoxin assays. Sampling plans have been developed for continuous lines, for 10 ton lots and for bag stacks. All of the recommended methods use samples sizes of 8 kg or more. Relevant papers should be consulted. For example, Codex has developed sampling plans for several commodities.

Sample Preparation

Entire samples of 8 kg or more should be comminuted in a vertical chopper or similar mill. Subsamples are then further processed.

Extraction

Extraction of subsamples, ideally of 500 g or more, employs a variety of mixed polar and nonpolar solvents, depending on the food matrix being analyzed. Methanol: water (80:20) is recommended for commodities such as maize, peanuts, and cottonseed.

Assays

The traditional methodology of thin layer chromatography is still in use, and is recommended for less developed economies as it is inexpensive and reliable. For acceptable/not acceptable testing, immunochemical methods are most frequently used. For advanced users such as high-volume

analytical laboratories or regulatory authorities, liquid chromatography, sometimes coupled with mass spectroscopy has become a normal practice. Limits of detection are now well below 1 $\mu g\ kg^{-1}$.

Levels in Foods

Levels of aflatoxins in foods are highly variable. However, only a few commodities are at serious risk if good agricultural and manufacturing practices are observed. Peanuts, maize, and cottonseed frequently contain unacceptable levels of aflatoxins, and assays are usually carried out at the wholesale level, and frequently in finished products as well. In developed countries, regulations are generally stringently applied on human foods and animal feeds that contain appreciable amounts of these commodities. Other closely watched commodities are tree nuts, especially pistachios and Brazil nuts, figs, and spices. However, although levels in these commodities may exceed regulatory limits from time to time, such levels seldom pose a long-term risk to human health.

Under inadequate storage conditions, other grains including sorghum and rice may also permit growth of *A. flavus* and aflatoxin production. These commodities have very high consumption levels in many communities, so adequate storage is of great importance.

Management Options – Limits in Foods

Good agricultural practice has been the standard method in developed countries for attempting to control aflatoxin levels in commodities, particularly maize and peanuts. Good agricultural practice involves good farm management, including weed control, optimal row and plant spacing, insect control, adequate water supplies, rapid and complete drying, removal of defects, and effective control of storage conditions. By themselves, these approaches are inadequate: too often drought stress or insect damage results in aflatoxin formation before harvest, out of farmer control, even in developed countries. The only effective control measure for aflatoxins remains end product testing.

Regulation of Aflatoxin Levels

Regulation of aflatoxin levels in foods commenced around 1970, using the then limit of detection, 5 $\mu g\ kg^{-1}$, as the permitted limit. It became clear that peanut producers could not reach that limit, even in developed economies, so higher limits were set up in peanut-exporting countries including the USA and Australia. As analytical techniques improved, with the introduction of liquid chromatography, lower limits were frequently set by importing countries, especially in Europe. Recent clearer understanding of aflatoxin toxicology has produced a compromise safe limit for human consumption of 15 $\mu g\ kg^{-1}$ of peanuts. Levels in most foodstuffs, where aflatoxin is less likely to occur, remain lower, usually 5 $\mu g\ kg^{-1}$ or less.

Although safe levels of aflatoxins have now been established and aflatoxin levels are closely controlled in developed countries, it has been estimated that up to 5 billion people worldwide are at risk from exposure to uncontrolled levels of aflatoxins in their diets.

Reducing Aflatoxin Levels

Reduction in aflatoxin levels in peanuts is accomplished by color sorting of individual kernels after shelling. The process was developed originally to reject commercially unacceptable discolored nuts, regardless of cause: but as fungal growth is a prime cause of discoloration, the process is also an effective nondestructive means of removing most nuts containing aflatoxins. Maize and fig samples are screened for the presence of aflatoxin by the examination of cracked kernels or fruit by ultraviolet light. No effective nonchemical testing techniques exist for cottonseed or pistachios and, as with other commodities, nondestructive chemical assays are not available.

It is a normal practice to assay aflatoxin levels in all consignments of peanuts and maize in major developed producing countries, often repeatedly, from intake to shellers to final product. Such controls rarely exist in less developed countries. Other commodities are similarly screened according to needs and markets.

Interventions

In recent years, several approaches have been put forward to reduce aflatoxin levels in foods and feeds. The most advanced of these is biocontrol by competitive exclusion, for both peanut and cotton crops. The technique, developed independently in the USA and Australia, relies on the fact that only approximately 40% of *A. flavus* strains produce aflatoxins. Selected nontoxigenic strains that are both competitive in the field and unlikely to revert to toxicity are introduced, in high numbers, into soils in fields where peanut or cotton crops are being grown. The nontoxigenic spores compete with the existing toxin producing spores in the soil for infection sites on developing nuts. In sufficiently high numbers, control can be very effective. This process is used commercially in the USA for peanuts and cotton. Pilot scale work is currently in progress on maize crops, and under development for some tree nuts.

Other reported experimental studies have aimed to improve farm efficiency, by providing advice and improved management for farmers in less developed countries. Some success has been achieved.

A different approach has resulted from the discovery that certain clays, among a variety of natural materials including activated charcoal and bentonite, adsorb aflatoxins very strongly. Animal studies have been very effective, and some human trials have yielded promising results. It is too early to say whether this approach will be effective in practice.

Some naturally occurring compounds are believed to be active against chemically induced cancers. Polyphenols from green tea and chlorophyllin, a derivative of chlorophyll, may have application here as dietary supplements.

See also: Mycotoxins: Deoxynivalenol and Other Trichothecenes; Foodborne Diseases: Overview of Chemical, Physical, and Other Significant Hazards; Mycotoxins: Fumonisins; Mycotoxins – General; Patulin; Ochratoxin A; Zearalenone

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Separation and quantitation of aflatoxins B and G using HPLC.

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Deoxynivalenol and Other Trichothecenes

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Glossary

Apoptosis Programed cell death that is a normal component of the development and health of multicellular organisms.

Conidium (pl. conidia) Microscopic asexual spores produced by molds (filamentous fungi with small fruiting bodies); the main means of propagating many species.

Macroconidium (pl. macroconidia) The larger of the two conidial types produced by *Fusarium* species, and characteristic of the genus.

Microconidium (pl. microconidia) The smaller of the two types produced by some *Fusarium* species, but also produced by a number of other related genera.

Chemical Characterization

Deoxynivalenol (DON) belongs to the family of chemicals known as the trichothecenes, sesquiterpenoid compounds characterized by an epoxy ring at the C-12,13 position. At least 100 trichothecene molecules are known, differentiated by hydroxy or acetyl groups and side chains. DON, still sometimes known as vomitoxin in the USA, is 12,13-epoxy-3,7,15-trihydroxy-trichothec-9-en-8-one, CAS number 51481-10-8 (Figure 1). It is the most commonly produced trichothecene in foodstuffs. Nivalenol (NIV), less commonly produced but more toxic, differs from DON by the substitution of a hydroxy group for the hydrogen atom at the C-4 position (Figure 1). The most toxic trichothecene is known as T-2 toxin. It differs from DON in several positions: at C-4, an acetyl ester in place of H; at C-7, H in place of OH; at C-8 an isovalerate ester in place of O; and at C-15, an acetyl ester in place of OH.

Fungal Sources, Physiology, and Ecology

DON and NIV are produced by *Fusarium graminearum* (often listed as *Gibberella zeae*, its sexual stage), *Fusarium culmorum*, and some related species.

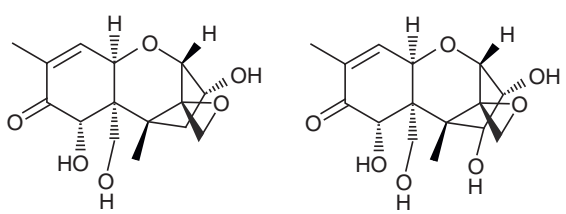

Figure 1 Structures of deoxynivalenol and nivalenol.

Fusarium graminearum grows rapidly on any standard mycological medium including Czapek yeast extract agar (CYA), malt extract agar (MEA), potato dextrose agar (PDA), and dichloran chloramphenicol peptide agar (DCPA). Colonies on CYA and MEA are colored grayish rose, grayish yellow or paler, with reverses orange red to yellowish brown. On PDA, colonies are colored yellowish brown to reddish brown, sometimes with a central mass of red brown to orange areas bearing macroconidia, with the reverse dark red (Figure 2).

Characteristic *Fusarium* conidia, macroconidia, are produced on DCPA or other specialist medium, usually with 5 septa, thick walled, straight to moderately curved, with the basal cell distinctly foot-shaped; microconidia are not produced.

Colonies of *F. culmorum* are similar to those of *F. graminearum*, but colored pale red to pastel red on CYA, MEA, and PDA, the reverse on CYA and PDA is pastel red to deep red, and on MEA brown to reddish brown. Macroconidia are relatively short, wide, and only slightly curved, with 4–5 septa, 30–45 μm long, with basal cells with a slight to definite notch. Microconidia are not produced.

Fusarium graminearum grows from below 5 °C to approximately 35 °C, and optimally at 25 °C. Growth occurs down to 0.90 water activity. *Fusarium culmorum* has a minimum temperature for growth near 0 °C, a maximum approximately 33 °C, and an optimum near 30 °C. It is capable of growth down to 0.87 water activity, lower than most other *Fusarium* species that have been studied. *Fusarium graminearum* occurs in maize, and both *F. graminearum* and *F. culmorum* in small grains, especially wheat and barley. These species are rank pathogens, invading plants and grains by causing diseases, known as Gibberella ear rot in maize and Fusarium head blight in wheat, barley, and triticale. Epidemics of Gibberella ear rot require the congruence of three factors: airborne or insectborne spores, inoculation at the susceptible time, and appropriate moisture and temperature. This disease is

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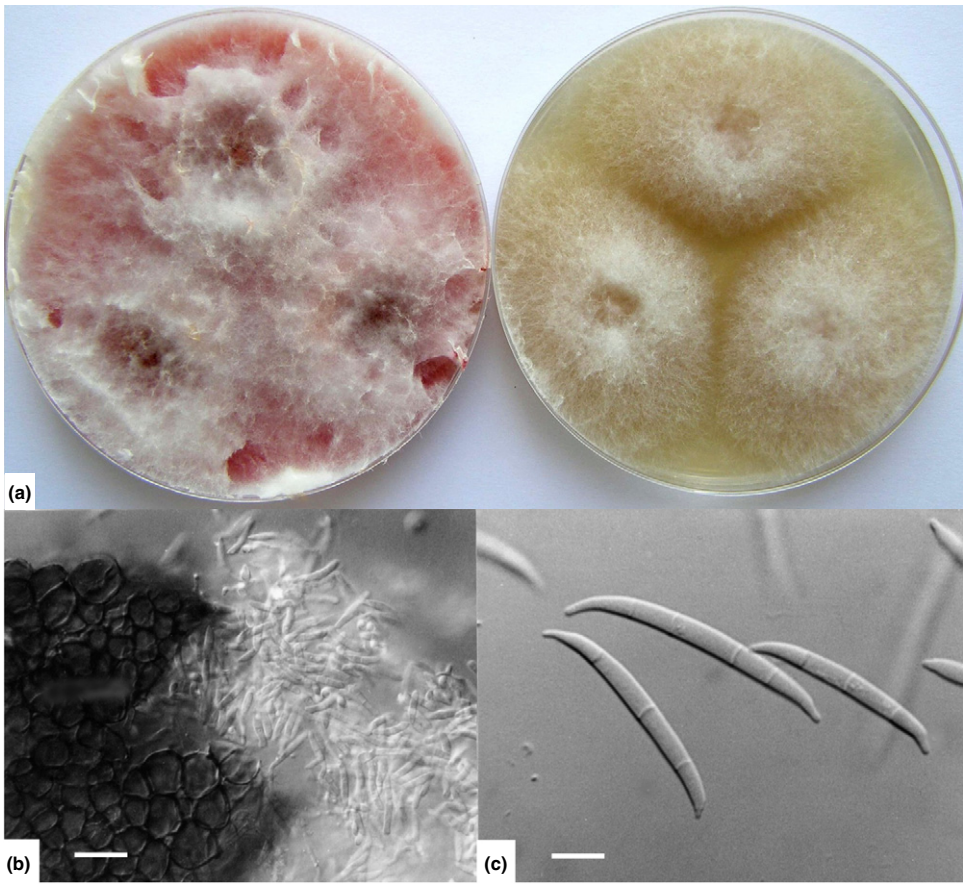

Figure 2 *Fusarium graminearum*. (a) Colonies on PDA and dichloran chloramphenicol peptone agar (DCPA), 7 days, 25 °C; (b) *Gibberella zeae* perithecium and ascospores, bar = 25 μm; (c) macroconidia, bar = 10 μm.

prevalent in north temperate climates especially in wet years, but is much less common in the tropics.

Fusarium head blight affects all commercial cultivars of wheat and barley. *Fusarium culmorum* always produces DON, but whether DON or NIV is produced by *F. graminearum* depends on the geographical origin of the fungal strain.

T-2 toxin is produced mainly by *Fusarium sporotrichioides*. This species grows rapidly on CYA, MEA, and PDA, producing white to pale pink mycelium, with reverse pale on CYA, violet brown on MEA and PDA. Macroconidia are similar to those of *F. graminearum*, but, unlike that species, abundant microconidia are also produced. *Fusarium sporotrichioides* grows under similar conditions to *F. graminearum*, except that its minimum temperature is −2 °C. T-2 toxin is produced on grains, but occurs only under cool conditions.

History

Before 1950, feed refusal in pigs in the US and human toxicoses in Japan and the USSR were separately found to be associated with consumption of small grains. In the USA, pigs became sick and sometimes died. Feeding trials soon established the source as blighted wheat grains and the source of toxicity as *F. graminearum*. This was confirmed by experimental inoculation and feeding. In Japan, it soon became clear the suspect grain – wheat, barley, and rice – was infected with *Fusarium* species, and that the disease, known as Akakabi-byo, had been occurring sporadically for half a century or more. The disease was characterized by nausea, vomiting, diarrhea, and sometimes other symptoms, but was rarely fatal. In the USSR, however, the disease called alimentary toxic aleukia, from the consumption of rye grain that had overwintered in the field due to wartime labor scarcity, killed several hundred thousand people, with symptoms like radiation poisoning.

Identification of the toxin or toxins involved in each of these outbreaks had to wait for improvements in chemical techniques. In the 1970s, the isolation and structural characterization of several trichothecene toxins took place in the USA and Japan. In the USA, DON, then termed vomitoxin, was shown unequivocally to be the cause of the pig disease, whereas in Japan, both DON and NIV were seen as the likely causes of Akakabi-byo. The USSR outbreak was eventually attributed to T-2 toxin produced mainly by *F. sporotrichioides*.

By the turn of the twenty-first century, more than 100 trichothecene molecules were known to occur naturally, produced by a number of plant pathogenic fungal genera. Of these genera, *Fusarium* is by far the most important trichothecene source. At this time, production of DON and

sometimes NIV by *F. graminearum* and *F. culmorum* are the main sources of trichothecenes in foods and feeds, and the main causes for concern.

Hazard Identification

Trichothecenes are potent inhibitors of protein synthesis. DON and other trichothecenes bind to ribosomes, interfering with normal ribosomal function by causing dysregulation of various proteins related to immune function and sometimes apoptosis. Toxicity of the particular molecule varies with conformation that depends on the particular side groups in the molecule. NIV is much more toxic than DON, but is produced in much lower quantities in grains, and so is much less important. T-2 toxin is the most toxic of these compounds when tested in cell lines, and equal to NIV by intraperitoneal injection. T-2 toxin is more than 10 times as toxic as DON by injection, and 70 times as toxic to cell lines. Early studies of DON toxicity used naturally toxic grain. This always included other naturally occurring compounds, providing erroneous results that indicated that DON was more toxic than has been established by assays using the pure compound. Nonetheless, DON is a significant mycotoxin because of its widespread occurrence in cereals and products derived from cereals.

The most obvious syndrome in animals caused by DON is feed refusal in pigs, accompanied by decreased weight gain, gastroenteritis, and effects on heart function and the immune system. DON may cause acute gastroenteritis in humans, where the main symptoms are nausea, vomiting, abdominal pain, diarrhea, and fever. Symptoms can develop within 30 min of exposure and are difficult to distinguish from effects due to bacteria, such as the emetic toxins from *Bacillus cereus*. Recovery is usually complete.

In 2001, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) reviewed the safety of DON and on the basis of a 2-year feeding study in mice concluded that DON was not carcinogenic.

Hazard Characterization

JECFA also evaluated other long-term effects from the same 2-year mice feeding study. Although the mean bodyweight of animals at the lowest dose was lower than that of controls, the difference was considered not to be biologically significant, and no toxicological changes were observed at this dose. JECFA established a provisional maximum tolerable daily intake (PMTDI) of 1 $\mu g\,kg^{-1}$ bodyweight on the basis of the no effect level of 100 μg per kg bodyweight per day in this study and a safety factor of 100. The committee concluded that intake at this level would not result in effects of DON on the immune system, growth, or reproduction.

Consumption of maize containing excessive levels of DON has been associated with numerous incidents of intoxication in China and India. Tens of thousands of people have sometimes been affected. In one episode in India, DON levels in wheat ranged from 0.4 to 8.4 $mg\,kg^{-1}$, whereas in China, intoxication was linked to wheat contaminated with 0.3 to 100 $mg\,kg^{-1}$ DON. In 2010, JECFA derived a group acute reference dose of 8 $\mu g\,kg^{-1}$ bodyweight for DON and its acetylated derivatives using the lowest lower limit on the benchmark dose for a 10% response of 0.21 mg per kg bodyweight per day for emesis in pigs. Limited data from human case reports indicated that dietary exposures to DON up to 50 μg per kg bodyweight per day are not likely to induce emesis.

Exposure Assessment

DON occurs worldwide in maize, wheat, and sometimes other small grains due to growth of *F. graminearum* and related species. JECFA has estimated that the total intake of DON in micrograms per kilogram of bodyweight per day to be 0.78 from the African diet, 1.2 from the Latin American diet, 1.4 from the European diet, 1.6 from the Far Eastern diet, and 2.4 from the Middle Eastern diet. The main source of intake in Europe, Latin America, and the Middle East is wheat (64–88% of total intake), whereas the sources in the other two regions are more varied: wheat, rice, and maize in the African region and wheat and rice in the Far East.

Risk Characterization

The main risk from DON is from chronic exposure, which has been estimated to exceed the PMTDI in many areas of the world. However, the reduction in exposure levels due to processing was not taken into account. For wheat, such reductions can be significant. Regarding acute exposure, the sporadic occurrence of outbreaks of acute gastroenteritis is of public health concern.

A potential risk to farm workers exists from DON inhalation. Grain dusts may contain quite high concentrations of DON. Air samples from Canadian grain elevators contained up to 2.6 $\mu g\,m^{-3}$ of DON. Airborne dust from the same sources contained up to 5.8 $mg\,kg^{-1}$ of DON, plus smaller amounts of T-2 toxin. Some evidence has been reported that grain farming may be associated with midterm pregnancy deliveries in northern Europe.

Chemical Analysis

Analysis for DON usually requires gas chromatography and mass spectroscopy. Modified DON, in the form of the 3-glucoside, is a problem because it is not assayed by the usual techniques. Extraction may use chloroform – ethyl acetate or 70% methanol, and cleanup is accomplished by filtering through a C18 or silica gel column.

Levels in Foods

DON is found in maize and small grains in all areas where these crops are grown, and particularly in wheat, the crop most commonly invaded by *F. graminearum*. It is especially prevalent in cooler areas where rainfall is higher, such as Canada, Europe, and Argentina, but less commonly occurs in drier, hotter areas such as Australia. Levels of DON and other related trichothecenes are usually lower than 1 $mg\,kg^{-1}$ in

foods, but can be much higher, potentially causing intoxication (see Section 'Risk Characterization').

A number of studies have shown that fungal infection rates are higher in crops planted in fields previously planted with maize, particularly when residues from those crops were left in the field. Once grains begin to dry, with the water activity reduced to <0.9, increases in levels of *Fusarium* mycotoxins rarely occur.

Favorable weather conditions are critical for infection to occur in wheat heads. Field observations have confirmed that temperature and moist conditions during anthesis and heading are the major factors of importance.

Management

In 2010, the US Food and Drug Administration issued revised guidelines for DON in foods and feeds as follows: for finished wheat products that may be consumed by humans, 1 mg kg^{-1}; for grains and byproducts for feedlot and dairy cattle, 10 mg kg^{-1}, except that for dairy cattle, the total DON content in feed should not exceed 5 mg kg^{-1}; in feed for pigs, 5 mg kg^{-1}, but not exceeding 20% of the total diet; and for all other animals, 5 mg kg^{-1}, not exceeding 40% of the total diet.

Some success has been achieved in controlling DON formation in wheat by the use of azole fungicides at anthesis. Forecasting systems to advice farmers of the likelihood of DON formation have been developed in Canada and Europe. Otherwise, control relies on reducing levels of *Fusarium* species in the field by good management and crop rotation.

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MYCOTOXINS

Fumonisins

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Glossary

Conidium (pl. conidia) Microscopic asexual spores produced by molds (filamentous fungi with small fruiting bodies); the main means of propagating many species.
Macroconidium (pl. macroconidia) The larger of the two conidial types produced by *Fusarium* species, and characteristic of the genus.
Microconidium (pl. microconidia) The smaller of the two types produced by some *Fusarium* species, but also produced by a number of other related genera.
Nixtamalization A centuries old process, used primarily in Central America, in which maize is soaked then cooked with ash or lime high in alkali.
Phialide A cell that produces conidia in basipetal succession, that is, from the tip, with the oldest cell furthest from the formation point, and without change in length of the phialide itself.

Chemical Characterization

Fumonisins are a family of compounds made up of a 19- or 20-carbon chain aliphatic acid backbone with amino or polyhydroxy side groups, two of which are esterified with propane tricarboxylic acid. This provides a hydrophobic/hydrophilic dichotomy unique among the mycotoxins. Fumonisins are structurally similar to the sphingoid base backbone of sphingolipids, important membrane constituents.

The most important fumonisin is known as fumonisin B_1 (CAS 116355-30-0), and has a molecular formula of $C_{34}H_{59}NO_{15}$, with a molecular weight of 721 (Figure 1). Most naturally occurring fumonisins belong to the B series, but some members of the C series, identical to the B series but with the terminal methyl group absent, have also been found in maize. Other much less common fumonisins, designated variously as A and P series, have been isolated from pure fungal cultures.

Fungal Sources, Physiology, and Ecology

Fumonisins are produced by *Fusarium verticillioides* (known in older literature as *Fusarium moniliforme)* and some closely related species, in particular *Fusarium proliferatum*. The principal habitat of these species is maize. It has been shown recently that some fumonisins are also produced by *Aspergillus niger*, an entirely unexpected source.

At 25 °C, *F. verticillioides* grows rapidly on any standard mycological medium including Czapek yeast extract agar, malt extract agar, and potato dextrose agar (PDA). Colonies are white to pale salmon colored, with low and often ropy mycelium and a powdery texture due to production of chains of microconidia. The reverse color on PDA is variable, pale salmon, greyish violet, brownish violet to deep violet. Slow growth occurs at 37 °C on these media (Figure 2).

Characteristic *Fusarium* spores, macroconidia, are produced on dichloran chloramphenicol peptone agar or other specialist media. Macroconidia are long (40–50 μm) and slender,

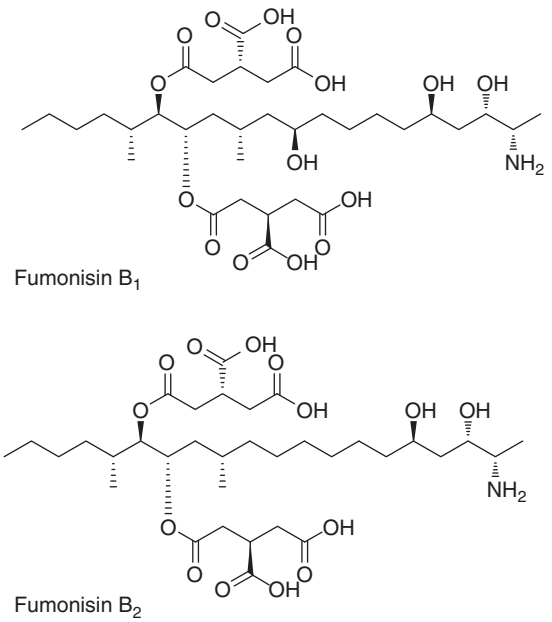

Figure 1 Structure of fumonisins B_1 and B_2.

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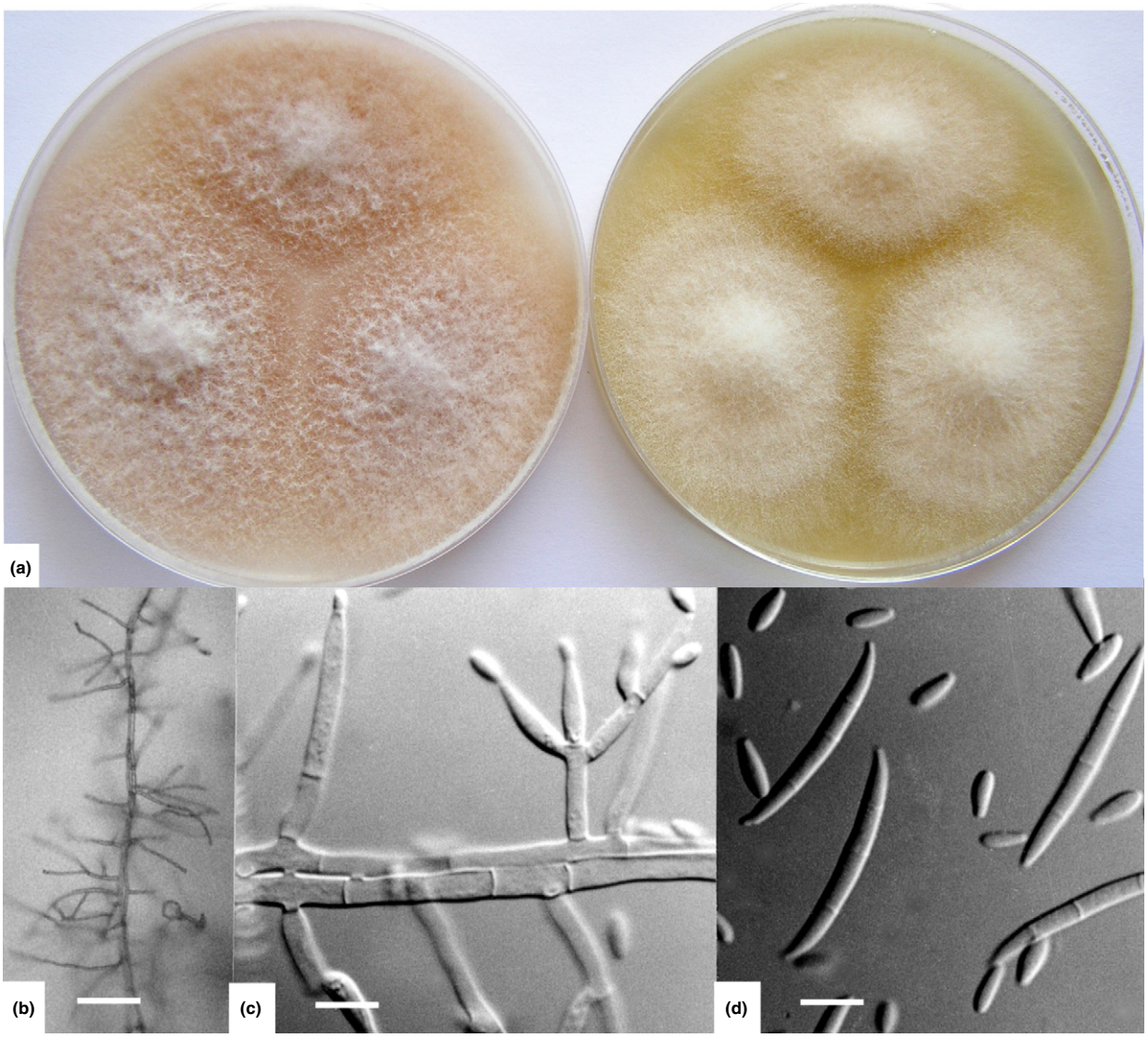

Figure 2 *Fusarium verticillioides*. (a) Colonies on potato dextrose agar and dichloran chloramphenicol peptone agar, 7 days, 25 °C; (b) phialides bearing chains of microconidia, bar = 50 μm; (c) phialides, bar = 10 μm; (d) macro- and microconidia, bar = 10 μm.

almost straight, thin walled, with foot shaped basal cells. Microconidia are pointed at both ends or club shaped, 7–10 μm long, and are produced in chains from long single phialides (fruiting cells). *Fusarium proliferatum* is very similar, but colonies on PDA lack purple colors in the reverse, and microconidia are produced from phialides with more than one fertile neck.

Fusarium verticillioides grows from approximately 3–37 °C, with an optimum near 25 °C. Growth is possible down to 0.87 water activity, but is very slow below 0.90. This species is able to grow under reduced oxygen tension, but not in its complete absence. Fumonisins are produced down to 0.92 water activity. The physiology of *F. proliferatum* is very similar.

Fusarium verticillioides is endemic in maize and grows in all regions, but is less common in cooler areas. It has been isolated from maize kernels wherever it has been sought. It causes both stalk and cob rots, and occurs at all stages in plant development, so it can rightly be considered a commensal in maize. *Fusarium verticillioides* also occurs in sorghum, rice, and millet. Reports exist of the occurrence of this species in many other crops, including causing spoilage of citrus, asparagus, yams, bananas, and pineapples. However, in light of the narrowing of the definition of this species (see Section History), many such claims need to be reexamined before being accepted as authentic.

History

By 1900, it was suspected that a lethal and widespread neurological disease in horses, mules, and donkeys in the US was associated with the feeding of maize. The disease was named leukoencephalomalacia, and was subsequently found

to occur very widely, in countries as far apart as USA, Mexico, South Africa, New Caledonia, and China. However, the cause remained elusive. In the 1970s, South African scientists recognized that the cause was a mycotoxin and, after 20 years of work, were able to isolate and characterize a new class of toxins, the fumonisins. Publication came comparatively recently, in 1998, and was followed by independent publication by scientists in New Caledonia the following year. Both groups of workers were able to demonstrate that fumonisins were directly responsible for the horse deaths by administering or feeding the pure compound to horses.

About 1990, a large scale, often fatal, outbreak of pulmonary disease in southeastern USA pigs occurred. South African experiments in the 1980s had indicated that maize containing fumonisins could cause such a syndrome. Experimental work soon confirmed that fumonisins were responsible.

For many years, the species primarily responsible for fumonisin production was known as *F. moniliforme*. However, due to confusion over the application of that name to a number of distinct species, in 2003 taxonomists agreed to solve the problem by taking up the older name *F. verticillioides* for the major fumonisin producer.

Hazard Identification and Characterization

Fumonisins are remarkable for the wide range of effects caused in animals and possibly in man. Fumonisins act by inhibiting the enzyme ceramide synthase, which causes accumulation of intermediates in the sphingolipid metabolism pathway, and also causes depletion of complex sphingolipids. This inhibition interferes with the binding of folate and some other proteins in cell membranes. The most dramatic effect occurs in horses, where the disease called equine leukoencephalomalacia occurs. This is a rapidly progressing disease that causes equine brains to liquefy. For horses, consumption of feed containing $>10\ \mathrm{mg\ kg^{-1}}$ fumonisin B_1 in the diet (equivalent to $0.2\ \mathrm{mg\ kg^{-1}}$ body weight per day) was associated with increased risk of developing this disease.

In pigs, fumonisins cause pulmonary edema, due to left ventricle heart failure, whereas in rats the primary effect is to cause liver cancer, but they also cause programmed cell death (apoptosis). It is unusual for a single toxin to have such diverse effects in different animal species, but careful investigations have confirmed that to be true in this case. The reasons for such diversity remain unclear.

In humans, fumonisins produced by *F. verticillioides* cause none of these animal syndromes, but are associated with esophageal cancer. Extensive studies in areas of low and high maize consumption in South Africa have suggested this connection. This disease is also prevalent in areas of China and occurs at significantly higher levels than background also in parts of Iran, northern Italy, Kenya, and a small area of the southern USA. In all of those areas consumption of maize and maize products are very high. There is also some evidence that high intake of fumonisins from maize are associated with neural tube defects such as spinal bifida in areas of Guatamala, South Africa, China and a population along the Texas, Mexican border.

Exposure Assessment

The primary source of fumonisins is maize. *Fusarium verticillioides* is endemic in maize, so fumonisins are of universal occurrence wherever maize is grown. Unacceptable levels occur only in plants that are drought stressed near harvest dates or where cobs are extensively damaged by insects. Intake can be high in countries where maize is a dietary staple, notably in Africa, some South and Central American countries and parts of China.

The discovery of fumonisin formation by *A. niger* means that fumonisins occur in many other food commodities, including grapes, wine, dried vine fruits, and probably coffee and cocoa. However, it seems likely that levels in these foods do not add significantly to exposure.

Risk Characterization

For the human population, a provisional maximum tolerable daily intake (PMTDI) of $2\ \mu\mathrm{g\ kg^{-1}}$ body weight per day has been established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). This PMTDI was maintained by JECFA at a meeting in 2011. The European Commission has established the same tolerable daily intake for fumonisins B_1, B_2, and B_3, alone or in combination.

Chemical Analysis

Analytical techniques have concentrated on the estimation of fumonisin B_1, as it is the most commonly occurring form. Screening methods in foods have been based either on thin-layer chromatography (TLC) after clean up, or more commonly on enzyme-linked immunsorbent assay (ELISA) tests.

The traditional methodology of TLC is still in use, and is recommended for less developed economies as it is inexpensive and reliable. For fumonisins a reversed phase system is preferable. It has been shown that derivatization with fluorescamine before spotting on the TLC plate was more effective than after elution. The preferred developing medium is methanol: 4% aqueous potassium chloride (70:30). A suitable developing solvent is benzene:methanol:acetic acid (18:1:1). Fumonisin B_1 is visualized under long-wave ultraviolet light as a greenish yellow spot with R_f approximately 0.35. Levels down to at least $1.0\ \mathrm{mg\ kg^{-1}}$ can be estimated.

ELISA techniques are also effective for fumonisin analysis. Although antibodies are raised against fumonisin B_1, cross reactivity usually occurs with fumonisins B_2 and B_3 as well. Commercial kits, using a range of methods, are available from several manufacturers.

For analysis by high-performance liquid chromatography, fumonisins lack suitable chromophores, so must be derivatized and detected by fluorescence. The most common derivatization is with orthophthalaldehyde precolumn. Liquid chromatography–mass spectrometry is increasingly being used, as derivatization is not necessary.

Levels in Foods

The occurrence of fumonisins in foods due to growth of *F. verticillioides* and *F. proliferatum* is of significance only in maize. Fumonisins have been detected in a variety of cereals due to other *Fusarium* species, and due to *A. niger* in grapes, wines, and other commodities, but levels are usually low. Excessive fumonisin levels occur when maize plants are drought stressed or suffer extensive insect damage. Maize is a widely consumed cereal, and a dietary staple in many regions of the world, that is, in parts of Africa, South and Central America, and China. Drought stress occurs quite commonly in all of these regions; hence, levels in foods frequently exceed regulatory limits. Maize consumption in parts of Africa has been increasing as this crop replaces traditional crops such as sorghum and millet, often in areas where drought stress occurs, and often outside the geographical regions for which the maize hybrids were developed and to which they are suited. In consequence, consumption of undesirable levels of fumonisins occurs quite frequently in some parts of the world.

Management Options – Limits in Foods

Regulatory Limits

The European Commission has set maximum permitted limits of 4 mg kg^{-1} total fumonisins in unprocessed maize and 200 μg kg^{-1} fumonisins in maize based foods and baby foods after processing. The US Food and Drug Administration has set guidelines for fumonisin levels in processed foods: degermed dry milled maize products, 2 mg kg^{-1} total fumonisins; dry milled maize bran, 4 mg kg^{-1}; and cleaned maize for popcorn, 3 mg kg^{-1}.

Preharvest Control

Fumonisins are produced in maize preharvest. Drought stress is the major factor causing production, though insect damage may also be important. Good agricultural practice, irrigation, and the use of Bt maize cultivars are all important in limiting fumonisin formation. Some progress has been made in breeding cultivars resistant to ear rot due to *F. verticillioides*.

Freshly harvested maize should be rapidly dried to a suitable moisture level, but in practice this is important only in the initial stages of drying because *Fusarium* species grow very slowly below approximately 0.9 water activity, so once the kernel moisture content has been reduced below that figure, fumonisin accumulation ceases. This frequently occurs in field drying before harvest of the cobs. For the same reason, fumonisins will not be produced in storage. Even if very high moisture occurs due to water ingress, competition with other microorganisms at such high water activities will prevent any significant increase in fumonisin levels.

Processing

In most geographical areas, the main methods for controlling fumonisin levels are visual inspection of lots for fungal damage, followed by fumonisin analyses, and rejection of lots that do not meet specifications.

In Central America, the process of nixtamalization is commonly used in preparation of meals based on maize. Nixtamalization is a centuries old process in which maize is soaked then cooked with ash or lime high in alkali. It removes almost all fumonisins, resulting in tortillas and other maize based foods being substantially free of these mycotoxins.

Maize is wet milled to obtain maize starch, germ, and fiber, where as dry milling produces bran, germ, and fractions of decreasing particle size – grits, corn meal, and flour. Fumonisins are not destroyed during these processes and are found in all fractions, with higher concentrations in bran and germ.

Processing at temperatures above 150 °C reduces fumonisin levels. Maize meal production, frying, baking, roasting, and alkaline cooking all have effects, dependent on the temperature actually attained. Extrusion processing is used extensively in the production of breakfast cereal, snack and textured foods based on maize meal. Extrusion temperatures of 160 °C or higher have a significant effect, especially if glucose is included.

See also: Foodborne Diseases: Overview of Chemical, Physical, and Other Significant Hazards. Mycotoxins: Mycotoxins – General; Ochratoxin A

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MYCOTOXINS

Ochratoxin A

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Glossary

Conidium (pl. conidia) Microscopic asexual spores produced by molds (filamentous fungi with small fruiting bodies); the main means of propagating many species.

Metabolite It is a chemical compound produced by a fungus or other organism during growth, development, or reproduction.

Chemical Characterization

Ochratoxin A (OTA; CAS 303-47-9) consists of a substituted isocoumarin moiety linked to phenylalanine (Figure 1). It is a white crystalline solid with intense ultraviolet (UV) fluorescence, green in acid, and blue under alkaline conditions, due to a closed or open lactone ring, respectively. This reaction is reversible

Fungal Sources, Physiology, and Ecology

OTA is produced by three well-defined groups of fungi. The first group, which may be termed the ocher colored Aspergilli, comprises *Aspergillus ochraceus, Aspergillus westerdijkiae, Aspergillus steynii*, and a few other (much less important) related species. This group is largely responsible for OTA production in coffee, and also in long stored grains. The second comprises two of the black spored Aspergilli, i.e., *Aspergillus carbonarius* and (much less frequently) the closely related and common species *Aspergillus niger*: these species are responsible for most OTA formation in grapes and grape products and, in some regions, in coffee. The third includes two *Penicillium* species, *Penicillium verrucosum* plus the closely related *Penicillium nordicum*. The well documented occurrence of OTA in temperate climate cereals results from the growth of *P. verrucosum*. *Penicillium nordicum* has been reported to produce OTA in cool stored processed meats.

Detection of any of these fungi requires growth on isolation media and identification on standard media, such as Czapek yeast extract agar (CYA) and malt extract agar (MEA) used for *Aspergillus* and *Penicillium* species.

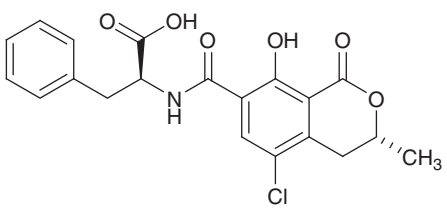

Figure 1 Structure of ochratoxin A (OTA).

Aspergillus ochraceus and Related Species

On CYA, after 1 week at 25 °C, *A. ochraceus* and closely related species produce colonies 40–55 mm in diameter with ocher (light yellow brown) conidia. These characteristics are distinctive for this group of species. *Aspergillus westerdijkiae* (Figure 2) and *A. steynii* are distinguished from *A. ochraceus* by the inability to grow on CYA at 37 °C: *A. westerdijkiae* produces finely roughened, spherical conidia and *A. steynii* smooth walled, ellipsoidal conidia, whereas those of *A. ochraceus* are smooth walled and spherical.

Apart from the difference in maximum growth temperatures, approximately 40 °C for *A. ochraceus* and approximately 5 °C lower for the other species, these three species have similar physiology so far as is known. All are likely to be able to grow down to approximately 0.77–0.80 water activity and over a wide pH range.

Although *A. ochraceus* is the oldest recognized species, recent studies indicate that *A. westerdijkiae* is much more common, and more likely to produce OTA. As *A. westerdijkiae* and *A. steynii* were separated from *A. ochraceus* only recently, studies reporting *A. ochraceus* before 2005 are likely to include all three species.

Ecologically, these three species are most often isolated from stored foods, reflecting their xerophilic nature. However, *A. westerdijkiae* has a major habitat in coffee beans, leading to OTA production.

Aspergillus carbonarius and *A. niger*

These two species produce rapidly growing colonies on CYA, usually 60 mm or more after 7 days at 25 °C, colored black or deep reddish brown. *Aspergillus carbonarius* (Figure 3) produces much larger conidia (6–7 μm in diameter) than *A. niger* (4–5 μm). *Aspergillus carbonarius* grows from approximately 10

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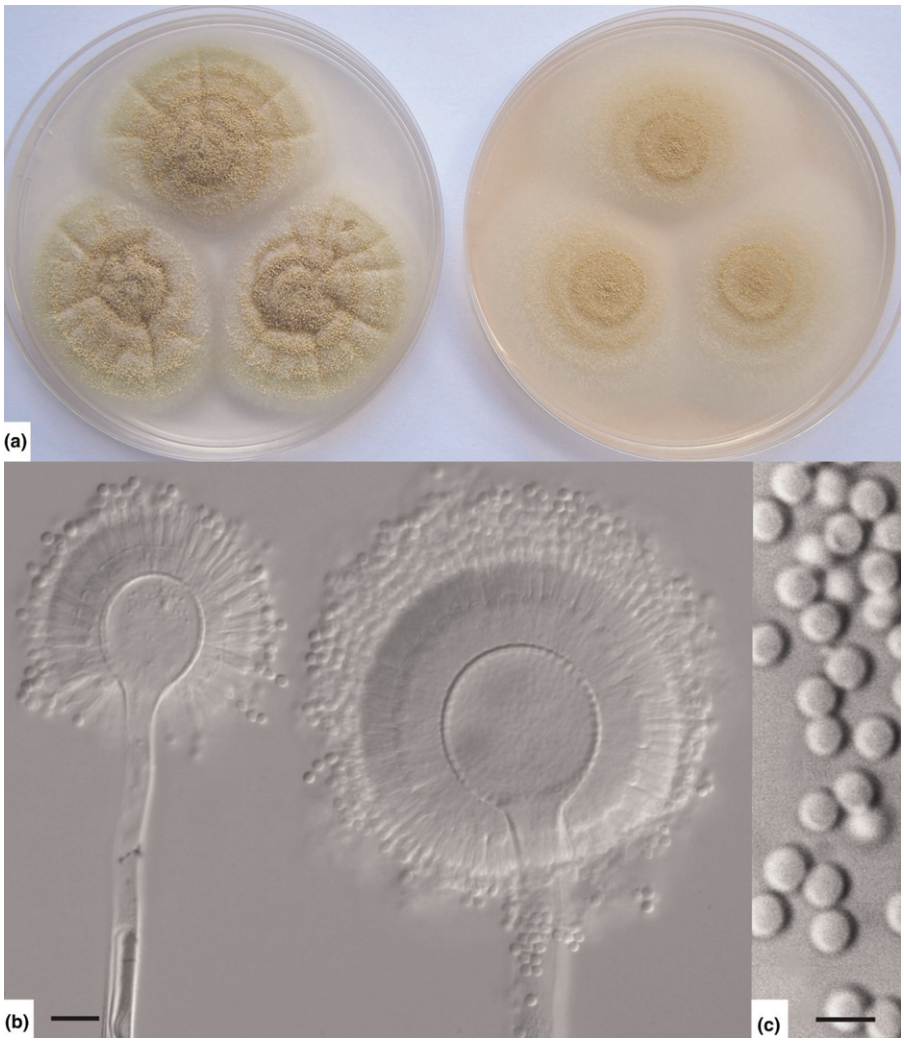

Figure 2 *Aspergillus westerdijkiae*. (a) Colonies on CYA and MEA, 7 days, 25 °C; (b) fruiting structures, bar = 10 μm; and (c) conidia, bar = 5 μm.

to 40 °C, and down to 0.85 water activity, whereas *A. niger* grows from approximately 8–45 °C and down to 0.8 water activity or slightly below.

These species have a major habitat in grapes, producing OTA in dried vine fruits and wines. They also occur in coffee in some growing regions.

Penicillium verrucosum and *P. nordicum*

Penicillium verrucosum (Figure 4) and *P. nordicum* are very similar, slowly growing species, reaching only 15–25 mm diameter on CYA after 7 days at 25 °C, and colored dull green to dark green. Both grow from approximately 0 to 30 °C and down to 0.80 water activity.

Penicillium verrucosum is endemic in European and Canadian wheat, barley, rye, and oats. It is a cold climate fungus, and is rarely isolated outside cool temperate zones or away from cereals. In contrast, *P. nordicum* grows mostly in proteinaceous foods, especially meat and cheese. Reflecting its low temperature profile, it has been isolated from foods cold stored for several weeks or more, but the significance of this for human health is unknown.

History

OTA was originally described as a metabolite of *A. ochraceus* from laboratory experiments on fungal toxicity in 1965, and production was detected from several related *Aspergillus* species soon after. However, natural occurrence, and practical importance, was first linked in 1973 with a *Penicillium* species, *Penicillium viridicatum*, later correctly identified as *P. verrucosum*. OTA was soon characterized as a potent kidney toxin. As a result, it was postulated quite early that OTA was a likely cause of Balkan endemic nephropathy, a kidney disease with a high mortality rate in certain areas of Bulgaria, Yugoslavia, Romania, and Tunisia. However, conclusive evidence of OTA involvement remains elusive, despite much effort. Recent work indicates that this syndrome is likely to result from contamination of wheat grains with seeds of the weed *Aristolochia*

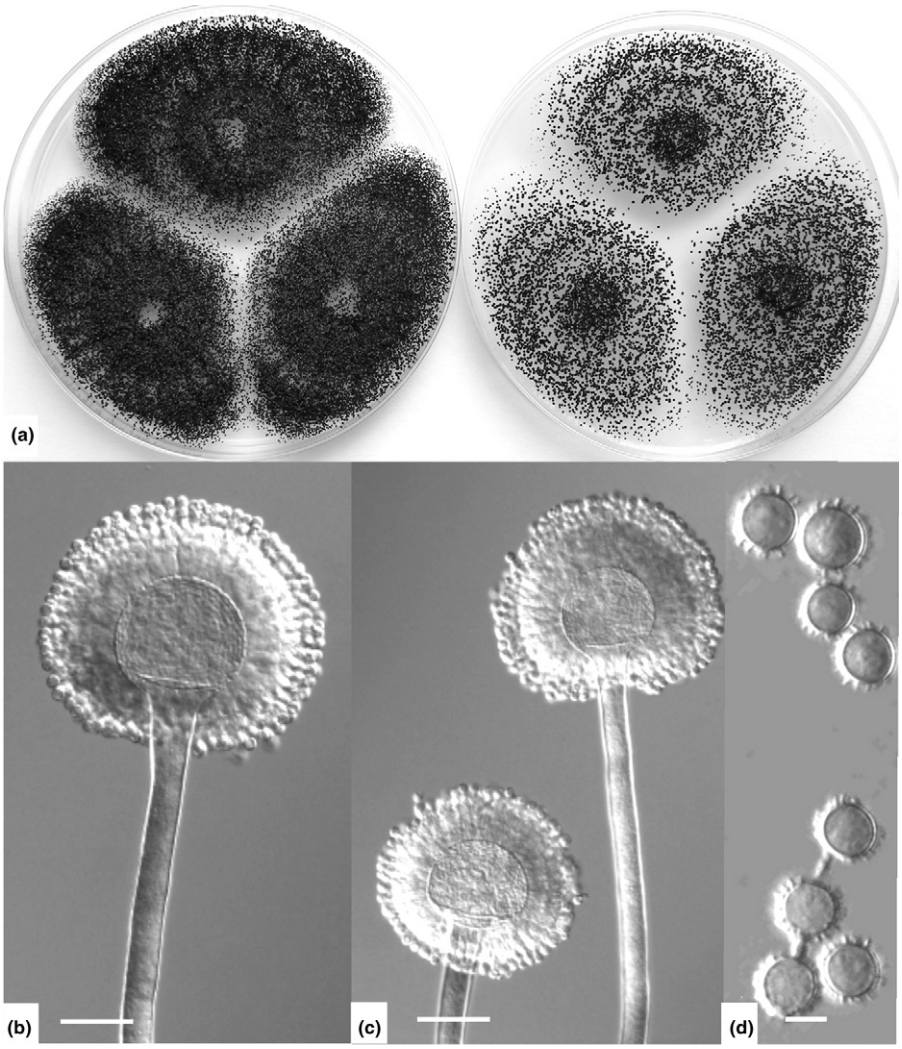

Figure 3 *Aspergillus carbonarius*. (a) Colonies on CYA and MEA, 7 days, 25 °C; (b, c) fruiting structures, bar = 10 μm; and (d) conidia, bar = 5 μm.

clematitis, endemic in affected areas, which commingle with the wheat before harvest.

Hazard Identification and Characterization

OTA is an acute and chronic nephrotoxin affecting kidney function in all animal species tested. OTA is readily absorbed through the intestines and, once it enters the blood stream, has a long half-life, up to 3 weeks in monkeys and 4 weeks or more in human volunteers. As fungi that produce OTA are very widespread in foods, the blood of healthy humans regularly contains detectable amounts of OTA. Because *P. verrucosum* has a restricted distribution, OTA intake as the result of growth of this species is confined almost entirely to Europe and northern North America. The occurrence of OTA in human blood from warmer regions of the world is the result of the growth of *Aspergillus* species in foods.

OTA also has carcinogenic properties, but the mechanism of carcinogenicity remains unknown. Reports by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) indicate that the carcinogenic effects in animals occur at higher doses than nephrotoxicity. Although OTA is demonstrably toxic to animals of all kinds, its effects in humans remain unclear and the subject of debate. Both genotoxic and non-genotoxic modes of action have been proposed. The International Agency for Research on Cancer has classified OTA as a possible human carcinogen (Group 2B), based on sufficient evidence of carcinogenicity in experimental animal studies and inadequate evidence in humans.

Exposure Assessment

People in Europe and Canada are exposed to OTA in barley and wheat and their products, especially bread, and also from offal products derived from animals, especially pigs, fed contaminated feed. OTA also occurs in beer, wine, coffee, cocoa and chocolate, and dried vine fruits, so people everywhere are exposed to this toxin, though exposure from these sources is lower than may

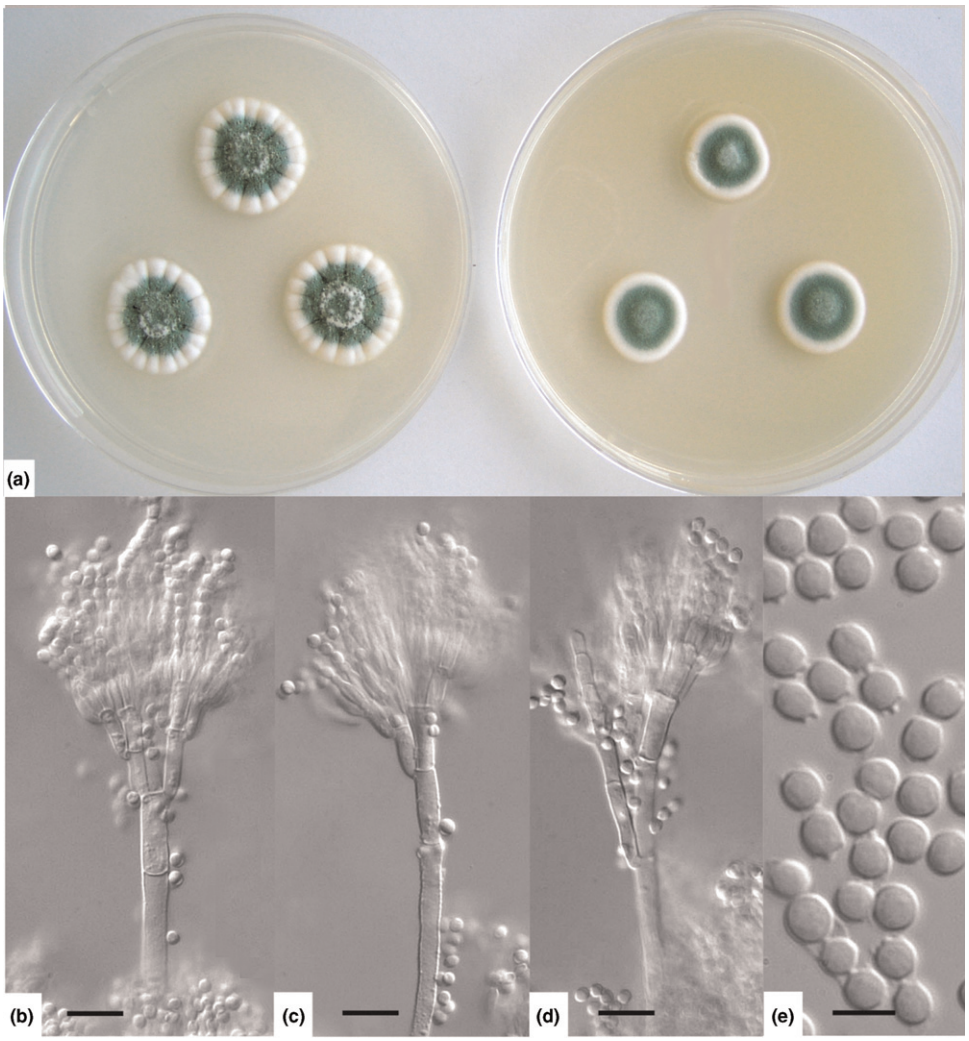

Figure 4 *Penicillium verrucosum*. (a) Colonies on CYA and MEA, 7 days, 25 °C; (b, c, d) fruiting structures, bar = 10 μm; and (e) conidia, bar = 5 μm.

occur from cereals. As wheat and barley crops from warmer climates, such as the US, South Africa, and Australia, are not infected by *P. verrucosum*, OTA intake is often much lower in regions outside Europe and Canada.

A large number of studies have now been published on OTA levels in human blood, although the correlation between blood level and measured food intake is still somewhat uncertain. Blood OTA levels are now considered to be the most reliable measure of human exposure. Published figures are quite variable, with reported levels sometimes exceeding 10 $\mu g\, l^{-1}$ in European individuals. Mean levels around the world commonly range from 0.2 to 1 $\mu g\, l^{-1}$. Often 50–100% of people sampled in any population have detectable levels of OTA in their blood.

Risk Characterization

In a recent evaluation, JECFA has maintained the provisional tolerable weekly intake (PTWI) of OTA at 100 $ng\, kg^{-1}$ of bodyweight based on the lowest observed adverse effect level of chronic toxicity in pigs. The European Food Safety Authority (EFSA) has recommended a PTWI of 120 $ng\, kg^{-1}$ bodyweight.

JECFA has assessed the dietary exposure to OTA in Europe from processed cereals as 8–17 and EFSA as 7–10 ng per kg of bodyweight per week, both well below the PTWI. Even with some additional intake from wines and coffee, the risk from intake of OTA in Europe appears to be acceptable. Intakes in other parts of the world are lower in the absence of appreciable levels in cereals.

Chemical Analysis

Analysis of OTA from foods is complicated by the facts that (1) it is unstable in alkali, which causes lactone ring opening, so any subsequent purification on an immunoaffinity column will be inaccurate and (2) OTA binds to protein, so purification by protein precipitation will also lead to serious inaccuracy.

Extraction

The AOAC International standard method uses phosphoric acid for acidification before extraction with chloroform. Acetonitrile:water is also used.

Purification

In the AOAC method, purification is carried out by absorption on a sodium bicarbonate–diatomaceous earth column, washing with hexane, then chloroform, followed by elution using acetic acid–benzene. Immunoaffinity columns are also recommended.

Assays

The traditional methodology of thin layer chromatography is still in use, and is recommended for less developed economies as it is inexpensive and reliable. A suitable developing solvent is benzene:methanol:acetic acid (18:1:1). OTA may be visualized under UV light. Confirmation is blue fluorescence after exposure to ammonia vapor or spraying with ethanolic sodium bicarbonate.

More modern methods for analysis are based on high performance liquid chromatography, with or without mass spectrometry.

Levels in Foods

The crops and foods affected by fungi producing OTA are quite specific. OTA is produced in temperate climate cereals (wheat and barley) by *P. verrucosum*. It is produced by *A. ochraceus* and related species, and by *A. carbonarius*, during drying or processing of coffee and cocoa, where the dominant causative fungus varies from region to region. Grapes are susceptible to infection by *A. carbonarius* just before or after harvest, due to skin splitting from rain or mechanical damage or infection by pathogenic fungi, especially *Rhizopus stolonifer* or powdery mildews. OTA may be produced just before harvest, during drying of raisins and other dried fruits, or in grape juice before crushing in wine making.

Management Options

Limits in Foods

The Codex Alimentarius Commission presented a recommendation to set a limit of 5 $\mu g\,kg^{-1}$ of OTA in cereals and cereal products, and a number of countries have adopted this limit, though it is still under discussion by Codex. The European Union has set a limit of 5 $\mu g\,kg^{-1}$ of OTA in unprocessed cereals and roasted coffee beans, 3 $\mu g\,kg^{-1}$ in processed cereals, and 10 $\mu g\,kg^{-1}$ in dried vine fruits and instant coffee. In wines and grape juice, the limit is 2 $\mu g\,kg^{-1}$, whereas infant foods must not contain more than 0.5 $\mu g\,kg^{-1}$.

Preharvest Control

Control of OTA levels before processing in foods relies on good agricultural practice. As none of the fungi that produce OTA are known to be systemic invaders or pathogens, the key to low levels in foods is rapid drying, and maintenance of low moisture, regardless of the crop. The problem in cereals in cool moist European climates is to accomplish rapid drying, and then to prevent moisture migration in silos. Coffee is grown under conditions often conducive to mist or rain at harvest, and again effective drying is the key to keeping OTA levels low. Good agricultural practice for vineyards includes disease and insect control, avoidance of mechanical damage before drying, rapid harvest and crushing for wines, and selection of cultivars resistant to splitting in late preharvest rain.

Processing

OTA is largely removed during the wine making process as it is bound to solid fractions and sediment. Some fining agents can also reduce OTA levels in wine. Red wines retain slightly more OTA than white wines, but, overall, the carryover from grapes into finished wine is between 1% and 8%.

Processing of wheat to make flour reduces OTA levels, though the process used greatly influences final levels in bread. Milling hard wheat to produce white flour produced approximately 65% reduction in OTA, and a further 10% decrease occurred during baking. Wholemeal flour and bread showed much less reduction in OTA during processing, as might be expected, because less of the grain is discarded.

Like most mycotoxins, OTA is relatively heat stable. In dry wheat, 50% inactivation at 100, 153, 200, and 250 °C required 707, 201, 12, and 6 min, respectively. In moist wheat, the corresponding inactivation times at 100, 150, and 200 °C were 145, 64, and 19 min, respectively. Complete destruction did not occur even at 250 °C.

Processing coffee beans, including roasting, drink preparation, and instant coffee production all reduce OTA levels in coffee. Dark roasting may remove up to 98% of OTA, but light roasts have much less effect.

See also: Foodborne Diseases: Overview of Chemical, Physical, and Other Significant Hazards

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MYCOTOXINS

Patulin

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Introduction

Patulin (CAS 149-29-1) is a furopyranone, 4-hydroxy-4H-furo[3,2-o]pyran-2(6H)-one, molecular formula $C_7H_6O_4$, molecular weight 154.12 (Figure 1). Patulin is stable in many kinds of foods, including apple and grape juices and dry maize, but not in wet maize, orange juice, flour, fermented juices, or cheese. Disappearance appears to be due to the reaction with sulfhydryl groups of amino acids or proteins, but that does not necessarily lead to a complete loss in biological activity. Patulin is moderately heat stable, surviving the pasteurization processes.

In the heady days after the discovery of penicillin, extensive searches were conducted for other fungal compounds that might be of therapeutic value. Many metabolites produced by *Penicillium* species were discovered and evaluated. For a short time, patulin was considered to be a possible cure for the common cold, but like most *Penicillium* metabolites apart from penicillin, it turned out to be too toxic to be useful as a drug. Patulin is considered as a mycotoxin.

Patulin is produced by 10 or more *Penicillium* species, including *P. carneum*, *P. paneum*, *P. glandicola*, and *P. griseofulvum*, all of which occur in foods from time to time, and also by the heat resistant spoilage fungus *Byssochlamys fulva*. However, the only patulin producer of significance for food safety is *P. expansum*.

P. expansum can be found in a variety of foods, but its importance lies in its ability to cause destructive rots in apples and pears. Mechanical damage or contact with a rotting fruit permits entry to fruit tissue. *P. expansum* forms brown, circular rots and produces patulin in the damaged tissue. In time, growth becomes visible as a blue colony, with a heaped-up appearance (Figure 2). The rot will consume a fruit in a few days at 25–30 °C.

On standard growth media such as Czapek yeast extract agar or malt extract agar (CYA), *P. expansum* forms moderately quickly growing colonies, 30–40 mm in diameter after 7 days at 25 °C. Conidial production is profuse, dull green, and often heaped in small tufts. The colony reverse on CYA is usually dark brown. The fruiting structures are very small, like little brushes, typical of species in *Penicillium* subgenus *Penicillium* (Figure 3).

P. expansum grows strongly at 0 °C and it has a maximum temperature for growth near 35 °C. The minimum water activity for germination is 0.82. *P. expansum* has a very low requirement for oxygen: concentrations as low as 1% oxygen have little effect on growth.

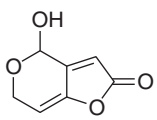

Figure 1 Chemical structure of patulin.

Hazard Identification

Consumers will always avoid rotten fruit, so patulin is not an issue in fresh fruit. However, harvesting for commercial juice manufacture often causes fruit damage, and may involve delays after picking that enable rot development, so apple juice is liable to contain patulin. Poor quality control, i.e., the use of rotting fruit in juice or cider manufacture, can result in high concentrations of patulin in products. For example, levels of up to 350 $\mu g\,l^{-1}$, 630 $\mu g\,l^{-1}$, 1770 $\mu g\,l^{-1}$, and even 3990 $\mu g\,l^{-1}$ have been reported in the literature. The worst results were obtained from apple juice sold at roadside stalls.

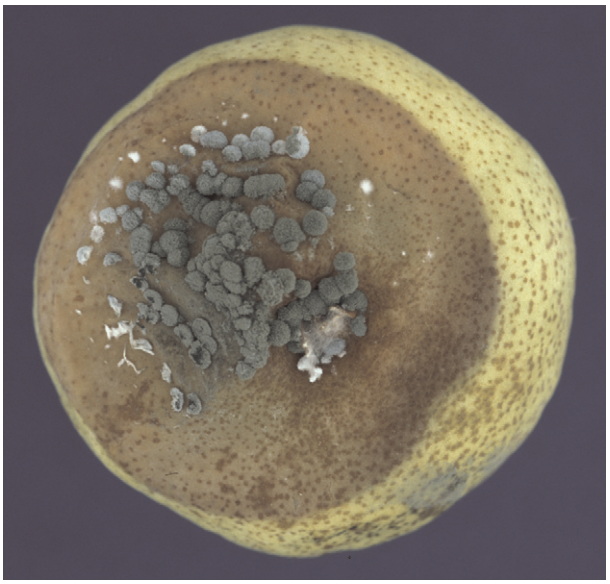

Figure 2 Apple rot due to the growth of *P. expansum*.

 doi:10.1016/B978-0-12-378612-8.00195-5

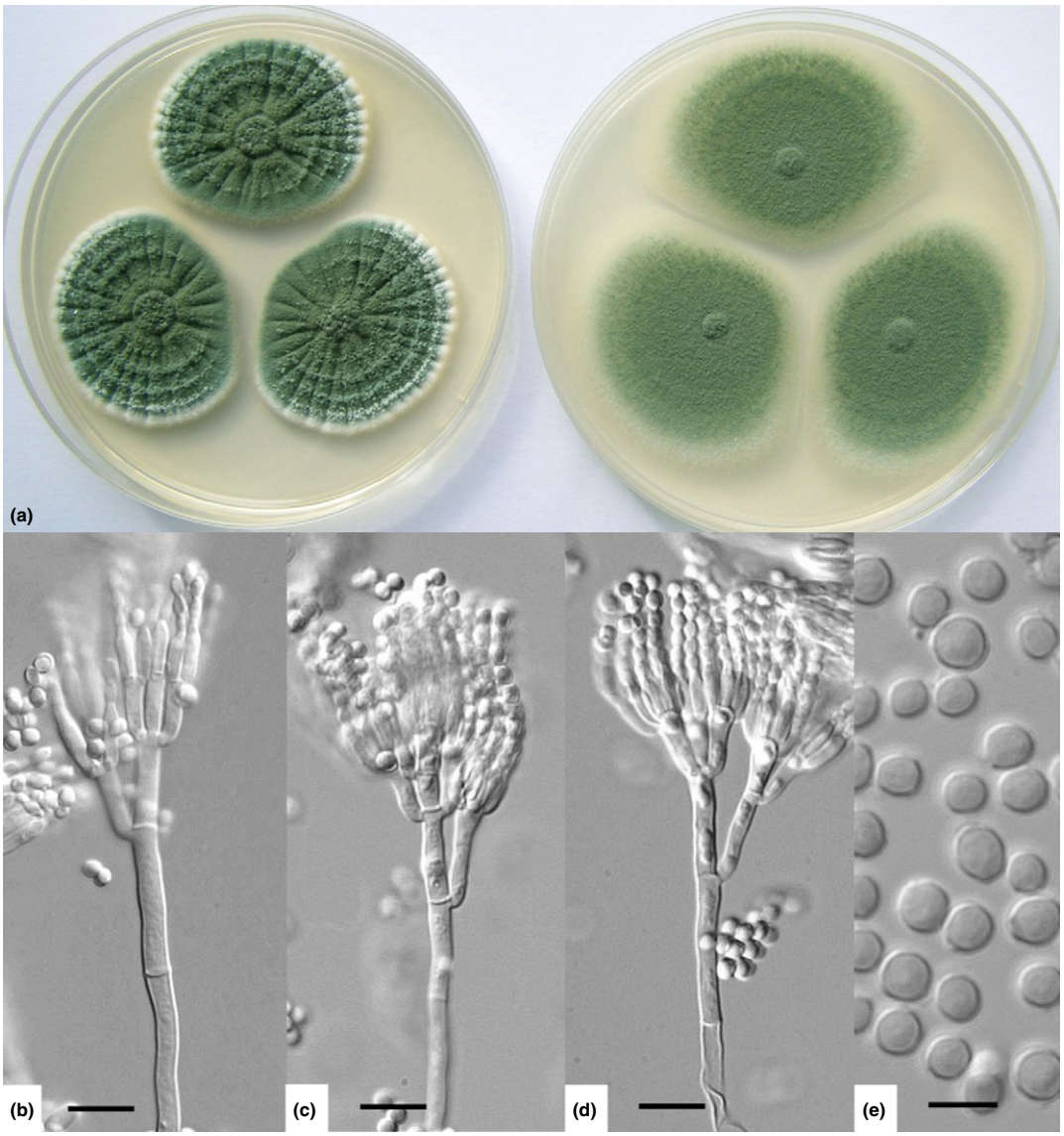

Figure 3 *P. expansum*. (a) Colonies on Czapek yeast extract agar and malt extract agar, 7 days at 25 °C; (b, c, d) fruiting structures, bar = 10 μm; (e) conidia, bar = 5 μm.

Hazard Characterization

Early indications that patulin is carcinogenic or genotoxic have not been confirmed, and effects were only produced at levels much higher than could be expected to occur in foods. However, as many people consume apple juice regularly, chronic toxicity to humans is an area of concern, especially as it is widely consumed by children as well as adults. The FAO/WHO Joint Expert Committee on Food Additives (JECFA) examined evidence for the toxicity of patulin in 1990 and 1995. In agreement with the International Agency for Research on Cancer, JECFA concluded that lack of evidence from studies in experimental animals prevented any evaluation of possible carcinogenicity in humans. However, JECFA noted that in short term studies, patulin could cause gastrointestinal problems, including hyperemia, distension, hemorrhage, or ulceration. The No Effect Level (NOEL) derived from a 13-week study in rats was 0.8 mg kg^{-1} bodyweight per day, based on slight impairment of kidney function and villous hyperemia in mild and high dose groups. The NOEL from a long term study of reproductive toxicity or carcinogenicity in rats was 43 μg kg^{-1} bodyweight per day. Doses above 1.5 mg kg^{-1} bodyweight per day produced maternal toxicity and an increase in the frequency of fetal resorptions, indicating that patulin can be embryotoxic at high dose levels, well above those seen in foods. Patulin also appears to have immunosuppressive properties at high doses. JECFA also concluded that patulin can be genotoxic, though data were variable and differences were observed between bacterial and mammalian cells.

JECFA established a provisional maximum tolerable daily intake (PTDI) of 0.4 μg kg^{-1} bodyweight per day.

Exposure Assessment and Risk Characterization

From the results of the US food surveys in 1994–96, the US Food and Drug Administration examined results from nearly 3000 samples of apple juice, both for sale as fresh juice and as ingredients for other foods. It was found that the mean patulin exposure for all age groups, 1–2 year old children, and children under 1 year was 0.14, 0.80, and 0.21 $\mu g\,kg^{-1}$ bodyweight per day, respectively. At the 90th percentile of exposure, the figures were 0.26, 1.7, and 0.40 $\mu g\,kg^{-1}$ bodyweight per day, respectively. Figures for 1–2 year old children exceeded the PTDI. However, if a limit of 50 $\mu g\,l^{-1}$ of patulin in apple juice was enforced, the figures for the 90th percentile of exposure were 0.10, 0.67, and 0.27 $\mu g\,kg^{-1}$ bodyweight per day, respectively. The figure for young children still exceeded the PTDI, but because of safety factors in that figure, intake levels would still be many times lower than the NOEL calculated by the JECFA.

In consequence, a regulatory limit of 50 $\mu g\,l^{-1}$ for patulin in apple juice and other apple products is recommended by the Codex Alimentarius Commission, and because of that adopted by a number of countries. Many others, including Canada and Australia, have not set limits. Two points are worth noting. First, in contrast to the other major mycotoxins, there have been no reported outbreaks in human or domestic animals associated with the ingestion of apple products containing patulin. Second, patulin intake is highest in temperate zone countries. As apples are a cool climate crop, patulin is more likely to be found in products in temperate zone countries than those in tropical regions.

Chemical Analysis

Although the determination of patulin levels in juices can be carried out by thin layer chromatography (TLC), it is recommended that high performance liquid chromatography (HPLC) be used where possible. TLC methods are imprecise because the recommended extraction by ethyl acetate also extracts other compounds such as 5-hydroxymethylfurfural, and the visualization methods are also relatively insensitive.

Risk Management

Although patulin is produced by a number of fungal species, the only practical concern is that from apple juice. In good commercial practice, rots, which contain nearly all of the patulin, are removed by hand culling, by water flumes or, most effectively, by high-pressure water jets before the apples are crushed. These measures provide effective control. Some patulin migrates from the rots to sound tissue, but residual levels are low. Fermentation of apple juice to produce alcoholic cider has been shown to reduce patulin levels by 90% or more. It is notable that in Australia, regulators use excessive patulin levels as an indicator of the use of poor quality raw materials in juice manufacture, rather than seeking prosecution for excessive levels of the mycotoxin.

See also: Foodborne Diseases: Overview of Chemical, Physical, and Other Significant Hazards

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MYCOTOXINS

Zearalenone

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Chemical Characterization

Zearalenone (ZEA) belongs to a large family of fungal metabolites described as the resorcylic acid lactone group. It has the formula 6-(10-hydroxy-6-oxo-*trans*-1-undecenyl)-*β*-resorcylic acid lactone, with a molecular formula of $C_{18}H_{22}O_5$ and a molecular weight of 318 (Figure 1). Its Chemical Abstracts Service (CAS) registry number is 17924-92-4.

Fungal Sources

ZEA is produced by the same *Fusarium* species that produces trichothecenes deoxynivalenol (DON) and nivalenol, i.e., *Fusarium graminearum* and *Fusarium culmorum*, and also by *Fusarium crookwellense* and some strains of *Fusarium equiseti* and *Fusarium semitectum*. Generally speaking, its formation occurs under the same conditions as trichothecene production, and the main sources are maize and small grains. As known so far, the conditions for ZEA production seen in these commodities are similar to trichothecene production, i.e., before harvest, mostly at temperatures between 10 and 30 °C and above 0.90 water activity.

History

In the 1950s and 1960s, some US states experienced severe epidemics of maize ear rot. When farmers used contaminated maize as a feed for pigs, a feed refusal and vomiting syndrome became evident, which led to the discovery of DON. Consumption of contaminated maize was also found to be associated with estrogenic effects in pigs. In females, symptoms included enlargement of mammary glands and genital organs, ovary atrophy, infertility, reduced litter size, and reduced piglet weight. In males, contaminated feed was associated with enlargement of mammary glands and atrophy of testes. In 1962, the estrogenic syndrome was reproduced in pigs by feeding maize on which a pure culture of *F. graminearum* had been grown. The syndrome was found to occur in mice also and the pure compound was characterized in 1966.

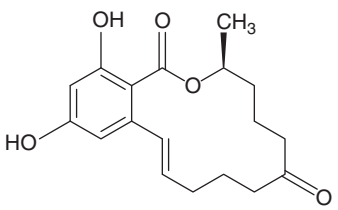

Figure 1 The chemical structure for ZEA. It has the formula 6-(10-hydroxy-6-oxo-*trans*-1-undecenyl)-*β*-resorcylic acid lactone, with a molecular formula of $C_{18}H_{22}O_5$.

Toxicology

ZEA has a low acute toxicity, a 50% lethal dose in rats and chickens exceeding 2 g kg^{-1} bodyweight, administered orally. However, much lower levels of ZEA and its metabolites possess estrogenic activity in experimental and farm animals. The most obvious problems are seen in pigs: doses of ZEA as low as 1–5 mg kg^{-1} can induce vulvovaginitis and vaginal and rectal prolapse in young female pigs. Ruminants, sheep and cattle, are more resistant, and it seems likely that rumen microorganisms are responsible for metabolizing ZEA to compounds of lower toxicity. Chickens are also comparatively resistant, tolerating up to 30 mg kg^{-1} ZEA in feed.

In one set of experiments in mice, ZEA ingestion resulted in an increased incidence of liver and pituitary tumors, consistent with a hormonal carcinogenic action. However, no effects were seen in rats, and the International Agency for Research on Cancer considered the evidence for animal carcinogenicity was limited. No studies on human carcinogenicity have been reported. The nature of its toxicity is not completely understood.

The metabolism of ZEA involves reduction of the 6-keto group, resulting in the formation of α- and *β*-zearalenol. α-Zearalenol has a greater estrogenic activity than ZEA. Some evidence has been put forward that ZEA is associated with the early onset of puberty and advanced growth of girls in Puerto Rico, Hungary, and Italy. However, its importance in human health is still poorly understood.

Both the Joint FAO/WHO Expert Committee on Food Additives and European Scientific Committee for Food have established a provisional maximum tolerable daily intake of ZEA and its metabolites at 0.5 μg per kg bodyweight per day.

Chemical Analysis

As with other mycotoxins, ZEA sampling should be conducted systematically to obtain representative samples. Samples are extracted with aqueous mixtures of methanol, acetonitrile, or ethyl acetate, followed by clean up. ZEA analyses can be

carried out effectively by thin layer chromatography, but high-performance liquid chromatography (HPLC) with fluorescence detection is now widely used. However, because of false positive results, HPLC coupled to mass spectroscopy is used to quantify and confirm the presence of ZEA in commodities. Rapid screening tests based on ZEA antibodies are also commercially available.

Occurrence and Regulation

ZEA occurs in the same situations as DON, i.e., as the result of growth of certain *Fusarium* species on maize and small grains. ZEA occurs in all regions of the world, but is less commonly found than DON. Levels in foods are usually less than 1 mg kg^{-1} in good quality grain, but in moldy material, concentrations of 10 mg kg^{-1} or more may occur. As with DON, levels are higher in crops grown in cooler, damper climates.

No internationally recognized limits for ZEA exist, with country regulations varying from 20 to 1000 μg kg^{-1} of food or animal feed, sometimes even higher.

Management

Control of ZEA in crops is similar to that for DON. However, as DON is the more potent compound, ZEA control is likely to be incidental.

See also: Environmental Contaminants: Environmental Estrogens – Hazard Characterization. Foodborne Diseases: Overview of Chemical, Physical, and Other Significant Hazards. Mycotoxins: Deoxynivalenol and Other Trichothecenes. Veterinary Drugs Residues: Anabolics

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ENVIRONMENTAL CONTAMINANTS

Contents

Dioxins, Furans, and Dioxin-like Polychlorinated Biphenyls

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Introduction

Polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans, and dioxin-like polychlorinated biphenyls (PCDD/Fs and dl-PCBs, 'dioxins' – Figure 1) are ubiquitously present in the environment and in human tissues even when there is no history of occupational or accidental exposure. Although exposure could occur through inhalation of air, dermal absorption, consumption of drinking water, and consumption of food, there is no doubt that food is by far the most significant input for most of the population where there is no history of occupational exposure. It has been estimated that dietary intake of 2,3,7,8-TCDD accounts for as much as 98% of human exposure. Over the past 15–20 years, many measurements have been made of dioxins in foods, which all lead to the conclusion that over 90% human exposure is normally from food.

Although the original focus was on just the PCDD/Fs, over the past decade or so, it has also become generally recognized that some of the PCBs also bind to the Ah-receptor and elicit dioxin-like biochemical and toxic responses, so that assessment of the health risks of exposure to dioxin-like chemicals must consider these PCBs as well as PCDD/Fs. More recently, attention is being given to other emerging contaminants such as brominated dioxins and biphenyls, polychlorinated (and brominated) naphthalenes, and other compounds which also might elicit dioxin-like toxicity. It is possible that bromine-containing compounds may become more important in time as a result of increased use of bromine in products such as flame retardants, and this use may be linked with increasing amount of bromine-containing chemicals with dioxin-like activity being released into the environment and thereby into our food and bodies.

This section addresses topics such as levels of dioxins in foods, the resulting intakes, geographical variations, trends over time, and the pathways by which PCDD/Fs and PCBs are transferred from the environment to the food chain. Some factors that influence the accuracy and comparability of analytical data are also discussed.

Toxicology and Health Consequences

Dioxins are carcinogenic and can also cause adverse developmental and reproductive effects. Their negative health impacts are linked to their metabolic resistance and lipophilic nature, i.e., their capacity to accumulate in fat tissue in animals and humans. Dioxin-like compounds are known endocrine disruptors. These adverse health impacts have not only been demonstrated by animal studies, but also been observed following accidental and significant short-term human exposure to dioxins. As with other pollutants, the developing fetus and also newborns are most sensitive to dioxin exposure (see World Health Organization (WHO) Fact Sheet No. 225 (2007) on 'Dioxins and their effects on human health').

All humans are faced with a certain 'background' exposure to dioxins. More than 90% of human exposure to dioxins typically occurs through the food supply, mainly fish, meat, and dairy products. Some groups of individuals experience

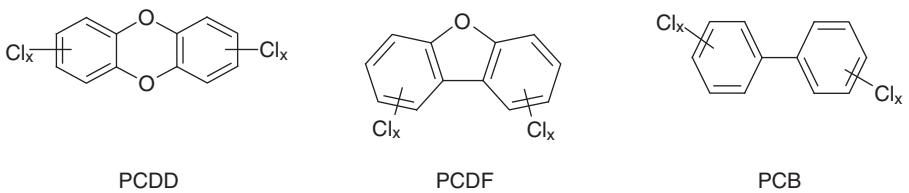

Figure 1 Structures of PCDDs, PCDFs, and PCBs.

higher exposure due to their occupation or because of their diets. Consumers may reduce their own exposure to dioxins by keeping a balanced and varied diet and limiting oily fish, liver, dairy, and other foods of animal origin. Individual strategies are, however, contentious, as oily fish, for example, is not only considered especially important for a healthy diet but also ranks among the foodstuffs most affected by dioxin contamination due to the pollution of the rivers and seas. This has been the subject of risk–benefit analysis, including that done by the UK Scientific Advisory Committee on Nutrition.

Analytical Methods

Analytical methods for dioxins fall into one of two broad categories: screening methods such as the chemically-activated luciferase expression (CALUX) bioassay and confirmatory methods such as gas chromatography coupled to high-resolution mass spectrometry (GC–HRMS). Both need extensive sample extraction and clean-up before they can be used. The CALUX bioassay uses a genetically modified cell line (rat hepatoma H4IIE GudLuc 1.1) that expresses the firefly luciferase on exposure to dioxins or dioxin-like compounds. The amount of luciferase is related to the amount of dioxin-like compounds in the exposure mixture and can be easily quantified with a luminometer. Confirmatory techniques using GC–HRMS involve adding known quantities of standards labeled with stable isotopes to the sample before analysis. Sample extracts are separated using GC and exact ratios of the compounds of interest and the known amount of isotopically labeled standard that was added are measured using HRMS.

Treatment and Interpretation of Analytical Data

Anyone who uses analytical data on dioxin-like chemicals, or the intake assessments derived from them, needs to remain aware of various issues that may influence the accuracy and comparability of the results. Even in some recent studies there are undoubtedly some issues related to the representativeness of sampling and to the accuracy of measured concentrations. Furthermore, interpretation of reported data is complicated by the differing ways in which compounds that are not actually detected and measured are represented in summations or averages.

Toxic Equivalency Factors

Because of the need to assess the risk from complex mixtures of dioxins, an approach has been adopted that assigns a factor relating to the relative potency of each congener, based on a comparison with the toxicity of 2,3,7,8-TCDD. Each chemical is assigned a toxic equivalency factor (TEF) related to the most toxic of the dioxins, 2,3,7,8-TCDD, which is given a TEF of 1. The total toxic equivalency of a mixture is the sum of the TEF-weighted concentration of each compound in the mixture, but there is some variation in the terminology used by different authors; total toxic equivalence (TEQ), summed TEQ, and Σ TEQ are self-explanatory, whereas the unqualified acronym, TEQ, is used by different authors to refer either to the total or to the TEF-weighted concentrations of individual compounds.

$$\text{TEQ} = \text{S}[\text{PCDD}_\text{i} \times \text{TEF}_\text{i}] + \text{S}[\text{PCDF}_\text{i} \times \text{TEF}_\text{i}] + \text{S}[\text{PCB}_\text{i} \times \text{TEF}_\text{i}]$$

Although consideration of total TEQs is essential, it is regrettable that many data are easily available only in this form. As discussed in the Section on PCDDs and PCDFs, total TEQ figures can have deficiencies and their interpretation is greatly facilitated by inspection of the underlying congener-specific data, especially when data from different laboratories are compared. The contribution of specific congeners to the total gives a particular pattern or 'fingerprint' which can be of great value for source identification during specific contamination incidents.

PCDDs and PCDFs

During the 1980s, a number of different TEF schemes were used. International toxic equivalency factors (I-TEFs) for PCDD/Fs were set in 1990 and were adopted by almost all scientists and regulatory authorities. A more recent system of TEFs, including TEFs for the dioxin-like PCBs (see Significance of TEFs for PCBs), set by the WHO in 1997 (WHO-TEFs) has been accepted by most authorities. This system is subject to periodic revision in light of new information about the toxicology of the various congeners, and revised TEFs were proposed in 2005. These have still not been incorporated into legislation. Previous TEFs were assigned in increments of 0.01, 0.05, 0.1, etc., but for this re-evaluation, it was decided to use half order of magnitude increments on a logarithmic scale of 0.03, 0.1, 0.3, etc. Changes were decided by the WHO expert panel for 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) (TEF 0.5→0.3), 1,2,3,7,8-pentachlorodibenzofuran (PeCDF) (TEF 0.05→0.03), octachlorodibenzo-p-dioxin and octachlorodibenzofuran (TEFs 0.0001→0.0003), 3,4,4#,5-tetrachlorbiphenyl (PCB81) (TEF 0.0001→0.0003), and 3,3#,4,4#,5,5#-hexachlorobiphenyl (PCB169) (TEF 0.01→0.03), and a single TEF value (0.00003) was given for all relevant mono-ortho-substituted PCBs. The idea is that TEFs will be subject to periodic review and new candidates with a similar mode of toxic action may be included in the scheme in future.

Significance of TEFs for PCBs

In many food samples, such as some fish, the TEQ contribution made by PCBs may equal or exceed that made by PCDD/Fs. In comparing or interpreting data expressed as TEQs, it is of paramount importance to note whether or not PCBs are included in the total.

It must be remembered that other PCBs have a variety of other biological effects; although consideration of 'dioxins' is incomplete without the inclusion of dioxin-like PCBs, this treatment is certainly not a sufficient response to PCBs in general.

Limits of Detection

In several types of food, it is common for the concentrations of some of the individual dioxins and PCBs to be below the analytical limits of detection (LOD). Inevitably, the sensitivity

of analyses in different laboratories varies, and so does the method of assessing and reporting the LOD. Frequently, quantitative results are reported for any congener giving a GC–HRMS peak with a signal-to-noise ratio (S/N) of, say, 3 or more. If no peak is detected, then an estimate is made of the concentration that would have produced this S/N. LODs so estimated vary between congeners and between different analyses and even between different times. Some laboratories work, instead, to a 'limit of quantitation' (LOQ) which, strictly, should be defined as the lowest concentration at which a specified measurement precision is achieved, as demonstrated in method validation studies, but which is, instead, sometimes taken to be a multiple of the LOD. Alternatively, some data are reported after applying an arbitrary, but consistent, 'reporting limit.' Different laboratories analyzing the same samples, or even interpreting the same raw data, may arrive at differing conclusions about which congeners are present at measurable concentrations, and at quite different LOD values for other congeners, even though their 'positive' results may be in good agreement.

When data include nondetects, then at least three different methods are in use for the calculation of total TEQ concentrations. These are the representation of nondetected congeners in subsequent calculations by: (1) a concentration of 0; (2) a concentration equal to the LOD; or (3) a concentration equal to $0.5 \times$ LOD. Even this may expand to five different methods if LODs and LOQs are differentiated.

The terms 'lower-bound' and 'upper-bound' are generally used to refer to the first two of these methods, which correspond, respectively, to the minimum concentration 'known' to be present and to a larger concentration which 'might' be present. The $0.5 \times$ LOD calculation gives a result which lies midway between these upper and lower bounds, and is sometimes referred to as 'median bound.' This often gives the appearance of better comparability, but if the upper-bound and lower-bound totals are far apart, as is sometimes the case, then the mid-point is not necessarily any closer to the true total TEQ concentration than is either extreme. Use of the $0.5 \times$ LOD convention in calculations from data in which the LOD itself varies between analyses can be particularly misleading.

Further complications may arise when results for specific congeners are averaged across a number of samples, with the total TEQ summation made using the average concentrations. In addition to using all of the above substitution approaches, some workers average only detected values to generate a statistic which is meaningless unless the frequency of detection is also taken into account.

All of these approaches have utility in some circumstances, if applied consistently, but comparisons of total TEQ data representing different detection limits and calculation methods can be extremely misleading.

Analytical Accuracy and Precision

All analytical measurements have, associated with them, a measurement uncertainty. Unfortunately, information on the accuracy and precision of measurements is often absent from reports of data on dioxins. The analysis of these substances in foods is particularly challenging because of the very low concentrations that are involved. Several studies have shown that good agreement can be achieved by highly expert laboratories, but the number of laboratories engaged in these analyses has increased dramatically in the past few years and there is a great variation in the quality of analysis available.

There exist a number of schemes that can be used to assess the performance of a laboratory. The largest of these schemes is the interlaboratory studies organized on an annual basis by the Norwegian Institute of Public Health. It can be seen that over the past decade the scheme has been in operation, the number of laboratories that participate has increased, and performance has generally improved. Samples with low amounts of dioxins represent the biggest challenge. There are also proficiency testing schemes organized by FAPAS (Food Analysis Performance Assessment Scheme) and a closed scheme organized by the EU Reference Laboratory where there is an obligation for National Reference Laboratories within the EU to take part.

Representativeness

The most accurate laboratory analysis can only give a result that represents the sample taken for analysis. How that sample is related to the broad food supply depends on both the quality and the intent of the sampling scheme.

Sampling to assess compliance with limits, whether statutory or guidelines, may not be appropriate for estimation of average intakes. For example, many studies have concentrated on domestic food production and have not included imports, or aim for broad geographical coverage without weighting by food production statistics. Any indication of localized contamination usually leads to more intensive sampling in the same location; small but significantly contaminated locations may, therefore, make a disproportionately large contribution to 'average' concentrations if these are based on all available data. Dietary habits and food consumption patterns may vary regionally and this should also be taken into account, for example, communities near the sea may consume more fish, and this may be caught locally. Some individuals may also have different dietary habits such as vegetarians, those growing their own produce, or those working in an industry giving free or cheaper access to certain food types. Food consumed outside the home, for example, in restaurants or staff canteens also needs to be considered.

For most foodstuffs, achievement of a representative result inescapably necessitates coverage of a large number of samples. However, because of the cost and difficulty of analysis, many studies are based on rather limited numbers of samples. Composite samples formed from many samples of each category of foodstuff are sometimes used; such pooling is usually an integral part of total diet study (TDS) schemes (see The UK TDS). The use of composite samples can be a cost-effective way of obtaining robust measures of average concentrations, but it does not furnish any information on the width and shape of the distribution of concentrations in the individual samples.

A further source of confusion is the varied definition of some terms used in food consumption surveys and databases.

The word 'cereal' in some reports includes simply grain and flour, in others cereal products which may include breads, cakes, and pastries prepared with animal fats, and sometimes various 'breakfast foods.'

Methods for Estimating Dietary Exposure

The most usual method of assessing average dietary intake is to multiply the consumption of each type of food by the concentrations found in corresponding food samples, and to add together the contributions from various components of the diet. For simple population averages, average concentration data can be combined with average food consumption data. For high consumers of particular foods that may be affected by contamination, for example, high fish eaters, then the average concentration data can be combined with high food consumption, for example, at the 95th or 97.5th percentile consumption of particular foods. If high food consumption could also be linked to contamination of hot-spots, for example, high eaters of fish caught locally from polluted waters, then it may be appropriate to combine high concentration data with high percentile consumption data.

A further option is the analysis of duplicate diets, whereby volunteers collect a duplicate portion of all food they consume over a period of time and this is used to produce a composite sample representing the diet.

There is a large amount of data available now for Europe, North America, Australia, and parts of Asia. Gaps still exist for Africa, and parts of Asia and South America, where relatively few studies have been conducted.

Tolerable Intakes

During the 1980s and early 1990s, a number of countries performed risk assessments and derived tolerable daily intakes (TDIs) of dioxins in the range of 1–10 pg kg^{-1} body weight (bw). A TDI is the maximum amount of a contaminant which can be eaten every day over a whole lifetime without incurring appreciable risk to health.

As the data on aspects of the toxicology of PCDD/Fs and PCBs have become more extensive and of better quality, views about the appropriate value of a TDI changed and values resulting from different assessments have become more consistent.

The WHO in 1998 concluded that the TDI was in the range of 1–4 pg TEQ kg^{-1} bw. At the end of May 2001, the Scientific Committee on Food (SCF), an expert committee that advises the European Commission, decided that the tolerable intake should be expressed on a weekly rather than a daily basis and set a tolerable weekly intake (TWI) of 14 pg WHO-TEQ kg^{-1} bw. The WHO/FAO Joint Expert Committee on Food Additives (JECFA) established a provisional tolerable monthly intake (PTMI) of 70 pg kg^{-1} bw per month in June 2001. These values are all of the same approximate value and are consistent with one another.

Legislation

The EU was the first body to set extensive and comprehensive limits for PCDD/Fs, and there is still little other legislation in place world-wide. Regulations came into force in July 2002 and included limits for PCDD/Fs in food and animal feed. Dioxin-like PCBs were later included in 2006. Proposals exist to include nondioxin-like PCBs and these are likely to be regulated later in 2010 or in 2011. This regulation was initially supported by a monitoring plan, which was implemented by all member states. The regulation is supported by strict performance criteria for analytical methods that are used and this is detailed in other legislation. Upper-bound results are used for monitoring purposes and for official control (see Limits of Detection).

The UK TDS

The TDS is a continuous market basket-type survey in which foods representing the average UK diet (based on Defra's Expenditure and Food Survey and trade statistics) are purchased, prepared, and combined into groups of similar foods for analysis. The TDS has been run since 1966 and has been used as a part of the UK monitoring program for chemicals in food. It allows the general UK population's average exposure to nonnutrients (i.e., contaminants such as heavy metals, dioxins, and pesticides), as well as intakes of some nutrients to be estimated. Used in conjunction with the Expenditure and Food Survey, the TDS has enabled trends over time to be established and assessments on the safety and quality of the food supply to be made.

Food samples representative of the UK diet are purchased throughout the year in 24 towns covering the UK, and 119 categories of foods are combined into 20 groups of similar foods (e.g., bread, poultry, milk, etc.) for analysis. The relative proportion of each food category within a group reflects its importance in the average UK household diet. Foods are grouped so that commodities known to be susceptible to contamination (e.g., offal and fish) are kept separate, as are foods which are consumed in large quantities (e.g., bread, potatoes, and milk). The quantities and relative proportions of each food that make up the total diet are largely based on data from the Expenditure and Food Survey and are updated annually to reflect changing eating habits.

Vegetables, Fruits, Pulses, and Grain

Growing plants may be exposed to PCDD/Fs and PCBs via soil, ground-water, and the air. With the exception of the *Cucurbitaceae* family (which includes zucchini or courgette) in which some uptake from soil has been demonstrated, absorption of dioxin-like compounds through plant roots and subsequent translocation does not occur to any significant extent. The outer layers of root crops may become contaminated by direct contact with soil particles, but this will normally be removed by peeling or washing. Contamination of the above-ground part of plants is considered to result largely from retention of airborne PCDD/Fs and PCBs, which

may include absorption from the vapor-phase by the waxy cuticle and retention of particulate-bound contaminants. The highest levels are therefore expected when a convoluted surface of high surface area is combined with a pronounced waxy cuticle.

Although contamination of plant material consumed by food-producing animals is a major part of the pathway from primary source to human dietary exposure, levels in vegetables, fruits, and grains consumed by humans are very low, immeasurably so for many laboratories. Many otherwise broadly based food surveys have omitted analysis of fruits and vegetables, either because of the assumption that their contribution to intake would be insignificant compared with that of fatty foods, or because of the very real analytical difficulties presented by the low concentrations of contaminants present. A number of intake assessments do, however, include quite large contributions for fruits and vegetables.

Data that have been reported need to be viewed with particular care because of issues of representativeness, detection limit, and accuracy. In the latter context, in contrast to animal tissues in which only the 2,3,7,8-substituted PCDD/Fs are found, many other congeners will be present in deposits on vegetation, at higher concentrations than those that contribute to the TEQ, and the concentrations in general will be lower because there is no bioaccumulation. Obtaining the required congener-specificity and sensitivity in the analysis becomes much more difficult in this case.

Animal Products

The main route for exposure of most food-producing animals to PCDD/Fs and PCBs is through their feed. Considerable progress has been made in understanding and modeling agricultural food chain accumulation, in which, for background contamination, the dominant pathway is that of atmospheric distribution and deposition onto vegetation. There is abundant evidence that proximity to point sources of atmospheric PCDD/Fs, such as incinerators operating below recommended temperatures and metal reclamation sites, can lead to markedly raised levels in milk and meat.

There have been a number of recent examples of high levels of contamination in manufactured feeds being carried over into foods. In Europe, much analytical and regulatory attention is now focused on animal feeds but little information is available on the relative importance, under 'background' conditions, of manufactured feeds versus atmospheric distribution.

There is some possibility that the use of sewage sludge for soil amendment could result in increased exposure of livestock, but recent experimental evidence indicates that carry-over of PCDD/Fs entering the feed as a result of sewage sludge fertilization is not significantly different from that for feed containing background levels of PCDD/Fs of atmospheric origin.

The use of pentachlorophenol-treated timber in animal housings has occasionally been found to result in increased levels of PCDD/Fs in cattle, and this was also a possibility for the recent contamination event associated with guar gum produced in India where the product was stored on treated pallets.

Milk and Milk Products

Excretion of PCDD/Fs and PCBs in milk is the main elimination pathway for these compounds in lactating cows. Since the first reports of the detection of PCDD/Fs in cow's milk in 1987, many surveys have been reported and probably more data have been generated on milk than any other foodstuff. Because milk from many animals is usually mixed together in the farm and then mixed with milk from further farms at the dairy, difficulties with representativeness of sampling are less than with other foods, and by sampling individual farms or individual dairies, data representing different sized areas can be obtained.

PCDD/Fs and PCBs are contained entirely in the milk fat. When expressed on a fat basis, milk products contain the same concentrations as the milk from which they were produced. Differences between milk and butter or cheese, reported in several surveys, may arise partly from issues of accuracy and representativeness, but may also occur because milk products are exported and imported to a greater extent and over greater distances than milk itself. Consequently, locally representative data for milk are not necessarily representative of milk products. A number of studies from the late 1980s and early 1990s demonstrated a marked localized influence on levels in milk produced in the vicinity of some incinerators and other point sources.

In terms of contribution to dietary intake, the estimated contribution from milk varies from approximately 15 to 40%.

Butter has been used as an integrative matrix to assess the difference in concentrations between global regions. This can be done because of the way milk is pooled before butter is made. In general, lower concentrations are found in butter from the Southern Hemisphere than in butter from more industrialized regions of the Northern Hemisphere.

Meat

Meat is a more heterogeneous group than milk and milk products and there are considerable differences in the species consumed by different ethnic groups and individuals. The 'meat products' classification included in a number of studies is poorly defined both in terms of the type and the proportion of meat included, and the representativeness of the resulting data is sometimes questionable.

Pork is usually found to have considerably lower levels of both PCDD/Fs and PCBs than beef. There is limited information on meat from other species.

Offal: There is increasing evidence that liver produced from farm animals such as cows, pigs, and sheep may contain elevated dioxin concentrations and exceed limits even when the livestock are given compliant animal feed and are exposed only to normal background levels of dioxin contamination in the environment. The dioxin concentrations in other commonly consumed tissues such as muscle, fat, kidney, etc., from the same animals will typically be well within regulatory limits. Because the limits were set on the basis of excluding the most contaminated foods from entering the food chain rather than on the basis of any health risk, they are under review and may be adjusted in light of this.

Eggs

It has been shown that PCDD/F concentrations in eggs depend on the type of housing; elevated wire cages gave the lowest concentrations whereas access to soil gave higher concentrations. This apparently results from intake of soil and organisms such as insects and annelids in which PCDD/Fs accumulate.

Fish

Although PCDD/Fs and PCBs are usually present in aquatic systems only at very low concentrations, bioaccumulation can result in significant concentrations in fish. As with terrestrial animals, the 2,3,7,8 substituted PCDD/F congeners dominate the pattern found in fish although this is not true of crustaceans and shellfish.

Fish comprise by far the most inhomogeneous food group due to the large number of different species used as food, with widely varying fat contents, different trophic positions, and the great variety of fishing grounds. In general, concentrations of chemicals such as PCDD/Fs and PCBs in fish depend on their fat contents, the extent to which the fish migrate, the number of times they spawn, and their ages, size, and feeding habits. For example, plaice are bottom-feeding fish and therefore may be more exposed to PCDD/Fs and PCBs bound to sediment. Herring has a relatively high fat content and is nonmigratory, which renders it more subject to localized contamination sources.

Consequently, the concentrations of PCDD/Fs and of PCBs are very varied. Because of the large seasonal variation in fat content of most fish, data are often expressed on a whole product basis, but units based on fat are also used, particularly when different species with different average fat contents are to be compared.

Certain fish species originating from the Baltic region are recognized as containing a high concentration of PCDD/Fs and PCBs. A significant proportion of oily fish from this region such as Baltic herring and Baltic salmon are unlikely to comply with the EU limit for PCDD/Fs, and this fish would, therefore, be excluded from the Swedish and Finnish diet. There are indications that such exclusion would have a negative health impact in Sweden and Finland, and consequently there is a local exemption to compliance with the legislation. Sweden and Finland have in place a system which informs consumers about the dietary recommendations for the consumption of fish from the Baltic region in order to avoid potential health risks.

Farmed fish are often fed on feed which contains fish meal. This has led to concerns that there is a possibility of biomagnification of PCDD/Fs and PCBs which are prevalent in fish, and there have been studies conducted that compare concentrations of these contaminants in farmed fish with wild fish of the same variety. Generally small differences have been found although these fall within the range of differences seen between different fish species.

River fish have been subject of more recent investigation partly because rivers are not usually as clean as the sea, and partly because there is an indication that people in some parts of the world are eating more river fish.

Miscellaneous Oils and Fats

Additional intake of fats arises from their use in cooking, or for direct consumption. The PCDD/F and PCB content of vegetable oils, as expected, has been found to be small but not negligible. Animal fats such as butter or lard contain higher concentrations of contaminants.

Food Processing and Cooking

PCDD/Fs and PCBs are stable chemicals that require temperatures far in excess of those encountered in cooking for their destruction. Decreases in concentration in meat during cooking have been observed. Thus in one study, pan-frying hamburger patties were found to reduce the amount of PCDD/Fs actually consumed by 40–50% if the pan fats and juices were discarded. In another, beef with PCDD/F incurred in an animal feeding trial was fried, grilled, barbecued, roasted using conventional and microwave ovens, and stewed in an open pan and pressure cooked. Concentration changes, on a whole product basis, were observed between raw and cooked product. However, in all cases these could be explained simply through changes arising from loss of water and elimination of PCDD/Fs with released fat. The total amounts of PCDD/Fs present in the system (including released fat) remained unchanged. Dietary intake of PCDD/Fs could, therefore, be reduced by the removal of visible fat from meat before cooking and by discarding any fat released from foods during the cooking process.

Reduction in the levels remaining in the tissue has also been reported for fish. It has also been demonstrated that removal of the skin from carp reduces the concentration of PCBs in the carp by approximately 50%.

Time Trends

In view of the great efforts made to identify and control or eliminate sources of PCDD/Fs, it would be both surprising and disappointing if a decrease in levels in foods was not evident, and such a decrease is readily apparent in data from several countries. A reduction, from a peak in the late 1960s to early 1970s, is also evident in human milk and human blood as well as in environmental samples. Levels of dioxin-like PCBs are also declining, but probably at a slower rate than those of PCDD/Fs, so that the proportion of the total TEQ intake attributable to PCBs is increasing. This presumably stems from the fact that PCB production ceased many years before effective control of PCDD/F formation in incineration, pulp bleaching, and other processes was achieved.

A decrease in intake can involve two contributions: first, from a decrease in the amount found in foods themselves, and second, it can arise from a shift in food consumption habits.

Animal Feeding Stuffs

Contamination of feeding stuffs has resulted in several recent 'incidents' which have precipitated considerable regulatory action in the EU.

In 1997, food control laboratories in Germany noticed a reversal of the downward trend in PCDD/Fs levels in milk, with average concentrations increasing over a short period from approximately 0.6 pg TEQ g^{-1} fat to 1.41 pg TEQ g^{-1} fat. An individual sample of milk was found to contain 7.86 pg TEQ g^{-1} fat. This increase was traced back to contaminated feed, and within the compound feed to the incorporation of Brazilian citrus pulp pellets. In the production of these pellets, approximately 2% lime is added and the most probable source of contamination was traced to be the use of a particular lime product formed as a byproduct in a chemical process.

The Belgian incident, which has been described in great detail, gained international notoriety. In March 1999, reduced hatch rates and increased mortality was observed in chickens. Some 2 months later, analysis showed high levels of PCDD/Fs in feeding stuffs and hens. It was eventually discovered that these were a concomitant of PCB contamination of fat. It has subsequently been established that approximately 25 l of PCB-containing transformer oil somehow became a contaminant of approximately 100 t of animal fat that was being recycled. Most of the fat was used to produce poultry feed, and some other to produce animal feeds. After incorporation into feeds, this affected some 20 000 t of poultry feed, 6000 t pig feed, and 400 t cattle feed, the former being contaminated at 811 ng WHO-TEQ kg^{-1} product. In two egg samples, concentrations as high as 266 and 713 pg WHO-TEQ g^{-1} fat were measured.

A further recent example of feed contamination, also discovered in Germany, was traced to the use of PCDD/F contaminated sawdust as a carrier for the incorporation of choline chloride into the feed.

More recently, in September 2008 a feed contamination incident occurred in Ireland, which subsequently led to contamination of pig herds and cattle herds supplied with this feed. The source of contamination was identified to be due to carry over of dioxins and PCBs from contaminated fuel used in a direct drying system in feed production. The animal feed was produced by direct (hot air) drying of raw baker's dough and left over bread products sent for recycling to a licensed feed mill. Two different types of feed materials were produced, bread-crumb, which was produced as pig feed, and biscuit, which was produced as cattle feed. The exhaust from the furnace used for drying, which was fired using contaminated oil, acted as transfer medium and the bread itself acted as filter adsorbing the contaminants from the circulating hot air. The concentrations found on a total TEQ basis ranged from 80 to 200 pg g^{-1} fat in the pork fat samples. Whereas PCBs were the dominant contaminants found, the PCDFs were of most toxicological concern.

Conclusion

PCDD/Fs and dioxin-like PCBs are present in foods throughout the world. Dietary intake is associated primarily with animal fats, offal, and with fish. The spread of concentrations in different examples of most animal products is rather dispersed. However, regionally representative averages are usually somewhat similar.

Most individuals consuming an 'average' mixed diet receive with it an intake of PCDD/Fs and dioxin-like PCBs of approximately 1.2–3.0 pg kg^{-1} bw per day. This is close to the currently recommended TDI, and a proportion of the population exceeds the TDI.

In Europe, the levels of these compounds in food are falling by approximately 50% each decade. It is clear that many adults will have had, for part of their lives, intakes well above current recommendations.

Regulation of primary sources of PCDD/Fs probably accounts for a significant proportion of the gradual decrease in the amount of these compounds in the environment, and the consequent downward trend of concentrations in food. Continued vigilance in the control of these emissions should help to ensure a sustained reduction in exposure.

However, there have been several specific incidents in which PCDD/Fs and PCBs have entered the food supply as a consequence of the use of contaminated animal feed. There could quite possibly have been incidents which have gone unnoticed because of the limited monitoring and surveillance programs that have been in place for these compounds. At the same time, the use of low-grade animal fat in feed can result in the recycling and biomagnification of dioxins and PCBs in food. Regulation of animal feeds, supported by surveillance programs, is therefore of considerable importance if dietary intakes are to be reduced to a minimum.

Individuals who wish to reduce their dietary intake of PCDD/Fs and PCBs can do so by reducing their consumption of animal fat, a choice that can have several health benefits. However, regular consumers of oily fish may receive a considerable part of their exposure to these compounds, possibly most of it, from this part of their diet which also has well-known benefits for health, and it is necessary to put any negative effects from exposure to dioxins and PCBs in context with the health benefits offered by the consumption of fish rich in unsaturated oils.

A holistic approach to food regulation will be increasingly needed in future. Toxicologists, nutritionists, environmental scientists, risk assessors, epidemiologists, analytical chemists, and others must all cooperate to examine the overall picture before decisions on regulation of foods are made.

See also: Disciplines Associated with Food Safety: Epidemiology; Food Safety Toxicology. Environmental Contaminants: Environmental Estrogens – Hazard Characterization. Foodborne Diseases: Overview of Chemical, Physical, and Other Significant Hazards. Institutions Involved in Food Safety: FAO/WHO Codex Alimentarius Commission (CAC); Food and Agriculture Organization of the United Nations (FAO); World Health Organization (WHO). Pesticide Residues: Organochlorines. Public Health Measures: Modern Approach to Food Safety Management: An Overview. Risk Analysis: Risk Communication: Chemical Hazards

Further Reading

Schecter A and Gasiewicz T (eds.) (2003) *Dioxins and Health*, 2nd edn Wiley, 0-471-43355-1.

World Health Organization (WHO) Fact Sheet No 25: Dioxins and their Effect on Human Health. http://www.who.int/mediacentre/factsheets/fs225/en/

Relevant Websites

http://ec.europa.eu/food/food/chemicalsafety/contaminants/dioxins_en.htm
European Commission, DG SANCO.
http://www.efsa.europa.eu/
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Food Standards Agency: Advice on fish consumption: benefits and risks.
http://www.dioxin20xx.org/
International Dioxin Symposium Website – Includes extended abstracts from conferences.

Environmental Estrogens – Hazard Characterization

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Glossary

Endocrine disrupting chemicals (EDCs) Exogenous substances that cause adverse health effects in an intact organism or its progeny consequent to changes in endocrine function.

Estrogens Physiological steroid hormones that regulate female reproductive function.

Metalloestrogens Inorganic xenoestrogens that can mimic or interfere in the action of physiological estrogens.

Phytoestrogens Nonsteroidal organic compounds found in plants that can mimic or interfere in the action of physiological estrogens (*phyto*: from the Greek word for plant).

Xenoestrogens Synthetic man-made organic compounds that can mimic or interfere in the action of physiological estrogens (*xeno*: from the Greek word for foreign).

Introduction

Estrogens are essential steroid hormones, which act at low concentrations as chemical messengers to regulate female reproductive function. The main physiological hormones, estrone (E_1), 17β-estradiol (E_2), and estriol (E_3), are synthesized from androstenedione and testosterone by aromatization in the premenopausal woman in the ovary (Figure 1). At the menopause, synthesis of estrogens by the ovary is reduced but production continues at a lower level from peripheral tissues and adrenal glands. Although estrogens act mainly within female reproductive tissues, they have also been shown

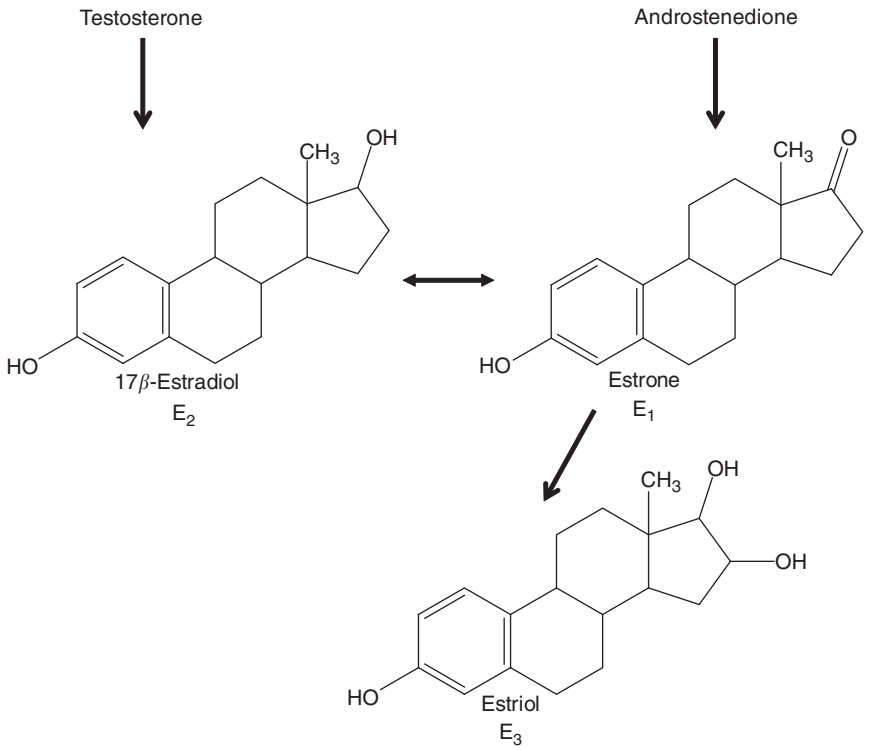

Figure 1 Structures of the physiological estrogens – E_1, E_2, and E_3.

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to influence bone, brain, liver, and the cardiovascular system and furthermore, may also play a role in hormonal signaling in the male as well as the female. Therefore, compounds ingested in the diet, which can mimic or interfere in estrogen action, have the potential to alter hormonal homeostasis in both women and men. Such compounds have been termed 'environmental estrogens'.

The ability of compounds in the diet to influence reproductive functions was first noted in the 1920s by pig farmers after feeding their animals on moldy grain, and concern was further stimulated in the 1940s by reports that sheep grazing on certain types of clover in western Australia became infertile ('clover disease'). More recent research has identified the estrogenic compounds in the moldy grain to be of microbial origin and these have been termed mycoestrogens. The estrogenic compounds in the clover have been identified as of plant origin and subsequently many other estrogenic compounds have been found in plant material and collectively these have been termed phytoestrogens. Over more recent decades, it has become evident that there are also many man-made chemicals, which can mimic estrogen action and enter the food chain from environmental contamination. Such compounds have been released into the environment from agricultural spraying (herbicides and insecticides) or as by-products of industrial processes and waste disposal (polychlorinated biphenyls (PCBs) and dioxin) and have been termed xenoestrogens. Most xenoestrogens are lipophilic and therefore pass up the food chain partitioned in animal fat where they accumulate at the top of the food chain in humans.

Molecular Basis of Estrogen Action

Binding to Cellular Receptors

Estrogens act in a target cell by binding to intracellular receptors, of which there are two types, estrogen receptor alpha (ERα) and ER beta (ERβ). ERα, for which the complementary deoxyribonucleic acid (cDNA) was cloned in 1985, is the so-called classical ER, but was named ERα after the cloning in 1996 of a second cDNA for a separate ER gene named ERβ. The two ER proteins share close homology, and all three physiological estrogens can bind to both albeit with differing affinities. In the 1990s, the crystal structures of these receptors showed that there was a ligand binding pocket within the ER protein, which is separated from the external environment, and 17β-estradiol was shown to be positioned specifically by hydrogen bonding of its 3- and 17-hydroxyl groups to amino acid side chains and by hydrophobic bonding of the remainder of the steroid ring to other amino acid side chains. Such interactions resulted in conformational changes to the ER protein, in particular to the folding of the helix 12 of the ER protein over the opening to the ligand binding pocket, and these changes in conformation resulted in activation of the ER within the cell.

Regulating Gene Expression

The main mechanism of action of ER within the cell resides in its ability to function as a ligand-activated transcription factor and therefore to regulate gene expression. This is termed the genomic mechanism of action (Figure 2). Binding

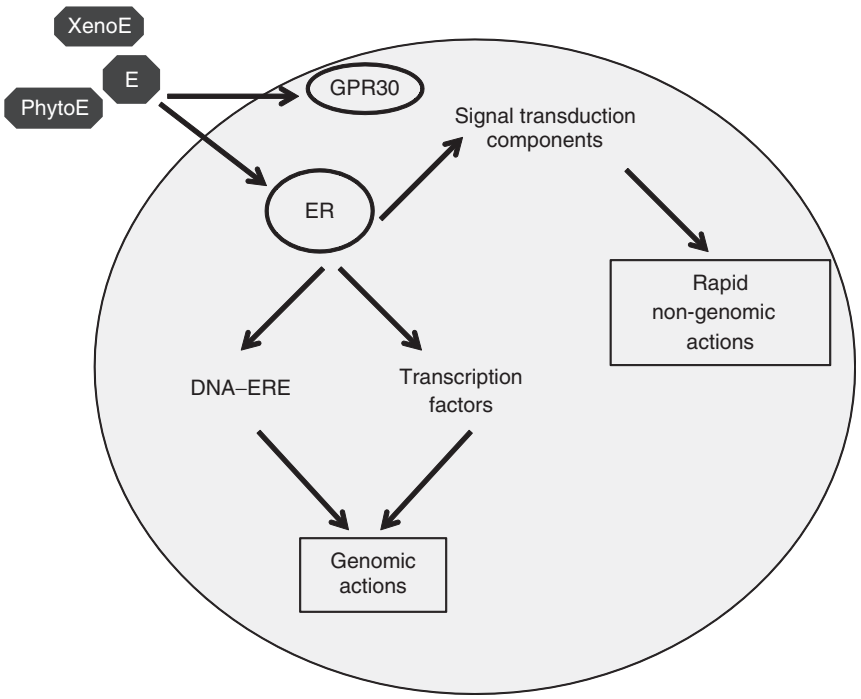

Figure 2 Effect of dietary compounds with estrogenic activity on intracellular mechanisms of estrogen action. Physiological estrogens (E) or dietary phytoestrogens (PhytoE)/xenoestrogens (XenoE) interact with intracellular ER, which can then either bind to estrogen response elements (ERE) in the DNA or to other transcription factors to bring about genomic actions. Interactions through the cell membrane G-protein coupled receptor (GPR30) or ER may also effect rapid nongenomic actions through signal transduction pathways.

of estrogen (ligand) to receptor results in release of associated chaperone proteins, increased receptor phosphorylation, and dimerization of ligand-receptor complexes. These ER dimers then bind directly to specific nucleotide sequences in the DNA termed ERE through regions within the ER protein, which form zinc finger motifs capable of interacting directly with the nucleotide strands of the DNA. Binding of ER to the ERE in the vicinity of estrogen-regulated genes results in an interaction of the ER with other coregulator proteins to modulate the activity of the transactivation complex and hence to alter levels of gene expression. Classical estrogen-regulated genes include genes for the trefoil growth factor pS2, progesterone receptor, and cathepsin D, which are all upregulated by estrogen. However, following the application of microarray technology, it is now known that estrogen can regulate the expression of hundreds of genes across a wide range of functional groupings, and more genes are downregulated (approximately 70%) than are upregulated by estradiol in studies using human breast cancer cells.

Other Mechanisms: Indirect Genomic and Non-Genomic

In addition to direct interactions of the ER protein with ERE in the DNA, ERs can also regulate gene expression through protein-protein interactions with other transcription factors. This so-called indirect genomic mechanism of action (Figure 2) was first shown from studies involving modulatory activity of ERβ on transcription factors of the Fos/Jun/AP-1 complex. Because AP-1 mediates cellular actions of growth factors, this has opened up understanding of how estrogen can influence the response of a cell to growth factors. Estrogen and growth factors have long been known to interact in the regulation of cell growth, but the mechanisms of action are only now beginning to be understood, and in addition to the genomic actions of estrogen, it seems that there is a further mechanism of interaction with growth factor pathways whereby estrogen can mediate fast cellular responses through interaction with cellular signaling phosphorylation cascades. This may be brought about through the estrogen–ER complex interacting at the cell surface with components of signal transduction such as mitogen-activated protein kinases or through binding of estrogen to the cell surface G-protein coupled receptor GPER (GPR30). This mechanism of estrogen action has been termed the nongenomic mechanism (Figure 2).

Molecular Actions of Environmental Estrogens

Environmental estrogens of the diet have been shown to bind to intracellular ERs and so mimic or interfere in the action of physiological estrogens. Their ability to act through such receptor-mediated mechanisms has challenged the concepts of classical toxicology because effects can occur at much lower concentrations and actions can be targeted within the cell through the receptor.

Phytoestrogens

Types and Source

Phytoestrogens are compounds, which occur naturally in plants and are therefore consumed in the diet through eating plant material. There are four main chemical classes: the isoflavones, the coumestans, prenyl flavonoids, and the lignans. Genistein and daidzein are isoflavones found in leguminous plants, especially not only soybeans but also lentils and chickpeas. Equol is a related compound derived from metabolism of daidzein, but ability for metabolic conversion varies between individual human subjects. Other isoflavones in food include glycitein, biochanin A, and formononetin. Coumestrol is a coumestan found in young sprouting legumes such as clover and alfalfa sprouts and is therefore found in many animal feedstuffs. Prenylated flavonoids, including 8-prenylnaringenin and 6-prenylnaringenin, are found in hops and products made from hops such as beer. Resveratrol is a polyphenolic compound found in grapes and therefore red wine. The lignans are found in many cereals especially linseed (flaxseed) and many fruits and vegetables, and include lariciresinol, isolariciresinol, matairesinol, and secoisolariciresinol together with their metabolites enterodiol and enterlactone.

Mechanisms of Action

Many of these compounds share structural similarities (Figure 3) to estradiol (Figure 1) and can therefore bind into the ligand binding domain of the ER enabling estrogenic responses in target cells possessing ER. Crystallographic studies have shown that in contrast to the classical binding characteristics of a substrate to its active site in an enzyme, the ligand binding domain of the ER is larger than the estradiol molecule, which explains why it can accommodate a range of different-sized molecules. There are some common features for binding, which include the presence of a para-hydroxy phenyl grouping, which is present either intrinsically or following metabolic conversion and enabling the hydrogen bonding to amino acids of the ligand binding pocket in an analogous manner to that described for the estradiol molecule.

As for any receptor-binding molecules, there can be a range of outcomes on binding. Compounds, which give responses equivalent to that of estradiol, are described as having full agonist activity; those giving similar responses but of a lesser magnitude to estradiol are said to have partial agonist activity. If a compound is able to reduce the response given in the presence of estradiol, then it is said to have antagonist activity. Studies *in vitro* testing purified compounds have shown that all phytoestrogens bind more weakly to the ER than does estradiol but with considerable variation in their relative binding affinity (RBA). For example, coumestrol binds to the ER with an RBA of only 10-fold lower than 17β-estradiol, but genistein has an RBA of approximately 1000-fold lower. This weaker binding affinity has resulted in the concept that phytoestrogens are 'weak' estrogens. However, when ligand efficacy is considered as well as ligand binding affinity, the efficacy of these compounds is not correspondingly low, and when sufficient concentration is present, both coumestrol and genistein can give the same response as estradiol

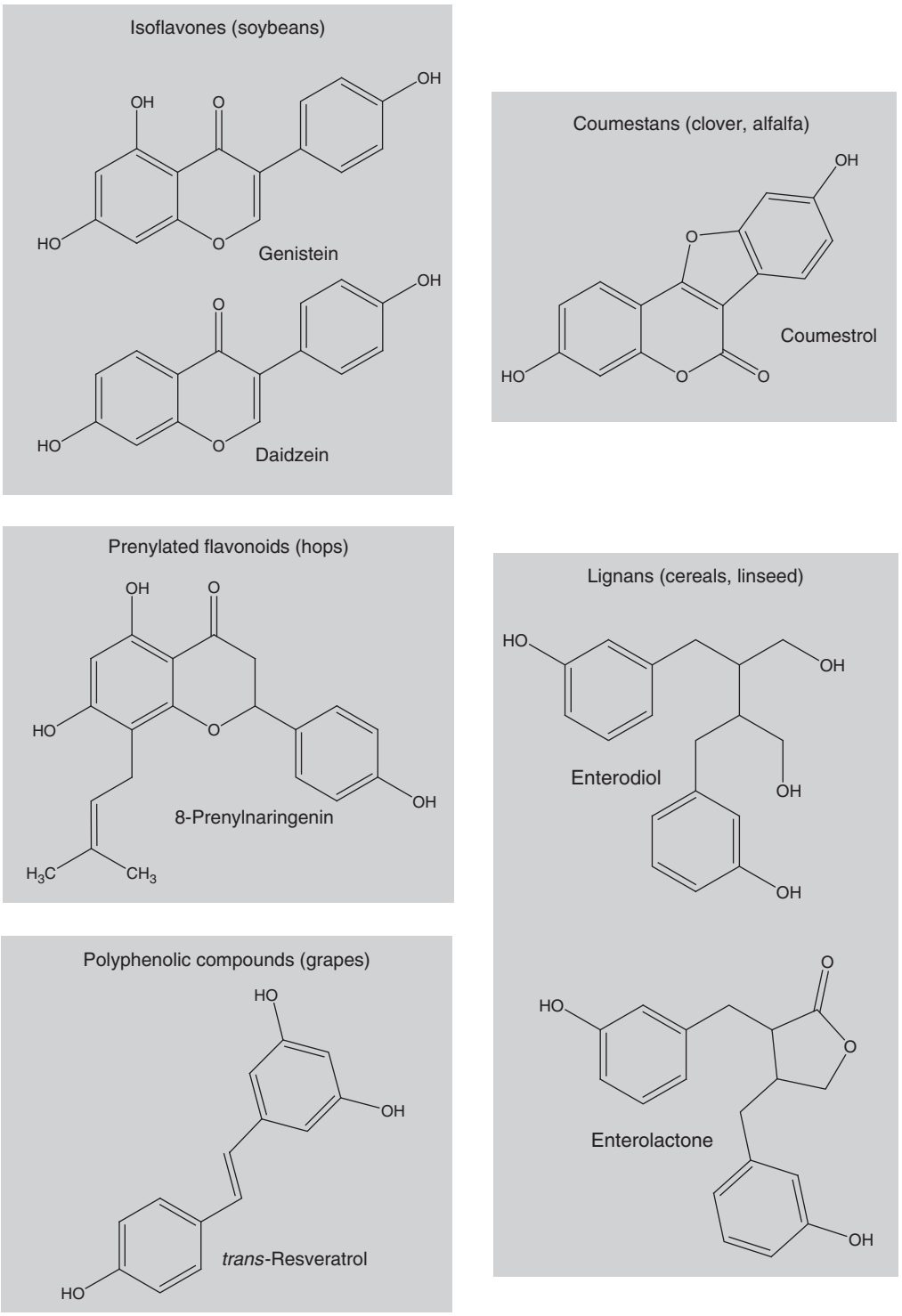

Figure 3 Source and structure of some phytoestrogens present in food.

when tested in cell culture assays on growth of estrogen-responsive cells. Assessment of effects of phytoestrogen needs, therefore, to include a measure of quantity present and not just an RBA value. Tests in cell culture have shown that some phytoestrogens with lower RBA values than genistein, such as resveratrol, are not able to give full agonist responses even at the highest testable concentrations. It is unclear whether this is due to solubility issues in that sufficient concentration cannot be achieved for the full agonist response but operationally, such compounds must be deemed as partial estrogen agonists. Despite theoretical assumptions, there is no evidence from *in vitro* testing for antagonist activity of phytoestrogens:

Inhibitory effects of phytoestrogens observed in cell culture at higher concentrations have been shown as not ER-mediated. However, it remains unexplained as to why this should contrast to the many rodent model experiments, which have shown that phytoestrogens, such as coumestrol, are able to antagonize estradiol-mediated response in the whole animal.

One interesting feature of the phytoestrogens was revealed in the late 1990s when some, such as genistein, were reported to bind with a stronger affinity to ERβ than to ERα. Because ERβ is distributed at low concentrations across many body tissues and ERα levels are not only relatively higher but also more specific in female reproductive tissues, beneficial physiological effects of phytoestrogens are thought to be ERβ-mediated, whereas adverse effects to be ERα-mediated. Because ERβ has also been considered as a tumor suppressor capable of ameliorating the adverse proliferative effects of ERα in cancer, phytoestrogens have become generally considered as beneficial selective ER modulators (SERMs). However, it must be remembered that the difference in RBA is relative not absolute and all phytoestrogens do still bind to both types of ER, so again effects have to be considered with a knowledge of both the relative levels of ERα and ERβ in target cells and the concentration of phytoestrogen in the target tissue.

In the Context of the Complexity of Plant Material

Research publications have now enabled relative specific binding affinities for ER to be tabulated for a range of phytoestrogens, and their relative efficacies have been described in terms of ability to regulate gene expression and/or cell growth in cell lines in culture and to influence reproductive functions in animal models. However, what remains still very poorly understood is how these compounds behave when in the context of the plant material in which they are consumed, and this may provide an explanation for apparently conflicting results between, for example, the known estrogenic activity of genistein and yet the lower incidence of breast cancer in populations consuming large quantities of soy-based products. Although, there may be other components in the soy plant material, which can act to prevent breast cancer development, it is also possible that the activity of genistein is influenced by the context of the plant extract. Diverse actions of genistein have been described in addition to estrogen receptor-mediated mechanisms including its ability to inhibit protein tyrosine kinases, cell cycle progression, DNA topoisomerase, angiogenesis, or to act as antioxidant. Actions of genistein, as indeed for many environmental estrogens, have been shown to be nonmonotonic and with responses, therefore, dependent on concentration, and it is now well established that while low concentrations of genistein can stimulate cell proliferation by an ER-mediated mechanism, higher concentrations can act in an inhibitory mechanism which is not ER-mediated. Furthermore, phytoestrogens can also produce estrogenic effects by modulating concentrations of endogenous estrogens through their ability to influence the activity of enzymes needed for synthesis of estrogens (e.g., aromatase) or of enzymes, that modulate bioavailability of estrogens (sulphatases and sulphotransferases).

Xenoestrogens

Types and Source

Xenoestrogens are synthetic chemicals released into the environment as pollutants from agricultural spraying (pesticides and herbicides), from industrial processes, and waste disposal (PCBs and dioxins). These persistent organochlorine pollutants are generally lipophilic and therefore passed up the food chain partitioned in animal fat. Although they can be found in fatty meat and fish, they are also found in dairy products (milk, butter, and cheese) in the fat content. These compounds are usually consumed in only small quantities but due to their lipophilic properties and long half-lives in the body, they tend to build up in body fat with age, being released only during times of weight loss during fasting or in females into milk during periods of lactation. This, of course, has implications for the diet of the baby who can consume a relatively high load of such compounds from the mother's milk.

Xenoestrogens may also enter food following storage procedures. Alkyl esters of *p*-hydroxybenzoic acid (parabens) are added as preservatives to some foods. Methylparaben, ethylparaben, propylparaben, and butylparaben have been shown to possess estrogenic activity in a range of *in vitro* and *in vivo* assays. On the grounds of endocrine and reproductive toxicity in dietary studies, the acceptable daily intake was withdrawn for propylparaben and butylparaben by the Joint Food and Agriculture Organization and World Health Organization Expert Committee on Food Additives in 2007. Although the other esters remain in use, it is thought that liver esterase activity should result in rapid metabolism and clearance when consumed by the oral route. Plastic containers are used widely for storage of food, and this can provide another source of exposure to estrogenic compounds such as bisphenol A and phthalate esters if they partition into fatty foods. Bisphenol A and some phthalate esters have been shown to possess estrogenic properties in assays *in vitro* and *in vivo*. Because of concern for endocrine disruption following exposure in young children, use of bisphenol A (**Figure 4**) in the manufacture of baby bottles has been ceased in Europe, Canada, and the USA.

Metalloestrogens

Although most compounds that mimic the action of estrogen have organic ring structures, some metal ions have also been shown capable of binding into the ligand binding domain of the ER and enabling estrogenic responses. Such metal ions include aluminum, antimony, arsenic, barium, cadmium, chromium, cobalt, copper, lead, mercury, nickel, selenium, tin, and vanadium, and these have been termed metalloestrogens.

Considerations of Exposure and Bioavailability

Physiological exposure to estrogens is a tightly regulated process such that men are exposed to only low levels, females to low levels before puberty and after menopause, and for females between puberty and menopause the levels cycle in

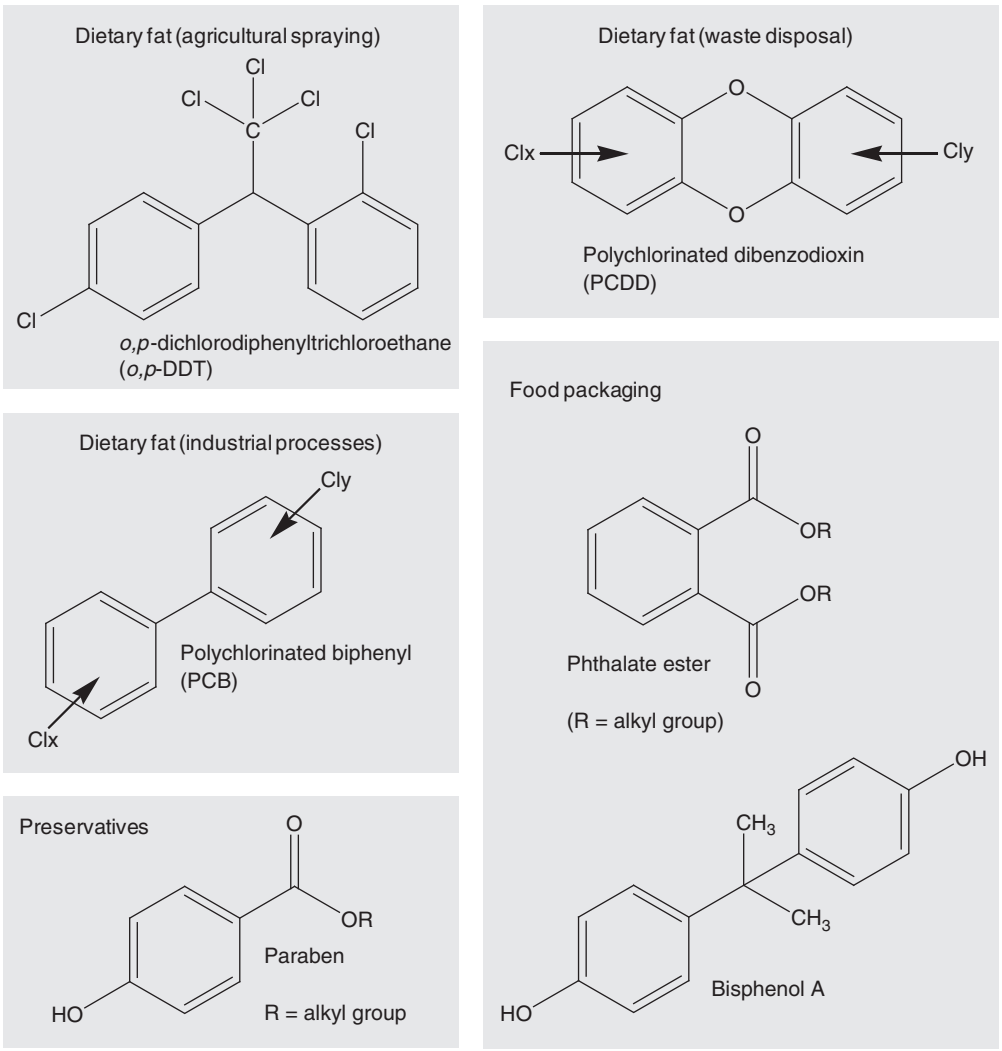

Figure 4 Source and structure of some xenoestrogens present in food.

conjunction with other hormones during the monthly menstrual cycle. By contrast, intake of environmental estrogenic compounds in diet is not linked to physiology, and whereas quantity, metabolism, and clearance may contribute to resulting effects, timing may also be important. Inappropriate exposure to estrogen has been linked to reproductive abnormalities and increased incidence of hormone-dependent cancers. The reproductive and oncological consequences for children born to mothers given the synthetic estrogen, diethylstilboestrol, to prevent miscarriage in early pregnancy in the 1970s has demonstrated the long-term consequences for both genders of exposure before birth to a potent synthetic estrogen.

Although intake of phytoestrogens will vary according to type and amount of plant material consumed, levels taken in the diet can be relatively high compared to intake of xenoestrogens. For example, soybean seeds have been reported to contain in the range 18–1382 mg kg^{-1} genistein and 68–1006 mg kg^{-1} daidzein, whereas total PCB levels have been measured mainly as less than 0.1 mg kg^{-1} in samples of dairy products and meat. However, by contrast, metabolism and clearance of phytoestrogens is also relatively high compared to the xenoestrogens, which due to their lipophilic properties tend to bioaccumulate in adipose tissue over a lifetime.

In addition to these considerations, bioavailability is another factor because many of these compounds are found conjugated to sugars or sulfate groups. Both genistein and daidzein can be detected in the plasma and urine of humans who consume soy foods, but the majority of these isoflavones circulate and are excreted in urine as mono- and di-sulfates, mono- and di-glucuronides, and sulphoglucuronides through conjugation at their 4′- and 7-hydroxyl groups. Glucuronides are the most abundant conjugates, but sulfates of genistein and daidzein have been shown to make up to 24% of the total isoflavone concentration in human plasma. At the target tissues, activity of phytoestrogens will depend not only on the enzymatic hydrolysis of the conjugates back to the aglycones by tissue sulphatases and β-glucuronidases but also on any intrinsic estrogenic activity of the conjugates themselves. Contrary to previous assumptions that conjugation would negate estrogenic activity, genistein glucuronides

have been reported to retain weak ER-binding capability and some monosulphates of genistein, daidzein, and equol to have greater estrogenic activity than the unconjugated phytoestrogen.

Consideration to Effects on Human Health

Much has been written and discussed concerning effects of consumption of compounds with estrogenic activity on human health. Most of the issues remain unresolved, but it is clear that there are likely to be both potential benefits and adverse consequences. On the basis that compounds of 'natural' origin are automatically assumed to be beneficial, whereas man-made compounds are automatically assumed to be adverse, society has responded in opposing ways to the plant-based and synthetic estrogenic compounds. Although both phytoestrogens and xenoestrogens display estrogenic activity in *in vitro* and animal models, society has generally chosen to positively embrace use of the phytoestrogens but to mistrust use of xenoestrogens. With this background, it is likely that the potential benefits of phytoestrogens have been often overstated and adverse effects underappreciated, whereas the opposite may be true for the xenoestrogens.

Female Reproductive Health

From the early studies showing loss of fertility in farm animals grazing on phytoestrogen-rich plants, it has been evident that phytoestrogen intake can influence female reproductive health. Many studies now show that isoflavone intake can increase cycle length and suppress levels of luteinizing hormone and follicle stimulating hormone, which suggests that soy foods should be consumed sparingly in women attempting to become pregnant.

However, intake of phytoestrogens through diet has been linked to a range of beneficial health outcomes, most notably reduction of menopausal symptoms and prevention of osteoporosis. This has stimulated a market for foods and food supplements rich in phytoestrogens on the basis of their potential health benefits. Publication of the million women study in 2002 reporting a link between the use of hormone replacement therapy (HRT) and increased incidence of breast cancer started a trend away from use of synthetic compounds to a search for more 'natural' alternatives. Dietary supplementation with phytoestrogen-rich plant material (such as soy, red clover, black cohosh, or aloe vera) has become, therefore, a favored option by many women as an alternative to HRT, despite the lack of control over quantity of intake or knowledge of long-term effects. Reports that hot flushes and night sweats are greater as perimenopausal side effects in Western than Asian countries where diets are higher in isoflavones has led to the widely held belief that consumption of soy phytoestrogens may offer a more 'natural' relief. However, as yet clinical trials have provided only limited evidence of benefit and there are no authoritative guidelines for women choosing to take such supplements.

Another consequence of reduced estrogen synthesis in the menopause is loss of bone density and consequent increase in risk of bone fracture. In view of their estrogenic properties, dietary supplementation with plant phytoestrogens has, therefore, also become popular in the hope of reducing osteoporosis. Clinical trials and animal experiments have given mixed results and any linkage between soy isoflavone intake and improved bone mineral density remains unresolved.

A clinical case report in 2008 has further linked soy consumption with abnormal endometrial bleeding, but it is not known to what extent this might be more widely associated with increasing incidence of endometriosis.

Male Reproductive Health

Owing to the estrogenic properties of phytoestrogens, questions still exist as to whether there may be adverse reproductive consequences associated with use of soy-based infant formulas for baby boys. Soy-based infant formulae have been used since the 1960s in the UK and have become popular as an alternative to cow's milk for babies with milk allergy. Daily intake of isoflavones by this route has been estimated as up to 4 mg kg^{-1} body weight in the infants, giving plasma isoflavone levels up to 1000 ng ml^{-1}, which is more than 10 000-fold higher than their own endogenous estrogen levels. The long-term effects of exposure to such high levels of estrogen early in life need to remain under investigation especially in the context of male reproductive health.

Cardiovascular Disease

Reduced risk of cardiovascular disease has been a suggested health benefit of soy food consumption, which was initially endorsed by the Food and Drug Administration in 1999 but later questioned by the American Heart Association in 2005. There are numerous risk factors for cardiovascular disease but the only consistent effect in human and animal studies of soy supplementation was a small (3% or less) reduction in levels of low density lipoprotein and interestingly, removal of isoflavones from the soy protein did not eliminate this effect suggesting a more complex link between soy intake and cardiovascular disease.

Obesity, Metabolic Syndrome, Diabetes

Obesogens are defined as compounds which cause weight gain. They may act directly to increase the number of fat cells or the storage of fat within the cells. Alternatively, they may act less directly through altering appetite, metabolic rate, or energy balance. Exposure to some persistent organic pollutants, phthalates, and bisphenol A can give rise to obesity in animal models, and such compounds are increasingly implicated in the rapid rise in obesity, metabolic syndrome, and diabetes in young people. Although mechanisms remain to be identified, it has been shown that some of these compounds can activate the peroxisome proliferator-activated receptor, and activation of this receptor is an important pathway for adipogenesis and obesity.

Hormone-Responsive Cancers

Consumption of soy-based diets in Eastern parts of the world, especially in Japan, where breast cancer incidence is low have led to the assumption that increased soy intake may prevent the development of breast cancer and that phytoestrogens possess antiestrogenic activity. However, epidemiological evidence and clinical trial results are often conflicting and suggest more complex linkage between phytoestrogen intake and breast cancer incidence. Animal models have shown that genistein is capable of reducing development of chemically induced mammary tumors in rats, especially in younger rats, but can stimulate growth of subcutaneous MCF-7-derived tumors in mice. Because studies *in vitro* have found no evidence for antiestrogenic activity of phytoestrogens, the animal studies could be interpreted as suggesting that preventative mechanisms of phytoestrogens act through their antioxidant properties, whereas estrogen agonist properties of phytoestrogens would support growth of estrogen-dependent mammary cancers already present.

There is justified concern surrounding the ability of xenoestrogens from food to bioaccumulate in human body fat, and epidemiological studies are beginning to identify an association with health risks. Understanding of the mechanisms by which such compounds may cause adverse effects has required an appreciation firstly that because they act through receptor-mediated mechanisms, effects occur at much lower concentrations than would be predicted from classical toxicology studies and secondly that single compounds do not act in isolation. The environmental reality is that hundreds of such compounds have now been measured in human body fat and consequences result from long-term exposure to low doses of multiple compounds, the overall composition of which will vary according to dietary and lifestyle choices.

Endocrine Therapy of Cancer

Reduction of estrogen action in the body through either inhibition of estrogen synthesis (aromatase inhibitors) or antagonism of estrogen action (antiestrogen) can be an effective way of reducing growth of endocrine-responsive cancers, and endocrine therapy has become an effective treatment for breast cancer. However, little attention has been given to the potential for environmental estrogens to interfere in the effectiveness of such therapy. Theoretically, it would seem counterproductive to reduce synthesis of estrogen in the body with aromatase inhibitor drugs on the one hand and yet on the other hand not to be aware of consumption of compounds with estrogenic activity in the diet. While it may be difficult to avoid estrogenic chemicals, which are contaminants of the diet, the decision to consume plant material containing high levels of phytoestrogen should be discouraged.

Conclusions

There are many compounds contained in food, both of endogenous and environmental origin, that can mimic or interfere in estrogen action with resulting implications for human health. Greater focus is, therefore, now needed on overall estrogenic load rather than on individual compounds. At the current time, most research has considered one compound at a time, but the environmental reality is that any one individual will consume varied mixtures of phytoestrogens and xenoestrogens, offering the potential for each individual to have high concentrations of different compounds but with a common mechanism of action. Assessment of the estrogenic load from cumulative exposures will need to take account not only of variations in quantity of intake but also absorption, metabolism, and clearance/bioaccumulation, which can be very different for the phytoestrogens and xenoestrogens, especially when considering fatty tissues such as the breast. Furthermore, since different estrogenic compounds have differing RBA for the ER, any functional assessment of concentrations in body tissues needs to be set in the context of the varied potencies of the compounds. In view of increasing reports of susceptibility to endocrine disruption *in utero* or in early life, more attention also needs to be given to exposure at different developmental and life stages. The transgenerational impact of some environmental estrogens suggests that greater attention needs to be given to the diet of women before and during pregnancy. Finally, because estrogenic compounds are no longer confined to diet, overall aggregate contribution from diet cannot be considered in isolation from similar compounds entering through other routes such as water, air, and dermal absorption of personal care products. At the current time, this holistic picture is lacking and measurement needs to be made of wider numbers of estrogenic compounds in any one tissue of an individual person, especially if health benefits are to be fully realized and long-term adverse health outcomes are to be avoided.

See also: Environmental Contaminants: Dioxins, Furans and Dioxin-like Polychlorinated Biphenyls. Foodborne Diseases: Overview of Chemical, Physical, and Other Significant Hazards. Mycotoxins: Zearalenone. Pesticide Residues: Organochlorines. Processing Contaminants: Polycyclic Aromatic Hydrocarbons (PAHs). Public Health Measures: Monitoring of Contaminants

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ENVIRONMENTAL CONTAMINANTS

Nitrate and Nitrite

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Glossary

Acceptable daily intake (ADI) A level of exposure without appreciable health risk when consumed everyday for a lifetime.
Lowest observed adverse effect level (LOAEL) The lowest concentration of a substance that is found to have no adverse effects on the test subject. These values are usually derived from chronic or subchronic animal studies using the most sensitive species for risk assessment and a dose–response relationship that includes levels producing adverse health effects.
No observed adverse effect level (NOAEL) The maximum concentration of a substance that is found to have no adverse effects on the test subject.
No observed effect level (NOEL) The maximum concentration of a substance that is found to have no effects on the test subject.
Risk assessment A framework to assess the health risk of exposure to a hazard; it comprises four steps: hazard identification, hazard characterization, exposure assessment and risk characterization.

Introduction

Nitrate and nitrite are ubiquitous in the environment and result from nitrogen fixation of dead and decaying plant and animal matter. The nitrite ion is relatively unstable and is readily oxidized to nitrate. Nitrate and nitrite are widely consumed from food and water by animals and humans, and are formed to a limited extent endogenously. Nitrate is used in agriculture as a fertilizer to replace the traditional use of livestock manure and in food processing as an approved food additive. Nitrate's toxicity *per se* is low, but the metabolites, nitrite, nitric oxide, and *N*-nitroso compounds make these substances of regulatory importance because of the potential for adverse health implications in humans and animals.

As food additives, nitrate and nitrite are used as antimicrobial agents, preservatives, and color fixatives in meat and fish. In the EU and when labeled 'for food use,' sodium nitrite may only be sold in a mixture with salt (NaCl) or a salt substitute (OJ L 61, 20.2. 1995, p27). Potassium nitrite is known as E249, sodium nitrite as E250, sodium nitrate as E251, and potassium nitrate as E252 (list of approved food additives see European Parliament and Council Directive No 95/2/EC as amended by Directive.

Recent research has shown that the conversion of nitrate to nitrite plays an important antimicrobial role in the stomach, and other nitrate metabolites also have important physiological/pharmacological roles. The potentially beneficial aspects of nitrate and nitrite are beyond the scope of this article.

This article will first consider the legislation of nitrate and nitrite with an emphasis on European Community legislation, the analytical techniques, and human and animal dietary exposure to both ions in Europe. The toxicology of both nitrate and nitrite will be summarized in laboratory animals, humans, and food-producing animals together with recent risk assessments from an animal health and public health point of view performed recently by the Scientific Panel on Contaminants in the Food Chain (CONTAM) of the European Food Safety Authority.

Legislation

Drinking Water

International efforts have been put in place to reduce and limit the occurrence of nitrate in water. The EU Council Directive 98/83/EC (OJ L 330, 5.12.1998, p. 32–54 corrigendum OJ L 111, 20.4.2001, p. 31) lays down the maximum permitted level of 50 mg nitrate and 0.5 mg nitrite per liter in drinking water. The standard for nitrate is in line with the international standard set by World Health Organization. For nitrite, a guideline value of 3 mg l^{-1} (short-term exposure) and provisional of 0.2 mg l^{-1} (long-term exposure) have been laid down at an international level.

Food

In the EU, maximum permitted levels for nitrate in different leafy vegetables (in particular lettuce and spinach) have been in place since 1997. The current maximum levels are laid down in Commission Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs (Official Journal L 364, 20.12.2006, p. 5–24). European Parliament and Council Directive No 95/2/EC (Official Journal L61, 18.3.1995, p. 1) as amended by Directive 2006/52/EC

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(Official Journal L204, 26.7.2006, p. 10–22) lays down maximum limits for nitrate and nitrite when used as food preservative.

Feed

European Parliament and Council Directive 2002/32/EC (Official Journal L 140, 30.5.2002), as amended by Commission directive 2010/6/EU (Official Journal L37, 10.2.2010, p. 29), lays down maximum permitted levels for nitrite as an undesirable substance in feed materials with the exception of silage and incomplete feedingstuffs and feed for dogs and cats. Nitrite is authorized as a preservative with a maximum content of 100 mg kg^{-1} in complete feed for dogs and cats and as an additive in silage (Community Register of Feed Additives pursuant to Regulation (EC) No 1831/2003, entry on sodium nitrite (Dogs, Cats) and sodium nitrite in silage.

Analytical Techniques, Occurrence in Food Sources, and Exposure Assessment

Different analytical methodologies and a variety of detection methods can be applied to determine nitrate and nitrite in various matrices such as water, food, and feedstuffs. Detection limits range from 0.001 to 1 mg l^{-1}. An overview of methods for the analysis of nitrate and nitrite in different matrices can be found in the recent International Agency for Research on Cancer (IARC) monograph No. 94 on nitrate and nitrite.

Human exposure to nitrate is mainly exogenous whereas exposure to nitrite is mainly endogenous via nitrate metabolism. In Europe, the main dietary sources of nitrate are vegetables, preserved meat, and drinking water, with vegetables being the most important source. Nitrate concentrations in vegetables can be influenced by several factors such as light intensity, storage, processing, and cooking. In a recent European survey, mean nitrate concentrations of 1 mg kg^{-1} (e.g., peas) to 4800 mg kg^{-1} (e.g., rucola) have been found. Leafy vegetables (e.g., lettuces and spinach) generally have higher nitrate concentrations compared with seeds or tubers (e.g., potatoes). The critical driver for a high dietary exposure to nitrate is not necessarily the absolute amount of vegetables consumed but the type of vegetable (e.g., leafy vegetables) and the concentration of nitrate related to the conditions of production. The largest source of nitrite is endogenous conversion from nitrate, and the exogenous contribution to nitrite through fruit and vegetables is less important. However, during storage, especially with home-prepared food, transformation of nitrate to nitrite can occur.

In farm animals, exposure to nitrite using the maximum authorized levels in feed (10 mg kg^{-1}) and the EU maximum limit of 0.5 mg l^{-1} in water has been estimated in the recent European Food Safety Authority (EFSA) assessment. Estimates of feed intake are based on typical feeding regimens within Europe that have formed the basis of previous EFSA exposure estimates in livestock. The overall nitrite exposure from feed was found to be 0.37 mg kg^{-1} nitrite body weight (b.w.) per day in pigs and 0.65 mg kg^{-1} nitrite b.w. per day in cattle (Official Journal L 140, 30.5.2002, Official Journal L37, 10.2.2010, p. 29, Community Register of Feed Additives pursuant to Regulation (EC) No 1831/2003, entry on sodium nitrite (Dogs, Cats) and sodium nitrite in silage) which are the most sensitive species to nitrite's toxicity, and these values would rise to 0.42 and 0.70 mg kg^{-1} nitrite b.w. per day, respectively, taking into account water intake as well (assuming the EU maximum level of 0.5 mg l^{-1}). With regard to human exposure, residues in fresh animal products (e.g., milk, meat, and eggs) represent only 3% of total daily dietary exposure to nitrite compared with other sources such as vegetables.

Toxicology and Health-Based Guidance Values

Laboratory Animals

Nitrite and nitrate have been studied extensively over the past half century. A full range of toxicity studies can be found in the literature spanning acute, subacute, and chronic as well as genotoxicity and somewhat limited reproductive toxicology. Toxicokinetic data are also available covering absorption, distribution, metabolism, and excretion. Not all of the work is to modern day standards but the results are sufficiently consistent to give confidence for the establishment of the toxicological profiles and also to understand the species variation in metabolic handling and interconversion of nitrate and nitrite. The rate of absorption of nitrate and nitrite varies between species, being relatively high in humans and rats but lower in ruminants. Both anions are quantitatively well absorbed from the stomach of monogastrics, and rumen in the case of ruminants. For nitrate, approximately 25% of the absorbed material is taken up by the salivary glands, bio-concentrated approximately 10-fold and secreted into the saliva. In the mouth, bacterial reduction of approximately 20% of the secreted nitrate leads to nitrite formation. In the stomach, under acidic conditions, nitrite is transformed to nitric oxide and other metabolites. Once absorbed, nitrite is rapidly distributed in the plasma with rapid binding to the erythrocytes. Nitrite, at normal physiological levels, is metabolized to nitrate. Most of the systemic nitrate is rapidly and extensively excreted in the urine, and thus does not accumulate in tissues. Significant nitrate salvage takes place through selective reabsorption from the kidney together with biliary and salivary recirculation.

For establishing the acceptable daily intake (ADI) values for nitrate and nitrite, the most recent Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluation used the following studies and endpoints (Table 1).

Acutely, nitrite is approximately 10-fold more toxic than nitrate and three main toxicological endpoints have been identified: equivocal evidence for carcinogenesis in female mice, hypertrophy of the adrenal zona glomerulosa in rats, and methemoglobin (MetHb) formation in a wide range of species including humans. MetHb formation results from a reaction of the nitrite metabolite nitric oxide with oxyhemoglobin. Predisposing factors include increased nitrite concentrations, intestinal infection together with gastric inflammation and low levels of nicotinamide adenine dinucleotide (NADH) cytochrome b5 methemoglobin reductase, which converts MetHb back to hemoglobin (Hb).

Table 1 Summary of NOELs from toxicological studies used to derive ADI values for nitrate and nitrite in the JECFA 2003 evaluation[a]

Type of study	*Toxicological endpoint*	*NOEL sodium salt/anion mg kg^{-1} b.w. day^{-1}*	*ADI sodium salt/anion mg kg^{-1} b.w. day^{-1}*
Nitrate			
Subchronic study in dogs (125 days)	Growth depression	500/370	5.0/3.7
2-year chronic study in rats	Growth depression	500/370	5.0/3.7
Nitrite			
2 years study in rats	Heart and lung toxicity	10/6.7	0.1/0.07

[a]Modified from EFSA, 2008.

Normal physiological MetHb background levels are in the range of 1–3%. Above 10% there is a reduction of oxygen transport, above 20% cyanosis and hypoxia can occur, and 50% becomes life threatening. Infants less than 3 months of age are more susceptible to MetHb. This is a consequence of 40–50% lower MetHb reductase levels compared with adults. Additionally, infants have an increased likelihood of intestinal infections where pathogenic bacteria can rapidly reduce nitrate to nitrite.

The first international evaluation of the risks associated with the ingestion of nitrate and nitrite was conducted by the JECFA in 1961. The former Scientific Committee for Food (SCF) of the EU reviewed the toxicological effects of nitrate and nitrite and established an ADI of 0–3.7 mg kg^{-1} b.w. for nitrate in 1990, retained the ADI in 1995 and derived an ADI of 0–0.06 mg kg^{-1} for nitrite. The JECFA completed its most recent review in 2002 and reconfirmed an ADI of 0–3.7 mg kg^{-1} b.w. for nitrate and set an ADI of 0–0.07 mg kg^{-1} b.w. for nitrite. In 2008, the CONTAM Panel noted that no new data were identified that would require a revision of the ADI values.

Food-Producing Animals

Feed and water constitute the main source of exposure to nitrite and nitrate in animals, which at excessive levels may cause adverse effects; these are principally due to methemoglobinemia especially in the young. The observed adverse effects of nitrite in livestock range from acute to chronic with varying degrees of severity and have been reviewed elsewhere. Clinical signs of acute toxicity predominate, which are generally associated with MetHb and include accelerated pulse, dyspnea, muscle tremors, weakness, vomiting, unstable gait, and cyanosis, and may include death. Symptoms of subchronic and chronic toxicity include reduction in feed intake, milk production in dairy animals, rough hair, and reduced weight gain or actual loss. Low fertility and abortion, the latter correlated with fetal hypoxia due to MetHb, can also occur. Reports of nitrite goitrogenicity have been reported in poultry, whereas nitrate has been shown to have this effect in swine, sheep, and cattle resulting from inhibition of iodine uptake from the thyroid gland by nitric oxide. Contributory factors to the adverse effects of nitrite and nitrate can result from intercurrent treatment with antibiotics which can impact both the salivary and gastro intestinal flora. Intoxication results from the interplay between a range of different exposure scenarios and susceptibility factors which include age, anatomical differences between monogastrics, ruminants, cecal/colonics, and differing physiological sensitivity. As an example, interspecies variability in MetHb reductase activity which converts MetHb to Hb has been estimated as a percentage of the human form for pigs (27%), horses (63%), cattle, cats and goats (90%), dogs (114%), sheep (150%), and rabbit (452%). As noted, the acute toxicity of nitrate is approximately 10-fold less than that of nitrite and this also applies to the potential for MetHb formation. This is because nitrite is active directly whereas nitrate first has to be interconverted to nitrite.

In monogastric animals, with the exception of rats and mice, an important source of nitrite is dietary nitrate that is systemically absorbed, secreted into the saliva, and then reduced to nitrite via nitrate reductase activity due to bacteria in the oral cavity and tongue. Such conversion is pH (alkali) dependent and, therefore, does not normally occur in the stomach of most monogastric animals due to relative acidity (pH <3.5). In contrast, the rumen of ruminants and the enlarged cecum and colon of horses are especially suited for nitrate reduction due to the dense microbial population and a relatively high pH (>5). Because herbivorous species, and in particular ruminants, feed on diets containing high levels of nitrate, they are potentially one of the most sensitive species to nitrite poisoning due to their innate ruminant ability for microbial conversion of nitrate to nitrite. However, under normal circumstances problems do not occur as the resultant nitrite is rapidly removed via bacterial fermentation for anabolism to produce ammonia, amino acids, and proteins. Problems occur only when this process becomes 'overloaded,' for example, by the consumption of contaminated feed or water containing abnormal amounts of nitrate or nitrite.

Evidence for the toxicity of nitrate and nitrite in livestock has been accumulated from a limited range of literature reports. Unlike well-controlled laboratory animal toxicology, the data reported were derived from a wide variety of husbandry practices, dietary regimes, and varied aggregate exposures from soil, water, feed, and forages. In certain instances to help fill data gaps, it was necessary to utilize published data on nitrate allowing a 10-fold ratio in acute toxicity to estimate the corresponding exposure to nitrite. Not all the data for all the species could be derived from vegetable feeding stuffs, some of the findings being taken from the direct dosing of sodium nitrite or nitrate as chemicals *per se*. As much of the data related to different exposures leading to adverse effects, no observed adverse effect levels (NOAELs) were estimated for the different livestock species using an uncertainty factor of 3 to convert lowest observed adverse effect levels (LOAELs) to NOAELs. Where LD50 data were the only figures available, for

Table 2 LOAELs and estimated NOAELs derived from the lowest exposure to nitrite (or nitrate) reported to induce toxicity in livestock and companion species[a]

Species	*Substrate*	*Toxic endpoint*	*LOAEL mg kg^{-1} b.w. day^{-1}*	*NOAEL*[b] mg kg^{-1} b.w. day^{-1}
Cattle[c]	Nitrate in feed (estimated to nitrite)	Not stated (MHb)	dc	3.3[d]
Calves	Nitrite *per se*	MHb	34	11
Sheep	Nitrite *per se*	Not stated (MHb)		10
Growing pigs	Nitrite in feed	MHb	10	3.3
Sows	Nitrite *per se*	Lack of developmental defects		17.2
Rabbits	Nitrite *per se*	Urinary hormone excretion changes	13.4	4.5
Poultry	Nitrite *per se*	Liver and kidney function	75	25
Horses[c]	Nitrate in feed (estimated to nitrite)	MHb	10	3.3[e]
Cats	Nitrite in food	MHb	69	23
Dogs	Nitrite *per se*	MHb	7.9	2.6
Fish (trout)[f]	Nitrite *per se*	MHb[4]		0.1[g]

[a]Modified from EFSA, 2009.
[b]A safety factor of 3 is applied to the LOAEL to derive the NOAEL value.
[c]Estimated from feed exposure to nitrate using a 10:1 ratio for nitrite:nitrate ratio.
[d]A safety factor of 10 is applied to nitrate LD 50 to derive the NOAEL value.
[e]No data for nitrite extrapolated from nitrate using factor of 10.
[f]Data reported on water.
[g]mg l^{-1}, MHb: methemoglobinemia.

nitrite or nitrate toxicity, the LOAEL was estimated to be 10% of the LD50 value.

The adverse effects of nitrite as demonstrated by the LOAELs derived in different livestock and companion animals (Table 2) broadly lie in the same range allowing for the different species sensitivities already discussed. Interpretation of the data is restricted as some findings relate to bolus administration of the nitrite or nitrate *per se* whereas other studies related to the nitrite or nitrate resulting from that contained within the feedingstuff. The latter scenario generally results in less toxicity and is more akin to the normal feeding and drinking habits of farmed livestock. However, the consequence of bolus dosing could be considered as the most conservative scenario. Of the food-producing livestock, cattle and pigs are known to be relatively sensitive to nitrite. This is at least partially explained by physiological factors, *vide supra* and confirmed by the figures estimated as NOAELs shown in Table 2.

Risk Assessment of Nitrate in Food and Nitrite in Food and Feed: Implications for Human and Animal Health

The risk-assessment process is conventionally divided into four sequential steps: hazard identification, hazard characterization, exposure assessment, and risk characterization. Risk assessment of chemicals in food differs from its counterpart in feed; the former aims to protect human health and the latter aims to protect both animal and human health. Generically, in feed risk assessment, animal health risks are estimated by comparing either the NOEL, NOAEL, or LOAEL values with the actual or estimated chemical exposure in animals. Human health risks are estimated using the occurrence data of the chemical of interest in animal-based products and consumption data of such animal products in humans. For food risk assessment, human exposure to the chemical is derived from occurrence data and human consumption of each food commodity and then compared with a health-based guidance value such as an ADI to conclude on the level of public health risk. In practice, once a chemical has been identified, its content in food measured through validated analytical techniques, its biological (toxicological) effects characterized in a target species and a safe level derived, one can relate the exposure to its biological effects for risk-assessment purposes.

The recent EFSA risk assessments for nitrate in food and nitrite in feed performed by the EFSA's CONTAM Panel are described below. Epidemiological evidence did not suggest a correlation between food or water nitrate intake and increased cancer risk; such evidence for nitrite intake is, to date, equivocal.

Risk assessment of dietary nitrate in food was addressed specifically with regard to the consumption of 400 g of mixed vegetables, as part of a healthy diet, at typical median nitrate concentration, for Europe, and other sources such as drinking water and cured meat. This conservative scenario gave a dietary exposure of 137 mg person^{-1} day^{-1}, which for a 60-kg human equates to 2.28 mg kg^{-1}day^{-1} which is within the ADI for nitrate of 3.7 mg kg^{-1} b.w. day^{-1}, and does not raise concerns for human health. In addition, because most people eat less than half of the recommended daily intake of 400 g of vegetables as fruit, which has relatively low nitrate levels (approximately 10 mg kg^{-1}), the actual dietary exposure would reduce to 81–106 mg day^{-1} nitrate for the majority of the EU population, i.e., 1.35–1.76 mg kg^{-1} day^{-1}. In addition, processing, for example, washing, peeling, and cooking, would also further decrease such exposure. The CONTAM Panel also took into account high consumers of leafy vegetables with high nitrite content, a small proportion of the population (2.5%) in some European countries, who may intermittently exceed the ADI for nitrate by two-fold. An

example would be the consumption of more than 47 g of rucola at one time assuming the median nitrate concentration level leading to an excursion above the ADI without taking into account any other source of nitrate exposure. However, normal intakes and exposure to nitrate from vegetables are unlikely to result in appreciable health risks. Exceeding the ADI is undesirable and occasional circumstances for which the ADI may be exceeded would need to be assessed on a case-by-case basis, for example, unfavorable local/home production conditions for vegetables high in nitrate or high vegetable consumers (such as rucola).

Risk assessment of nitrite in animal feed was first addressed from the animal health point of view. Total daily nitrite intakes for sensitive species such as pigs and cattle with a NOAEL of 3.3 mg kg^{-1} b.w. day^{-1} in both species were estimated using the maximum exposure level in complete feed according to the current EU legislation (10 mg kg^{-1}) and typical feeding regimens within the EU and maximum nitrite level in forages from member states (cattle). Overall, nitrite intakes in both species were 9-fold and 5-fold below the NOAEL with exposures of 0.37 and 0.65 mg kg^{-1} b.w. day^{-1}, respectively (excluding endogenous formation of nitrite). The CONTAM panel concluded that these levels of exposure do not raise concerns for animal health provided that livestock are husbanded under good agricultural practices. Additionally, the awareness of the livestock producer, in particular regarding conditions that may lead to nitrite poisoning (high nitrate levels in forages and the inter-conversion of nitrate to nitrite) further contributes to the protection of livestock health. With regard to the human health aspect, resulting from the consumption of animal products, typical daily human dietary exposure to nitrite from fresh animal products (e.g., milk, meat, and eggs) represents only 2.9% of total background daily dietary exposure, and such low nitrite levels do not raise concern for human health.

Conclusions and Future Perspectives

Nitrate and nitrite are widely consumed by animals and humans mostly through food from plant sources (i.e., forages and fruit/vegetables) and water, and are also formed to a limited extent endogenously. Nitrate is relatively nontoxic but is readily metabolized to more toxic species such as nitrite. The recent EFSA risk assessment for nitrate in food and nitrite in feed did not raise concerns concerning human or animal health. Additionally, a risk–benefit assessment weighing the risk of nitrate toxicity and the benefit of fruit/vegetable consumption was performed and showed that the beneficial effects of the consumption of 400 g day^{-1} fruit/vegetables, as part of a healthy diet, prevailed. For a future perspective on this, the assessment of the balance between health risks and health benefits requires an extension of the current risk assessment approach to additionally allow a scientific evaluation of health benefits. This should utilize harmonized science-based methodology to allow for a quantitative weighing of risks and benefits with a 'common currency.' The outcomes of both risk and benefit assessment and their weighing should be provided to risk managers together with a detailed description of the assumptions and uncertainties in order to optimize subsequent decision-making. Several EU-funded groups such as BRAFO are currently working in this field.

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ENVIRONMENTAL CONTAMINANTS

Perchlorate

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Glossary

Exposure Assessment The qualitative or quantitative evaluation of the likely intake of biological, chemical, and physical agents via food as well as exposures from other sources if relevant.

Goiter A greatly enlarged thyroid gland.

Hazard characterization The qualitative or quantitative evaluation of the nature of the adverse health effects associated with biological, chemical, and physical agents that may be present in food. For chemical agents, a dose–response assessment should be performed. For biological or physical agents, a dose–response assessment should be performed if the data are obtainable.

Hazard identification The identification of biological, chemical, and physical agents capable of causing adverse health effects and which may be present in a particular food or group of foods.

Hypothyroidism Abnormally deficient activity of the thyroid gland.

Provisional maximum tolerable daily intake (PMTDI) The endpoint used for contaminants with no cumulative properties. Its value represents permissible human exposure as a result of the natural occurrence of the substance in food and in drinking water. In the case of trace elements that are both essential nutrients and unavoidable constituents of food, a range is expressed, the lower value representing the level of essentiality and the upper value the PMTDI.

Reference dose (RfD) An estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime.

Risk characterization The qualitative or quantitative estimation, including attendant uncertainties, of the probability of occurrence and severity of known or potential adverse health effects in a given population, based on hazard identification, hazard characterization, and exposure assessment.

Background

Perchlorate (ClO_4^-) is an inorganic anion that has a molecular weight of 99 g mol^{-1}. This inorganic anion is produced naturally and through anthropogenic processes. From both of these processes the anion is found and used in the form of a salt. The most common perchlorate salts are ammonium perchlorate (NH_4ClO_4), lithium perchlorate ($LiClO_4$), potassium perchlorate ($KClO_4$), and magnesium perchlorate ($Mg(ClO_4)_2$). The chemical abstract numbers (CAS No.) for these perchlorate salts are 7790-98-9 for ammonium perchlorate, 7791-03-9 for lithium perchlorate, 7778-74-7 for potassium perchlorate, and 10034-81-8 for magnesium perchlorate. Ammonium perchlorate and potassium perchlorate are used as an oxidizing agent in rocket propellants and with the remaining salts are found in other items (e.g., explosives, road flares, fireworks, and car airbags), occur naturally in some fertilizers, and potash. The use of these perchlorate salts and the disposal of these salts provide a pathway for them to enter into the soil and ground and surface water allowing solvation to occur. The solvation liberates this inorganic anion from its salt and it is able to persist in the environment because it is kinetically stable and degrades slowly over time.

Naturally occurring perchlorate salts have been predominately found in arid regions. The general explanation for the occurrence of perchlorate salts in arid regions results from a photochemical reaction in the atmosphere. The proposed photochemical reaction involves sodium chloride from land or sea that is blown in the atmosphere. The sodium chloride reacts with ozone to form a perchlorate salt. After the perchlorate salt is formed, it dissolves in precipitation and returns to the earth surface. Over a period of time, as the rate of perchlorate salt deposition on the surface becomes greater than the rate of perchlorate salt being dissolved results in perchlorate salt becoming incorporated in the composition of the soil and ground.

The arid regions where the greatest perchlorate deposits are formed are located in South America, specifically in the following countries: Chile, Peru, and Bolivia. Of the three countries, the Atacama Desert in Chile has gained the most attention because the caliche has been mined and exported throughout the world for almost 200 years. The composition of the caliche is gypsum, sodium, and salitre (sodium nitrate ($NaNO_3$) and potassium nitrate (KNO_3)). The perchlorate salt is found as a contaminant in the salitre portion of caliche. It is documented that large quantities of Chilean salitre were

imported to the US during the 1800s to be used as a fertilizer, as a source of iodine, for saltpeter used in gunpowder, and as a feedstock to make nitric acid, explosives, and fireworks.

Besides, perchlorate salts being found in arid regions in South America, it has been found in potash from worldwide evaporate deposits. Potash is potassium carbonate that is derived from wood-ash and various dissolved minerals. Potash is formed in evaporite deposits as parts of the formation of evaporate minerals. The majority of potash, mined and milled, is used for feedstock in making fertilizers. Other uses of potash are for the development of potassium-bearing chemicals and reagents, use in deicing salt solution, detergents, and pharmaceuticals.

The anthropogenically produced perchlorate salts and perchlorate-containing chemicals are produced in large-scale facilities and in laboratory settings. The common reaction used to produce sodium perchlorate involves the electrolysis of sodium chlorate. The reaction used to produce potassium perchlorate or ammonium perchlorate involves an additional step to the electrolysis of sodium chlorate by adding a base, resulting in a precipitate containing either potassium perchlorate or ammonium perchlorate. The large-scale production of perchlorate salts and perchlorate-containing chemicals began in 1890 in Masebo, Sweden, by Stockholm Superfostfat Fabrisk AB. The earliest documented large-scale production of perchlorate salts and perchlorate-containing chemicals in the US was located in Niagara Falls, New York, in 1910 by Oldbury Electro-Chemical. The initial production of perchlorate salts and perchlorate-containing chemicals was limited to ammonium perchlorate, sodium perchlorate, potassium perchlorate, and perchloric acid. These compounds were used primarily in fireworks and railroad signals. Later in the mid-1940s during World War II, perchlorate salts and perchlorate-containing chemicals' production began to increase. Specifically, potassium perchlorate production increased because of its use as an oxidizing agent in solid propellant for missiles and rockets. Later in the 1950s, ammonium perchlorate began to replace potassium perchlorate as the oxidizing agent in solid propellant in larger rocket motors. In the 1960s, ammonium perchlorate was used in NASA's space shuttle booster rockets and in the rocket motors for satellite vehicle. In addition, ammonium, lithium, potassium, and magnesium perchlorate are used in the manufacture of flares, fireworks, explosives, and airbag inflators, and herbicides. Potassium perchlorate has been used as a human medicine and used primarily in the 1950s and 1960s to treat hyperthyroidism.

The occurrence of perchlorate contamination found in the soil and ground and surface water has been the topic of some discussions. It has been suggested that the use of fertilizers contaminated with perchlorate salts and the disposal methods of products using perchlorate salts are pathways for the contamination found in the soil and ground and surface water. It has been argued that the latter pathway is the major route for environmental contamination (NAS 2005). The open-burn/open-detonation or hydromining and hog-out processes were the disposal methods that resulted in contamination of the environment. These disposal methods resulted in the high concentrations found in some soils, groundwater, drinking water, and irrigation water.

Hazard Identification and Characterization

Perchlorate is one of several chemicals that has the ability to disrupt thyroid gland function at pharmacological doses (0.02, 0.1, and 0.5 $mg\,kg^{-1}\,day^{-1}$). From the Greer *et al.* (2002) study, perchlorate at these doses can competitively inhibit iodide uptake from the blood by the sodium–iodide symporter (NIS), a glycoprotein expressed in the outer basolateral membrane of the thyroid follicular cells, into the apical membrane. The inhibition of iodide uptake by perchlorate reduces the amount of iodide that is available for organification, which is the oxidation of iodide by thyroid peroxidase and bound to the thyroglubin. With the reduction of iodide, the availability of thyroglubin needed to synthesize thyroid hormones and the resulting concentration of thyroxine (T_4) and triiodothyronine (T_3) are lowered. Because the concentrations of these hormones are reduced, the brain triggers the release of thyrotropin-releasing hormone (TRH) in the hypothalamus, which, in turn, causes the release of thyrotropin, also known as thyroid-stimulating hormone (TSH) from the anterior pituitary gland. The TSH initiates the production of thyroid hormones by the NIS transporting iodide into the thyroid for the synthesis of T_3 and T_4. If the reduction of iodine is continuous and the uptake of iodine is hindered, it may cause the thyroid to become enlarged (goiter). If the disruption of iodide uptake by the thyroid continues it may result in hypothyroidism which has adverse implications for structural and functional brain development in the fetus, infant and child and metabolism and the functioning of cardiovascular, gastrointestinal, skeletal, neuromuscular and reproductive systems in adults.

In the mid-1990s, the US Environmental Protection Agency (EPA) along with other federal government agencies investigated the potential health effects of perchlorate levels in soil, groundwater, and drinking water around the US. In 2002, EPA asked the National Academy of Sciences (NAS) to review the relevant scientific literature and key finding underlying the EPA's 2002 Toxicological Review. After 3 years of review, the NAS recommended to EPA that a reference dose (RfD) of 0.7 $\mu g\,kg^{-1}\,bw\,day^{-1}$ for perchlorate anions from the Greer *et al.* (2002) study, with the application of an uncertainty factor of 10 would protect the most sensitive population – the fetuses of pregnant women who might have hypothyroidism or iodine deficiency. The RfD calculated by the NAS used a point of departure (POD) based on a no observed effect level (NOEL) of 0.007 $mg\,kg^{-1}\,bw\,day^{-1}$ from the Greer *et al.* (2002) study. The NAS committee discussed the use of the NOEL POD as a different approach from the traditional approach of using the no observed adverse effect level (NOAEL) as the POD for determining an RfD. The NAS committee explained that using a nonadverse effect that precedes the adverse event is a conservative health-protective approach to address the perchlorate risk. Because EPA's adoption of the NAS recommended RfD in 2005, there have been additional studies in the literature by Amitai *et al.* (2007) and Blount *et al.* (2009), which have further investigated the link between perchlorate, iodine status, and thyroid function in newborns, pregnant woman, and adults. In early 2010, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) of the World Health Organization (WHO) evaluated perchlorate.

From their evaluation of perchlorate, the JECFA committee derived a provisional maximum tolerable daily intake (PMDTI) of 0.01 mg kg^{-1} bw day^{-1} from their dose–response analysis. The JECFA committee dose–response modeling used the Greer *et al.* (2002) data and a critical size of 50% inhibition of iodide as the benchmark response. The rationale for using this specific critical size was based on the Greer data showing no associated changes in TSH and thyroid hormones at 50% inhibition of iodide. From the JECFA dose–response model, the committee was able to derive a POD of 0.1 mg kg^{-1} bw day^{-1}, which was four-fold lesser than the 0.4 mg kg^{-1} bw day^{-1} dose from the Greer study that is estimated at sustained exposure to trigger hypothyroidism in normal adults. Finally, the committee applied an uncertainty factor of 10 to their POD for the protection of vulnerable groups – such as pregnant women, fetuses, neonates and young infants, those with iodine-deficient diets, and those with clinical or subclinical hypothyroidism.

Method of Analysis

The most common methodology of analysis and sampling perchlorate levels in foods is ion chromatography – tandem mass spectrometry (IC–MS/MS). This method of analysis is rapid, sensitive, and specific that reduces the amount of sample needed to analyze a food matrix for perchlorate. This methodology consists of a triple-stage quadrupole mass spectrometer, equipped with electrospray ionization (ESI) in the negative ion mode that is used to detect perchlorate anion. An $^{18}O_4$-labeled perchlorate anion internal standard is used to correct for any matrix effects.

Another method of analysis used to analyze perchlorate levels is ion chromatography conductivity detection. This method of analysis uses the EPA method 314 EPA (1999) for determining perchlorate ions in drinking water. Unfortunately, this method of analysis is not as sensitive as the IC–MS/MS in handling complicated food matrices. This system consists of an isocratic pump, an eluent generator, a continuous regenerating trap column, a conductivity detector, guard and separation column pair, and a suppressor.

Exposure

Since the early 2000s, the US government, academia, and international organizations have developed individual food surveys that have collected and sampled foods suspected to have perchlorate levels in them. The analytical results of these collection and sampling efforts are shown in Table 1. This table provides 865 analytical results from the scientific literature that represent foods from the US and Canada. Table 1 also provides for the comparison of perchlorate levels in 8 commodities. Shown in Table 1 are the weighted mean (mean of reported mean levels weighted by number of sample) perchlorate levels. In vegetables, the weighted mean perchlorate level range was 10.3–129.5 μg kg^{-1} and in fruits it demonstrated a range of 5–34.1 μg kg^{-1}. The milk samples demonstrated a weighted mean perchlorate level of 5.8 μg kg^{-1}. Rice had a weighted mean perchlorate level of 0.5 μg kg^{-1}. The individual commodities and their respective perchlorate levels results were compared and 6 of the 8 commodities showed fairly good agreement. The commodities that showed fairly good agreement were milk, iceberg lettuce, oranges, carrots, grapes, and rice. In other commodities (spinach and cantaloupe), perchlorate levels varied.

Several national authorities, academia, and international organizations have derived dietary exposures for perchlorate from their individual collection and sampling of foods. The general methodology for these exposure estimates involved using representative consumption survey data and the individual analytical results for perchlorate level for each food. The exposure estimates derived from their methodology resulted in perchlorate dietary exposure estimates either from a specific targeted food or a perchlorate dietary exposure from a Total Diet Study (TDS).

The US perchlorate dietary exposure estimates, derived from the targeted sampling, provide excellent information about the dietary exposure for the individuals that consume specific food but does not provide a picture of the overall dietary exposure to perchlorate. Shown in Table 2 are perchlorate dietary exposure estimates from targeted foods. Shown in Table 2 are the mean and the 90th percentile perchlorate exposure estimates for three subpopulations, all ages, children, and females. As mentioned earlier in the hazard characterization, children and females represent the most sensitive subpopulations to the adverse health effects of perchlorate. Table 2 displays perchlorate dietary exposure estimates from regions that are suspected to have high contamination of perchlorate in the environment. The perchlorate dietary exposure estimates from the USFDA and Sanchez *et al.* (2009) show good agreement. These perchlorate dietary exposure estimates were derived using US consumption data from the Continuing Survey of Food Intakes by Individuals (CSFII) 1994–1998 and sampled foods known to have high concentration of perchlorate.

Health Canada derived an average perchlorate dietary exposure estimates from consumption data from their Nutrition Canada Food Consumption Survey which is a 24-h dietary recall survey and analytical levels for perchlorate in foods from retail establishments in Ottawa. The foods analyzed by Health Canada were of domestic and imported origin. The averaged exposure for women of child bearing age showed good agreement with the Sanchez *et al.* (2009). In the case of children, the Canadian children exposures were different with the USFDA and Sanchez *et al.* (2009) exposures based on the limited foods analyzed.

The dietary perchlorate exposure estimates derived from the FDA's TDS are shown in Table 3. In general, the TDS provides an overall estimate of the average diet in a specific country. The TDS is often referred to as a market basket study in which 200–300 core foods are collected and analyzed to determine the levels of various contaminants and nutrients in those foods. Often in the TDS, the population is divided into age–gender groups to estimate dietary exposure for the respective groups. In the case of perchlorate, the FDA's mean and the 90th percentile dietary perchlorate exposure estimate for 14 age–gender groups are shown in Table 3 and has a range of 0.09–0.45 μg kg^{-1} bw day^{-1} for the mean and 0.176–0.734 μg kg^{-1} bw day^{-1} for the 90th percentile. For comparison, the results from the USFDA TDS adult age–gender groups were compared with results derived

Table 1 Perchlorate levels in selected foods in the literature

Commodity	*Number of samples*	*Mean ($\mu g\ kg^{-1}$)*	*Source*
Milk	47	2	Kirk *et al.* (2005)
	125	5.8	FDA exploratory samples (2004/2005)
	8	7	Murray *et al.* (2008)
	41	5.8	Sanchez *et al.* (2009)
	Weighted mean 5.1 $\mu g\ kg^{-1}$		
Vegetables			
Lettuce, iceberg	63	7.4	Sanchez *et al.* (2005)
	43	8.1	FDA exploratory samples (2004/2005)
	4	2.1	Murray *et al.* (2008)
	144	13.1	Sanchez *et al.* (2009)
	Weighted mean 10.3 $\mu g\ kg^{-1}$		
Spinach	10	85.1	Sanchez *et al.* (2005)
	36	115	FDA exploratory samples (2004/2005)
	4	40	FDA TDS Murray *et al.* (2008)
	6	133	Wang *et al.* (2009)
	16	211	Sanchez *et al.* (2009)
	Weighted mean 129.5 $\mu g\ kg^{-1}$		
Carrot	59	15.8	FDA exploratory samples (2004/2005)
	12	1.31	Wang *et al.* (2009)
	30	29	Sanchez *et al.* (2009)
	6	6.75	Murray *et al.* (2008)
	Weighted mean 17.4 $\mu g\ kg^{-1}$		
Fruits			
Cantaloupe	48	28.6	FDA exploratory samples (2004/2005)
	4	24.4	Murray *et al.* (2008)
	18	56.1	Wang *et al.* (2009)
	Weighted mean 34.1 $\mu g\ kg^{-1}$		
Orange	28	7.4	Sanchez *et al.* (2006)
	10	3.4	FDA exploratory samples (2004/2005)
	4	2.72	Murray *et al.* (2008)
	12	0.5	Wang *et al.* (2009)
	21	5.5	Sanchez *et al.* (2009)
	Weighted mean 5 $\mu g\ kg^{-1}$		
Grapes	12	27.7	Wang *et al.* (2009)
	12	8.51	FDA exploratory samples (2004/2005)
	15	30.8	Sanchez *et al.* (2009)
	4	54.675	Murray *et al.* (2008)
	Weighted mean 25.9 $\mu g\ kg^{-1}$		
Grains			
Rice	19	0.5	FDA exploratory samples (2004/2005)
	4	0.5	Murray *et al.* (2008)
	Weighted mean 0.5 $\mu g\ kg^{-1}$		

Table 2 Estimated perchlorate exposure for three subpopulations found in the literature from similar targeted sampling

	All ages	*Children*	*Females*	*Source*
Mean	0.05	0.17	0.04	FDA exploratory samples (2004/2005)
	0.05	0.182	0.029	Sanchez *et al.* (2009)
		0.0366	0.0279	Wang *et al.* (2009)
90th percentile	0.12	0.34	0.07	FDA exploratory samples (2004/2005)
	0.113	0.349	0.063	Sanchez *et al.* (2009)

from urinary perchlorate levels from the 2001–2002 NHANES survey participants who were 20 years and more by Blount *et al.* (2007) and their results at the mean was 0.066 $\mu g\ kg^{-1}$ bw day^{-1} and the 90th percentile was 0.234 $\mu g\ kg^{-1}$ bw day^{-1} for these adults which demonstrated close agreement with the TDS results from the adult age–gender groups.

Table 3 Estimated mean and 90th percentile perchlorate exposure from TDS

Age–gender groups	*Mean* ($\mu g\ kg^{-1}\ bw\ day^{-1}$)	*90th percentile* ($\mu g\ kg^{-1}\ bw\ day^{-1}$)
M/F 6–11 months	0.305	0.554
M/F 2 yrs	0.446	0.734
M/F 6 yrs	0.278	0.437
M/F 10 yrs	0.181	0.322
F 14–16 yrs	0.101	0.204
M 14–16 yrs	0.118	0.230
F 25–30 yrs	0.100	0.191
M 25–30 yrs	0.111	0.208
F 40–45 yrs	0.093	0.183
M 40–45 yrs	0.093	0.165
F 60–65 yrs	0.094	0.189
M 60–65 yrs	0.095	0.177
F 70+ yrs	0.107	0.207
M 70+ yrs	0.098	0.176

Risk Characterization

Perchlorate has been evaluated by the NAS and JECFA. The results from these evaluation resulted in an RfD of 0.7 μg kg^{-1} bw day^{-1} by NAS and PMDTI for perchlorate 0.01 mg kg^{-1} bw day^{-1} by JECFA. Both committees acknowledge that perchlorate has a very short life and is rapidly cleared from the body and their evaluations included a 10-fold safety factor of protection for pregnant women, fetuses, neonates and young infants, those with iodine-deficient diets, and those with clinical or subclinical hypothyroidism.

Finally, the reference values derived by NAS and JECFA are compared with dietary perchlorate exposure from the USFDA TDS assessment in **Table 3**. The estimated dietary perchlorate exposure provided in **Table 3** shows that the mean for all age–gender groups from the USFDA TDS is below the RfD and PMDTI. The estimated perchlorate exposure estimates for 13 of the 14 age–gender groups at the 90th percentile were below the NAS RfD, with children of 2 years of age having a perchlorate exposure equal to the RfD. When these age–gender groups were compared with the PMDTI at the 90th percentile, all these groups were well below 0.1 mg kg^{-1} bw day^{-1}.

See also: Disciplines Associated with Food Safety: Epidemiology; Food Safety Toxicology. Institutions Involved in Food Safety: World Health Organization (WHO). Public Health Measures: Monitoring of Contaminants. Risk Analysis: Risk Assessment: Chemical Hazards; Risk Assessment: Principles, Methods, and Applications. Veterinary Drugs Residues: Control of Helminths

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TOXIC METALS

Contents

Arsenic

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Glossary

Benchmark dose – hlower confidence limit (BMDL) The lower boundary of the confidence interval on the benchmark dose (BMD). The BMD is the dose of a substance associated with a specified low incidence of risk, generally in the range of 1-10% of a health effect.
Bioavailability The degree or rate at which a substance is absorbed into a living system or is made available at the site of physiological activity.
Biomarkers Indicators of signaling events in biological systems or samples. They have been used as markers of exposure, effect, and susceptibility.
Carcinogen A chemical capable of inducing cancer.
Minimal risk level (MRL) An estimate of daily human exposure to a hazardous substance that is likely to be without an appreciable risk of adverse noncancer health effects over a specified route and duration of exposure.
Provisional tolerable weekly intake (PTWI) The end-point used by the Joint FAO/WHO Expert Committee on Food Additives that represents the permissible human weekly exposure to those contaminants unavoidably associated with the consumption of otherwise wholesome and nutritious food.

Chemical

Arsenic (CAS no. 7440-38-2), arsenic trioxide (As_2O_3, CAS no. 1327-53-3), arsenic pentoxide (As_2O_5, CAS no. 1303–28-2), sodium arsenite ($NaAsO_2$, CAS no. 7784-46-5), disodium arsenate (Na_2HAsO_4, CAS no. 7778-43-0).

Background

Arsenic is a naturally occurring element that is released from volcanoes and the erosion of arsenic-containing mineral deposits. Human activities such as mining; burning of coal, oil, gasoline, and wood; and the use of arsenic compounds, primarily chromate copper arsenate (CCA), as medicines, pesticides, herbicides, and wood preservatives, also contribute to its environmental contamination. Arsenic exists in many chemical forms and valency states (-3, 0, $+3$, and $+5$). Low concentrations can be found in air, water, soil, and food. The background soil content of arsenic varies widely, typically ranging from 1 to 40 ppm, with an average of 5 ppm. Arsenic concentration in natural surface and groundwater is generally approximately 1 ppb, but may exceed 1 ppm in contaminated areas or areas with high soil arsenic. For example, naturally occurring arsenic-contaminated groundwater has severely affected people in Bangladesh where 35–70 million people have been chronically exposed to elevated arsenic in drinking water. This is the result of tube wells installed more than 30 years ago to tap groundwater as a source of pathogen-free drinking water to prevent infectious diseases.

The primary forms of arsenic found in drinking water are arsenite ($+3$) and arsenate ($+5$), the inorganic forms. Sea water typically contains 1–2 ppb arsenic. Ambient air arsenic background concentrations generally range from less than 1 to 3 ng m^{-3}, but concentrations in an urban area may go up to 100 ng m^{-3}. Food arsenic levels usually range from 20 to 140 μg kg^{-1}. However, higher total arsenic levels are found in seaweed, seafood, mushroom, rice and rice products, and some meat. Most arsenic present in seafood is as the organic form (arsenobetaine and arsenocholine) which is considered to be nontoxic. In general, inorganic arsenic is the most toxic form with trivalent arsenic being more toxic than pentavalent arsenic.

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Hazard Identification and Characterization

Arsenobetaine, the major form of arsenic in most seafood and fish, is considered to be of no toxicological concern because it is not metabolized and is excreted intact by humans. Arsenosugars and arsenolipids are metabolized to dimethylarsinate in humans, but no specific toxicological information is available. Soluble inorganic arsenic is rapidly and well absorbed (80–90%) after ingestion, distributed throughout the body, metabolized by methylation, and excreted mainly in urine. Organic arsenic is also well absorbed, generally by more than 70%.

Two basic processes are involved in the metabolism of inorganic arsenic: (1) reduction/oxidation reactions that interconvert As^{+3} and As^{+5} and (2) methylation reaction, which converts arsenite to monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA). The latter facilitates the excretion of inorganic arsenic from the body as both MMA and DMA are more readily excreted in urine. Inorganic arsenic can also be excreted directly in the urine. In contrast, with the exception of arsenosugars, ingested organic arsenicals such as MMA, DMA, and arsenobetaine, do not readily enter the cell, undergo limited metabolism, and are excreted unchanged in the urine. High variability of arsenic metabolism and toxicokinetics has been reported among different species, populations, and individuals. Some species (marmoset monkey, guinea-pig, chimpanzee) have minimal or no arsenic methylation capability. In humans, inorganic arsenic is extensively methylated and its metabolites are excreted primarily in the urine. Age, gender, and smoking contribute only minimally to the large individual variations in arsenic methylation in humans. The relative proportions of arsenic metabolites in human urine are usually 40–75% DMA, 20–25% inorganic arsenic, and 15–25% MMA. Similar urinary metabolic profiles were reported among family members. An increase in DMA excretion was observed in individuals with a specific allele on a gene, suggesting its possible association with a genotype that protects against arsenic toxicity. Other than genetic polymorphisms and wide differences in methyltransferase activities, nutritional status (protein, selenium, and folate) can also influence methylation capacity. Urine arsenic is commonly used as a measure of recent exposure. Arsenic levels in hair and nails have been shown to provide reliable biomarkers for long-term chronic exposure to arsenic in humans.

Inorganic arsenic binds to the sulfhydryl groups of cellular proteins, inhibiting the pyruvate and succinate oxidative pathways. It also competes with phosphorus in the oxidative phosphorylation process. Although chronic exposure to inorganic arsenic has been associated with cancers in humans, the exact underlying molecular mechanisms are not clear. Several modes of action (MOA) of inorganic arsenic in carcinogenesis have been proposed, including: induction of oxidative stress; genotoxicity as induction of mutations and chromosomal aberrations; modulation of signal transduction and apoptosis (growth factors, cell proliferation, and promotion); and alterations in gene expressions via hyper- and hypomethylation of DNA. Arsenic does not directly react with DNA, and it is also probable that more than one of these mechanisms are involved. Recent evidence has proved that arsenic activates Hedgehog signaling, a key oncogenic signaling pathway, and also showed that there is a strong positive correlation between arsenic exposure and high levels of Hedgehog activity in a cohort of bladder cancer patients. This is the first report to suggest that activation of Hedgehog signaling may be in part involved in arsenic-induced cancer.

Ingestion of large doses of arsenic can be fatal. The oral lethal dose of arsenic trioxide is reported to be between 70 and 180 mg day^{-1}. The estimated minimum lethal dose in humans ranges from 1 to 3 mg As per kg of bodyweight (bw) per day. Poisoning may appear with daily doses of inorganic arsenic as low as a few mg for a short period of time, for example, weeks. An estimated daily exposure of 1.3–3.6 mg arsenic from the consumption contaminated dried milk for a few weeks resulted in fever, insomnia, and anorexia in more than 12 000 infants (130 deaths) in Japan. Many hospitalized patients also showed liver swelling, anemia, and changes in electrocardiograms. In another episode, more than 200 adults were poisoned by contaminated soy sauce with an estimated daily exposure of 3 mg of arsenic for 2–3 weeks. Depending on dose and duration of exposure, adverse health effects caused by inorganic arsenic can occur in many organs. Symptoms of acute exposure to arsenic in drinking water at doses of 0.2 mg kg^{-1} day^{-1} or above usually occur within the first several hours. Essentially, all cases of short-term high-dose exposure to inorganic arsenic show clinical signs of gastrointestinal effects.

Short-term exposure (weeks/months) to elevated arsenic (0.06 $mg^{-1}day^{-1}$) in drinking water can result in gastrointestinal effects, such as abdominal pain, vomiting, diarrhea, and muscular cramping; hematological effects, such as anemia and leucopenia; and peripheral neuropathy, such as numbness, burning or tingling sensations, or pain in the extremities. A metallic taste, garlic odor in breath and feces, and salvation may also be present. These short-term effects are generally reversible when the exposure is terminated.

For chronic exposure, lower lethal doses of 0.014–0.05 mg As $kg^{-1}day^{-1}$ in drinking water have been reported. Chronic exposure to arsenic in drinking water typically causes specific dermal effects. Diffuse or spotted hyperpigmentation followed by palmer–planter hyperkeratosis occurs after 6 months to 36 months of ingestion of high doses of arsenic (0.04 mg $kg^{-1}day^{-1}$) or 5–15 years of ingestion of low doses of arsenic (0.01 mg $kg^{-1}day^{-1}$ or higher). Chronic exposure to 0.02 mg $kg^{-1}day^{-1}$ or higher has been shown to cause perturbed porphyrin metabolism and irreversible hypertension. In addition to skin lesions, chronic exposure to arsenic is also associated with other health outcomes including peripheral vascular, cardiovascular, diabetes mellitus, peripheral neuropathy, diseases of the respiratory system, negative impacts on fetal and infant development (low birth weight), and cancers (skin and internal organs).

Inorganic arsenic is a listed as a human carcinogen by many national agencies and international organizations. It is a multisite carcinogen; numerous epidemiologic studies provide evidence associating of oral exposure to inorganic arsenic via drinking water with different types of cancers including the skin, urinary bladder, lung, kidney, liver, and prostate. This link between these cancers and arsenic exposure in drinking water was observed in many populations of the world, including Taiwan, Japan, Chile, Argentina, Bangladesh, India, and China. In 2010, The International Agency for Research on Cancer (IARC) concluded that there was sufficient evidence to show

that skin lesions and cancers of the urinary bladder, lung, and skin were caused by arsenic in drinking water, and limited evidence for cancers of the kidney, liver, and prostate.

Assessment of human cancer risk related to the exposure of total inorganic arsenic is limited. Because only drinking water arsenic was measured in most available epidemiological studies the information on total dietary exposure was lacking. In 2010, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) withdrew its provisional tolerable weekly intake (PTWI) for arsenic because evidence from human epidemiology data indicated that it did not protect human health. Therefore, using a range of assumptions to extrapolate drinking water arsenic concentration to total dietary inorganic arsenic exposure, JECFA has determined the 95% confidence limit of the benchmark dose ($BMDL_{005}$) for 0.5% increased incidence of lung cancer over background for inorganic arsenic to be 3.0 $\mu g\ kg^{-1}\ day^{-1}$ (2–7 $\mu g\ kg^{-1}\ day^{-1}$ based on the range of estimated total dietary exposure). Similarly, based on the studies showing statistically significant association of drinking water arsenic concentration below 100 $\mu g\ l^{-1}$ and its adverse effects in humans, the European Food Safety Authority (EFSA) panel on Contaminants in the Food Chain (CONTAM Panel) selected a benchmark response of 1% extra risk and calculated a range of values of the $BMDL_{01}$ instead of a single reference point for inorganic arsenic. The $BMDL_{01}$ values for the various health endpoints, skin lesions, cancers of the skin, urinary bladder, and lung ranged from 0.3 to 8 $\mu g\ kg^{-1}day^{-1}$.

Based on animal studies, Agency for Toxic Substances and Disease Registry (ATSDR) has derived an intermediate oral minimal risk level (MRL) of 0.1 $mg\ kg^{-1}\ day^{-1}$ for MMA for diarrhea in rats exposed to dietary MMA for 13 weeks, a chronic oral MRL of 0.01 $mg\ kg^{-1}\ day^{-1}$ for MMA for increased incidence of progressive nephropathy in male mice exposed to dietary MMA for 2 years, and a chronic oral MRL of 0.02 $mg\ kg^{-1}\ day^{-1}$ for DMA for increased vacuolization of the urothelium in the urinary bladder of female mice exposed to dietary DMA for 2 years. In a 2-year rat bioassay, DMA has been shown to promote carcinogenesis in the urinary bladder but not in other tissues. Cytotoxicity and increased cell proliferation were involved rather than direct DNA damage. However, the relevance of these findings to humans has not been established especially because the rats eliminate DMA much slower than other species, including humans.

Methods of Analysis

Sample preparation is required for the determination of arsenic in food. Acid digestion is usually used for total arsenic determination, whereas milder extraction combined with chemical separation of inorganic and organic arsenic, or chromatographic separation of arsenic species with on-line selective detector of arsenic are needed for the determination of arsenic species from food. A variety of instrumental techniques for the determination of arsenic are available. These include hydride generation atomic absorption spectrometry (HG-AAS), high-performance liquid chromatography (HPLC)–HG-AAS, HPLC combined with hydride generation atomic fluorescence spectrometry (HPLC–HG-AFS), inductively coupled plasma atomic emission spectrometry (ICP-AES), inductively coupled plasma mass spectrometry (ICP-MS), HPLC–ICP-MS, neutron activation analysis (NAA), and electro-thermal atomization laser-excited atomic fluorescence spectrometry (ETA-LEAFS). For arsenic species determination in food, HPLC–ICP-MS is by far the most common and useful method.

Exposure

For the general population who do not smoke, the most common route of exposure to arsenic is through ingestion, and drinking water and food are the major sources. Depending on arsenic levels in drinking water and food, exposure can vary greatly in different regions. In regions with high natural arsenic levels, drinking water can be the major contributor of inorganic arsenic in the diet. Whereas, in regions where arsenic concentration in the drinking water is low, less than 50 $\mu g\ l^{-1}$, food can be the major contributor. Seafood/fish products contribute the most to dietary total arsenic exposure while cereal and grain products contribute the most of inorganic arsenic. Because rice is generally grown in paddy soil under flooded conditions, it accumulates arsenic from the soil and water more than other cereal grains; its arsenic content is approximately 10 times higher than that of other grains. The predominant arsenic species in rice are inorganic arsenic and DMA. The bioavailability of greenhouse-grown rice in which approximately 86% arsenic is present as DMA was determined to be low: only 33% of total rice arsenic was absorbed in an *in vivo* swine model. Total and inorganic arsenic content in rice varies greatly, ranging from 0.03 to 0.54 and 0.01 to 0.41 $mg\ kg^{-1}$, respectively, and can be influenced by geographic location, cultivar, arsenic levels in irrigation water, soil arsenic, use of arsenic-containing fertilizer and pesticides, etc. The percentage of inorganic arsenic as total arsenic in rice ranged from 11% to 90% or 100%. A recent report indicated that even though Chinese rice has lower inorganic arsenic content than that from other countries, it can contribute significantly to human inorganic arsenic exposure due to high consumption of rice as a staple food.

Dietary exposure to inorganic arsenic has been estimated to be 0.13–0.56 $\mu g\ kg^{-1}day^{-1}$ for average consumers and 0.37–1.22 $\mu g\ kg^{-1}day^{-1}$ for 95th percentile consumers across 19 European countries. This was calculated based on the assumption that the overall average of the proportion of inorganic arsenic to total arsenic for food other than fish and seafood was 70% and a fixed inorganic value of 0.03 $mg\ g^{-1}$ in fish and 0.1 $mg\ kg^{-1}$ in seafood, and using the lower bound and upper bound concentrations. Recently, Xue *et al*. using a probabilistic modeling with the Stochastic Human Exposure and Dose Simulation Dietary model (SHEDS-Dietary) and based on data from the National Health and Nutrition Examination Survey, reported that in the US, the estimated dietary exposures to total and inorganic arsenic were 0.36 and 0.05 $\mu g\ kg^{-1}day^{-1}$, respectively for the mean, and 1.40 and 0.19 $\mu g\ kg^{-1}day^{-1}$, respectively for the 95th percentile.

Risk Characterization

Inorganic arsenic has been identified as a human carcinogen based on numerous epidemiological studies associated with

arsenic levels in drinking water. Food can contribute significantly to total dietary inorganic arsenic exposure in people who are high consumers of rice or algae-based products. Water, depending on arsenic levels, used in food, cooking/preparation and possibly irrigation of crops, particularly rice, can also contribute to arsenic in food. More information on bioavailability and speciation data for different food is needed for improving exposure estimation and for more accurate risk assessment. The estimated mean background dietary exposures to inorganic arsenic in the US and various European and Asian countries were reported to vary from 0.1 to 3.0 μg $kg^{-1}day^{-1}$ that are near or at the range of the $BMDL_{01}$ and $BMDL_{005}$ values identified by EFSA and JECFA, respectively. This suggests that certain populations may have an increase in risk depending on their particular dietary habits.

See also: Disciplines Associated with Food Safety: Epidemiology; Food Safety Toxicology. Foodborne Diseases: Overview of Chemical, Physical, and Other Significant Hazards. Institutions Involved in Food Safety: World Health Organization (WHO). Public Health Measures: Monitoring of Contaminants. Risk Analysis: Risk Assessment: Chemical Hazards; Risk Assessment: Principles, Methods, and Applications. Veterinary Drugs Residues: Control of Helminths

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TOXIC METALS

Cadmium

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Glossary

Beta-2-microglobulin A small protein normally found on the surface of many nucleated cells. It is normally filtered through the glomerulus and gets reabsorbed by renal tubules back into the blood. In case of tubular damage such as in chronic cadmium toxicity, the reabsorption is impaired, resulting in elevated level in urine.

***Itai-Itai* disease** A bone disease caused by industrial cadmium pollution of the food and water supply, found in the polluted Jinzu River basin in Japan. The major symptoms of *Itai-Itai* disease include pain in the joints and the back, resulting from the cadmium-induced bone damage.

Metallothionein A type of low molecular weight, cysteine-rich protein that binds with metals such as copper, cadmium, and zinc.

Provisional tolerable weekly/monthly intake (PTWI/PTMI) An estimate of the amount of a contaminant that can be ingested over a lifetime without appreciable risk.

Tubular proteinuria An increase of urinary excretion of low molecular weight plasma proteins, such as beta-2-microglobulin (β2MG) and retinol-binding protein (RBP). It is an early sign of cadmium-induced renal dysfunction through chronic exposure.

Chemical Names

Cadmium (CAS Number: 7440-43-9); Metallic cadmium, Inorganic cadmium, such as cadmium oxide and cadmium sulfide.

Background

Cadmium is a transition bivalent metal naturally occurring in the earth's crust, and is typically found in ores with other metals such as zinc, lead, and copper. Cadmium has been used widely in the industry as an electroplating agent, a color pigment, and a plastic stabilizer since the 1930s. In many countries including the US, these traditional applications have declined because of its health effects and issues of environmental contamination. Today, cadmium is mainly used in the manufacture of nickel–cadmium batteries and cadmium telluride solar panels.

Cadmium can enter the environment in many ways. Metal mining, smelting, and burning of coal and household waste can release cadmium into the air. Cadmium can enter water from waste water disposal in related industries, and can enter soil from car exhaust, fertilizer usage, and the deposition from the air. High levels of cadmium have been found in the soil close to hazardous waste sites because of spills and leaks. As a chemical element, cadmium does not break down in water and soil, where it can be taken up by plants, fish, and animals.

Hazard Identification and Characterization

Cadmium has no known biological function in animals and humans. Occupational exposure to cadmium, mainly through inhalation, can result in metal fume fever, chemical pneumonitis, pulmonary edema, and lung cancer. In the general population, long-term exposure to cadmium from tobacco smoking and consumption of contaminated food and water can lead to adverse health effects including renal damage and bone demineralization.

In humans, the absorption of cadmium from the gastrointestinal (GI) tract is approximately 5% in adults. Bioavailability of cadmium is lower in zinc and iron-rich foods such as oysters, because these two ions compete with cadmium for absorption from the GI tract. Iron deficiency results in an increased absorption of cadmium, and it has been reported that the divalent metal transporter I (DMTI) acts as the transporter of cadmium. Dietary absorption of cadmium is also favored by deficiencies of zinc, calcium, and protein. Women generally have a higher body burden of cadmium and are more susceptible to cadmium toxicity compared to men. Some studies suggest that the absorption of cadmium from diet in newborns and infants can be seven to eight-folds higher than that in adults.

Cadmium induces and binds with metallothionein (MT) primarily in the liver, and a small amount of the cadmium–MT complex is then transported to the kidneys. The binding of cadmium to MT prevents the free cadmium ions from exerting their toxic effects. However, because of the small molecular weight of MT, the complex can be easily reabsorbed by the renal tubules and then degraded by lysosomes. The degradation releases unbound cadmium, which induces the tubular cells to produce more MT. When the free cadmium ions exceed a certain level at which the newly synthesized MT is insufficient to neutralize them, tubular damages start

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to occur. This cadmium level is called the 'critical value,' and is commonly quoted as 200 μg g^{-1} renal cortex.

In nonoccupational exposures in humans, cadmium is found mainly in liver (50%) and kidney (15%), with a small amount in bone. Blood cadmium levels are low, usually less than 1 μg l^{-1} and the level in the breast milk is even less. Cadmium does not readily cross the placenta and the blood–brain barrier. Its excretion routes include urinary and fecal, but only approximately 0.001% of the body burden is excreted per day. The biological half-life of cadmium in humans is extremely long, approximately 10–30 years.

In experimental animals, chronic oral exposure to cadmium can result in kidney damage, decrease in bone calcium levels, and adverse effects on developmental, neurobehavioral, and immune systems. Cadmium also causes tumors at multiple tissue sites by various routes of exposure.

In humans, acute toxicity is a rare event. Exposures to high concentrations of cadmium from heavily contaminated food or beverages can result in GI symptoms including nausea, vomiting, and abdominal pain. The 'no observed adverse effect level' (NOAEL) of a single oral dose is estimated to be 3 mg elemental cadmium per person, and the reported lethal doses range from 350 to 8900 mg.

The chronic toxic effects of cadmium are a much greater concern than the acute exposures because they require lower exposure levels and occur more frequently. One of the major toxic effects of low-level oral cadmium exposure is renal injury. Cadmium accumulation in proximal tubules causes histopathological changes in tubular cells and glomeruli, resulting in impairment of renal function as reflected by tubular proteinuria. The most commonly used biomarkers of cadmium-related renal effects include the urinary levels of beta-2-microglobulin (β2MG) and retinol-binding protein (RBP), and the activity of *N*-acetyl-beta-glucosaminidase (NAG) as indicators of tubular toxicity, whereas the urinary albumin, transferrin, and glomerular filtration rate (GFR) are used as indicators of glomerular damages. Cadmium-induced nephrotoxicity has been associated with increased mortality rates in many epidemiological studies, and it may also potentiate diabetes-induced effects on the kidneys.

Long-term consumption of cadmium-contaminated rice caused *Itai-Itai* disease in postmenopausal women in Japan, characterized by severe osteomalacia and osteoporosis, bone deformities, and concomitant renal dysfunction. Cadmium levels lower than what was seen in Japan have been associated with skeletal effects such as bone mineral loss, decreased bone density, and increased bone fragility and fracture.

Some epidemiological evidence suggests that cadmium may be an etiologic agent for cardiovascular disease including hypertension, yet the associations are weak and inconsistent with unclear mechanisms. The relationship between abnormal behavior and decreased intelligence in children and adults exposed to cadmium was also reported, but typically complicated by exposure to other toxic metals. As noted previously, the blood–brain barrier severely limits cadmium access to the central nervous system.

Cadmium and cadmium compounds have been classified as known human carcinogens by the International Agency for Research on Cancer (IARC) and the National Toxicology Program (NTP). Most of the evidence is based on the occupational exposure through inhalation, which has been most clearly associated with lung cancer. Some epidemiologic studies have reported an association between chronic cadmium exposure and cancers of the breast, endometrial, kidney, pancreas, and urinary bladder. The mechanism of cadmium carcinogenesis is not clearly understood.

A Provisional Tolerable Weekly Intake (PTWI) for cadmium of 7 μg kg^{-1} bw was established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1988 and has been widely accepted. At its 73rd meeting in 2010, JECFA reevaluated cadmium and established a new Provisional Tolerable Monthly Intake (PTMI) of 25 μg kg^{-1} bw, which is equivalent to 0.8 μg kg^{-1} bw day^{-1}. This PTMI, derived based on the renal biomarker β2MG, is intended to protect 95% of the population by age of 50 years without having adverse renal effects. US Agency for Toxic Substances and Disease Registry (ATSDR) established a chronic-duration oral minimal risk level (MRL) of 0.1 μg cadmium kg^{-1} bw day^{-1} in 2008. In the same year, US EPA published a Reference Dose (RfD) of 0.5 μg kg^{-1} bw day^{-1} from water and 1 μg kg^{-1} bw day^{-1} from food based on the critical value of 200 μg g^{-1} renal cortex. This RfD is equivalent to the previous JECFA PTWI.

Methods of Analysis

Quantitative determination of cadmium can be performed using a variety of methods, among which atomic absorption spectroscopy (AAS) is the most commonly used method to quantify cadmium levels in food samples. Other analytical methods include inductively coupled plasma techniques using optical emission spectroscopy (ICP-OES) or mass spectrometry (ICP-MS). In the US Total Diet Study (TDS), the standard method used to measure cadmium level in foodstuffs is graphite furnace AAS (GFAAS). In the European Union (EU), it is required that an acceptable method should have the limit of detection (LOD) less than 1/10 of the maximum level (ML), and the limit of quantification (LOQ) less than 1/5 of the ML.

Exposure

The presence of cadmium in food results from contamination of soil and water. Cadmium can be taken up and accumulated in plants and animals. Concentrations of cadmium in food vary widely among food categories and geographic regions. As consented at the 73rd JECFA meeting, the average occurrences of cadmium range from less than 0.0001 to 0.04 mg kg^{-1} in most food categories. Higher concentrations ranging from 0.1 to 4.8 mg kg^{-1} were reported for certain foods that accumulate relatively high levels of cadmium, such as shellfish/mollusks, animal offal, oilseeds, and vegetables, especially wild mushrooms. Tobacco and cereal grains have the ability to concentrate cadmium to levels of 10–150 μg Cd kg^{-1}. In contrast, eggs and milk usually have lower levels of cadmium. The contribution of each food category in cadmium intake from the total diet varies by countries and regions due to the differences in cadmium occurrences and dietary habits. For instance, the main dietary sources of cadmium in Japan are rice, vegetables/

seaweed, and seafood, whereas cereals/grains, animal offal and vegetables/nuts/pulses are the main sources in Europe.

Food is the major source of cadmium for the general population, especially in nonsmokers. Cigarette smoking can significantly increase the life-time body burden of cadmium. According to the data submitted to JECFA at its 73rd meeting (2010), the estimates for the mean cadmium exposure in adults ranged from 2.2 to 12.0 μg kg^{-1} bw per month in different countries/regions. The cadmium exposures for high consumers, as reported by European Food Safety Authority (EFSA), Lebanon, and the USA, ranged from 6.9 to 12.1 μg kg^{-1} bw per month. For Australia and the USA, mean dietary exposure for children 0.5–12 years of age ranged from 2.7 to 12.9 μg kg^{-1} bw per month. Dietary exposure for vegetarians, according to EFSA's hypothetical model that replaced meat and fish with nuts and oilseed in equal amounts, was estimated to be 21.6 μg kg^{-1} bw per month.

Risk Characterization

Given the results of the dietary exposure assessments described above, the estimated dietary cadmium intake for all age groups, including consumers with high exposures and subgroups with special dietary habits, are below the JECFA PTMI of 25 μg kg^{-1} bw. The risk of adverse renal effects from dietary cadmium exposure is low in the general population, but susceptible populations such as smokers (high background exposure levels), vegetarians, and individuals who frequently eat rice grown in heavily contaminated soil may be at increased risk of adverse renal effects.

See also: Analytical Methods: Overview of Methods of Analysis for Chemical Hazards. Public Health Measures: Monitoring of Contaminants. Risk Analysis: Risk Assessment: Chemical Hazards

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TOXIC METALS

Lead

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Glossary

Acute toxicity Adverse effects of a substance that result either from a single exposure or from multiple exposures in a short space of time (usually less than 24 hours).
Cardiovascular system Organ system that passes nutrients, gases, hormones, blood cells, etc., to and from cells in the body to help fight diseases and help stabilize body temperature and pH to maintain homeostasis.
Chronic toxicity Property of a substance that has toxic effects on a living organism, when that organism is exposed to the substance continuously or repeatedly.
Electrothermal atomic absorption spectrometry (ETAAS) A type of atomic absorption spectrometry where the sample is atomized using a probe which is rapidly heated by passing a current through it. The probe often is either a graphite tube or a tungsten coil.
Inductively coupled plasma mass spectrometry (ICP-MS) A type of mass spectrometry that is highly sensitive and capable of the determination of a range of metals and several non-metals at concentrations below one part in 10^{12} (part per trillion). It is based on coupling together with inductively coupled plasma as a method of producing ions (ionization) with a mass spectrometer as a method of separating and detecting the ions.
Intelligence quotient (IQ) It is a score derived from one of several different standardized tests designed to assess intelligence. Current IQ tests are constructed so that the median score is set to 100 and a standard deviation to 15.
Osteoporosis Thinning of bone tissue and loss of bone density over time.
Systolic blood pressure (SBP) Maximum blood pressure during each heart beat.
Thiol group A functional group that contains a sulfur atom bonded to a hydrogen atom.

Background

Lead is a ubiquitous element that occurs in the environment due to natural and anthropogenic pathways/sources. Dietary exposures can be elevated because of the use of lead compounds such as those used in paint, gasoline, and solder. Absorption of lead from the gastrointestinal tract is influenced by physiological factors (e.g., age, fasting, calcium and iron status, and pregnancy). In particular, absorption is higher in children than in adults and is lower in the presence of food. Absorbed lead is transferred to soft tissues, including liver and kidney, and to bone where it accumulates with age. The half-life is approximately 30 days in blood and much longer in bone (10–30 years). Conditions, such as pregnancy and osteoporosis, result in bone resorption and elevated blood levels. Lead readily crosses the placenta and is transferred into breast milk. Urine and feces are the major routes of excretion. Lead binds to thiol groups and other ligands in proteins and its toxicity is attributed to inhibition of a number of important enzymes (e.g., heme synthesis) and to interference with calcium, magnesium, and zinc homeostasis. For infants and children, a chronic dietary exposure of 30 $\mu g\,day^{-1}$ is estimated to correspond to a decrease of 1 intelligent quotient (IQ) point. In adults, a dietary lead exposure of 80 $\mu g\,day^{-1}$ is estimated to correspond to an increase in systolic blood pressure of 1 mm Hg. Reductions in population blood levels have primarily occurred because of the elimination of lead fuel additives, lead solder in food cans, and lead additives in paint.

Hazard Identification and Characterization

Blood is the tissue used most frequently to estimate exposure to lead, and blood lead levels generally reflect exposure in recent months. However, if the level of exposure is relatively stable, then blood lead level is a good indicator of exposure over a protracted period of time. Longitudinal surveys in some countries have shown substantial reductions in population blood lead levels in recent decades.

The acute toxicity of lead is low whereas chronic oral exposure has detrimental effects on multiple organs, including the kidneys and liver, and systems, including the cardiovascular, hematological, immune, reproductive, and nervous systems. Lead elicits a wide range of effects, including various neurological and behavioral effects, mortality (mainly due to cardiovascular diseases), impaired renal function, hypertension, impaired fertility and adverse pregnancy outcomes, delayed sexual maturation, and impaired dental health. High doses of inorganic lead compounds are carcinogenic in

experimental animals, causing renal and brain tumors. Results of genotoxicity studies and the inhibition of DNA-repair indicate a non-DNA-reactive mode of action.

For children, the weight of evidence and consistency across studies is greatest for the association of blood lead levels with impaired neurodevelopment, specifically reduction of intelligence as measured by the IQ. This effect has been associated with lower blood lead concentrations than those associated with the effects observed in other organ systems. Although the estimated IQ decrease per microgram per deciliter ($\mu g\ dl^{-1}$) of blood lead is small when viewed as the impact on an individual child (6.9 points over the range of 2.4–30 $\mu g\ dl^{-1}$), the decrement is considered to be important when interpreted as a reduction in population IQ. The most significant impact on population IQ would be at the tails of the IQ distribution such that there would be more children considered to have less than normal IQ and there would be less children deemed to be of superior intelligence. The lead-associated reduction in IQ may also be a marker for other adverse neurodevelopmental effects for which the evidence is not as robust, but which have been observed in children at approximately the same blood lead levels (e.g., executive dysfunction and fine motor deficit).

For adults, the adverse effect for which the weight of evidence is greatest and most consistent is that associated with an increase in blood pressure. As with the IQ detriment, the increase is small when viewed as the effect on an individual's blood pressure, but important when viewed as a shift in the distribution of blood pressure within a population. Increased blood pressure is clearly associated with increased risk of cardiovascular mortality.

Methods of Analysis

Sample preparation used most frequently for the determination of lead in food is acid digestion with strong oxidants in open or closed vessels. Microwave-assisted acid digestion is also used, which allows the use of large sample masses (1–2 g) under controlled temperature and pressure conditions, reducing contamination and avoiding loss of the element during mineralization. The analytical methods of choice for the determination of lead in food and blood are electrothermal atomic absorption spectrometry (ETAAS) and inductively coupled plasma mass spectrometry (ICP-MS). In the past decade, many technical improvements have been made to ETAAS, which allow for the determination in food at the low microgram per kilogram level. ICP-MS is increasingly used in food laboratories owing to its capability to perform multielement measurements in a wide variety of foods. The use of dynamic reaction cell (DRC)-ICP-MS has allowed further refinements resulting in the determination of lead in food at levels lower than 0.1 $\mu g\ kg^{-1}$. In blood, the methods are well established and the level of detection (LOD) of 0.1 $ng\ ml^{-1}$ level is adequate to quantify lead in blood.

Exposure

Lead is taken up from soil into food crops, and the sources of lead in food may also include soil remaining in or on the food, atmospheric deposition, water, contact with lead-containing processing equipment, and packaging. Food categories with the highest frequency of detectable lead include: meat, especially offal, organ meats, and wild game; shellfish (particularly bivalves); cocoa; tea; cereal products; milk; and vegetables. Most foods that contain lead do so in the low parts per billion (ppb) range, however some, like cocoa, organ meats, tea, and coffee, can have somewhat higher averages of more than 100 ppb. In adults, mean exposures tend to range from 0.02 to 3 microgram per kilogram of body weight per day ($\mu g\ kg^{-1}\ bw\ day^{-1}$) whereas higher percentile levels of exposure range from 0.06 to 2.4 $\mu g\ kg^{-1}\ bw\ day^{-1}$. Children's mean exposures range from 0.03 to 9 $\mu g\ kg\ bw\ day^{-1}$. Estimated high end percentile exposures for children range from 0.2 to 8.2 $\mu g\ kg^{-1}\ bw\ day^{-1}$. Overall blood lead levels are useful biomarkers of exposure and reflect dietary exposures as well as exposures from other pathways such as inhalation. The contribution of dietary exposure to the total body burden as measured by blood lead levels will of course vary between countries depending on the individual contributions of nondietary lead sources and pathways.

Risk Characterization

Because there have been many epidemiological studies of the relationship between blood lead levels and children's IQ, characterization of the dose–response relationship requires integration of multiple study results. The Lanphear *et al.* (2005) meta-analysis included 1333 children enrolled in seven longitudinal cohort studies conducted in several countries, who were followed from birth or early infancy to 5 – 10 years of age. The use of a log-linear model produced an estimated IQ decline of 6.9 points in concurrent blood lead level over a range of 2.4–30 $\mu g\ dl^{-1}$. The slope of the inverse association between IQ and concurrent blood lead level was steeper among children with a maximum observed (at any time point) blood lead level below 7.5 $\mu g\ dl^{-1}$ than it was among children with a blood lead level of 7.5 $\mu g/dl^{-1}$ or higher.

The relationship between blood lead levels and dietary exposure to lead has been estimated to be between 0.05 and 0.16 $\mu g\ dl^{-1}$ of lead in blood per 1 $\mu g\ day^{-1}$ of dietary lead exposure. This range is based on toxicokinetic analyses of data on Scottish infants exposed to lead in drinking water.

Dietary lead exposures associated with a range of decreases in IQ (i.e., 0.5 – 3 IQ points) were estimated by using a Monte Carlo simulation, where the resulting confidence interval (CI) reflects the uncertainties in both the dose–response modeling of blood lead levels and the extrapolation to dietary exposure. The chronic dietary exposure corresponding to a decrease of 1 IQ point was estimated to be 30 μg of lead per day, with a 5–95% CI ranging from 4 to 208 $\mu g\ day^{-1}$.

Although the relationship between blood lead levels and children's IQ is roughly linear, there is some evidence that the largest effects occur in the range of 5–15 $\mu g\ dl^{-1}$. To the extent that the dose–response relationship is nonlinear, the incremental effect of dietary exposure is somewhat dependent on the extent to which there are other exposures to lead (e.g., drinking water, air, soil, and paint). Therefore, a linear

estimate (i.e., 1 IQ point per 30 μg day^{-1}) may underestimate the impact in the range of 50 – 150 μg day^{-1}, and overestimate the impact on a level below 50 and above 150 μg day^{-1}.

For adults, increased systolic blood pressure is the most sensitive end-point. A linear slope of 0.28 mm Hg per 1 μg dl^{-1} (5–95% CI 0.03 – 0.53 mm Hg per μg dl^{-1}) relating increases in systolic blood pressure as a function of blood lead level has been derived by averaging the estimates from four different studies. Blood lead levels were converted to dietary exposures using a blood level range of 0.02 – 0.07 μg dl^{-1} per 1 μg day^{-1} of dietary lead exposure. Dietary lead exposure corresponding to an increase in systolic blood pressure of 1 mmHg was estimated to be 80 μg day^{-1}, or approximately 1.2 μg kg^{-1} bw day^{-1}. As the relationship appears to be linear, the increases in blood pressure associated with other dietary exposures are proportional. Published studies used by World Health Organization in estimating the global burden of disease attributable to lead indicate that relative risks of ischemic heart disease and cerebrovascular stroke associated with small increases in blood pressure (0.4 – 3.7 mm Hg systolic blood pressure) have been estimated to be in the range of 1.0–1.4, with higher relative risks at younger ages.

Summary

Neurodevelopmental effects in the fetus, infant, and child are clearly the most sensitive adverse health outcomes of exposure to lead. Estimated mean dietary exposure for children aged approximately 1–4 years ranges from 0.03 to 9 μg kg bw day^{-1}. The health impact at the lower end of this range is calculated to be associated with a population decrease of 0.5 IQ point. The higher end of the exposure range is associated with a population decrease of 3 IQ points, which is demonstrable of some public health concern. For adults, the mean dietary lead exposure estimates range from 0.02 to 3.0 μg kg bw day^{-1}. The lower end of this range is considerably below the exposure level of 1.2 μg kg^{-1} bw day^{-1} which is associated with a population increase in systolic blood pressure of 1 mm Hg. At the higher end of the exposure range, a population increase of approximately 2 mm Hg in systolic blood pressure would be expected to occur. An increase of this magnitude has been associated, in a large meta-analysis, with modest increases in the risks of ischemic heart disease and cerebrovascular stroke. These estimates are of less magnitude and public health concern than that estimated for the neurodevelopmental effects observed in children.

See also: Disciplines Associated with Food Safety: Epidemiology; Food Safety Toxicology. Institutions Involved in Food Safety: World Health Organization (WHO). Public Health Measures: Monitoring of Contaminants. Risk Analysis: Risk Assessment: Chemical Hazards; Risk Assessment: Principles, Methods, and Applications. Veterinary Drugs Residues: Control of Helminths

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TOXIC METALS

Mercury

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Glossary

Exposure Contact with a substance by swallowing, breathing, or touching the skin or eyes. Exposure may be short term (acute exposure), of intermediate duration, or long term (chronic exposure).
Hazard A biological, chemical, or physical agent in, or condition of, food with the potential to cause an adverse health effect.
Hazard characterization The qualitative and/or quantitative evaluation of the nature of the adverse health effects associated with biological, chemical, and physical agents that may be present in food. For chemical agents, a dose–response assessment should be performed. For biological or physical agents, a dose–response assessment should be performed if the data are obtainable.
Hazard identification Qualitative evaluation of the adverse effects of a substance on humans or other organisms of concern.
In vivo Within a living organism or body, e.g., some toxicity testing is done on whole animals, such as rats or mice.
Minimum risk level (MRL) An estimate of daily human exposure to a hazardous substance at or below which that substance is unlikely to pose a measurable risk of harmful (adverse), noncancerous effects. MRLs are calculated for a route of exposure (inhalation or oral) over a specified time period (acute, intermediate, or chronic). MRLs should not be used as predictors of harmful (adverse) health effects.
Provisional tolerable weekly intake (PTWI) An endpoint used for food contaminants such as heavy metals with cumulative properties. Its value represents permissible human weekly exposure to those contaminants unavoidably associated with the consumption of otherwise wholesome and nutritious foods.
Reference dose (RfD) An estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime.
Risk characterization The qualitative and/or quantitative estimation, including attendant uncertainties, of the probability of occurrence and severity of known or potential adverse health effects in a given population based on hazard identification, hazard characterization, and exposure assessment.

Chemical Names

Elemental/metallic mercury, Chemical Abstracts Service Registry (CAS) Number: 7439-97-6; inorganic mercury (e.g., mercuric chloride, CAS Number: 7439-97-6, and mercurous chloride, CAS Number: 10112-91-1); organic mercury (e.g., methylmercury, CAS Number: 22967-92-6).

Background

Mercury (Hg) is released into the environment from both natural and anthropogenic sources. Mercury has been used commercially and medically for thousands of years. The ancient Greeks, Romans, and Egyptians used mercury (in the form of mercuric sulfide) for cosmetic and decorative purposes, and in ointments. In the Middle Ages, mercuric salts were used in the treatment of vermin, lice, and most notably syphilis, when Paracelsus noted that a little might be useful but too much could be fatal, leading to his immortal dictum "the dose makes the poison." In the 18th century, the extensive use of mercuric nitrate in the hat-making industry led to the 'mad as a hatter syndrome'.

In the 1940s and 1950s, phenylmercury or inorganic mercury salts were known as causative agents of acrodynia, also known as pink disease. In the 1960s, exposures to methylmercury were noted in Minamata Bay and Niigata, Japan, from consumption of contaminated fish, and in Iraq through consumption of grain treated with a methylmercury-based fungicide. A cosmetic cream from Mexico, called 'Crema de Belleza-Manning,' sold in the US in 1996 was also found to contain mercurous chloride.

In spite of its potential risks, mercury continues to be used in a multitude of products and processes all over the world owing to its unique properties. Mercury is utilized in the electrical industry, dentistry (dental amalgams), numerous industrial processes, including the production of chlorine and caustic soda, in nuclear reactors, as an antifungal agent for wood processing, a solvent for reactive and precious metal,

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as a preservative of pharmaceutical products, and in energy-saving fluorescent light bulbs.

Hazard Identification and Characterization

Mercury occurs in three basic forms: elemental mercury (metallic), inorganic mercury, and organic mercury (primarily methylmercury). Depending on the dose each form can be toxic to humans, although they behave differently in terms of absorption into the body and the degree to which they migrate to organs. As a result, the toxicity of mercury is dependent on its chemical form.

Elemental mercury is relatively inert and not readily taken up from the gastrointestinal tract in vertebrates, but it is volatile and its vapor is toxic. Effects on the nervous system appear to be the most sensitive toxicological endpoint observed following exposure to elemental mercury.

Inorganic mercury is reported to have hematological, hepatic, renal, reproductive, genotoxic, and general toxicity as well as organ-specific effects in rats and/or mice treated with inorganic mercury in the form of mercuric chloride. Although chronic exposure of mice and rats to mercuric chloride produced some indication of carcinogenicity in male rats, International Agency for Research on Cancer (IARC) reported that there was limited evidence in experimental animals for the carcinogenicity of mercuric chloride.

Exposure to elemental mercury through food is virtually nonexistent. The general population is exposed to methylmercury and inorganic mercury through the diet. However, only a small percentage of inorganic mercury in food is absorbed after ingestion, and it is also considerably less toxic compared to methylmercury. As methylmercury is the most toxic form to which humans are exposed via food, this review focuses only on the potential risk of methylmercury.

Methylmercury is highly toxic to humans. Exposure to methylmercury is known to result in adverse effects in several organ systems (nervous system, kidney, liver, and reproductive organs), with neurotoxicity considered the most sensitive endpoint. Dietary methylmercury is almost completely absorbed into the blood, bound to red blood cells, and distributed to all tissues, including the brain, and is able to cross the blood–brain and the placental barriers. Both neurotoxicity and nephrotoxicity have been associated with acute methylmercury poisoning incidents in humans. Epidemics of poisoning following high-dose exposures to methylmercury in Japan and Iraq demonstrated that neurotoxicity is the health effect of greatest concern when methylmercury exposure of the fetus occurs. Epidemiological studies conducted in New Zealand and the Faroe Islands reported that chronic, low-dose prenatal methylmercury exposure from maternal consumption of fish was associated with more subtle endpoints of neurological outcomes in children. Furthermore, data from animal studies, including studies on nonhuman primates, indicate that the developing nervous system is a sensitive target organ for low-dose methylmercury exposure. Methylmercury has been classified as a possible human carcinogen, based on inadequate data in humans and limited evidence of carcinogenicity in animals.

Unlike elemental or inorganic forms, methylmercury is almost completely absorbed from the gastrointestinal tract and is distributed to all tissues. Methylmercury can be transferred from blood to mother's milk and hair. The concentration of mercury in hair can be used as an indicator of previous exposures, e.g., during pregnancy, although signs of exposure may not be apparent at that time. Methylmercury accumulates in the brain and is demethylated into inorganic mercury with a half-life of approximately 55 days. Animal studies have shown that inorganic mercury deposits formed in the brain play an important role in methylmercury neurotoxicity.

A wide range of adverse health effects has been observed in humans following methylmercury exposure, the severity largely depending upon the magnitude of the dose and the duration of exposure. Methylmercury has been reported to be highly toxic particularly to the nervous system, and the developing brain is thought to be the most sensitive target organ for methylmercury toxicity. As methylmercury is toxic to the developing nervous system, fetuses are especially susceptible. Therefore, the population at highest risk is the fetus and infants of women of child-bearing age who consume fish and seafood. To protect the fetus during the early stages of development, women of child-bearing age are also considered at high risk. Several epidemiological studies have shown that nervous system domains involving fine motor function, attention, verbal learning, and memory can be affected. Furthermore, recent studies suggest that chronic exposure to methylmercury may have toxic effects on the immune and cardiovascular systems.

There are established tolerable intake limits for methylmercury. The United States Environmental Protection Agency (US EPA) established a reference dose (RfD) of 0.1 μg per kg body weight per day methylmercury (0.7 μg per kg body weight per week). The United States Agency for Toxic Substances and Disease Registry (US ATSDR) derived a minimum risk level (MRL) of 0.3 μg per kg bw per day based on the neurodevelopmental effects in a study where children were exposed *in utero* to methylmercury from maternal fish consumption. In 2003, based on assessment of epidemiological studies from the Seychelles and the Faroe Islands that investigated the relationship between maternal exposure to mercury and impaired neurodevelopment in their children, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) recommended a provisional tolerable weekly intake (PTWI) for methylmercury of 1.6 μg per kg bw per week (equivalent to 0.23 μg per kg bw per day of methylmercury) in order to sufficiently protect the developing fetus. Similar tolerable intake limit for methylmercury was also established by other regulatory authorities (e.g., Health Canada, which adopted a provisional tolerable daily intake (PTDI) of 0.20 μg per kg bw per day).

Methods of Analysis

The most commonly used analytical methods for the quantification of total mercury in food are cold vapor atomic absorption spectrometry (CV-AAS) or cold vapor atomic fluorescence spectrometry (CV-AFS), and inductively coupled plasma mass spectrometry (ICP-MS) techniques.

Gas chromatography (GC) has been the most widely used technique for the separation of mercury species in food although high performance liquid chromatograph (HPLC) is increasingly being used. Detection by CV-AAS ($10\ \mu g\ kg^{-1}$), CV-AFS ($1\ \mu g\ kg^{-1}$), microwave-induced plasma atomic emission spectrometry (MIP-AES) or ICP-AES ($5\ \mu g\ kg^{-1}$), MS ($40\ \mu g\ kg^{-1}$), and ICP-MS ($<3\ \mu g\ kg^{-1}$) methods all have sufficient sensitivity for food samples. HPLC/ICP/MS have the additional advantage of permitting separation and quantitation of individual mercury species. The advantages of MS and ICP-MS are their multielement and multi-isotope capabilities, whereas CV-AAS and CV-AFS have the advantage of being comparatively low cost and simple operations.

Exposure

The principal source of human exposure to methylmercury is fish and seafood consumption. Food sources other than fish and seafood products may contain mercury, but mostly in the form of inorganic mercury. The contribution to methylmercury exposure from nonfish and nonseafood is considered to be insignificant. Available data collected on mercury in fish are largely for total mercury rather than methylmercury. The proportion of total mercury contributed by methylmercury generally ranges between 75% and 100% depending on species of fish, size, age, and diet. Dietary exposures to methylmercury and inorganic mercury are fairly similar.

Long-lived predatory fish contain the highest levels of methylmercury, and are significant sources of human exposure to methylmercury. For instance, large fish, such as shark, swordfish, king mackerel, and tilefish have average concentrations of 1.0, 1.0, 0.73, and 1.45 parts per million (ppm) of mercury, respectively; collectively accounting for six-tenths of 1% of US consumption. The average concentration of methylmercury for all commercial fish in the US marketplace is reported to be 0.086 ppm.

All forms of mercury entering the aquatic environment, as a result of anthropogenic or natural sources, are converted into methylmercury by microorganisms and subsequently concentrated in fish and other aquatic species. Methylmercury can have long half-life in fish; thus, large older fish, particularly predatory species, which are high in the food chain, will have accumulated considerably more methylmercury than small younger fish. Therefore, population groups that rely heavily on consumption of these species will have higher dietary intakes than populations with a high intake of fish containing low levels of methylmercury. Factors to be considered in estimating methylmercury exposure and risk from fish consumption include the species of fish consumed, the concentrations of methylmercury in the fish, the quantity of fish consumed, and how frequently fish is consumed.

Risk Characterization

Both the JECFA and the US National Research Council (NRC) evaluations concluded that the developing brain is the most sensitive target organ for methylmercury toxicity. Therefore, pregnant women or women who may become pregnant within the next year are considered to be the most susceptible population because of the risk to the embryo and fetus. The JECFA PTWI of 1.6 μg per kg bw is considered to be sufficiently protective of the developing fetus. Based on current evidence it is clear that infants and children aged up to 17 years are no more sensitive to the adverse effects of methylmercury than the embryo or fetus and may be less so. Nevertheless, available data do not allow the identification of a level of intake higher than the existing PTWI that would not pose a risk of developmental neurotoxicity. Hence, the existing PTWI established for the fetus applies also to children. However, intakes of up to approximately two times higher than this PTWI would not pose any risk of neurotoxicity in the adult population.

For the average consumer, the estimated dietary exposure to methylmercury is reported to be below the PTWI of 1.6 μg per kg bw per week established by the JECFA, but in some cases, high percentile consumers of fish may approach or exceed this value. Populations who frequently consume large predatory fish such as swordfish and shark may have a considerably higher intake of methylmercury and exceed the PTWI.

Methylmercury is the most toxic form of mercury. Although fish and seafood consumption is the main source of exposure to methylmercury, the benefits and risks of their consumption should be balanced, as fish and seafood are important sources of energy, protein, and a variety of essential nutrients, such as vitamins, trace elements, and fatty acids. Therefore, it is highly advisable to optimize the contribution of fish and seafood products to a healthy diet, while at the same time minimizing the exposure to methylmercury.

See also: Analytical Methods: Overview of Methods of Analysis for Chemical Hazards. Foodborne Diseases: Overview of Chemical, Physical, and Other Significant Hazards. Public Health Measures: Monitoring of Contaminants. Safety of Food and Beverages: Seafood

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TOXIC METALS

Trace Metals – Chromium, Nickel, Copper, and Aluminum

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Glossary

Carcinogen A chemical capable of inducing cancer.
Homeostasis The property of a system that enables the body's internal environment to be kept stable.
In vitro In an artificial environment, i.e., glass, outside a living organism.
In vivo Occurring or carried out within a living organism.
Metalloprotein A protein that contains a metal ion cofactor.
Mutagenicity An agent that can induce or increase the frequency of mutation in an organism.

Background and Nutritional Aspects

Chromium

Chromium is a ubiquitous element naturally found in rocks, animals, plants, and volcanic soils. Chromium enters the environment mostly in the chromium(III) and chromium(VI) forms as a result of natural processes and human activities, such as chromate production, chrome plating, and leather tanning. Chromium(III) is an essential nutrient that helps the body use sugar, protein, and fat and its shortage in the diet may result in weight loss or decreased growth, improper function of the nervous system, and a diabetic-like condition. There was no sufficient evidence to set an estimated average requirement (EAR) for chromium. Therefore, an adequate intake (AI) was set based on estimated mean intakes. The AI is 35 and 25 μg day^{-1} for young men and women, respectively. Absorption through exposure via the diet and water is consistently low, with values reported in the range of 1–5%. Few serious adverse effects have been associated with excess intake of chromium from food. Therefore, a tolerable upper intake level (UL) was not established.

Nickel

Nickel is a hard, silvery-white lustrous metal and has properties that make it very desirable for combining with other metals, such as iron, copper, chromium, and zinc. Nickel is the 24th most abundant element, found in the Earth's crust, meteorites, and on the ocean floor. It can be found in a variety of oxidation states ranging from 0 to IV. However, Ni(II) is the only oxidation state occurring in ordinary chemistry. Ni(II) forms a wide variety of compounds ranging from simple inorganic complexes (salts) to complexes with various organic ligands. Some plants can take up and accumulate nickel but it does not concentrate much in animals, including fish. There is no established recommended dietary allowance (RDA) for nickel. Common amounts included in dietary supplements range from 35 to 100 μg day^{-1}.

Copper

Copper is a reddish-orange, soft, ductile, and malleable metal that occurs naturally in rock, soil, and water sediment. Most copper compounds occur in $+1$ or $+2$ valence states. Copper is primarily used as a metal or an alloy (e.g., brass, bronze, and gun metal). Copper sulfate is used as a fungicide, algicide, and nutritional supplement. It has been used as a conductor of heat and electricity, building material, and constituent of various metal alloys for thousands of years. Average copper level in human body is 1.4–2.1 mg kg^{-1}. The main criterion used to estimate the EAR for copper is a combination of indicators, including plasma copper and ceruloplasmin concentrations, erythrocyte superoxide dismutase activity, and platelet copper concentration in controlled human depletion/repletion studies. The recommended dietary allowance (RDA) for adults is 900 μg day^{-1}. The median intake of copper from food in the USA is approximately 1.0–1.6 mg day^{-1} for adult men and women and the UL for adults is 10 000 μg day^{-1} (10 mg day^{-1}), which is a value based on protection from liver damage as the critical adverse effect. Copper is an essential element for good health and its deficiency can produce anemia-like symptoms, neutropenia, bone abnormalities, hypopigmentation, impaired growth, increased incidence of infections, osteoporosis, hyperthyroidism, and abnormalities in glucose and cholesterol metabolism. Absorption and excretion feedback mechanisms normally prevent chronic copper toxicity in humans. However, an accumulation of copper in body tissues can occur in rare cases of Wilson's disease.

Aluminum

At approximately 8.8%, aluminum is the third most abundant element in the Earth's crust after oxygen and silicon. It is also

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present in air, water, and many foods, but not in its metallic form. Rather, aluminum is found in the environment as aluminum compounds, where the aluminum is bound with other chemical species such as silica, oxides, hydroxides, sodium, and fluoride, and as complexes with organic matter. As it is a major constituent of the Earth's crust, aluminum enters environmental media naturally through the weathering of rocks and minerals. Foods naturally high in aluminum include potatoes, spinach, and tea. Processed dairy products, flour, and infant formula may be high in aluminum if they contain aluminum-based food additives. As a lightweight metal with a melting point of 649.8 °C, it is being used increasingly for a number of different and important uses such as: food additives; cooking utensils; food packaging; drugs (antacids); consumer products (antiperspirants, aluminum foil); in the treatment of drinking water as coagulants to reduce organic matter, color, turbidity, and microorganism levels; as a structural material in the construction, automotive, and aircraft industries; and in the production of metal alloys, in the electric industry.

Hazard Identification and Characterization

Hazard identification and characterization are concerned with the inherent properties of a chemical that gives rise to its potential to cause adverse biological effects. These are described for chromium, nickel, copper, and aluminum in the following sections.

Chromium

Not considered a health hazard, chromium(III) in trace levels is an essential nutrient needed by the body for normal energy metabolism that plays a role in glucose, fat, and protein metabolism. The biologically active form of chromium exists as a complex of chromium(III), nicotinic acid, and possibly the amino acids glycine, cysteine, and glutamic acid to form glucose tolerance factor. Absorption of chromium by the oral route ranges from essentially zero for the water-insoluble chromium(III) compound chromic oxide to 10% for potassium chromate. A large amount of absorbed chromium is accumulated in the bone, liver, kidney, and spleen. Although chromium(VI) is a strong oxidizing agent and may damage the kidney, liver, and blood cells through oxidation reactions, the lethal dose 50% for chromium(VI) ranges between 50 and 150 mg per kg bw. The International Agency for Research on Cancer (IARC) has determined that chromium(VI) is carcinogenic to humans and is characterized by significant lethality after acute exposures by the oral, dermal, and inhalation routes. Acute oral toxicity ranges from 1.9 to 3.3 mg per kg bw per day.

Hexavalent chromium is characterized by significant lethality after acute exposures by the oral, dermal, and inhalation routes. Hexavalent chromium also produces significant skin irritation and dermal sensitization. Relative bioavailability of hexavalent chromium through ingestion is also low. The World Health Organization (WHO)-recommended maximum allowable concentration in drinking water for chromium(VI) is 0.05 mg kg^{-1}. Hexavalent chromium is rapidly taken up by cells through the sulfate transport system. Once inside the cell, chromium(VI) is quickly reduced to its trivalent form by cellular reductants, including ascorbic acid, glutathione, flavoenzymes, and riboflavin. Reported effects in humans included oral ulcers, diarrhea, abdominal pain, indigestion, vomiting, leukocytosis, and presence of immature neutrophils by drinking contaminated well water with chromium(VI) concentrations of 20 mg l^{-1}, and an estimated exposure dose of 0.57 mg kg^{-1} day^{-1}. Some chromium(VI) compounds are potent oxidizing agents, such as potassium dichromate and chromium trioxide, and are thus strong irritants of mucosal tissue. Effects included metabolic acidosis, acute tubular necrosis, kidney failure, and death.

In general, hexavalent chromium has given positive results in *in vitro* mutagenicity tests, whereas trivalent chromium compounds have been negative. However, chromium picolinate is more likely to cause deoxyribonucleic acid damage and mutation than other forms of trivalent chromium in mammalian cells. The significance of these observations is unclear and no *in vivo* genotoxicity data are available. For some occupational sources of chromium exposure (e.g., ferrochromium industry and manufacture of chrome pigments) and for some occupations (e.g., leather tannery workers and chromium platters) increased risks have been observed, but almost invariably in the epidemiologic studies the available data do not permit discrimination between the simultaneous exposure to trivalent chromium and hexavalent chromium. Although the chromium compound that increases the risk of lung cancer and sinonasal cancer is yet to be identified, there is a fairly general agreement that hexavalent species are responsible for these diseases, and that the trivalent and metallic species are not. For cancers other than those of the lungs and sinonasal cavity, no consistent pattern of cancer risk has been demonstrated in workers exposed to chromium compounds.

Nickel

Nickel compounds can be grouped according to their solubility in water: Soluble compounds include nickel chloride, nickel sulfate, and nickel nitrate, and less-soluble compounds include nickel oxide and nickel subsulfide. Both the soluble and less-soluble nickel compounds are important with regard to all relevant routes of exposure. Generally, the soluble compounds are considered more toxic than the less-soluble compounds, although the less-soluble compounds are more likely to be carcinogenic at the site of deposition. Nickel sulfate inhalation causes lung and nasal cancer in animals and humans, but the data on carcinogenicity via oral administration of nickel salts is limited. Orally ingested nickel salts can cause adverse effects on kidneys, spleen, lungs, and the myeloid system in experimental animals. Furthermore, perinatal mortality was reported to be increased in the offspring of female rats ingesting nickel salts, even at the lowest administered dose (1.3 mg per kg bw per day of nickel). Contact with nickel sulfate can irritate and burn the skin and eyes. Contact dermatitis, which results from dermal exposure to nickel, is the most prevalent effect of nickel in the general population. Dermatitis resulting from nickel allergy is well reported in the literature.

After ingestion of various nickel compounds in food, beverages, or drinking water, it is absorbed by the gastrointestinal tract at a rate that depends on the chemical form and the solubility. Absorption may also be suppressed by binding or chelating substances, competitive inhibitors, or redox reagents. In contrast, absorption is often enhanced by substances that increase pH, solubility, or oxidation, or by chelating agents that are actively absorbed. Although soluble nickel compounds (nickel sulfate, nickel chloride, and nickel nitrate) are better absorbed than relatively insoluble ones, the contribution of some slightly soluble compounds (nickel metal, nickel carbonate, nickel oxides, and nickel sulfides) to the total nickel absorption may be more considerable, as they are more soluble in the acidic gastric fluids. The absorption of nickel following administration of the drinking water to fasting individuals might be as high as up to approximately 25–27% and approximately 1–6% when administered to nonfasting individuals. Most nickel in food remains unabsorbed in the alimentary tract and passes through into the feces.

In human serum, 34% of the nickel is present with albumin, 26% as a complex associated with a nickel metalloprotein (nickeloplasmin), and 40% as ultrafiltrable material. In human serum, it is present as ultrafiltrable material, associated with albumin, and associated with nickeloplasmin. Limited information exists on tissue distribution in humans. Nickel compounds are considered as human carcinogens based on epidemiological studies, mechanistic information, and evidence from animal studies. The overall findings indicate that nickel ions generated in target cells are determinants for the carcinogenic process.

Copper

Copper is an essential nutrient and incorporated into many metalloenzymes functions, such as hemoglobin formation, drug metabolism, carbohydrate metabolism, catecholamine biosynthesis, the crosslinking of collagen, elastin, hair keratin, and the antioxidant defense mechanism. Copper-dependent enzymes function to reduce activated oxygen species or molecular oxygen, such as cytochrome oxidase, superoxide dismutase, ferroxidases, monoamine oxidase, and dopamine β-monooxygenase. Copper is easily absorbed by the stomach and small intestine. Once nutritional requirements are compensated, there are several mechanisms that prevent copper overload. Excess copper is absorbed into gastrointestinal mucosal cells. Copper that eludes binding to intestinal metallothionein is transported to the liver. It is stored in the liver bound to liver metallothionein, from which it is ultimately released into bile and excreted in the feces. Although copper homeostasis plays an important role in the prevention of copper toxicity, exposure to excessive levels of copper can result in a number of adverse health effects including liver and kidney damage, anemia, immunotoxicity, and developmental toxicity. Many of these effects are consistent with oxidative damage to membranes or macromolecules. Copper can bind to the sulfhydryl groups of several enzymes, such as glucose-6-phosphatase and glutathione reductase, thus interfering with their protection of cells from free radical damage. Gastrointestinal distress is one of the most commonly reported adverse health effect of copper. Nausea, vomiting, and/or abdominal pain have been reported, usually occurring shortly after drinking a copper sulfate solution, beverages that were stored in a copper or untinned brass container, or first-drawn water (water that sat in the pipe overnight). The observed effects are not usually persistent and gastrointestinal effects have not been linked with other health effects. The liver is also a sensitive target of toxicity resulting in necrosis and fibrosis. Liver effects have been observed in individuals diagnosed with Wilson's disease, Indian childhood cirrhosis, or idiopathic copper toxicosis.

Following ingestion of copper, copper levels in the blood rise rapidly. The copper is mainly bound to albumin. There is some indication that albumin plays a passive role in copper transport. It carries a large portion of the exchangeable copper in the circulation and releasing this to other carriers for actual cell-specific uptake. There is also evidence that transcuprein is another plasma protein carrier. Thus, dietary copper is transported to, and enters, the liver and kidney. Copper then reemerges into the plasma bound to the ceruloplasmin, which tightly binds six or seven copper atoms and is the most abundant copper protein in the plasma. Copper is transported from the liver to other tissues via ceruloplasmin.

Numerous factors may affect copper absorption, such as the amount of copper in the diet, competition with other metals, including zinc, iron, and cadmium, and age. The absorption of copper appears to be inversely related to the amount of copper in the gastrointestinal tract. In humans, the amount of stored copper does not appear to influence copper absorption. It has been observed that abnormally high serum copper levels are found in patients with many types of progressive tumors. Depriving malignant tumors of their copper supply may be a potent antiangiogenesis strategy for stabilizing patients with advanced cancer. The liver is the primary organ of copper-induced toxicity in humans and other target organs include bone and the central nervous and immune systems. Based on the WHO values established for human copper intake, the acceptable daily intake (ADI) – or more appropriately designated as 'upper limit for copper intake' – for copper(I) and (II) variants was established by the European Food Safety Authority (EFSA) at 0.15 mg copper per kg bw per day.

Aluminum

Aluminum is used for everything from medications to door frames and car bodies. Aluminum was not considered harmful to human health because of its relatively low bioavailability. However, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) reduced the provisional tolerable weekly intake (PTWI) value for aluminum from 7 to 2 mg per kg bw per week. Oral intake of foodstuffs would appear to be the most important source of aluminum. The hematopoietic system, the nervous system, and bone are three organ systems that are implicated in the toxic effects of aluminum. Increased aluminum exposure can be compensated for by excretion via intestines and normal, healthy kidneys. Body balance is maintained because aluminum is cleared both in feces and in urine. Aluminum is removed from the bloodstream by the

kidneys and other organs. Kidney insufficiency, however, was shown to result in increased aluminum concentrations in the kidneys of dialysis patients. Aluminum produces toxic effects at the cell membrane by altering the physical properties of the membrane, interfering with the function of voltage-activated ionic channels, and altering the secretion of transmitters. Aluminum can affect various key processes in the nucleus, cytoplasm, and mitochondria, such as glucose metabolism, signal transduction, neurotransmitter synthesis, phosphorylation and dephosphorylation of cytoskeletal proteins, slow axonal transport of neurofilament proteins, and inhibition of nucleotide activity. There is no indication from any studies and animal bioassays that aluminum is carcinogenic. Aluminum is neurotoxic to humans under certain specific exposures. To date there is no convincing evidence that aluminum plays a role in Alzheimer's disease. Humans with decreased renal function face a high risk of developing renal osteodystrophic osteomalacia and dialysis encephalopathy from levels of aluminum in food and drinking water, whereas healthy individuals face no risk.

Gastrointestinal absorption of aluminum is low, generally in the range of 0.1–0.4% in humans. Bioavailability of aluminum varies depending mainly on the chemical form of the ingested compound (i.e., type of anion) and the concurrent exposure to dietary chelators, such as citric acid, ascorbic acid, or lactic acid. The total body load of aluminum in healthy human subjects is approximately 30–50 mg. Normal levels of aluminum in serum are approximately 1–3 $\mu g\,l^{-1}$. Of the total body load of aluminum, approximately one-half is in the skeleton and one-fourth is in the lungs.

Methods of Analysis

Chromium

Several methods are available for the analysis of chromium in different biological media. The four most frequently used methods for determining low levels of chromium in biological samples are neutron activation analysis, mass spectrometry, graphite spark atomic emission spectroscopy (AES), and graphite furnace atomic absorption spectroscopy (GFAAS). GFAAS is readily available in conventional laboratories, and this method is capable of determining chromium levels in biological samples when an appropriate background correction method is used. Contamination and chromium loss in environmental samples during sample collection, storage, and pretreatment should be avoided for biological samples.

Nickel

Analytical methods that determine nickel in biological materials are the same as those used for environmental samples. The most common methods determine the total nickel content of the sample instead of the particular nickel compound that may be present. Methodological differences are a function of the nickel level in the sample, digestion procedure required to solubilize the sample, and the level of potentially interfering substances that may be present. Either wet ashing with sulfuric acid or dry ashing through dissolution of the ash with dilute sulfuric or hydrochloric acid is generally a satisfactory method to detect nickel in tissue or food. Another methodological approach utilizes digestion of biological samples with nitric acid that can also be followed by treatment with hydrogen peroxide to remove residual biological material. As the digestion procedures require the use of strong acids and substances with explosion hazards, all safety procedures should be carefully reviewed before the analyses are completed. As nickel is normally present at very low levels in biological samples, sensitive and selective methods are required to determine trace nickel levels in these samples accurately. The AAS and inductively coupled plasma-AES (ICP-AES) are the most common methods.

Copper

Determining specific copper compounds and complexes in samples is difficult. Copper in biological materials such as hair and nails can be determined by using suitable procedures for dissolving the sample matrix and employing the same analytical techniques as with blood and tissue. These methods determine the total amount of copper in the sample. The methodology for analyzing biological material is similar to that used for environmental samples. The most commonly employed methods use atomic absorption spectroscopy (AAS) or ICP-AES. If determination of dissolved and suspended copper is required, samples should be filtered using a 0.45 μm membrane filter. Other analytical methods used for copper analysis include X-ray fluorescence, anodic stripping voltammetry, neutron activation analysis, photon-induced X-ray emission, as well as chemical derivatization, followed by gas chromatographic or liquid chromatographic analysis.

Aluminum

Aluminum is reacted with pyrocatechol violet followed by spectrometric measurement of the resulting colored complex. The method is restricted to the determination of the aquated cations and other forms of aluminum readily converted to the cationic form by acidification. The limit of detection is 2 $\mu g\,l^{-1}$. The limit of detection for the determination of aluminum by ICP-AES ranges from 40 to 100 $\mu g\,l^{-1}$. Flame AAS and GFAAS methods are applicable for the determination of aluminum in water at concentrations of 5–100 and 0.01–0.1 $mg\,l^{-1}$, respectively. The working range of the graphite furnace AAS method can be shifted to higher concentrations by either dilution of the sample or using a smaller sample volume.

In the determination of trace metals, major concerns are contamination and loss. Contamination can be introduced from impurities in reagents and containers as well as from laboratory dust. Losses may also occur due to adsorption onto containers.

Exposure

Chromium

Drinking water and foods are major sources of dietary exposure to chromium. However, the levels of chromium in

air and water are generally low. Fish do not accumulate much chromium in their bodies from water. Drinking water contains generally less than 0.002 mg kg^{-1}. Contaminated well water may contain chromium(VI). Chromium(III) occurs naturally in many fresh vegetables, fruits, meat, yeast, and grain. Different methods of processing, storage, and preparation can alter the chromium content of food. Acidic foods in contact with stainless steel cans or cooking utensils might contain higher levels of chromium because of leaching from stainless steel. Refining processes used to make white bread or sugar can decrease chromium levels. Chromium(III) is an essential nutrient for humans. On average, adults in the US take in an estimated 60 μg of chromium daily from food. People may also be exposed to chromium by using consumer products, such as household utensils, wood preservatives, cement, cleaning products, textiles, and tanned leather.

Chromium(III) is a widely used supplement used to prevent or treat chromium deficiency. Chromium chloride, chromium nicotinate, chromium picolinate, high-chromium yeast, and chromium citrate were not associated with adverse effects at doses of 15 mg per kg bw per day of chromium. Increased levels of tissue chromium indicated that absorption had occurred. Higher doses of chromium (approximately 100 mg per kg bw per day) are associated with reproductive and developmental effects, although these may be secondary to parental toxicity.

Nickel

Nickel usually enters groundwater and surface waters from dissolution of rocks and soils, biological cycles, atmospheric fallout, industrial processes, and waste disposal. Nickel leached from dump sites can contribute to nickel contamination of the aquifer, with potential ecotoxicity. Acid rain has a tendency to mobilize nickel from soil and increase the nickel concentration in groundwater, leading eventually to increased uptake and possible toxicity in microorganisms, plants, and animals. Drinking water usually contains less than 20 μg l^{-1} nickel. Much higher concentrations may occur due to pollution of the water supply or leaching from nickel-containing pipes and nickel-plated faucets.

The concentration of nickel in the water of rivers and lakes is usually very low (less than 0.01 mg kg^{-1}) compared with that in soil (between 4 and 80 mg kg^{-1}).

For the general population, the major source of nickel exposure is food and the water that contains nickel. Contact with trace amounts of nickel on a daily basis is through diet. Foods such as chocolate, soybeans, oatmeal, bananas, barley, beans, cabbage, some nuts, baking powder, and cocoa powder are the major source of exposure to nickel. Nickel is present in tobacco at average contents of 2.2 and 2.3 μg per cigarette and is present in tobacco smoke. Skin absorption can happen from coins, hairpins, jewelry, prosthetic joints, heart valves, and nickel plating. Some cooking utensils can also introduce nickel into the body.

Children may also be exposed to nickel by eating soil, although this is rare. Exposure of an unborn child to nickel is through the transfer of nickel from the mother's blood to fetal blood. Likewise, nursing infants are exposed to nickel through the transfer of nickel from the mother to breast milk. However, the concentration of nickel in breast milk is either similar or less than that in infant formulas and cow's milk. Our daily intake of nickel from drinking water is only approximately 2 μg. Inhalation accounts for 0.1 and 1 μg nickel per day, excluding nickel in tobacco smoke. Exposure to nickel also occurs when touching coins and other metals containing nickel. Reference values for nickel in healthy adults is 0.2 μg l^{-1} in serum and 1–3 μg l^{-1} in urine. The most consistently reported adverse effects resulting from exposure to nickel are contact dermatitis and respiratory effects, including cancer. The IARC has determined that some nickel compounds are carcinogenic to humans and that metallic nickel may possibly be carcinogenic to humans. The Environmental Protection Agency (EPA) has determined that nickel refinery dust and nickel sulfides are human carcinogens.

The EFSA has estimated that the intake of nickel from the average European diet is estimated to be approximately 150 μg day^{-1} (approximately 2.5 μg per kg bw per day), but may reach 900 μg day^{-1} (approximately 15 μg per kg bw per day) or more, when large amounts of food items with high nickel contents are consumed. In addition, first-run drinking water, which may contain up to 1.0 mg l^{-1}, and leaching from kitchen utensils into food may also contribute to nickel intake. Intakes of 150 and 900 μg day^{-1} are approximately 500- and 90-fold lower, respectively, than the lowest dose reported to cause adverse effects in rats. Average intakes from food are about one-third of the lowest intake reported to aggravate hand eczema in nickel-sensitized subjects.

Copper

The mean concentration of copper in soil in the US ranges from 5 to 70 mg kg^{-1}. The estimated daily intake of copper from food is 1.0–1.3 mg kg^{-1} day^{-1} for adults. The primary source of copper intake is the diet; however, the amount of copper in the diet usually does not exceed the average dietary requirements for copper. Drinking water is the primary source of excess copper, especially in water that is first drawn in the morning after sitting in copper piping and brass faucets overnight. Populations living near sources of copper emissions, such as copper smelters and refineries, and workers in these and other industries may also be exposed to high levels of copper in dust by inhalation. Copper concentrations in soils near copper emission sources could be sufficiently high to result in significantly high intakes of copper in young children who ingest soil. Exposure to high levels of copper will result in the same types of effects in children and adults.

The average daily dietary intake of copper from food is approximately 2 mg day^{-1}. The dietary intake of copper may be greater than this average for those individuals who regularly consume organ meats such as liver and kidney, oysters or clams, nuts, seeds, cocoa powder, avocadoes, mushrooms, legumes, and bran and germ portions of grains. These intakes, however, are not expected to exceed the ADI of 0.15 mg per kg bw per day. However, the EFSA has estimated the aggregate exposure from all sources that shows, in particular for children, the overall dietary copper exposure is not negligible and may be significantly higher. Additional potential sources of

dietary copper intakes have not been considered. Moreover, the estimate is not representative for all consumers, and therefore situations may arise where the overall copper intake of some consumer groups exceeds the toxicological reference value.

Aluminum

There is little evidence that aluminum is acutely toxic by oral exposure despite its widespread occurrence in drinking water, fruit juices, wine, and beer, articles of daily use that are made of aluminum, cosmetics and pharmaceuticals such as local therapeutic agents, antidiarrheal drugs or antacids. The use of aluminum cookware, utensils, and wrappings can increase the amount of aluminum in food; however, the magnitude of this increase is generally not of practical importance. Despite aluminum being so ubiquitous in our environment, there is little evidence that any of these sources are contributing to increased levels of aluminum in tissues. At exposure levels of approximately 70–300 mg per kg bw per day in drinking water, the main observed effect is change in body weight gain.

It is impossible to give an average value for the exposure of the general population worldwide to aluminum. Daily intake by humans is estimated to be 1–10 mg. People consume little aluminum from drinking water, which generally does not exceed 0.1 mg l^{-1} even when the water is treated with aluminum salts, for example, alum. The EPA has set a limit of 0.05–0.2 mg l^{-1} for aluminum in drinking water.

The major source of aluminum is food, but levels can vary widely due to dietary habits, aluminum-containing food additives in the food supply and use of aluminum utensils and cooking equipment. Some potential sources of bioavailable aluminum are as follows: alum-treated drinking water, food additives (free-flowing agent in salt, rising agent in some baking powders, and hardening agent for pickles and candied fruits), many antacids, buffered aspirins, abrasives in some toothpastes, beer and soft drinks in aluminum drink cans (aluminum cans are resin-lined but carbonated drinks attack the lining and corrode the aluminum during storage), food cooked in aluminum trays and foils, use of aluminum cookware and kitchen utensils, use of carbonated drink makers and aluminum components in coffee percolators.

Risk Characterization

Chromium

Chromium(III) is an essential mineral and is widely present in a variety of foods. Intake of chromium(III) by the general population is considered adequate. However, the additional intake of chromium(III) supplements for nutritional purposes is not considered to be of concern provided that the amount of total chromium does not exceed 250 μg day^{-1}, the value established by the WHO for supplemental intake of chromium(III). However, exposure to chromium(VI), which is a genotoxic carcinogen, should be maintained as low as reasonably achievable.

Nickel

Although certain compounds of nickel are quite toxic, dietary exposure to these is not a public health concern. The most common adverse health effect in the general population is an allergic reaction to metallic nickel. Nickel is a potent skin sensitizer. The main toxic endpoint for nickel in humans is the aggravation of nickel sensitization, for which a threshold is unclear but which is possible at the levels of nickel found in food. It is therefore not possible to set a safe upper level or guidance for supplemental intake of nickel.

The intake of nickel from the average diet is estimated to be approximately 150 μg day^{-1} (approximately 2.5 μg per kg bw per day), but may reach 0.9 mg day^{-1} (approximately 0.015 mg per kg bw per day) or more, when large amounts of food items with high nickel contents are consumed. In addition, first-run drinking water, which may contain up to 1.0 mg l^{-1}, and leaching from kitchen utensils into food may also contribute to nickel intake. Intakes of 150 and 900 μg day^{-1} are approximately 500- and 90-fold lower, respectively, than the lowest dose reported to cause adverse effects in rats. Average intakes from food are about one-third of the lowest intake reported to aggravate hand eczema in nickel-sensitized subjects.

Copper

Copper is an essential nutrient and the WHO recommends a minimal acceptable copper intake of approximately 1.3 mg day^{-1}. Copper is kept under tight homeostatic control to prevent the accumulation of excess amounts. Acute copper toxicity in humans is rare due to the emetic properties and unpleasant taste of the compounds. There are relatively few data on lower level or chronic oral copper exposure in humans. In the general human population, the key adverse effects usually associated with excess copper intake are gastrointestinal, and can occur as a result of food or beverage contamination. The EFSA has established an ADI of 0.15 mg per kg bw per day. Although dietary intake of copper was below this level, the estimated aggregate exposure assessment from all sources was not negligible. While the estimate is not representative for all European consumers, the overall copper intake of most consumer groups is not expected to exceed the UL reference value of 10 mg day^{-1}.

Aluminum

For adults, the estimates of mean dietary exposure to aluminum-containing food additives from consumption of cereals and cereal-based products are up to the PTWI of 2 mg per kg bw per week, as established by JECFA in 2011. Dietary exposure of children to aluminum-containing food additives, including high dietary exposures (e.g., 90th or 95th percentile), are estimated to be 2-fold higher than the PTWI. For potassium aluminum silicate-based pearlescent pigments at the Codex maximum proposed use levels and using conservative estimates, anticipated dietary exposure at the highest range of estimates could be 200 times higher than the PTWI.

Although aluminum can accumulate in the body, consumption of aluminum-containing food additives would be acceptable if the total dietary exposure to aluminum is below the PTWI. To this end, provisions for food additives containing aluminum should be reviewed so that exposure to aluminum from all sources does not exceed the PTWI for aluminum compounds of 2 mg per kg bw from all sources.

See also: Foodborne Diseases: Overview of Chemical, Physical, and Other Significant Hazards. Institutions Involved in Food Safety: World Health Organization (WHO). Public Health Measures: Monitoring of Contaminants. Risk Analysis: Risk Assessment: Chemical Hazards; Risk Assessment: Principles, Methods, and Applications

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PROCESSING CONTAMINANTS

Contents

Acrylamide

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Glossary

Carcinogen A substance that is directly involved in causing cancer. This may be due to its ability to damage the genome or to disruption of cellular metabolic processes.

Maillard reaction The Maillard reaction is named after the French scientist Louis Camille Maillard (1878–1936), and comprises a complex series of reactions between amino acids and reducing sugars, usually at increased temperatures.

Neurotoxic compound A substance that alters the normal activity of the nervous system in such a way as to cause damage to nervous tissue.

Precursor A compound that participates in the chemical reaction that produces another compound.

Reducing sugar Any sugar that either has an aldehyde group or is capable of forming one through isomerization.

History, Background, and Sources

In 1997, tunnel workers at Hallandsås, in the south of Sweden, were occupationally exposed to acrylamide. They were examined and compared with a nonexposed control group. Surprisingly, it was found that the control group had elevated levels of acrylamide adducts in the blood. Later it was shown that rats fed with a fried diet had elevated levels of acrylamide adducts, indicating that food could be a source of acrylamide. In 2002, the Swedish National Food Administration announced that heat-treated starch-rich foods contained high levels of acrylamide, a compound that has been shown to be neurotoxic to humans and classed as probably carcinogenic to humans (Group 2A). The potential carcinogenicity of acrylamide in humans together with the fact that high amounts, up to a milligram per kilogram, could be formed in certain foods, caused great concern for national authorities and the food industry. This led to the initiation of extensive research activity to find ways of controlling and minimizing acrylamide formation and to obtain data on which to base recommendations for reduction strategies.

The aim of cooking is to increase the palatability and digestibility of foods, to kill possibly harmful microorganisms and to provide variety to our diet. At temperatures above 120 °C, the Maillard reaction takes place, which produces characteristic flavor, aroma, and color. Acrylamide has been shown to be formed via the Maillard reaction as a result of the reaction between the reducing sugars glucose and fructose, and the amino acid asparagine. Potatoes contain relatively high levels of these precursors and, therefore, high levels of acrylamide can be formed in, for example, crisps, but acrylamide is also found in bread, cookies, and coffee. Until more is known about the role of acrylamide in human health it would be prudent to decrease the intake of acrylamide. Considerable research has been devoted to increasing our knowledge concerning the factors that favor acrylamide formation and to find means of reducing its presence in heat-treated foods.

Chemistry, Industrial Use, and Factors Influencing the Formation

Acrylamide Chemistry and Industrial Use

Acrylamide (2-propenamide, CAS number 79–06–1) is a nonflammable, odorless, and white crystalline compound. The molecular structure is shown in Figure 1, and some chemical properties are listed in Table 1.

Acrylamide has been produced industrially since the 1950s. It is mainly used for the production of polyacrylamide which, among other things, is used for drinking water clarification, for waste water treatment, in soil stabilization, and as a grouting agent. Polyacrylamide gels are also used in the paper and textile industries, in ore processing and in cosmetics, and are widely used in research laboratories to separate proteins and other compounds by electrophoresis.

Reaction Pathways for Acrylamide Formation

After the finding of acrylamide in heat-treated foods, several model experiments were performed to elucidate the precursors and reaction routes. It was proposed early that acrylamide is formed via the Maillard reaction, and it was soon demonstrated that formation of acrylamide requires the amino acid asparagine and a carbonyl compound, for example, glucose or fructose. By using labeled asparagine and glucose it was shown that the main reaction pathway is through condensation of the precursors to form a Schiff base that undergoes decarboxylation and a series of rearrangements to eventually form acrylamide. However, the exact pathway from the Schiff base is dependent on the carbonyl source in the reaction and several pathways have been proposed.

Minor amounts of acrylamide can be formed via the Maillard reaction from other amino acids such as glutamine or methionine. Other possible reaction pathways have been suggested, for example, from acrylic acid and ammonia. Acrylic acid may be formed via the Maillard reaction through degradation of aspartic acid or through the oxidation of acrolein, resulting from the oxidation of fatty acids or glycerol. However, this pathway may be limited due to the requirement for ammonia, which reacts readily with carbonyls and other Maillard intermediates. Another minor route is formation from 3-aminopropionamide, which is an intermediate in the Maillard reaction, but can also be formed in foods during storage through enzymatic decarboxylation of asparagine.

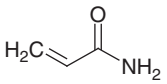

Figure 1 Molecular structure of acrylamide.

Table 1 Some properties of acrylamide

CAS number	*79–06–1*
Molecular formula	C_3H_5NO
Molecular weight	71.08 g mol^{-1}
Boiling point	125 °C at 25 mmHg
Melting point	84.5 °C
Solubility	Soluble in water, alcohols, acetone, and acetonitrile
Reactivity	Reacts with acids, bases, and oxidizing agents
Stability	May polymerize violently at temperature above the melting point

Acrylamide Formation in Model Systems

Several model systems have been used to elucidate factors affecting acrylamide formation, such as temperature and time, water content, the concentration of sugars and amino acids, and pH. These are the same parameters that affect the formation of flavor and color. However, in some studies extreme conditions have been used which are not realistic for food production.

Temperature and Time

Acrylamide starts to form in high amounts at temperatures above 120 °C. Depending on the model system studied, the acrylamide yield reaches a maximum at approximately 160–180 °C. However, the yield of acrylamide in model systems is not very high. Approximately 0.1–0.3% of the asparagine on a molar basis is converted to acrylamide. Acrylamide content increases with time, although a decrease in acrylamide has been observed in a dry glucose/asparagine system after prolonged heating.

Water Content

The formation of acrylamide is dependent on the water content of the system. During roasting and frying water evaporates from the surface. Acrylamide is formed at a higher degree when the surface starts to dry out and the surface temperature rises.

Precursors

The contents of acrylamide precursors: asparagine and reducing sugars (glucose and fructose), are critical factors in acrylamide formation. Using chemical model systems, it has been found that both asparagine and sugars are equally important in determining the reaction rate. Sucrose is not a reducing sugar, but can decompose to glucose and fructose at higher temperatures, and may thus contribute to acrylamide formation to some extent.

pH

The Maillard reaction is pH dependent. In most cases the reaction rate has been found to increase with increasing pH. In a buffered model system with equimolar amounts of asparagine and glucose, more acrylamide was formed at pH 7 and 8 than at lower pH. Lowering the pH favors protonation and may inhibit the formation of the Schiff base necessary for acrylamide formation.

Additives

Divalent cations such as Ca^{2+} and Mg^{2+} have been shown to reduce acrylamide formation. The mechanism responsible is probably covalent binding between the ions and asparagine,

and thus preventing the molecule from participating in acrylamide formation. The addition of Ca^{2+} to a fructose/asparagine model system was found to completely inhibit acrylamide formation.

Other amino acids apart from asparagine can participate in the Maillard reaction and thus competitive reactions can occur. The addition of various amino acids, for example, glycine, cysteine, and lysine to model systems, has been shown to lower the production of acrylamide.

Pretreatment of a potato snack model with the enzyme asparaginase for 30 min at room temperature reduced the asparagine content by 88%; the amount of acrylamide was reduced by 99% when the snack was microwaved on high power for 10 min. When asparaginase was added to fresh homogenized potato and incubated for 30 min at 37 °C, a reduction of up to 97% of acrylamide was obtained after heating at 180 °C for 20 min.

Acrylamide Formation in Heated Potato Products

Potatoes (*Solanum tuberosum L.*) are one of the most widely consumed carbohydrate-rich staple foods in large parts of the world. In Europe, the traditional means of preparation has been to boil fresh potatoes. During recent decades the consumption of fresh potatoes has decreased and there has been an increase in the consumption of processed commercial products, for example, deep-fried and roasted potato products such as crisps and French fries. In some countries, the economic significance of fried and roasted products is higher than that of fresh potatoes.

The acrylamide precursors are naturally occurring in potatoes and the levels differ between varieties and with storage conditions. The genotype of the potato, together with soil, fertilization, and weather conditions during growth, influence the initial sugar content, but the content of free amino acids, for example, asparagine that is the main nitrogen source in potato tubers, is mainly influenced by genotype.

Changes in Potatoes During Storage

Potatoes are often stored for long periods before distribution to retailers or the food industry. Directly after harvest the potatoes should preferably be stored at approximately 15 °C, for 2–3 weeks to heal the wounds arising from harvest and handling. After this period the storage temperature is successively lowered. Storage at 3–4 °C reduces respiration, prevents shrinkage, and sprouting and reduces the risk for storage diseases. However, storage at temperatures below 8 °C causes so-called cold-induced sweetening, i.e., degradation of starch to reducing sugars. A common way to inhibit sprouting at storage temperatures approximately 8 °C is to treat the potatoes with sprout-inhibiting compounds, for example, chlorpropham (isopropyl-N [3-chlorophenyl] carbamate). The conversion of starch to sugars is a reversible process and the sugar levels can be lowered by 'reconditioning' the tubers, i.e., storage for 2–3 weeks at approximately 15 °C. Interestingly, there are potato clones that are more resistant to cold-induced sweetening and therefore can be stored at low temperatures.

Crisp Production

Potato crisps are thin slices, approximately 1.5 mm thick, deep-fried in oil. The industrial production of potato crisps includes several steps: the choice of raw material, washing, slicing, rinsing, and deep-fat frying in a continuous fryer at 150–180 °C. A blanching step, i.e., heating potato slices in water before frying is sometimes used to reduce high levels of sugars, thus avoiding too dark crisp color. However, too high removal of precursors may affect product quality negatively. During frying a crust is formed, and the amino acids and sugars in the outer parts of the potato slices react to form a variety of compounds that are characteristic for the flavor and color of the crisps. French fries are produced in a similar way, but the shape is different.

During deep-frying, heat is efficiently transferred to the potato pieces through convection by the surrounding oil. Deep-fat frying is a more efficient way of cooking than, for example, oven roasting where heat is transferred through radiation and air convection although the oven temperature may be higher ($\sim$200 °C). Deep-frying results in drying of the slices and crust formation. The moisture content decreases from approximately 75% in the potato slices to below 2% in the crisps. As long as water is evaporating from the slices, the surface temperature remains at approximately 100 °C. When the surface starts to dry, and the crust is formed, the temperature increases and high amounts of Maillard products are formed.

Not all potato varieties are suitable for crisp production, because there are high demands on dry matter content and concentrations of reducing sugars. The dry matter content should be high as the yield of potato crisps is directly in proportion to the solids content. The concentration of reducing sugars should be low to avoid excessive browning, and the sugar content of the potatoes should remain low during prolonged storage.

Mitigation Strategies for Heated Potato Products

Much research on acrylamide formation in potato products has been devoted to mitigation strategies including soaking, blanching, addition of various compounds to the blanching water, and coatings. Because acrylamide is formed via the Maillard reaction it is important that the mitigation strategies do not negatively affect the formation of the desired flavor and color compounds. Literature data from some laboratory-scale mitigation studies on potato slices/crisps are compiled in Table 2. The table presents an overview, but it is difficult to exactly compare the results because different time/temperatures have been used and sometimes data on processing conditions are missing.

Good correlations between concentrations of acrylamide and reducing sugars have been obtained in several studies on potato crisps or French fries and in some studies a low content of asparagine gave reduced levels of acrylamide. Thus, the choice of potato variety is crucial to ensure that the acrylamide content is as low as possible, i.e., potatoes should be low in both reducing sugars and asparagines.

Temperature and time are interacting factors, as has been demonstrated in both model systems and potato products. Lowering the temperature may require longer frying times in order to obtain a product that is acceptable to consumers. Lowering the frying temperature or reducing the frying time will reduce the formation of acrylamide in potato products. Deep-frying of potato slices at 150 °C instead of 190 °C was found to give almost 10 times lower levels of acrylamide.

Table 2 Some literature data of mitigation strategies for acrylamide in potato crisps/slices

Mitigation strategy	*Reduction/effect*
Soaking, 50 °C	60%
Blanching, 85 °C for 3.5 min	49–75%
Soaking (60 min at 20 °C) in citric acid (0.05 M)	50%
Soaking (60 min at 20 °C) in acetic acid (0.15 M)	90%
Blanching (3 min at 70 °C) in citric acid (0.05 M)	49%
Blanching (3 min at 70 °C) in acetic acid (0.15 M)	49%
Postdrying (75 min at 105 °C)	69%
Soaking 40, 90 min	20–38%
Immersion in citric acid, 10 or 20 g l^{-1} for 30 min	70%
Blanching in combination with predrying	44%
Organic acids	Significant reduction
Addition of L-lysine, glycine, NaCl, and Ca^{2+}	Lowering effect
Combination citric or acetic acid/L-lysine or glycine	Lowering effect
Addition of $NaHSO_3$, $CaCl_2$, and L-cysteine in blanching water	Reduced acrylamide in the order cysteine > $CaCl_2$ > $NaHSO_3$
Addition of glycine or glutamine during blanching	30%
Coating with chick pea flour	Threefold reduction
Addition of antioxidants	No significant difference
Addition of rosemary herbs to frying oil	25%
Vacuum frying 8 min at 118 °C, vacuum pressure 10 Torr	95%

Note that the products have been heated/fried at different time/temperature combinations.

The precursor content can be significantly reduced by blanching. Blanching has been shown to significantly reduce acrylamide formation in potato crisps/slices or French fries. Soaking is another method used. Soaking is performed at a lower temperature than blanching but based on the same principle, i.e., the leakage of precursors. Blanching is more efficient, because higher temperatures make cell membranes more permeable and enhance the extraction of sugars and asparagine. Blanching has been combined with other methods such as predrying or postdrying to shorten the frying time and thus minimize acrylamide formation.

The Maillard reaction is pH dependent, and considerable research has therefore been directed toward the addition of organic acids, for example, citric acid and acetic acid to blanching or soaking water.

The effect of different additives has been studied on potato products. Dipping potato strips in a $CaCl_2$ solution has been found to inhibit acrylamide formation by up to 95%, whereas the addition of $CaCl_2$ to blanching water at different concentrations before frying, was found to reduce acrylamide in potato crisps to undetectable levels. However, other studies show conflicting results and further research is needed.

The addition of glycine or glutamine to blanching water was found to reduce acrylamide in potato crisps by approximately 30%, compared with a nonblanched control batch. The addition of different concentrations of cysteine to blanching water reduced the acrylamide content in potato crisps to undetectable levels, whereas the content of acrylamide was reduced by the addition of glycine or lysine by between 58% and 89%, compared with blanched controls, depending on the concentration.

In potato slices coated with chick pea flour, the acrylamide content was reduced to one-third, and this was suggested to be due to the protective effect of proteins, for example, complex formation of proteins and starch at the surface of the slice, making the sugars less available for acrylamide formation. Contradictory data have been observed from many studies, for example, soaking in water containing antioxidants before frying had no significant effect on acrylamide content, but the addition of rosemary to frying oil reduced acrylamide content in potato slices by 25%.

Vacuum frying has also been studied as a means of reducing the acrylamide content in crisps. Reducing the boiling point of the oil lowers the frying temperature, and the acrylamide content was reduced by 63% when the crisps were fried at temperatures between 125 and 140 °C. Neither the texture nor the flavor was significantly different from normal frying, whereas the color was mostly darker than the traditionally fried crisps.

Sensory Properties

A trained sensory panel has been used to study the sensory properties of potato crisps after the addition of various salts, organic acids, or amino acids. After blanching in 0.025 M citric acid a sour taste was reported, whereas the taste of crisps treated with acetic or lactic acid were not significantly different from normally blanched crisps. The addition of acetic acid- + lysine gave a pop-corn-like taste, which was not accepted by the panel. $CaCl_2$ gave a more crispy texture, although too high concentrations led to a bitter aftertaste. Crisps blanched in water containing acetic acid or citric acid had a lighter color, whereas the addition of glycine or lysine gave darker crisps. These results show that the additives and their concentrations must be carefully chosen so as not to negatively affect the flavor, color, or texture of the product.

Hazard Identification and Characterization

Until the discovery of acrylamide in foods, only occupational exposure to acrylamide was thought to represent a significant health risk. Today the health effect of dietary exposure to acrylamide is still an open question. Once acrylamide is absorbed from the diet, it is rapidly and extensively absorbed from the gastrointestinal tract and distributed to various tissues. Its double bonds are oxidized to a reactive compound, glycidamide, by the enzyme system cytochrome P450 2E1, see Figure 2. Both acrylamide and glycidamide are conjugated to glutathione and transformed to mercapturic acid derivatives, which are excreted in the urine. Protein binding occurs with both acrylamide and glycidamide. Hemoglobin adducts are often used as a tool in risk evaluation of acrylamide as biomarkers of the internal dose. Glycidamide is more reactive with DNA and forms adducts with the nucleotides guanine and adenine.

The primary concern following exposure to acrylamide via food is a possible cancer risk. Glycidamide is the molecule considered to be responsible for the carcinogenic effects because it can interact with DNA. Acrylamide is carcinogenic in animal experiments; for example, its metabolite glycidamide has shown an increased incidence of small intestinal tumors in mice injected with acrylamide. Long-term studies on rats given oral administration of acrylamide via drinking water showed increased incidences of both thyroid and mammary tumors. Besides being carcinogenic, acrylamide is also neurotoxic to experimental animals and humans, and has been shown to be genotoxic and to give reproductive and developmental effects in experimental animals.

Epidemiological studies have earlier been conducted on occupationally exposed subjects. After the discovery of the presence of acrylamide in different foods, several studies on the cancer risk associated with acrylamide in foods have been carried out on European populations. However, the studies show conflicting results and point to the difficulties of addressing low-level exposure of hazardous compounds from the diet. For appropriate measurements, the studies should be designed especially for investigating the carcinogenic risk of acrylamide, and the exposure assessments should be validated, and all major dietary sources of acrylamide should be taken into account. Furthermore, misclassification may weaken a hypothetical association between acrylamide exposure and cancer. Another problem is that the power of the studies should be high enough to detect small increases in risk expected from the lowest to the highest exposure groups.

There are no human studies on the carcinogenicity of acrylamide as a base for hazard characterization. However, dose descriptors such as BMDL10 (the lower confidence limit on the benchmark dose) for the most sensitive tumor response from rodent bioassays can be used to compare the risk characterization, and this approach has been recommended by the European Food Safety Authority (EFSA) for compounds that are both carcinogenic and genotoxic and has also been used by the Joint Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Committee on Food Additives (JECFA) in its evaluation of acrylamide.

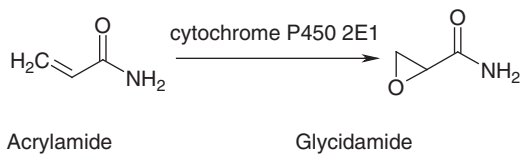

Figure 2 Conversion of acrylamide to glycidamide.

Methods of Analysis of Acrylamide in Foods

The analysis of acrylamide in various foods is difficult as the concentrations can vary from undetectable levels to several milligrams per kilogram, and other substances in the food matrix may cause interference. Several methods have been developed to analyze acrylamide in different foods. High-performance liquid chromatography (HPLC) and gas chromatography (GC) combined with mass spectrometry (MS) are the most commonly used methods for separation and detection. Most of these methods have a detection limit of 10–30 $\mu g\ kg^{-1}$.

A typical extraction procedure includes homogenization of the food sample and extraction with water. Additional steps are sometimes included, for example, defatting of the sample by the addition of isohexane, or precipitation of proteins with Carrez reagents. In an optimization study it was shown that acrylamide was efficiently extracted from various foods, for example, potato crisps, crisp bread, and coffee, using water, and that neither the addition of alcohol nor the removal of fat had any significant effect on the extraction yield. Labeled isotopes, for example, $[^{13}C_3]$-, $[^{13}C_1]$-, and $[^{2}H_3]$-acrylamide, can be added as internal standards to check for losses of acrylamide during the extraction and clean-up procedures. Subsequent clean up of the samples is required, and solid-phase extraction (SPE) is generally used. Acrylamide does not easily bind to any of the conventional sorbents, so the advantage of SPE is mainly the retention of interfering substances.

The choice of analytical column in HPLC analysis is important to achieve sufficiently long retention times because acrylamide is very soluble in water. Graphitic carbon columns and water with a small amount of an organic modifier as mobile phase are most common.

The detection of acrylamide using HPLC–MS is usually carried out using mass spectrometers run in positive electrospray ionization mode. Fragmentation of the ions (MS/MS) is often used to increase sensitivity and selectivity. Protonated acrylamide (m/z 72) gives a fragment at m/z 55, due to the loss of NH_3. This fragment is commonly used for quantification of acrylamide and compared to the corresponding fragment (m/z 58) of an isotope labeled standard such as $[^{2}H_3]$-acrylamide.

HPLC–UV is sometimes used for acrylamide determination, although the selectivity and sensitivity are rather poor. Nevertheless, this method can be used for acrylamide analysis when levels are high and the sensitivity is of less importance.

GC–MS analysis of acrylamide can be performed with or without derivatization. Bromination of acrylamide results in a molecule that is more volatile and less polar, and therefore suitable for GC. Bromination results in a higher molecular weight which improves the MS features. GC–MS can also be performed without bromination, but due to the polarity and low volatility of acrylamide this is not an optimal method and the sample clean up is more demanding.

Computer vision-based image analysis of the color of crisps or French fries has been developed to estimate acrylamide levels. This approach may be useful for online determination of acrylamide during food production. Calibration for each potato variety is important because the variety may influence the color when fried.

Levels in Foods and the Total Diet

Acrylamide has been found at microgram per kilogram levels in a wide range of heat-treated foods, for example, potato products, breakfast cereals, rice, bread, cookies, coffee, prunes, and olives. The highest amounts of acrylamide are generally found in carbohydrate-rich foods. Fried protein-rich foods such as hamburgers, chicken, and fish contain very low levels. Acrylamide has not been found in boiled food. The acrylamide content in some potato products, bread, and breakfast cereals are given in Table 3. There is a great variability not only between different food groups, but also within groups. This is probably due to differences in precursor content, ingredients, and processing conditions. Differences in sampling procedures, for example, if only one brand is analyzed or if the result is a mixture of different brands, may also affect the results. Studies on the stability of acrylamide in foods have revealed that the content in coffee and cacao powder decreased significantly during storage for more than 3 months.

The levels of acrylamide in wheat bread are linearly related to the content of asparagine in the flour, which in turn may vary considerably. In addition, long leavening times reduces the content of acrylamide in the bread. Other factors that affect the amounts of acrylamide in bread and cookies are the amount of reducing sugars and ammonium carbonate in baking powder.

Table 3 Levels of acrylamide in some foods, 2008 (European Food Safety Authority, report)

Food item	*Acrylamide ($\mu g\ kg^{-1}$)*		
	Mean	*Median*	*Maximum*
Potatoes			
Chips (French fries)	278	211	2466
Crisps	614	416	4382
Home-cooked products	180	65	3025
Bread/cereals			
Biscuits	205	120	1940
Bread, soft	38	15	528
Bread, crisp	234	109	1538
Breakfast cereals	160	74	2072
Others			
Coffee, roasted	206	167	1524

Exposure Assessment

Intake estimates vary between populations. Some estimates of the daily intake of acrylamide in different countries and different populations/age groups are presented in Table 4.

The estimated mean intake values in Table 4 range from 0.2 to 1.4 μg kg^{-1} body weight per day. The differences in

Table 4 Examples of estimated intake in different populations

Exposure assessment (year)	*Population/age group*	*Mean*	*90th percentile*
FAO/WHO (2000)		0.3–0.8	
EU/SCF (2002)		0.2–0.4	
Germany (2002)	15–18	1.1	
Switzerland (2002)	16–57	0.28	
France (2002)	>15	0.5	
	2–14	1.4	
Sweden (2002)	18–74	0.45	
Norway (2003)	Males	0.49	1.01
	Females	0.46	0.86
	Boys 9	0.36	0.72
	Girls 9	0.32	0.61
	Boys 13	0.52	1.35
	Girls 13	0.49	1.20
Denmark (2007)	Children 10–13	0.5	
	Adults	0.3	
FDA, USA (2004)	>2	0.43	0.92
	2–5	1.06	2.31
The Netherlands (2003)	1–6	1.04	0.8
	7–18	0.71	0.7
	1–97	0.48	0.2
Belgium (2004)	13–18	0.51	
	Boys 13–18	0.64	
	Girls 13–18	0.46	

The intake is given in micrograms per kilogram body weight per day.
SCF, Scientific Committee on Food.

estimated acrylamide intake are due to several factors, for example, different dietary habits, cooking traditions, and processing techniques. Other factors include dissimilarities in the dietary surveys performed, i.e., how the food intake was recorded, the age group, and how the food items were grouped. In addition, little information on the acrylamide content in home-cooked food is available, which adds to uncertainties in the estimates. Owing to these differences it is difficult to compare intake levels between countries.

There is a variation in consumption patterns between people of different ages: younger people probably tend to eat more snacks like potato crisps than older people, resulting in a higher intake of acrylamide when expressed per kilogram body weight. There is also a tendency for males to have a higher intake than females.

The contributions of different food groups to the intake of acrylamide vary between countries. An example from Sweden shows that a large contribution is from coffee, 23% and the combined contribution from potato crisps, French fries, and other fried potato products is 44%. In Denmark the contribution from coffee is almost the same as in Sweden, 24%, whereas the contribution from fried potato products and crisps is 54%. In a Dutch study the contribution from potato crisps was 31% (age 1–97 years).

Risk Characterization

The risk assessment process consists of four parts: hazard identification, hazard characterization, exposure assessment, and risk characterization. Hazard identification aims to determine the qualitative nature of the adverse effects by a contaminant (genotoxicity, carcinogenicity, neurotoxicity etc.). Hazard characterization determines the relationship between the dose and the incidence of effect. This could be investigated by establishing dose–response relationships. The aim of exposure assessments is to determine the amount of contaminant an individual is likely to receive. Several factors such as dietary habits, lifestyles and cultures could influence this. The final phase of the risk assessment process is risk characterization, which integrates the three processes mentioned above, and determines the probability of an adverse effect by a contaminant to a human population.

For acrylamide the exposure assessment is based on intake estimates, which includes the collection of food consumption data, for example, through food records or household surveys; analysis of the levels of acrylamide, grouping of food items and the choice of modeling of exposure. Biomarkers in urine and blood may also be used to evaluate and confirm the intake. If possible, exposure assessments based on dietary questionnaires should be combined with measurements of biomarkers of exposure. Different methods can be used to model the exposure, for example, point estimates or probabilistic modeling. Owing to the lack of data on the adverse effects of acrylamide on humans, data from animal experiments are required to perform risk characterization. Based on estimates of the intake, and extrapolation of the results from animal experiments, the lifetime cancer risk in humans has been estimated to be between 0.7 and 4.5 per 1000 individuals, depending on the method used.

The risk to human health arising from the intake of low levels of acrylamide during a lifetime of exposure is still unclear. Epidemiological studies have not shown any evidence of increased cancer risk in humans due to acrylamide in food. However, epidemiological studies are often not sufficient to completely disprove an increased risk of cancer. The studies that have been made were not designed to investigate the carcinogenic effect of acrylamide and in some of them not all dietary sources of acrylamide have been taken into account. Furthermore, a comparison of intake estimates based on food frequency questionnaires with hemoglobin adducts showed large overlaps.

Managing the Risk

Scenario analyses performed within an EC project, HEATOX, indicated a possible maximum acrylamide reduction of 40% if all known mitigation efforts were applied. This shows that it is important that the efforts to find more efficient minimizing tools are continued. Advice on consumption can be a complementary way of reducing the intake. This points to the need to consider a management strategy combining regulatory means with consumption advice and risk communication activities. The food industry and catering are the places in the food chain where mitigation efforts would be most efficient and they could benefit from advices in the Acrylamide Toolbox that has been developed by the Confederation of the Food and Drink Industries of the EU.

A large number of foods contributing to acrylamide intake are industrially produced whereas the contribution from home cooking is probably quite small in the general population. Acrylamide exposure from home cooking comes primarily from potato products with some addition of toasted and homemade bread. Home cooking, especially potato products, can generate a very large variation even for the same product, which makes it difficult to predict the real intake from home-cooked foods. Individuals with a high consumption and preference for specific hard fried foods might constitute high exposure risk groups. At the home-cooking level, avoiding overcooking is probably by far the most important action to recommend. Other advice includes clear and accurate cooking instructions on the packaging of prefried products, and clear and accurate instructions for fryers for domestic use. French fries and roast potatoes should be cooked to a golden yellow rather than golden-brown color and bread be toasted to the lightest color acceptable.

See also: Disciplines Associated with Food Safety: Food Safety Toxicology. Foodborne Diseases: Overview of Chemical, Physical, and Other Significant Hazards

Further Reading

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Advanced Glycation End Products (AGEs)

R Kent and J Uribarri, The Mount Sinai School of Medicine, New York, NY, USA

Glossary

Adducts A product of addition of two or more distinct molecules, resulting in a single product containing all components of original molecules.

Advanced glycation end product (AGE) Proteins that become glycated after exposure to sugars.

AGE receptor 1 (AGER1) A 48-kDa receptor protein, which when bound by AGE leads to suppression of AGE-induced oxidative stress.

Glycation The nonenyzymatic bonding of a free amino group with a sugar molecule.

Homeostasis model assessment (HOMA) An index using fasting blood glucose and insulin levels used to estimate insulin resistance.

8-Isoprostane A prostaglandin-like compound, which is a biomarker of oxidative stress (OS). It is formed *in vivo* by the free radical-catalyzed peroxidation of essential fatty acids.

Maillard reaction A form of nonenzymatic browning that results from a chemical reaction between an amino acid and the reactive carbonyl group of a reducing sugar.

Oxidative stress An imbalance of prooxidants and antioxidants, resulting from either an increase in oxidizing species or a decrease in antioxidant defenses.

Receptor for AGEs (RAGE) A multiligand receptor of the immunoglobulin superfamily, which when bound leads to activation of inflammatory pathways and increases in OS via intracellular signaling.

Tumor necrosis factor α (TNF-α) An inflammatory cytokine secreted by several cell types, but by macrophages in particular. TNF-α stimulates a cascade of inflammatory cytokines and increases vascular permeability.

Introduction

Modern 'Western' society has brought with itself profound changes in lifestyle, but at the same time a much greater prevalence of chronic diseases, such as cardiovascular disease (CVD), the metabolic syndrome, insulin resistance (IR), obesity, and type-2 diabetes mellitus (T2D). All these diseases seem to have in common, elevated levels of markers of inflammation and oxidative stress (OS). Of the many components of modern lifestyle, which alone or in combination with other factors may play a role in increasing inflammation and OS, diet is a predominant one. Western dietary patterns that emphasize higher consumption of red and processed meats, sweets and desserts, potatoes and French fries, and refined grains have been linked to increased inflammation and OS.

Of the many dietary factors that may be associated with inflammation and OS, particular interest has been focused on a specific group of food-derived proinflammatory and prooxidant compounds, the so-called advanced glycation end products (AGEs). Although AGEs have been widely recognized as important factors in the pathogenesis of diabetic complications, the importance of AGEs of dietary origin as a factor in human disease has been largely unappreciated. In this article, human data that have accumulated in the past two decades on the role of food-derived AGEs in causing chronic human disease are presented.

What are Advanced Glycation End Products or AGEs?

AGEs are a large group of heterogeneous and highly reactive compounds that form through different pathways. They may form by the spontaneous, nonenzymatic reaction of reducing sugars with free amino groups in amino acids. A series of intermediary reactions leads finally to the irreversible formation of AGEs. This is the classic Maillard reaction also called the browning reaction. Alternatively, AGEs can also form through a variety of other reactions, including oxidation of sugars, lipids, and amino acids, which create reactive aldehydes that eventually also form AGEs (Figure 1).

The same AGEs that form endogenously in the above-mentioned reactions can also form in any system in nature wherever the needed reagents are present. For example, AGEs also form spontaneously in food, but cooking the food at high temperatures markedly increases their formation. A portion of the AGEs contained in the foods we eat gets absorbed and becomes incorporated into the body AGE pool with exogenous compounds being indistinguishable from their endogenous counterparts.

Despite the identification of numerous AGE compounds, the elucidation of the structure of pathogenic AGEs remains elusive. Pentosidine, carboxymethyllysine (CML), and methylglyoxal (MG) derivatives are among the better-characterized compounds and they are often used as AGE markers.

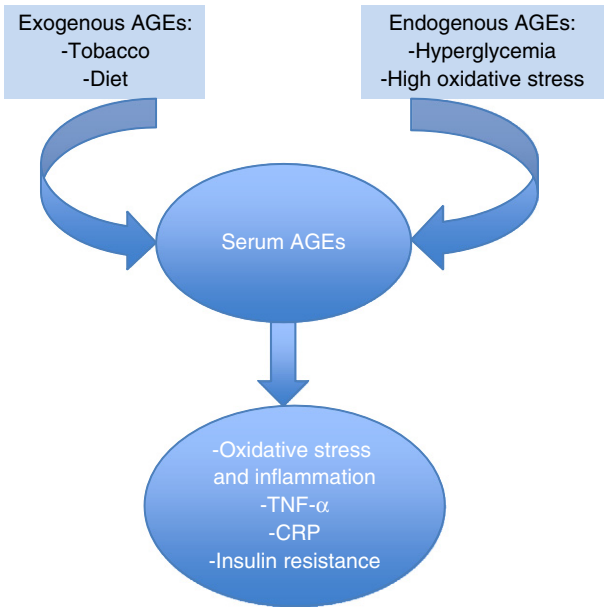

Figure 1 Sources and possible effects of advanced glycation end products (AGEs).

Chemically and immunologically distinct AGEs can coexist on the same carrier proteins. There are many methods to measure AGEs. Detection of their fluorescence capacity is simple but nonspecific. More specific methods include immunoassays and high-performance liquid chromatography/mass spectrometry, each with its own advantages and disadvantages.

AGEs' Interaction Leading to OS and Inflammation

AGEs produce biologic effects through two general mechanisms. First, they induce crosslinking of proteins altering directly their structure and therefore their function. For example, glycation of collagen fibers in the skin and vessel wall changes the characteristics of these proteins and their holding tissues. This produces wrinkling under the skin and stiffness of arterial walls.

AGEs also activate several intracellular pathways acting through receptor and nonreceptor mechanisms. AGEs interact with different cell receptors. The interactions with some of them, such as receptor for AGEs (RAGEs) and EGFR lead to the stimulation of intracellular pathways that eventually increase reactive oxygen species and proinflammatory cytokines, such as TNF-α. The stimulation of other receptors, such as AGE receptor 1 (AGER1), increases AGE breakdown and increase sirtuin 1 (SIRT1) activity and overall decreases cellular proinflammatory activity. AGER1 has been found to be directly associated with the levels of circulating AGEs in healthy subjects. Interestingly, AGER1 levels are much suppressed in subjects who are exposed to a chronic high OS state, such as chronic kidney disease (CKD) and diabetes mellitus patients.

The steady state blood AGE level at any particular point in time reflects the balance of endogenous and exogenous sources and elimination via tissue degradation and renal elimination. AGE elimination is dependent on several mechanisms. MG, for example, is effectively catabolized by the enzymes glyoxalase I and II. Circulating proteins such as lysozyme bind AGEs increasing macrophage endocytosis and degradation of AGEs and increasing their renal excretion. AGE peptides are filtered by the glomerulus and reabsorbed to a variable degree by proximal tubular cells.

Food-Derived AGEs

AGEs form spontaneously in foods, during storage at room temperature, but their rate of formation markedly accelerates as temperature increases during cooking. Besides temperature, other factors known to affect AGE generation in food include nutrient composition, amount of water, pH, presence of trace metals, and the duration of cooking. Food scientists have learned over many decades how to manipulate cooking techniques to get the exact color, aroma, and taste of food they desire, but have been unaware of the potential hazards.

Recently, two large databases with the contents of CML, a commonly measured AGE, in more than 500 food items have become available. These databases allow estimating daily dietary AGE intake as well as designing diets with variable AGE content. These databases show that foods rich in both protein and fat, mostly of animal origin, and cooked at high and dry heat, such as in broiling, grilling, frying, and roasting, tend to be the richest dietary sources of AGEs, whereas low-fat carbohydrate-rich foods tend to be relatively low in AGEs. This may reflect the fact that the AGEs in the diet are generated not only by the Maillard reaction but also by interactions between oxidized lipids and protein; such reactions are known to give rise to AGEs, such as CML. As these fat-rich foods are the ones commonly consumed in the US, most people are constantly ingesting a diet with high-AGE content. Based on the analysis of 3-day food records from a group of 90 healthy subjects eating their usual foods, the average dietary AGE intake was estimated at 16 ± 5 AGE Eq units per day (mean $\pm$ SD).

Knowledge about the effect of cooking temperature, duration of cooking, use of water, and pH of the solution in the generation of food AGEs is of great importance in developing culinary techniques, which allow reduction of food AGE content without necessarily changing the type and quantity of foods consumed. The essential concept here is that it is the way of cooking and not the actual nutrient composition of the food that determines its AGE content; this can be more easily illustrated with an example in which the AGE content of food is expressed in arbitrary AGE kilounits per serving. For example, a 90 oz beefsteak contains 720 AGE kilounits when raw, 2199 kilounits when stewed, and 6731 kilounits when broiled. Moreover, temperature and method of cooking appear to be more critical to AGE formation than the duration of cooking; for example, meat samples broiled or grilled at 230 °C for shorter durations have higher AGE content when compared with samples boiled in liquid media at 100 °C for longer periods. Stewing or steam cooking of meat, which maintains food moisture during cooking, will generate much less AGE than broiling or frying. Marinating will also have an AGE-reducing effect by lowering the local pH of food (pH effect of lemon or vinegar).

Tobacco smoke is another largely ignored exogenous source of AGEs. Tobacco processed in the presence of

reducing sugars leads to the formation of AGEs. Combustion of these adducts during smoking gives rise to reactive AGE formation.

In Vitro Data

Extracts of food containing AGEs tested *in vitro* showed the same biologic properties previously described for endogenous AGEs, namely, protein crosslinks and induction of inflammation and OS. Importantly, these properties persisted when the AGEs were absorbed into the bloodstream after ingestion. For example, LDL obtained from diabetic subjects maintained on a high-AGE diet promoted a marked increase in NF-κB activity in cultured human endothelial cells, compared with LDL obtained from diabetic patients maintained on a low-AGE diet.

Experimental Data

A high dietary AGE intake in mice has been shown to predispose to many chronic diseases, including diabetes types 1 and 2, IR, atherosclerotic disease, and renal disease. More importantly, the institution of a low-AGE diet prevented or significantly ameliorated all the above-mentioned conditions.

Human Data

Observational Data

A number of studies have addressed the clinical relevance of the *in vitro* and animal data discussed in section Experimental Data. A cross-sectional analysis that included a large number of healthy subjects of different age groups demonstrated that dietary AGE intake, adjusted for calories and nutrient intake, significantly correlated with levels of serum CML, MG, 8-isoprostanes (marker of OS), VCAM-1 (marker of endothelial dysfunction), and mononuclear TNF-α, among other inflammatory markers across different age groups. These findings suggest that persons who have diets high in AGEs have relatively high levels of circulating AGEs, which may exceed the capacity of native defenses to remove or neutralize them eventually causing a high OS, a common denominator for most chronic diseases. When some of these subjects were followed for several months without any specific interventions, the spontaneous changes in dietary AGE intake over time also correlated positively with changes in serum AGEs, 8-isoprostane, and TNF-α levels during the same period.

A close association between dietary and blood levels of AGEs, 8-isoprostane, and several markers of inflammation has also been shown in diabetic patients. A recent study of T2D patients of two different ethnic groups (Mexicans and non-Hispanic whites) showed that dietary AGE intake was associated with their risk of CVD. An association between dietary AGE intake and circulating AGE levels was also found in a large population of dialysis patients.

Effects of Acute Oral AGE Loads

A group of diabetic patients and five healthy subjects were challenged with a single meal of egg white, cooked with (AGE-rich) or without fructose. The AGE-rich meal, but not the control meal, produced significant elevations in serum AGE levels in direct proportion to the amount ingested over the next few hours. Only one-third of the absorbed AGEs were detected in the urine over the next 48 h. This study was the first one to clearly demonstrate that food-derived AGEs are absorbed along the gastrointestinal (GI) tract in humans, although the exact mechanism of absorption remains uncertain.

An acute antiendothelial effect of dietary AGEs was demonstrated by administering a single oral dose of a high-AGE beverage to both healthy subjects and diabetic patients. Within 90 min, those drinking the high-AGE beverage showed increased serum AGE levels as well as increased levels of plasminogen activator inhibitor-1 (an inhibitor of fibrinolysis produced by the endothelium) and transient impairment of flow-mediated vasodilatation, both noninvasive indices of endothelial function. These antiendothelial effects were prevented if the subjects were pretreated with an AGE inhibitor (benfotiamine). A single high-AGE solid meal given to diabetic patients also markedly impaired flow-mediated vasodilatation compared with an isocaloric low-AGE meal. As endothelial dysfunction is the earliest abnormality in atherosclerosis, such findings provide a probable mechanistic link between dietary AGE content and CVD.

Clinical Trials with a Low-AGE Diet

Low-AGE Intervention in Healthy Subjects

A group of healthy subjects, equally divided among young and older age groups, were randomly assigned to either their own usual diet or a low-AGE diet. The AGE-restricted diets were otherwise of similar caloric, nutrient, and micronutrient content as at baseline. To reach these goals, participants received detailed instructions on how to prepare their food at home by a study dietitian who was in frequent telephone contact with them. The subjects were required to have a habitually high/normal dietary AGE intake as a condition to participate in the study. After 4 months, significant reductions in serum AGE (both CML and MG) levels were noted, with parallel reductions in plasma levels of 8-isoprostanes, VCAM-1, and peripheral mononuclear cell-derived TNF-α, below baseline values. There was no age difference in the response to the dietary intervention.

Another randomized, crossover study with 62 healthy volunteers compared the effect of two diets, one based on mild steam cooking (low AGE) and the other one on high temperature cooking (high AGE), each one followed for 1 month. The low-AGE diet significantly decreased circulating CML levels and improved insulin sensitivity as assessed by the homeostasis model assessment (HOMA) index.

Low-AGE Intervention in Diabetic Patients

Randomized studies on the effect of an AGE-restricted diet have been performed in two groups of diabetic patients. The first one was a crossover study between low- and regular-AGE diets for a period of 6 weeks. Meals were prepared in the

medical center clinical research unit kitchen, and patients picked them up twice a week during the duration of the study. Patients in the low-AGE diet experienced decreased levels of circulating AGEs (both CML and MG), VCAM-1, C-reactive protein (CRP), and TNF-α (32). Notably, circulating AGE levels decreased by as much as 40% despite same degree of diabetic control. Before this study, high serum AGE levels in diabetic patients were thought to result exclusively from endogenous overproduction owing to hyperglycemia, and therefore this significant fall while maintaining overall same glycemic control was unexpected.

In the second study, patients were randomized to follow either their regular-AGE diet or a low-AGE diet for 4 months (12). A study dietitian followed the patients closely and instructed them how to prepare their own meals at home. At the end of the study, patients in the low-AGE diet showed not only decreased circulating levels of AGEs, 8-isoprostane, RAGE, and TNF-α but also decreased HOMA and increased AGER1 and SIRT1. The reduction of HOMA, which implies improvement of insulin sensitivity, raises a very important consideration. Although it is widely recognized that hyperglycemia in diabetes can increase endogenous production of AGEs, this study suggests that AGEs in turn have an important role in modifying IR and therefore diabetes. This is supported by previous mice experiments showing that high-AGE diet can induce IR and diabetes mellitus, whereas a low-AGE diet has the opposite effect. This newly uncovered information on AGEs and IR needs to be further confirmed in more clinical trials, but clearly opens a big opportunity for a safe, inexpensive, and effective dietary modulation to prevent or ameliorate diabetes.

The effect of an AGE-restricted diet increasing AGER1 and SIRT1 levels in these patients deserves further comments. Other studies have shown that CKD and T2D patients have depressed levels of these two markers of innate immunity. The restoration of their levels following the low-AGE diet demonstrates that the previous suppression was because of an environmental factor, most likely the high AGE-induced high OS. These results may be interpreted as indicating that a high-AGE diet, when sustained, eventually will suppress defensive innate mechanisms, such as AGER1 and SIRT1, which initiates a vicious cycle of further OS increase. The fact that both defense mechanisms can be improved by a simple dietary intervention, both in diabetes and CKD patients (see below), is good news in terms of therapeutic potential.

Low-AGE Intervention Study in CKD Patients

To obtain a measure of the relative impact of the intervention on subjects with different renal abilities to handle oxidants, a subgroup of patients with established CKD not yet on dialysis were challenged with the AGE-restricted diet. The effects of the low-AGE diet in these CKD patients mimicked those in healthy participants (reduction in serum AGEs and in markers of inflammation and OS) and in diabetic patients.

Low AGE Intervention in ESRD Patients on Peritoneal Dialysis

An AGE-restricted diet has also been tested for 4 weeks in a group of patients having nondiabetic end-stage renal disease (ESRD) on maintenance peritoneal dialysis. The results again showed a significant reduction of serum AGEs and CRP.

Diabetic CKD Subjects on a Regular AGE Diet but Exposed to an Agent That Binds AGEs in the GI tract

A group of 20 diabetic CKD patients were studied in a crossover design with one therapy of sevelamer carbonate (1600 mg tid with meals) and another one of calcium carbonate (1200 mg tid with meals) for 8 weeks each. Sevelamer therapy, in contrast to calcium carbonate, reproduced all the previous findings observed on the low dietary AGE intervention, namely, reduced circulating levels of AGEs, 8-isoprostane, TNF-α, all of which were high and increased AGER1 and SIRT1, both of which were low. The study also showed independently that sevelamer, not calcium carbonate, binds AGEs quite effectively *in vitro* and presumably in patients with diabetic CKD. The AGE-binding capacity of sevelamer is most likely responsible for these results, which require confirmation in a larger number of subjects.

Summary

All the above data strongly suggest that dietary AGEs are significant contributors to the body AGE pool and that chronic exposure to these exogenous prooxidant substances gradually erodes native defenses, setting the stage for abnormally high OS and inflammation, precursors of chronic diseases. Real-life dietary patterns are complex and include the simultaneous presence of many factors other than AGEs. Some of these dietary factors are proinflammatory such as fats and AGEs, whereas others are clearly anti-inflammatory such as polyphenols and a variety of antioxidants. The final biologic effect of dietary patterns most likely reflects the combined influence of all these factors, each one of them acting in a different direction and with different intensity. It is the combination of different types of foods and their respective amounts consumed over the day that is important rather than any specific food item with AGE content. Detailed information on how to initiate and maintain a low-AGE diet has been published. Available scientific evidence strongly indicates that restriction of dietary AGEs is a safe and feasible dietary intervention that effectively reverses increased inflammation and OS, both in health and in the disease state.

See also: Food Additives: Antioxidants; Food Additives – General; Preservatives

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Relevant Website

http://theage-lessway.com/
The AGE-Less Way.

PROCESSING CONTAMINANTS

Benzene

A Becalski, Health Canada, Ottawa, ON, Canada

Glossary

Aromatic hydrocarbon A hydrocarbon that contains one or more benzene rings.

Gas chromatography/mass spectrometry (GC/MS) A technique, comprising a gas chromatograph (GC) coupled to a mass spectrometer (MS). Using this apparatus complex mixtures of chemicals may be separated, identified and quantified.

Headspace analysis A technique whereby the vapors in the gas above and in equilibrium with a solid or liquid is sampled, as opposed to sampling only the solid or the liquid.

Log octanol–water partitioning coefficient (log K_{ow}) The octanol–water partition coefficient (K_{ow}) is a measure of the equilibrium concentration of a compound between octanol and water and indicates the potential for partitioning into organic matter. K_{ow} is inversely related to the solubility of a compound in water. Log K_{ow} is generally used as a relative indicator of the tendency of an organic compound to adsorb to soil.

Solid-phase microextraction (SPME) A method for collection of volatile compounds in a headspace on a solid fiber for subsequent analysis of the trapped volatiles using a technique like gas chromatography.

Contaminant Definition

Benzene is an organic chemical compound that has the molecular formula C_6H_6 and structure as given in the figure below:

Benzene is the simplest member of monocyclic aromatic hydrocarbons (sometimes abbreviated as MAHs). At room temperature, benzene is a volatile liquid lighter than water and has a boiling point of 80.1 °C. It exhibits slight water solubility at approximately 1 g l^{-1}, but is fully miscible with fats and oils. It has a log K_{ow} value of 2.13.

Toxicity

The International Agency for Research on Cancer has determined that benzene is carcinogenic to humans. The human cancer induced by long-term exposure to high levels of benzene in air is predominantly acute myelogenous leukemia. In animals, benzene is a multiple-site carcinogen by both the inhalation and oral routes. More than 90% of ingested benzene is absorbed in the digestive tract.

Absorbed benzene is distributed throughout the body and tends to partition in fatty tissues. However, at low-exposure levels (as those which are found in the general population), benzene is rapidly metabolized in the liver and excreted mostly as conjugated urinary metabolites.

Use and Occurrence in Environment

Benzene is both an anthropogenically produced and a naturally occurring chemical. It is an important industrial chemical and a precursor in the production of other chemicals, plastics, synthetic rubber, dyes, etc. Benzene is a natural constituent of crude oil (up to 3%), and benzene content in the gasoline is approximately 1%.

Trace amounts of benzene may result when carbon-rich materials undergo incomplete combustion, for example, in forest fires and volcanic emissions. Outdoor air may contain low levels of benzene, typically at a few microgram per cubic meter, from multiple sources: exhaust from motor vehicles; handling of gasoline, tobacco smoke, wood smoke; and from industrial emissions. Urban areas, where the concentration of benzene might exceed 10 μg m^{-3}, are more affected. Indoor air might also be contaminated by vapors from products that might contain benzene, such as gasoline, glues, paints, rubber, waxes, etc. Water and soil are also affected by atmospheric deposition and by spills of benzene-containing materials. Benzene does not bioaccumulate to a significant degree.

Occurrence in Food

Benzene can be introduced to foods by three principal routes:

1. In the absence of good manufacturing, storage, and cooking practices, benzene can be introduced into food as an environmental contaminant.
2. Benzene can be a contaminant present in certain food additives or certain flavors added into foods.

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3. Benzene can occur in food due to the process-induced changes resulting from high-temperature chemical transformations, ionizing radiation, and from the reaction of added or naturally occurring precursors.

One or more of these sources might be associated with the presence of benzene in food.

Analysis

A number of analytical techniques have been developed for the quantitative determination of benzene. Most of these methods were developed for multiresidue analysis (e.g., benzene, toluene, ethyl benzene and xylenes (BTEX); or other volatile organic chemicals (VOCs)) and they include benzene as a target analyte. Several new methods specifically targeting benzene, some of which use solid-phase microextraction (SPME), were recently developed.

Static headspace sampling followed by gas chromatography/mass spectrometry (GC/MS) is the predominant technique used to determine benzene in foods. Before headspace analysis, solid and viscous foods may need to be homogenized by blending with water. To avoid benzene losses, samples should be chilled to 0 °C before homogenization and processed as quickly as feasible. Benzene in food is usually quantified by isotope dilution method with a d_6-benzene (or $^{13}C_6$-benzene) as an internal standard. The limit of detection is typically in the range of 0.1–1.0 ppb (microgram per kilogram or microgram per liter).

Levels of Benzene in Foods

The occurrence of benzene in foods has been investigated by targeted sampling and market basket sampling as part of total diet studies (TDSs). In general, TDS data and data from targeted sampling show that benzene concentrations in food seldom exceed low ppb (microgram per kilogram or microgram per liter) levels.

TDS and General Surveys

In 1993, UK conducted a TDS investigation of VOCs in 20 food groups collected from 10 UK locations. Benzene concentrations in these food groups ranged from less than 1 ppb in fruit products to 18 ppb in offal.

In a 1995 survey conducted in USA, 22 raw and cooked foods were analyzed. Most of the foods were found to contain less than 1 ppb benzene with the exception of peanuts and canned olives, which were found to contain approximately 2 ppb benzene. In another 1995 survey in USA, benzene was detected in 15 of the 37 foods and beverages analyzed at levels ranging from 0.5 to 9 ppb.

Targeted Surveys

Studies Focused on Environmental Contamination

Once released into the atmosphere, benzene may enter foods via absorption at different points in the food supply chain. Owing to the excellent solubility of benzene in fats and oils, lipid-rich foods are most affected. The sorption of benzene in lipids is rapid as the partition coefficient of lipids/air for benzene is approximately 1000. Infrequently, water contaminated by benzene might also introduce benzene into foods.

In a study conducted in 1996 in UK, 114 retail packs of fatty foods were purchased from shops attached to gasoline stations and stalls on busy roads (such samples were expected to contain higher concentrations of benzene in the air). Shops from the same locations believed to be remote from sources of aromatic hydrocarbons served as a control.

In most of the retail packs analyzed, the center subsamples showed lower concentrations than the surface subsamples. Such distribution indicated that contamination did not occur before or during processing, as the concentrations of benzene would likely be constant throughout the bulk of the food. Instead, it pointed to a passive absorption of benzene from the atmosphere after manufacturing.

However, there were no significant differences of benzene concentration between locations. The maximum concentrations found (calculated after correction for nonhomogeneity) were: bacon 13, cheese 13, butter 16, margarine 17, and lard 10 ppb.

In a study conducted in 1996 in Switzerland, the benzene concentrations in certain virgin olive oils were found to be approximately 50 ppb. A parallel investigation demonstrated that uptake of benzene by olives from ambient outdoor air in the olive groves would result in concentration of only approximately 1 ppb of benzene in the oil.

It was concluded that the production of the oil, and also the collection, transport, and storage of olives were responsible for the increase in the concentration of benzene, predominantly as a result of uptake from the air.

Another study of commercial seed oils showed an average concentration of benzene of 3.9 ppb indicating the ubiquity of traces of this contaminant in oils. It thus appears that approximately 5–10 ppb of benzene in a virgin olive oil might be unavoidable due to the use of motorized equipment during the olive processing.

In a 1996 survey of 24 fruits and vegetables, benzene was found at concentrations ranging from 27 to 56 ppb (dry weight) in the peels of apples, kiwifruit, and oranges. No benzene was found in the pulp of those fruits or the remaining fruits and vegetables.

In addition, benzene can be introduced to foods by a contact with contaminated equipment. For example, contamination of a mineral water (at the level of 10–20 ppb) occurred as a result of an improperly maintained filter. This contamination incident resulted in a worldwide recall of more than 100 million bottles of Perrier mineral water. Other sources of benzene contamination include its migration via contact of food with plastic material harboring traces of benzene.

Studies Focused on Contamination Caused by Substances Added to Foods

Solvent extraction of vegetable oils with hexane or other organic solvents contaminated with benzene can introduce benzene into the oil. In India, relatively high concentrations of benzene were found in refined and unrefined soy oil, i.e., 3.1 and 32 ppm, respectively.

Substances intentionally added to food may contain benzene due to the manner in which they were produced. Liquid smoke flavoring obtained through the incomplete combustion of wood is an example. Benzene was found in two liquid smoke products, at concentration of 21 and 121 ppb.

Studies Focused on Contamination Induced by Processing

Benzene can be introduced into food as a result of grilling over an open flame, especially with wood or charcoal. Benzene concentrations greater than 10 mg m^{-3} were detected in flue gases from glowing charcoal. Benzene can also be introduced into food by smoking as benzene can reach a concentration of approximately 2 mg m^{-3} in an alder wood smoke chamber.

Roasting foods at high temperatures can cause the pyrolysis of organic matter resulting in benzene formation. This occurs either from the recombination of intermediates or by the degradation of compounds containing a benzene moiety, such as phenylalanine.

Most of the data on VOCs released during roasting are reported for roasted coffee beans. Benzene concentrations in roasted coffee beans were estimated to be approximately 0.1–0.15 ppm as compared with 0.1 ppb in brewed coffee.

Trace levels of a few ppb of benzene can occur in some irradiated foods, presumably from radiolytic cleavage of phenylalanine. Some studies also report low-level benzene formation from irradiation of model solutions of benzoate and experimentally prepared foods preserved with benzoate.

In the early 1990s, it was found that benzene could form in certain beverages containing potassium or sodium benzoate and ascorbic acid. Ascorbic acid might be either naturally occurring in fruit juice or added as a preservative or nutrient. Benzoate is used as an antimicrobial agent but it also occurs naturally in many fruits. In cranberries and lingonberries, concentrations of 480 ppm and 600–1300 ppm have been reported. Benzoic acid can also be found in other foods, for example, cinnamon, at concentrations up to few hundred parts per million. When used as a preservative, benzoate may be added to foods at a level of up to 1000 ppm. Benzene forms through a series of reactions mediated by catalytic amounts of iron or copper salts, which produce hydrogen peroxide (H_2O_2). Iron (Fe^{2+}) or copper (Cu^{+}) ions then react with H_2O_2 (Fenton's reaction) to form the hydroxyl radical.

The hydroxyl radical might subsequently react with benzoic acid to form an unstable benzoic acid radical that readily loses CO_2 to form benzene. In the 2000–10 decade, several targeted surveys were conducted to investigate the amount of benzene found in beverages and other foods containing added or naturally occurring benzoate. In some countries, beverages purchased from a retail stores beyond the manufacturer's sell-by-dates contained even higher levels of benzene, up to 263 ppb (Table 1).

Mitigation

Environmental Contamination

Generally, only low-benzene concentrations are found in food. Exposure of foods, especially food rich in lipids, to the air grossly contaminated with benzene could dramatically increase these otherwise low concentrations. Owing to the ubiquity of benzene in the environment, the contamination potential is substantial.

The most likely contamination route is via fugitive emissions of gasoline, especially in confined spaces. These emissions might include accidental spills, losses in refueling, gasoline vapor escaping during engine starts, or from parked hot engines, etc. Even a small amount of gasoline (a few milliliters) could seriously contaminate food stored in a confined space. Such contamination, although significant, might not be detectable by olfactory means.

Good practices must also include preventing contact of food with the exhaust of internal combustion engines, especially gasoline engines without catalytic converters. Shipping and storage of foods should only be done using containers appropriate for that purpose. For example, containers (e.g., freight containers) whose interiors are contaminated by benzene residues (from gasoline, paints, or glues) must be avoided. To prevent uptake of benzene from ambient air, lipid-rich foods should be packaged in container/membrane, which is impervious to benzene and does not release benzene by itself.

Contamination Caused by Substances Added to Foods

The uptake of benzene in grilled foods could be potentially reduced by switching to alternative fuels. One study showed that benzene emissions from flue gases could be reduced by an order of magnitude, depending on the composition of the charcoal used during grilling.

Contamination Induced by Processing

With the exception of beverages, efforts to mitigate process-induced benzene contamination in food have not been deemed necessary. Both the International Council of Beverages Associations and the American Beverage Association have published guidelines that describe mitigation strategies that can be used by the beverage industry to reformulate the affected products. Benzene formation in beverages requires four reactants – benzoate, ascorbic acid, trace metal ions, and oxygen. In principle, the exclusion of any one of these reactants would be an option that could be used to mitigate benzene formation.

Chelating agents, such as ethylenediaminetetraacetic acid (EDTA), may be added to beverages containing benzoate and/or ascorbic acid to reduce the potential for benzene formation; however, EDTA may be less effective in products fortified with calcium. In addition, formulating beverages with sugars, instead of artificial sweeteners, might also help to reduce benzene formation.

Other factors that can reduce *in situ* formation of benzene in beverages are: minimizing exposure of products to high temperatures and/or UV light during the manufacture, storage, distribution, and shelf-life of beverages. In response to these findings, the beverage industry reformulated affected products to eliminate or minimize benzene formation.

Exposure

Dietary exposure to benzene is considered to be of low significance when compared with the total intake of benzene from

Table 1 Levels of benzene found in surveys of beverages

Country	*Year*	*Concentrations found (ppb)*	*Comments*
Australia	2006	1–40	Of 68 beverages, 29 were found to contain benzene concentration higher than 1 ppb, 4 samples contained benzene higher than 5 ppb, and 5 samples contained benzene higher than 10 ppb with a maximum of 40 ppb
Belgium	2008	0.3–11	One hundred thirty-four samples of soft drinks were analyzed, of which 10 samples contained benzene higher than 1 ppb, whereas 1 sample contained 11 ppb of benzene
Canada	2006	1–23	A survey of 124 soft drinks and beverages was conducted. Approximately 60% of the beverages analyzed were found to contain less than 1 ppb benzene. Six products were found to contain benzene higher than the Canadian guideline of 5 ppb for benzene in drinking water; 2 of those products were found to contain benzene higher than the WHO guideline of 10 ppb in drinking water. The highest benzene concentration found was 23 ppb in a low-calorie soft drink specifically marketed to children
Canada	2007	0.06–9.1	Data from the survey of 139 samples of soft drinks indicate that for most of the products, the average benzene concentration has decreased compared with the 2006 survey. The highest concentration found in a ready-to-drink beverage was 9.1 ppb
Ireland	2006	1–17	Of 76 samples of drinks, 7 samples contained benzene at or higher than 1 ppb, only 2 diet products were found to contain benzene higher than 10 ppb; the highest concentration found was 17 ppb
Ireland	2007	1–3.9	Nine of the 63 samples contained benzene higher than 1 ppb
Germany	2008	0.1–42	Seven of the 313 soft drink samples contained benzene higher than 1 ppb, only one sample exceeded 10 ppb guideline. Twenty-nine of the 33 samples of carrot juice for infants contained benzene higher than 1 ppb, with a maximum of 4.6 ppb. The average benzene concentration found in 451 beverages was below the European drinking-water limit of 1 ppb
South Korea	2006	5.7–88	Of 30 beverage samples, 27 products were found to contain benzene
UK	2006	1–28	Of the 150 soft drinks, 107 did not contain benzene higher than 1 ppb. Most of these beverages contained benzoate and ascorbic acid. Thirty-eight samples were found to contain benzene that ranged from 1 to 10 ppb. Four products had benzene concentrations higher than 10 ppb. The highest benzene concentration found was 28 ppb in a diet soft drink
US	2006–7	1–89	In the survey, 199 soft drinks and other beverages were analyzed. More than 90% of the samples analyzed did not contain benzene concentration higher than 5 ppb and most samples contained less than 1 ppb. Eighteen beverages were found to contain benzene above the US EPA MCL of 5 ppb for drinking water.

MCL, Maximum contaminant level; US EPA, US Environmental Protection Agency.

all sources. Several studies concluded that inhalation of contaminated air was the primary route of nonoccupational benzene exposure for nonsmokers, and mainstream cigarette smoke was the primary route for smokers. The average inhalation exposures were estimated to be approximately 3.3 μg per kg of bodyweight per day (0.2 mg day^{-1}, 60 kg adult) for nonsmokers and 33.3 μg per kg of bodyweight per day (2 mg day^{-1}, 60 kg adult) for smokers. These data were based on the benzene concentration in ambient outdoor/indoor air combined with the exposure from gasoline-powered machinery.

Estimates of dietary exposures vary significantly from 3 to 3000 ng per kg of bodyweight per day, with most estimates in the lower range. The variability is caused by using different consumption scenarios. Although these values are not consistent, it is generally considered that dietary intake of benzene is a minor source of total benzene exposure. Many studies point to the likely dietary exposure in the range of 50–200 ng per kg of bodyweight per day (3–12 μg day^{-1}, 60 kg adult). Dietary exposure of benzene would therefore constitute only a few percent of the total exposure to benzene. Mitigation strategies implemented by the beverage industry in 2006 should reduce the dietary exposure to benzene formed in drinks containing benzoate.

Health Risk

The variability of the dietary exposure and the human relevance of an estimated low-dose cancer effect level have resulted in some uncertainty in the assessed health risk from dietary exposure to benzene. However, the risk assessments conducted by several international organizations point to a likely hypothetical risk of cancer, which is defined as a unit risk value over a lifetime, to be in the range of 10^{-5}–10^{-6} (one in one hundred thousands to one in a million).

Risk Management

In general, the low concentrations of benzene found in foods have not generated the level of concern that would warrant the

establishment of formal risk management procedures or regulatory limits. Regulatory limits for benzene in food and beverages have not been established, except for bottled water where the Food and Drug Administration has adopted the US Environmental Protection Agency's maximum contaminant level (MCL) of 5 ppb as a quality standard. The European and Australian standard for benzene in drinking water is 1 ppb, the Canadian guideline is 5 ppb, and the World Health Organization's guideline is 10 ppb.

Recent reports of elevated levels of benzene in soft drinks and beverages initiated a series of actions by food safety organizations and the beverage industry. Actions included verification of the findings, identification of the source of contamination, evaluation of the health risk, and reduction of the concentrations to levels considered to meet acceptable quality standards. These actions were guided by the respective drinking water standards.

In North America and in several other jurisdictions, manufacturers were notified, products with excessive benzene concentration were withdrawn or discontinued, and other products were reformulated to keep the products 'as low as reasonably achievable' in benzene.

Conclusion

Environmental contamination and process-induced contamination are the most important sources of benzene in foods. Nonetheless, levels of benzene in food are generally low. The available data suggest that dietary exposure to benzene, and resultant health risks, are negligible in comparison with the overall exposure from environmental sources.

See also: Food Additives: Flavors and Flavor Enhancers; Preservatives. Foodborne Diseases: Overview of Chemical, Physical, and Other Significant Hazards. Food Technologies: Sterilization

Further Reading

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Biogenic Amines

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Glossary

Biogenic amines Also known as biologically active amines, these are nitrogenous low-molecular weight substances of biological origin that can be found in nearly all types of foods in a wide and variable range of concentrations. Dietary biologically active amines can be classified into three different groups according to their origin and chemical structure: aromatic biogenic amines, aliphatic diamines and natural polyamines. Biogenic amines are mainly produced by decarboxylation of the precursor amino acids by specific microbial enzymes.

Cheese reaction A hypertensive response than can occur when subjects treated with monoamino oxidase inhibitor (MAOI) drugs consume foods or beverages rich in tyramine (a biogenic amine resulting from bacterial decarboxylation of tyrosine). Originally, the reaction was attributed to tyramine-rich cheese, though the mechanism of action is common for all types of food containing high amounts of tyramine.

Decarboxylation reaction A chemical reaction in which a carboxyl group (–COOH) is split off from a compound as carbon dioxide (CO_2). Enzymes that catalyze decarboxylation reactions are called decarboxylases, and convert amino acids to amines. A wide variety of decarboxylases occur in living organisms. Decarboxylases responsible for the formation of biogenic amines (i.e., histamine, tyramine, putrescine, cadaverine, phenylethylamine, and tryptamine) are able to convert specific amino acids (i.e., histidine, tyrosine, ornithine, lysine, phenylalanine, and tryptophan, respectively).

Histamine fish poisoning (HFP) A foodborne chemical intoxication caused by the consumption of fish containing high contents of histamine, a biogenic amine derived from bacterial decarboxylation, usually associated with spoilage. HFP formerly called scombrotoxin poisoning because of the frequent association of the illness with the consumption of spoiled scombroid fish such as tuna and mackerel. Histamine intoxication can also occur if other food containing high amounts of histamine is consumed.

Histamine intolerance Health disturbance mainly due to an impaired degradation of histamine ingested through foods. Usually, histamine intolerance is associated with a reduced diamino oxidase (DAO) activity due genetic constitution, physiological status, and intake of DAO-clocking drugs. Under these circumstances, an excess of histamine absorption may cause numerous symptoms mimicking an allergic reaction, including headache, diarrhea, congestion of the nose, asthmatic wheezing, hypotension, arrhythmia, urticaria, pruritus, and flushing.

Biogenic Amines: Classification and Origin

Several nitrogenous low-molecular weight substances with biological functions in animals, plants, and microorganisms are included within the term 'biologically active amines.' Biologically active amines can be classified into several groups according to their biosynthetic pathway and chemical structure, as shown in Figure 1. In food, the biologically active amines, known as (exogenous) biogenic amines, include tyramine, histamine, phenylethylamine, tryptamine, putrescine, and cadaverine, and they are mainly derived from the bacterial decarboxylation of precursor amino acids. Putrescine can also be formed through the deamination of agmatine, an arginine-derived polyamine.

Physiologically natural (endogenous) amines, including the polyamines spermidine and spermine, are not associated with the microbial decarboxylase activity but their intracellular biosynthesis in living organisms involves the incorporation of aminopropyl groups into their precursor putrescine. Some of the above-mentioned biogenic amines can also be synthesized *de novo* in animal and plant tissues and at low concentrations in food can be considered physiologically natural.

Both biogenic amines and polyamines can be found in nearly all types of foods in a wide and variable range of concentrations. Despite biogenic amines of the microbiological origin being the focus of this article, it is worth mentioning that the natural polyamines spermidine and spermine constitute the main amines occurring in fresh products (such as fruit and vegetables, milk, meat, and fish) where they have a physiological role associated with cell growth and proliferation. Polyamine content varies depending on the food type (Table 1). The main polyamine in plant-based products is spermidine, whereas spermine content is higher in foods derived from animals. Apart from the small

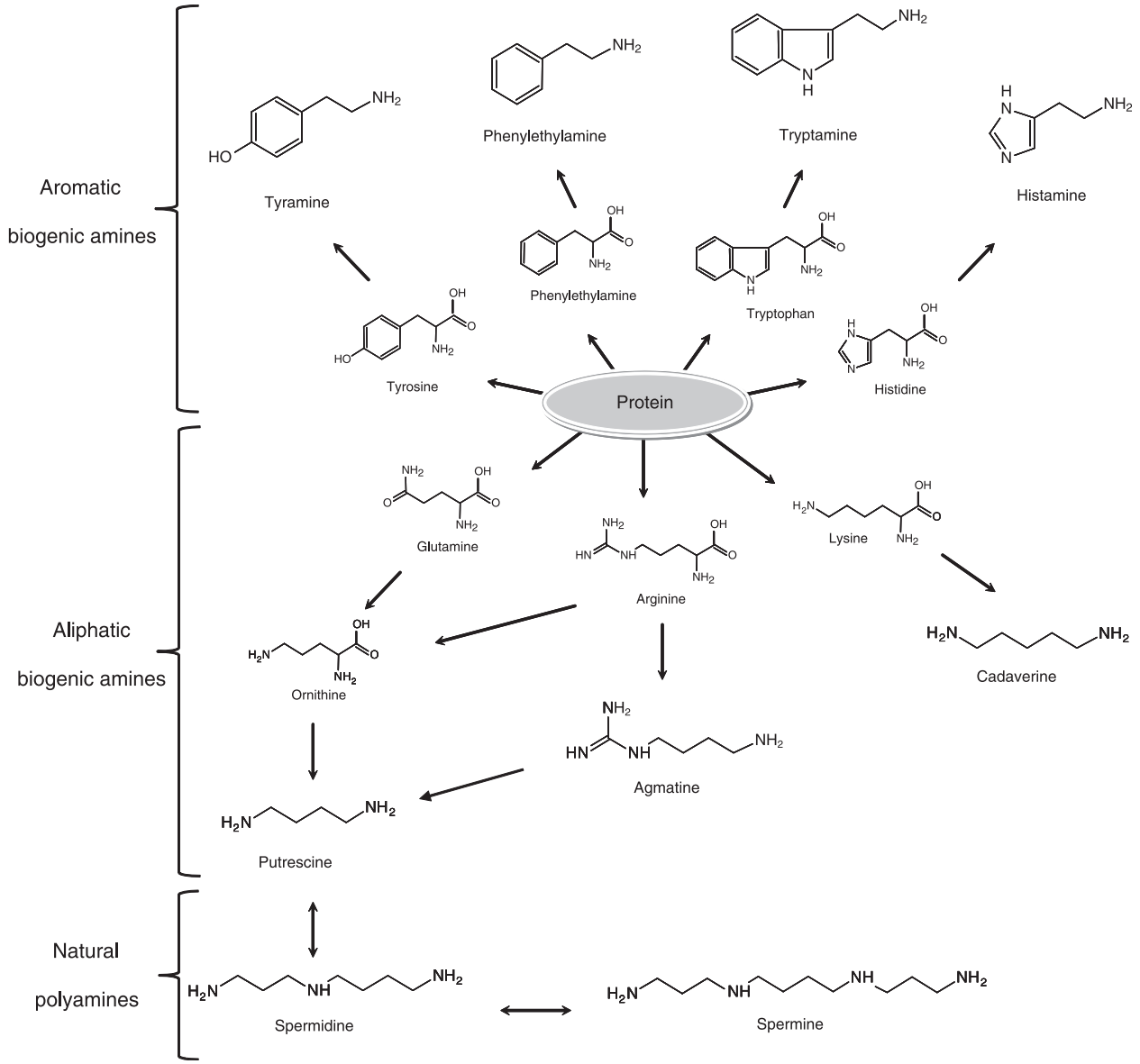

Figure 1 Classification of biologically active amines.

amounts of putrescine, other amines like histamine may occur physiologically in some fresh vegetables (e.g., spinach) as well as products made from blood or liver.

Biogenic amines are found in a wide variety of foods and beverages in which microbial growth and activity has been allowed to occur during storage or production, for example, fish and seafood products, meat and meat products, cheese, fermented vegetables, and beer and wine (Table 1). The accumulation of biogenic amines in food requires the concurrence of several factors, the most important of which are: The occurrence of microorganisms bearing decarboxylase enzymes, the availability of precursor amino acids and favorable environmental conditions for the growth or activity of aminogenic microorganisms.

Several bacterial groups associated with food spoilage and/or with a technological role are known to be able to decarboxylate amino acids and produce one or more biogenic amines to a variable extent. Though the ability to decarboxylate certain amino acids is a strain-dependent property, the ability to produce specific biogenic amines is reported in some bacterial families or genera more often than others. *Enterobacteriaceae*, including mesophilic and psychrotolerant *Morganella*, *Enterobacter*, *Hafnia*, *Proteus*, etc., and *Photobacterium phosphoreum*, are the most prolific histamine-producing bacteria in fish. Many enterobacteria strains isolated from meat and vegetables have been shown to produce diamines, putrescine and cadaverine, and some particular strains have also been described as histamine producers, although less often and to a lesser extent than the enterobacteria isolated from fish products.

Tyramine is usually associated with Gram-positive microorganisms; among these, *Enterococcus* is one of the most frequent and intensive tyramine producers. Other lactic acid bacteria (including lactobacilli, lactococci, pediococci, oenococci, leuconostoc, carnobacteria, etc.) are also meaningful

Table 1 Biogenic amine content (mg kg^{-1}) found in different food categories[a]. Data is presented as: the mean, (standard deviation), *median*, and minimum–maximum

	n	*Spermidine*	*Spermine*	*Histamine*	*Putrescine*	*Cadaverine*	*Tyramine*	*Phenylethylamine*	*Tryptamine*	*Agmatine*
Fruits, vegetables, and plant-based products										
Fruits (e.g., orange, apple, and kiwi)	136	4.65 (2.83)	0.95 (1.97)	0.07 (0.20)	12.54 (26.03)	–[c]	0.72 (1.16)	0.13 (0.32)	0.35 (0.31)	–
		4.25	*ND*[b]	*ND*	*1.90*	–	*ND*	*ND*	*ND*	–
		0.78–15.79	ND–8.88	ND–2.51	ND–173.81	–	ND–11.52	ND–1.67	ND–7.94	–
Nuts (e.g., almond, pine nuts, and pistachios)	41	22.91 (7.17)	14.29 (9.32)	0.45 (1.23)	4.50 (4.00)	–	0.06 (0.17)	0.03 (0.06)	0.71 (1.97)	0.18 (0.33)
		24.79	*12.32*	*ND*	*2.96*	–	*ND*	*ND*	*ND*	*ND*
		6.21–48.06	1.98–37.81	ND–11.86	ND–39.51	–	ND–2.63	ND–0.82	ND–11.86	ND–5.11
Vegetables (e.g., lettuce, tomato, potato, and spinach)	98	16.45 (18.53)	2.72 (3.17)	2.82 (7.43)	6.13 (7.89)	0.01 (0.06)	0.95 (1.49)	0.11 (0.29)	0.31 (0.89)	0.32 (0.77)
		11.81	*1.59*	*ND*	*3.69*	*ND*	*ND*	*ND*	*ND*	*ND*
		1.13–108.22	ND–15.87	ND–69.72	ND–48.09	ND–0.43	ND–14.06	ND–1.40	ND–3.21	ND–3.75
Legumes (e.g., lentils, beans, and chickpeas)	11	30.82 (8.57)	14.26 (26.24)	–	2.80 (1.82)	–	–	–	–	0.30 (0.53)
		15.02	*14.93*	–	*2.95*	–	–	–	–	*ND*
		11.04–85.32	5.1–32.36	–	0.35–6.39	–	–	–	–	ND–1.68
Cereals (e.g., bread, wheat, oats, and rice)	28	55.14 (30.34)	15.47 (119.77)	0.12 (0.33)	6.04 (11.36)	0.09 (0.024)	–	–	–	2.09 (5.25)
		4.55	*4.21*	*ND*	*2.19*	*ND*	–	–	–	*ND*
		0.18–327.58	ND–84.21	ND–0.89	ND–31.89	ND–0.69	–	–	–	ND–15.88
Chocolate	25	2.01 (1.66)	2.10 (0.35)	0.58 (0.44)	0.39 (0.54)	0.79 (1.11)	2.19 (2.13)	1.53 (1.29)	0.49 (0.39)	0.79 (0.57)
		0.47	*0.53*	*0.17*	*0.37*	*0.68*	*0.73*	*1.03*	*0.17*	*0.92*
		0.26–4.65	1.20–2.72	0.16–0.56	ND–1.98	ND–2.78	0.23–5.85	0.26–4.38	0.45–1.26	ND–2.98
Spices (e.g., black pepper and red pepper)	12	11.37 (11.71)	3.45 (3.36)	–	3.31 (2.57)	1.38 (1.51)	4.45 (5.57)	0.87 (1.36)	–	2.56 (3.19)
		32.72	*8.70*	–	*6.77*	*3.34*	*15.76*	*2.93*	–	*7.79*
		0.65–32.73	ND–8.97	–	ND–8.31	ND–3.42	0.47–15.84	ND–3.60	–	ND–8.10
Alcoholic beverages										
Highly fermented beer	49	0.40 (0.20)	0.72 (0.50)	0.82 (2.21)	4.24 (2.61)	0.96 (3.29)	3.91 (7.50)	0.25 (0.69)	0.11 (0.16)	10.76 (2.59)
		ND	*0.76*	*0.59*	*4.30*	*0.44*	*3.03*	*ND*	*ND*	*11.11*
		ND–3.40	ND–4.65	ND–17.00	ND–12.40	ND–31.40	0.55–46.80	ND–2.20	ND–1.75	ND–40.90
Low fermented beer	88	0.13 (0.48)	0.63 (0.42)	1.57 (0.31)	4.34 (2.30)	1.89 (1.00)	7.24 (1.07)	0.54 (0.22)	0.16 (0.12)	6.18 (5.99)
		ND	*0.45*	*0.85*	*4.60*	*0.45*	*3.60*	*0.40*	*ND*	*6.20*
		ND–3.90	ND–6.30	ND–14.00	ND–9.70	ND–17.70	1.15–36.40	ND–8.35	ND–2.75	ND–19.40
Spontaneously fermented beer	7	–	0.44 (0.22)	7.25 (3.14)	5.79 (1.50)	9.55 (7.93)	18.41 (11.12)	0.25 (0.19)	0.15 (0.30)	7.75 (4.89)
		–	*0.45*	*4.63*	*4.73*	*4.15*	*16.79*	*0.20*	*ND*	*8.73*
		–	ND–0.70	2.6–21.60	2.9–14.50	3.1–20.95	2.45–41.25	ND–0.65	ND–1.80	1.05–18.8
White wine	83	–	–	1.24 (1.69)	–	–	1.25 (1.07)	–	–	–
		–	–	*0.45*	–	–	*0.46*	–	–	–
		–	–	0.10–13.00	–	–	ND–7.50	–	–	–
Red wine	260	–	–	3.81 (3.51)	–	–	2.57 (1.62)	–	–	–
		–	–	*1.90*	–	–	*2.40*	–	–	–
		–	–	0.09–55.00	–	–	ND–15.85	–	–	–

(*Continued*)

Table 1 Continued

	n	*Spermidine*	*Spermine*	*Histamine*	*Putrescine*	*Cadaverine*	*Tyramine*	*Phenylethylamine*	*Tryptamine*	*Agmatine*
Fish and seafood products										
Fresh fish	136	3.54 (2.81)	6.52 (6.15)	0.79 (0.71)	0.95 (0.61)	0.99 (0.96)	0.54 (0.51)	0.03 (0.07)	0.06 (0.15)	2.25 (2.42)
		2.51	*5.93*	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
		ND–11.90	ND–37.30	ND–36.55	ND–4.90	ND–33.65	ND–17.10	ND–1.70	ND–5.85	ND–43.50
Canned fish	96	0.90 (2.01)	2.41 (5.39)	14.42 (16.03)	0.10 (0.22)	0.21 (0.47)	2.25 (1.37)	0.09 (0.21)	0.21 (0.48)	0.21 (0.48)
		ND	*ND*	*5.93*	*ND*	*ND*	*1.10*	*ND*	*ND*	*ND*
		ND–19.50	ND–35.20	ND–657.05	ND–2.20	ND–12.00	ND–41.15	ND–7.30	ND–12.90	ND–10.40
Semi-preserved fish	49	2.16 (1.48)	4.52 (2.89)	3.48 (3.37)	4.15 (2.37)	12.51 (8.40)	14.60 (11.90)	0.56 (0.97)	0.37 (0.56)	14.76 (8.50)
		1.72	*3.15*	*2.18*	*4.26*	*10.98*	*14.53*	*ND*	*ND*	*9.31*
		0.37–11.80	ND–9.4	ND–34.90	ND–21.15	ND–55.80	ND–88.50	ND–9.20	ND–10.05	ND–52.85
Meat and meat products										
Fresh meat	6	3.05 (0.07)	36.65 (4.45)	–	–	–	–	–	–	–
		ND	*ND*	–	–	–	–	–	–	–
		0.80–4.50	27.3–44.6	–	–	–	–	–	–	–
Cooked meat	48	3.00 (0.95)	19.93 (4.18)	0.30 (0.26)	1.25 (0.32)	6.01 (5.45)	9.14 (2.17)	0.07 (0.09)	0.25 (0.06)	1.77 (1.94)
		2.63	*18.00*	*ND*	*0.78*	*1.70*	*2.50*	*ND*	*ND*	*ND*
		1.00 8.90	6.45–87.80	ND–4.80	ND–12.40	ND–40.00	ND–78.10	ND–1.40	ND–5.10	ND–27.60
Cured meat	23	5.66 (0.96)	35.75 (8.21)	12.98 (37.64)	3.89 (3.95)	27.85 (68.74)	7.21 (13.26)	0.31 (0.54)	0.23 (0.77)	1.61 (1.14)
		5.60	*35.10*	*0.80*	*2.30*	*1.80*	*0.70*	*0.00*	*ND*	*1.40*
		4.10–7.30	24.90–62.10	ND–150	ND–17.40	ND–305.00	ND–46.50	ND–2.00	ND–2.90	ND–3.50
Dry-fermented sausages	209	4.88 (1.64)	20.67 (7.22)	32.15 (14.22)	94.05 (24.41)	40.55 (13.52)	180.95 (25.34)	8.54 (3.74)	16.33 (2.40)	0.18 (0.18)
		3.57	*19.31*	*8.03*	*36.50*	*11.30*	*143.74*	*3.34*	*8.21*	*ND*
		ND–32.10	ND–87.15	ND–357.70	ND–537.05	ND–658.05	0.51–742.60	ND–181.83	ND–194.09	ND–7.65
Dairy products										
Unripened cheese	20	0.28 (0.28)	0.21 (0.33)	–	0.43 (0.78)	0.26 (0.48)	0.05 (0.16)	–	–	–
		0.31	*ND*	–	*ND*	*ND*	*ND*	–	–	–
		ND–0.82	ND–1.12	–	ND–3.10	ND–1.50	ND–0.56	–	–	–
Raw milk cheese	20	7.39 (12.87)	1.73 (5.33)	59.37 (106.74)	129.55 (212.69)	55.36 (82.93)	154.82 (168.99)	6.45 (9.49)	7.40 (10.40)	3.82 (0.01)
		0.15	*ND*	*18.38*	*27.84*	*29.22*	*125.86*	*ND*	*0.28*	*ND*
		ND–39.43	ND–21.07	ND–389.86	ND–666.92	1.27–368.22	ND–602.07	ND–28.98	ND–32.62	ND–27.13
Pasteurized milk cheese	20	7.81 (10.31)	3.51 (5.57)	18.05 (38.23)	87.03 (151.70)	57.55 (107.14)	78.10 (81.58)	7.13 (7.30)	6.41 (10.43)	3.32 (6.36)
		3.14	*1.25*	*4.59*	*23.17*	*10.59*	*67.49*	*5.46*	*2.25*	*ND*
		ND–41.94	ND–18.11	ND–162.03	ND–610.46	0.44–355.91	ND–241.45	ND–28.92	ND–44.57	ND–21.96

[a]Data collected from the surveys and studies conducted in our laboratory from 1990 to 2009 (most of these data have been published in peer-review articles).
[b]ND: Not detected.
[c]Not determined.

aminogenic organisms not only as tyramine producers but also as producers of other aromatic (e.g., phenylethylamine) and aliphatic biogenic amines. Although staphylococci are less frequently reported to be powerful aminogenic organisms, the decarboxylase activity has also been reported in some strains.

Other microorganisms belonging to the genera *Clostridium*, *Vibrio*, *Acinetobacter*, *Plesiomonas*, *Pseudomonas*, *Aeromonas*, and *Bacillus*, among others, have also been found to produce biogenic amines.

The function of decarboxylase enzymes in bacterial metabolism is not fully understood, although it has been described as one of the primary emergency systems involved in the acid stress response. Decarboxylases work in cooperation with a membrane antiporter protein, thus enabling amino acids to be transported into the cell and biogenic amines excreted out of the cell. Because decarboxylation consumes a proton, biogenic amine formation contributes to the regulation of intracellular pH and it may also help to increase the pH of the extracellular medium with low buffer capacity. Apart from the alkalization effect, amino acid decarboxylation can also induce metabolic energy generation through a proton motive force.

Decarboxylase synthesis and activity are induced at acidic pH and generally by the presence of precursor amino acids. Therefore, foods containing high levels of precursor may be more susceptible to biogenic amine accumulation, as is the case of histamine accumulation in scombroid fish species that are rich in free histidine. Except for the pyruvoyl-dependent histidine-decarboxylase of Gram-positive bacteria, decarboxylase enzymes require pyridoxal-5′-phosphate (a form of vitamin B_6) as a cofactor. Besides the presence of the cofactor, the proteolysis phenomena and/or yeast autolysis occurring in products undergoing fermentation may also offer a favorable amino acid concentration for biogenic amine production.

In general, biogenic amine accumulation can be expected in almost all food products that contain proteins or certain amounts of amino acids and are subjected to conditions that allow the microbial activity to occur (such as storage and fermentation). Nevertheless, the total content of the different amines strongly depends on the nature of the product, the microorganisms present and the environmental conditions. In perishable products, temperature and storage time probably have a major impact on the accumulation of biogenic amines, mainly not only via the growth of potentially aminogenic organisms but also through the expression of metabolic activity. Other factors such as pH, atmosphere, food ingredients (salt, sugar, etc.), preservatives, and preservation processes, etc., also play a role in the modulation of the aminogenesis process. The influence of these environmental factors is not always well known and it may even be controversial. For instance, despite the fact that an acidic pH may enhance the bacterial-decarboxylase activity, a rapid and sharp acidification in food fermentation inhibits contaminant bacteria and the consequent formation of biogenic amines. Therefore, the type and the amount of biogenic amines in a product depends on multiple and complex variables that may interact among each other, making it difficult to characterize the effects of each technological factor on the aminogenesis during food fermentation, ripening, and storage.

Relevance of Biogenic Amines in Food

Biogenic amines in food are relevant from both the safety and quality point of view. Some biogenic amines have bioactive properties and have been associated with certain adverse health reactions. Nowadays, however, the presence of biogenic amines in food is significant from a hygienic and technological perspective, as their occurrence may be a sign of the unwanted microbial activity and reduced microbiological quality.

Toxicological Risks of Biogenic Amines

Although the health concerns are well documented, information concerning the mechanisms, the toxic doses and the individual factors governing the severity of the reactions is inconsistent. At the moment, it is not possible to perform an accurate and complete risk assessment, mainly because there is no sufficient data available to reasonably estimate the level of illness caused by exposure to dietary biogenic amines. However, the risk profile approach in relation to biogenic amines in food can be applied to describe food safety concerns, as recently done by the European Food Safety Authority (EFSA) BIOHAZ panel (for more details see references in the Further Reading section).

Hazard Identification

Histamine, tyramine, and to a lesser extent phenylethylamine are the main dietary biogenic amines associated with several acute adverse reactions in consumers (Table 2). The bioactivity of biogenic amines mainly involves vasoactive reactions, although they can also be active at a neurological level as neurotransmitters. The health problems that may occur following the ingestion of food containing biologically active amines include histaminic intoxication (also known as histamine fish poisoning (HFP)), food intolerances due to enteral histaminosis, interactions between tyramine and MAOI drugs, and food-induced migraines.

Histamine, as a local hormone and neurotransmitter, has a physiological role in several functions including gastric-acid secretion and local immune response. In animals, histamine causes vasodilatation and subsequent hypotension as well as dermal (flushing and pruritus), gastrointestinal (diarrhea, cramps, and vomiting) and neurological (headache and dizziness) disorders, mediated through the specific histamine receptors of cellular membranes. The most globally widespread form of histamine toxicity is the so-called HFP, which accounts for up to one-third of the seafood-borne outbreaks in humans in countries where statistics are available (e.g., Wales, England, USA, etc.). Many species of marine fish, particularly those containing high concentrations of free histidine (i.e., *Scombridae*, *Scomberesocidae*, mahi-mahi, escolar, sardines, garfish, etc.), have been incriminated in HFP outbreaks. Histamine intoxication and intolerance may also be caused by other histamine-rich food (e.g., cheese, meat products, etc.), although knowledge in this area is even more scarce than for HFP.

Tyramine and phenylethylamine are vasoconstrictive, leading to increased blood pressure if a higher than physiologically normal concentration reaches the blood stream.

Table 2 Hazard identification

	Histamine	*Tyramine (β-phenylethylamine)*	*Putrescine, cadaverine*
Mechanisms of toxicity (acute)	Vasodilatation and psychoactive	Vasoconstriction (release of adrenaline)	Saturation of intestinal barriers (if ratio is 5:1)
Clinical symptoms	Cardiovascular (flushing, urticaria, hypotension, edema, and headache) Gastrointestinal (cramps, diarrhea, and vomiting) Neurological (pain and itching)	Increase in blood pressure, fever, perspiration, and vomiting. Pupil dilatation, lacrimation, etc. Headache and migraine	Enhances the effects of other biogenic amines
Duration	1 min–3 h 'incubation' 3–6 h duration (<12 h)	Few min 'incubation' 10 min–6 h duration	–
Severity	Low to moderate	Low to severe	–
Outbreaks	Mean annual rate (cases/year/million people) of histamine fish poisoning varies among countries, for example, from 31 in Hawaii, USA; 4.9 in Denmark; 3.1 in New Zealand; 2.5 in France; 2.1 in Finland; 1.5 in Taiwan; 1.1 in Japan; <1 in UK, Norway, Switzerland, South Africa, Australia, USA, Sweden, Canada, Netherlands, Philippines	Limited information is available due to a lack of official records	–

Besides hypertension, tyramine and phenylethylamine may also provoke headache, perspiration, vomiting, pupil dilatation, etc. Hypertensive crises and migraines caused by the consumption of tyramine-rich food have occasionally been recorded in scientific publications, but there is limited official epidemiological data available in this respect.

The clinical signs following consumption of biogenic amine (both histamine and tyramine) appear between 30 min and few hours after ingestion and usually disappear within 24 h. The severity of the reactions varies, but it may be considered mild because medical attention is only occasionally required. The mild nature of the symptoms, together with misdiagnosis and the lack of mandatory or adequate systems for reporting these food-borne diseases, account for the poor, or lack of, statistics on the incidence of intoxication due to dietary amines.

The diamines putrescine and cadaverine are not toxic on their own, but they may enhance the absorption of vasoactive amines due to the saturation of intestinal barriers. It has been suggested that the mechanisms involve competition for the mucin attachment sites and detoxification enzymes.

Biologically active amines present in food products have been recognized as precursors of nitroso compounds with the potential carcinogenic activity, which constitutes an indirect additional risk associated with dietary amines. Nitrosamines result from the action of nitrite on secondary amines, which in turn may derive from primary amines (such as aliphatic diamines and polyamines) through a cyclation reaction under certain conditions. However, according to the available information, dietary sources of nitrite and *N*-nitroso compounds, including those derived from biogenic amines, contribute little to the body pool of nitroso compounds; instead the main source comes from the bacterial and mammalian metabolism of ingested nitrates.

Hazard Characterization

Even though there is compelling evidence concerning the involvement of biogenic amines as causative agents of the above-mentioned adverse food reactions, the varying amounts in the products responsible makes these hazards difficult to characterize. There is also a great deal of variability and uncertainty regarding the factors that influence the human clinical response to a given quantity of a biogenic amine. Besides the genetic disposition or the physiological status, several environmental factors such as diet and medication can temporarily modify individual susceptibility. The capacity of detoxifying enzymes (monoamine (MAO) and diamine oxidases (DAO), as well as histamine *N*-methyltransferase (HMT)) at intestinal and hepatic level may be reduced by food components, such as other amines (diamines and polyamines), alcohol and its metabolite acetaldehyde, phenols, etc. However, diet (especially fat and proteins) may reduce biogenic amine bioavailability. The influence of certain medication seems to be far more critical, because it is not only the well-known antidepressant and antiparkinson MAOI drugs that increase susceptibility to biogenic amines but also a considerable number of other commonly used drugs (e.g., acetylcysteine, clavulanic acid, metoclopramide, verapamil, isoniazid, cephalosporins, etc.) also present DAO-inhibition as a side effect.

Bibliographical information regarding the dose of histamine that can trigger toxic reactions is not consistent. From epidemiological studies, most incidents of HFP involve fish with high histamine content (600–3000 mg kg^{-1}). The attack rates are also variable, from 10% to 80% depending on the outbreak. Some reviews and textbooks have suggested guideline levels to define the oral toxicity of histamine (some recorded in Table 3), which reflect 'expert opinion,' but they may be inadequately supported by specific clinical studies. Unfortunately, histamine poisoning has hardly ever been reproduced in clinical studies. The limited results of toxicological studies, usually performed with a reduced number of individuals, have also been compiled in Table 3.

The specific toxicity derived from the vasopressor effect of tyramine can be found from clinical studies focused on the

Table 3 Hazard characterization

	Dose–response relationship (toxic threshold)	*Population affected*
Histamine		
Histamine poisoning (enteral histaminosis)	5–6 mg threshold level	
	8–40 mg light intoxication	
	70–1000 mg moderate intoxication	
	1500–4000 severe intoxication	General – all population; higher sensitivity in DAO deficient individuals
	8–40 mg slight	
	>40 mg moderate	
	>100 mg severe	
	8–40 mg mild poisoning	
	70–1000 mg moderate intensity disorders	
	1500–4000 mg severe incidents	
	100–180 mg in a meal	25–50% of tested volunteers
	75 mg pure histamine (in peppermint tea)	50% of tested volunteers (healthy females without history of food intolerance)
	90 mg in a meal	25% of tested volunteers
	120 mg (intraduodenal administration)	70% of patients suffering from chronic urticaria (susceptible volunteers)
Tyramine (β-phenylethylamine)		
Hypertensive crisis	200–2000 mg TY ($ED_{50} \approx 1400$ mg)	Healthy population (100%) (50%)
PD30[a] dose of TY causing up to 30 mm Hg SBP increase	6 mg mild symptoms	Classical MAOI[c]
	10–25 mg severe	
	50–100 mg tolerated	RIMA (3rd generation of MAOI: reversible/selective)
Migraine[b]	100 mg TY	80% migraine sufferers (0–15% nonmigrainous)
	3 mg PHE	50% migraine sufferers
	5 mg PHE	Intoxication symptoms

[a]Increase of systolic blood pressure up to 30 mm Hg by a given dose of tyramine. Only those clinical studies with human volunteers, in which tyramine is administered orally in a meal have been considered.

[b]Scientific literature (from 1967 to 2001) is barely able to prove the relationship between tyramine and migraine.

[c]The following potentiation factors have been described for MAOI drugs: tranylcypromine 20–56; clorgyline 10; brofaromine 10; phenelzine 13; moclobemide 5–7; befloxatone 5–8; selegiline 2–5.

study of the interaction between dietary tyramine and MAOI drugs. These studies prove that the intestinal barriers to tyramine in nonmedicated healthy volunteers (placebo groups) are quite effective, because 200–2000 mg of tyramine is needed to cause a minimal pressure response (Table 3). For those individuals medicated with MAOI drugs, lesser amounts of dietary tyramine can produce similar effects. The intensity of the MAOI-enhancing action depends on the nature of the drugs (see Table 3 for enhancing factors associated with several MAOI compounds). Third generation MAOI drugs have progressed in safety compared with previous ones, because their action is reversible or selective for the MAO-A or MAO-B isoenzyme.

Tyramine and phenylethylamine have also been identified as causative agents of certain migraines. However, migraine is a multifactorial problem that is not only affected by one dietary component but also by other environmental, physiological, and psychological factors. Limited research has been performed in the past, specifically on the threshold dose of biogenic amines triggering migraine (Table 3), yet the accuracy of their experimental design is doubtful and the results can hardly be considered conclusive.

Exposure Assessment

Table 1 shows some data on the biologically active amine contents of different types of food products. It shows the average value, standard deviation, median, and range, reflecting the wide variability that is characteristic of the presence of biogenic amines in food.

Fresh, unspoiled food usually contains low levels of biogenic amines, if any. Some products, such as fruit and vegetables, have a significant biogenic amine content as a natural constituent. Nevertheless, fermented foods constitute the major dietary source of biogenic amines. The manufacture of these products presents favorable conditions for the bacterial production of biogenic amines, though the type and amounts accumulated vary widely between products, producers, and even between batches of a specific product. In fermented foods, besides the potential aminogenic activity of certain bacteria responsible for the fermentation, the accumulation of biogenic amines can also stem from the unwanted activity of contaminant bacteria present in the raw materials.

Among fermented foods, cheese and dry-fermented sausages are generally associated with the presence of large amounts of biogenic amines, especially tyramine, ranging from

not detected to values higher than 600 mg kg^{-1}. The diamines putrescine and cadaverine are also quite common in fermented products, though the levels may vary more and are often lower than tyramine. The occurrence of histamine varies even more. The amounts of histamine reported in the literature are relatively low in the majority of the sausage and cheese samples, though in some cases histamine levels are notably high. The occurrence of lower amounts of other biogenic amines, such as phenylethylamine and tryptamine, is generally accompanied by a high accumulation of tyramine and/or diamines.

In fermented beverages (e.g., wine and beer), tyramine and histamine contents are much lower than those reported for other fermented foods, because their contents usually do not exceed 5 mg l^{-1}, although in some cases the histamine levels have reached approximately 50 mg l^{-1}. Putrescine has also been described, with a natural (from grapes, malt, and hops) and microbial origin. By contrast, cadaverine has hardly been found in wine but occasionally described in beer.

To assess consumer exposure to biogenic amines in food and the potential risks, it is important to consider that multiple sources of biogenic amines with a variable range of concentrations are possible. Moreover, a reasonable estimate of the quantity (i.e., serving) of the different food products consumed in a meal should be taken into account. Although the overall exposure is difficult to estimate, it is clear that certain food combinations, for example, a meal based on fermented sausages and cheese, accompanied by beer or wine, would be highly probable to reach hazardous biogenic amine thresholds, especially in sensitive individuals.

In a recent assessment published by the EFSA, the biogenic amine contents and the food consumption patterns were combined to provide the exposure values in terms of mg per day as an estimation of a high exposure scenario. For histamine, the food categories which pose a major risk for consumers were fish and fish products (nonfermented) followed by fermented sausages, cheese, and fish sauces. For tyramine, beer (due to the occasional high consumption values recorded), cheese, fermented sausages, and fermented fish were identified as the major exposure sources.

Biogenic Amine Index (BAI) to Assess Food Hygienic Quality

As a result of their microbiological origin, biogenic amines have been used as criteria to assess the hygienic quality of fresh food not only fish and meat but also food products in which the bacterial activity is related to contamination incidents. Significant accumulation of biogenic amines generally occurs before the appearance of sensorial signs of spoilage. Moreover, biogenic amines are resistant to many physical and chemical processes, which is an advantage compared to other hygienic quality indicators (e.g., microbial counts) that can be inactivated by a number of food-processing procedures. The application of a BAI, which includes multiple amines, may increase the specificity and selectivity compared to a single biogenic amine. Most of the BAI consists of a sum of biogenic amines, mainly diamines and histamine, but tyramine has also been considered because it usually increases during fish and meat storage. The natural polyamines spermine, spermidine, and agmatine have also been included in some BAI, dividing the amount of biogenic amines. There is no general agreement on BAI values that denote spoilage because the production of amines depends on the product and many other factors. Thus, the type of microbial populations present in a particular food responsible for alteration can change the profile of amines formed.

In general, the application of a BAI as a criterion for the hygienic quality assessment of fermented products (e.g., fermented sausages, cheese, etc.) is more difficult because the occurrence of biogenic amines cannot be directly and exclusively associated with the quality of the raw materials.

Although there are no legal guidelines that restrict the presence of biogenic amines in wine, it has been reported that different countries have applied specific histamine thresholds to reject wine imports: 2 mg l^{-1} in Germany, 5–6 mg l^{-1} in Belgium, 8 mg l^{-1} in France, and 10 mg l^{-1} in Switzerland.

Legislation

Currently from a legal perspective, maximum legal limits are only set for histamine in certain fish and seafood products, mainly from fish species associated with high levels of histidine (Table 4). The maximum histamine levels permitted and the sampling plans differ depending on the country, with the USA having the most restrictive.

Analytical Methods

Determination of Biogenic Amines in Foods

From an analytical perspective, determining the biologically active amines in food and beverages is not a straightforward issue, mainly due to the variety of chemical structures, the wide range of concentrations and the complexity of most of the food matrices (e.g., high content of free nitrogen compounds and high fat content). Determining the biogenic amines and polyamines in food usually involves two well-differentiated phases: Their extraction from the food sample and their analytical determination.

The aim of extracting amines is to separate them from other potentially interfering compounds and it is an important step to obtain accurate results. The most widely used extraction solvents include acid solutions, such as hydrochloric acid (e.g., 0.1 M), trichloroacetic acid (e.g., 5–10%), and perchloric acid (e.g., 0.4–0.6 M) as well as organic solvents such as methanol, acetone, acetonitrile–perchloric acid, or dichloromethane–perchloric acid.

Amine determination can be carried out using a variety of approaches including biologic, enzymatic, spectrofluorometric, and chromatographic procedures. The last of these are used extensively because several amines can be determined simultaneously with high resolution, sensitivity and versatility, and require a simple treatment of samples. Thin layer chromatography (TLC), gas chromatography, and micellar liquid chromatography methods have been reported in the analysis of biogenic amines. However, high-performance liquid chromatography (HPLC), and more recently, ultrahigh liquid chromatography with reverse-phase columns or ion-exchange columns are the most frequently reported procedures in the

Table 4 Regulations regarding histamine levels in fish and seafood products

Region/Country (regulation reference)	*Criteria*		*Sampling plan*	*Method of analysis*	*Products*
USA (CPG 7108.24)	Defect action level	50 mg kg^{-1}	$n=18$ fish/lot[a]	Fluorimetric AOAC 977.13 (1990)	Many fish species including tuna, escolar, oilfish, marlins, etc. www.cfsan.fda.gov/~comm/haccp4c1.html
	Hazard action level	500 mg kg^{-1}			
EU (Regulation EC 2073/2005)	Safety criterion	$m=100$ mg kg^{-1}	$n=9$ samples; average histamine below m, $c=2$ samples out of 9 are allowed to have histamine concentration between m and M	HPLC Male *et al.* (1996)	Fish species with a high amount of free histidine, i.e., species of the families Scombridae, Clupeidae, Engraulidae, Coryphaenidae, Pomatomidae, and Scomberesocidae
		$M=200$ mg kg^{-1} $m=200$ mg kg^{-1} $M=400$ mg kg^{-1}			Fish products that have undergone enzyme maturation treatment in brine, manufactured from fish species with a high amount of free histidine
South Africa (Government Notice No. R 490)	Decomposition Unsafe	100 mg kg^{-1} 200 mg kg^{-1}		Fluorimetric AOAC 977.13 (1990)	Partly cooked or uncooked seawater and freshwater foods such as prawns, shrimps, crayfish, lobsters, crab meat, oysters, mussels, clams, eels, or fish
Australia (Standard 2.2.3)		200 mg kg^{-1}		Fluorimetric AOAC 977.13 (1990)	Fish and fish products

[a]Fish should be analyzed individually or composited into, for example, 3 composites (6 fish each), then critical limit reduced accordingly, for example, from 50 to 17 mg kg^{-1}.

literature. Biologically active amines are usually detected using fluorometry, UV absorption, multichannel diode array UV, and mass spectrometry. Amine detection usually requires derivatization because most of them have low-absorption coefficients or quantum yields. The most widely used reagents to perform amine derivatization include 5-dimethylamino-1-naphtalene-sulfonyl chloride (dansyl chloride, DnCl), *o*-phthaldialdehyde (OPA), and *N*-acetylcysteine. Derivatization can be applied using pre-, post-, or on-column procedures, depending on the reagent. The reference HPLC procedure indicated by the current EU regulation to determine histamine in fish permits the detection and quantification of histamine and other biogenic amines following their extraction with perchloric acid and dansyl chloride derivatization.

Currently, a biological method based on the histamine-induced contraction of guinea pig ileum, is still recognized by the Association of Official Analytical Chemists (AOAC) as an official method for histamine determination in fish and seafood. However, in addition to the above-mentioned instrumental and biological procedures, other analytical procedures are available and recognized as more economical, less time-consuming and more straightforward techniques, especially for routine screening or controls. For instance, the official method in the USA is the AOAC procedure based on methanol–histamine extraction, purification through an ion-exchange column and fluorescence measurement following derivatization with OPA.

Enzymatic procedures involve the use of amine-specific enzymes such as DAO, MAO, and HMT, that recognize and rapidly transform the substrate into another measurable product quantified amperometrically or by colorimetry. For example, such biosensors have been used to estimate the tyramine content during the bacterial spoilage of meat, or estimate the total concentrations of histamine, cadaverine, and putrescine accumulated in fish during storage.

The immunological approach has also been developed for the analysis of histamine. Commercial kits are available to analyze histamine from aqueous food extracts (e.g., fish, cheese, or sausage) through an enzyme immunoassay. However, the antibodies used in these tests require chemical derivatization of histamine before analysis, using toxic reagents (*p*-benzoquinone) and being also time-consuming. Alternatively, polyclonal antihistamine antibodies that recognize intact histamine have been included in commercial competitive direct enzyme-linked immunosorbent test kits, which seem to provide results comparable with the official fluorometric or HPLC methods.

Determination of Microbial Amino Acid Decarboxylase Capability

Several methods have been described to study the amino acid decarboxylase activity or, at the end, biogenic amine production by microorganisms. Whether or not certain enzymes exist in a particular bacterium depends on its genetic capabilities. Based on polymerase chain reaction (PCR) procedures, specific bacteria showing aminogenic potential can be detected with high speed and sensitivity. The PCR detection

system is based on the use of primers defined according to similarities in the deoxyribose nucleic acid sequence of the genes corresponding to the specific decarboxylase enzyme (e.g., histidine, tyrosine, ornithine, and lysine decarboxylase). However, the presence of the particular amino acid decarboxylase gene does not prove that whether the enzyme will be functional or amines will be formed. Similarly, the PCR test cannot determine whether a food product will contain low or high amine levels. However, the test helps to estimate the potential risk of biogenic amine formation.

As an alternative to molecular methods, assays on growing or resting cells may be performed. The composition of the medium used to grow the microorganism is of great importance because the expression and activity of decarboxylase enzymes depend on environmental factors. One extensively used screening procedure involves the use of a differential medium containing a pH indicator, such as bromocresol purple, and the addition of the precursor amino acids. In a positive result, the medium turns a purple color in response to a pH shift caused by the production of the more alkaline biogenic amines from the precursor amino acids. Occurrence of false-positives is possible due to the formation of alkaline compounds different from biogenic amines, and false-negatives may occur as a consequence of the acid production from the fermentative activity. Therefore, a suitable, more specific and sensitive alternative would perform a quantitative analysis of the biogenic amines potentially formed in the culture broth using one of the chemical, immunological or instrumental procedures described above (e.g., HPLC or TLC).

Control Measures

In the field of food safety, the presence of histamine seems to be the greatest concern associated with biogenic amines. Other amines in food may be of minor concern for the general healthy population, especially compared to the current problems regarding emerging pathogens. However, no positive effect can be attributed to food biogenic amines and furthermore, a proportion of the population may be vulnerable to food intolerances or drug interactions. Therefore, efforts to reduce the occurrence of biogenic amines in food deserve to be prioritized and the challenge for the food industry to offer products with biogenic amine levels as low as possible is justified. This last objective is consistent with the current trends that aim to minimize the risks, optimize the quality of food and even increase the prestige of food products through an added value with respect to consumer health. In this case, the added value is achieved through the reduction or elimination of biogenic amines as undesirable compounds. Moreover, the absence (or very low levels) of biogenic amines in foods could be considered as a quality attribute derived from optimal hygienic and technological practices.

Current knowledge about the actual causes of biogenic amine formation in certain foods has made it possible to design measures to prevent or at least reduce their accumulation during the manufacture and storage of food. Product improvement can be achieved using the following approaches:

(a) Assurance and improvement of the hygienic quality of raw materials and production processes because contaminant microbiota are responsible for biogenic amine formation in many products. Therefore, in addition to good manufacturing practices, food quality and safety management based on hazard analysis and critical control points is essential. The time/temperature binomial is the most critical and determinant risk factor in biogenic amine formation in most fresh and lightly preserved meat and seafood products, as well as in the raw materials (of animal and plant origin) used in the preparation of cooked, ripened, or fermented products. Therefore, storage temperature constitutes the most important control measure to limit biogenic amine accumulation. Other factors, such as packaging atmosphere, salt and other preservatives also inhibit or reduce the potential of aminogenic activity and should be taken into account with regards to lightly preserved fish, meat, and vegetables.

Predictive models of biogenic amines in perishable products, as a function of time and temperature, could be used to avoid storage conditions and time periods resulting in hazardous products. This approach has already been developed for histamine accumulation in seafood, which is particularly associated with growth and histamine formation by *Morganella psychrotolerans* and *Morganella morganii*. The model, included in the Seafood Spoilage and Safety Predictor tool (SSSP v3.1, 2009) provides valuable predictions to determine critical combinations of storage time and temperature enabling to control histaminogenesis in fish.

In the case of fermented foods or beverages, one of the most critical point is also the hygienic status of the raw materials because they are the main source of microbiota that will subsequently be present during fermentation. The hygienic quality of the raw materials may be improved by decreasing microbial charge through sterilization or pasteurization, a common practice in the cheese-making industry. However, in fermented meat products, temperature causes detrimental changes in the raw materials and it is therefore not possible to apply conventional heat treatments. Alternative nonthermal technologies may provide solutions in this case. The application of high-pressure treatments to the raw materials (milk and meat) could be effective in improving the hygienic status and thus reducing the biogenic amine accumulation without changing the sensory properties significantly.

(b) Degradation of biogenic amines once they have been formed, for example, through the use of amine-oxidase positive organisms. Attempts to reduce biogenic amine contents through product irradiation have also been described. Nevertheless, the efficiency of these procedures has not been fully demonstrated and they may even be controversial because in some cases the elimination of biogenic amines could cover up improper hygienic practices.

(c) Implementation of production techniques that inhibit the formation and accumulation of these substances. For foods in which fermenting microbiota may also show the aminogenic activity, the implementation of specific technological procedures can result in effective control measures, along with good hygienic and manufacturing practices.

To minimize biogenic amine accumulation during food fermentation and ripening, the growth and activity of potential aminogenic microorganisms must be reduced. A suitable sausage formulation (by adjusting the type and amount of fermentable sugar, spices, and preservatives) and technological

parameters (temperature and relative humidity) contribute to a proper and quick selection of desired fermentative microbiota. The growth and fermentative activity of these bacteria (mainly lactic acid bacteria) limits the action of contaminant microorganisms and their potential biogenic amine formation. Nevertheless, spontaneous fermentation can also produce notable amounts of biogenic amines. It has been widely demonstrated that the use of accurately selected microorganisms added as starter cultures constitutes one of the most effective control measures for biogenic amines in fermented products. To achieve this, starter cultures must lack aminogenic potential, but they must also be competitive and well adapted to the product fermentation environment. The effectiveness of starter cultures is dependent on the hygienic quality of the raw materials, because the starter culture will compete with the initial microbial load during product fermentation. In addition, technological conditions such as product formulation and processing temperature must be accurately applied to ensure optimal implantation of the starter culture and thus limit the growth and activity of undesired microorganisms.

Within this framework, the so-called 'low-histamine technology' applied to fermented beverages (e.g., wine) has been described, which is based on the assurance of the hygienic quality of the raw materials, the addition of selected starter cultures and the use of specific production techniques that inhibit the formation of histamine. This technology has already been implemented successfully in the manufacturing of traditional wines in certain countries, for instance Switzerland and Spain. The challenge facing the food industry is to extend this technology to other biogenic amines and other products. Indeed, the implementation of 'low biogenic amine technologies' will enable food manufacturers to produce safe and high-quality amine-free food products.

See also: Foodborne Diseases: Overview of Chemical, Physical, and other Significant Hazards

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PROCESSING CONTAMINANTS

Chloropropanols and Related Esters

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Glossary

EFSA The European Food Safety Authority, an EU risk-assessment body for food and feed safety. It provides independent scientific advice to the European Commission, national authorities, the food industry, and other stakeholders.

Gas chromatography (GC) A means of separating volatile compounds by passing a stream of gas through a narrow heated glass tube coated on the inside with an absorptive phase.

Headspace GC A means of sampling volatile compounds from food for analysis by GC.

Mass spectrometry (MS) An instrumental means of detecting, identifying, and measuring compounds by producing and separating ions. Used as a detector for gas chromatography/mass spectrometry (GC–MS).

Chloropropanols

Chloropropanols are contaminants of food safety concern that are produced by chlorination of glycerol or glycerides by reaction with chloride ions. They were initially associated with the flavor-enhancing ingredient hydrolyzed vegetable protein (HVP or acid-HVP) in which they were formed by the action of strong hydrochloric acid during the manufacturing process, thus they have been classified and known as food-processing contaminants. Later, simple chloropropanols were found to be formed under the milder conditions of domestic cooking, and fatty acid esters of chloropropanols were found to be produced during the refining of edible vegetable oils.

A range of chloropropanols were found in HVP by Professor Jan Velisek at the Institute of Chemical Technology in the Czech Republic in 1979. HVP is made by the treatment of vegetable materials such as defatted oil seeds (soy, rapeseed, and peanut), and protein from maize, wheat, and rice with strong (4–6 M) hydrochloric acid at high temperature and pressure, typically 8 h at 100–130 °C. The acid hydrolyzes the proteins to give a range of small molecules including amino acids and peptides with flavor-enhancing properties. The reaction mixture is neutralized with sodium hydroxide or sodium carbonate to give a salty solution that was used to help flavor soups, stocks, and savory foods such as extruded corn snacks.

The chloropropanols were found to have been formed by the reaction of chloride with glycerides in the fat associated with the proteinaceous material. Chlorine atoms replaced some of the fatty acids in the triacylglycerols to form a mixture of compounds including 1- and 2-monochloropropanols, 2- and 3-monochloropropanediols (2-MCPD and 3-MCPD), 1,3- and 2,3-dichloropropanols (1,3-DCP and 2,3-DCP), and fatty acid esters of these compounds. The structures of the major compounds are shown in Figure 1. They can be considered as analogs of glycerol, and are closely associated with the toxic nonchlorinated compound glycidol (3-hydroxy-1,2-epoxypropane). 1,3-DCP is a known carcinogen and 3-MCPD is suspected to be so. Glycidol is a genotoxic

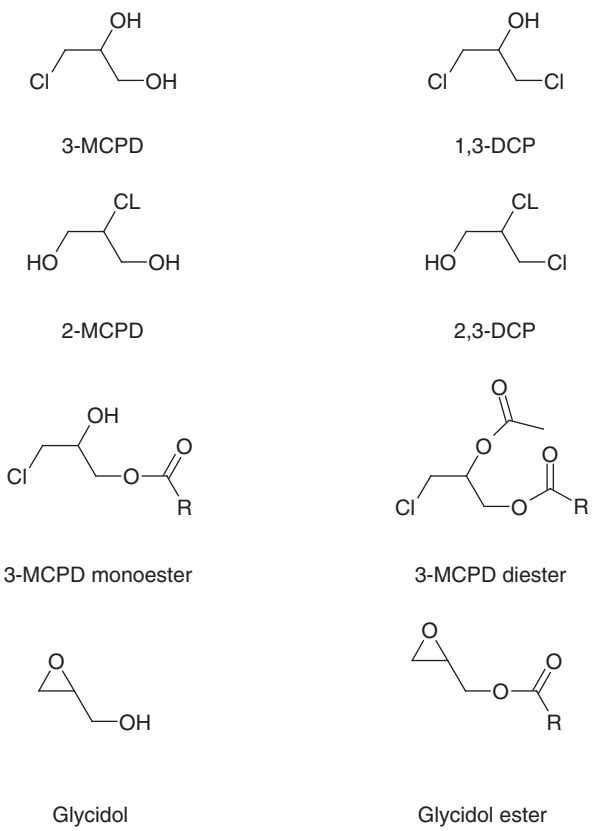

Figure 1 Structures of the major chloropropanols.

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carcinogen in animals and has been classified as probably carcinogenic to humans by the International Agency for Research on Cancer. It is highly reactive and difficult to measure and has not been the subject of much study. Glycidol esters with fatty acids are, however, associated with MCPD esters and have become of considerable interest.

MCPD esters exist as either mono- or diesters of each of the fatty acids found in oils, as there are about seven common fatty acids, and taking into account the possible positional isomers there are approximately 100 different MCPD esters.

The discovery of chloropropanols in acid-HVP caused concern because many chlorinated compounds are toxic and it was known that 1,3-DCP was a genotoxic carcinogen. Toxicity testing began on the other major chloropropanol, 3-MCPD, and simultaneously the industry took steps to remove the chloropropanols from acid-HVP. The chloropropanol esters were not problematic as they were insoluble in the aqueous acid-HVP solution. The monochloro- and dichloropropanols were volatile and could be removed by evaporating the acid-HVP solution to give a dry product. Monochloropropanediols were more difficult to remove but a major reduction was achieved by careful control of the acid hydrolysis step and incorporating a postproduction alkaline hydrolysis step that cleaved the chlorine from the molecules to form harmless glycerol.

Improvements in analytical methods required to detect the lower levels of 3-MCPD in acid-HVP led to the discovery that it could also be found in certain foods, particularly those that contained salt and fat and were heated or had long storage periods, such as cereal products, canned fish, and salamis. High levels were reported in some soy sauces, although this was probably due to the use of poor quality acid-HVP.

In 2006, it was reported that fatty acid esters of 3-MCPD were present at high levels in refined vegetable oils. These compounds had been somewhat neglected since their discovery in acid-HVP, apart from an early report of their occurrence in some samples of goat's milk. Refined vegetable oils are heavily used within the food industry. Palm oil is particularly popular on account of its low price and adaptability; unfortunately relatively high levels of chloroesters are formed in palm oil on refining. The large number of fatty acids and the possibility of mono- and diesterification of the glycerol skeleton led to the formation of many MCPD esters, including both 3-MCPD and 2-MCPD monoesters and diesters, in which the fatty acid profiles matched those in the oil. They are collectively known as MCPD-esters or bound MCPD, although few bound forms other than fatty acid esters have been reported.

It is known that the esters are formed during the oil refining process and mostly in the deodorization stage. In this the vegetable oils are treated with steam at high temperature and pressure to remove small molecules and free fatty acids that would otherwise taint the oil. It has been demonstrated that the agents responsible for MCPD ester formations are, very probably, naturally occurring inorganic and organic chlorine compounds present naturally in the oilseed that release chlorinating species in the deodorizer. There is concern over the potential for MCPD esters to contribute to the dietary intake of 2- and 3-MCPD from their hydrolysis by lipases in the gastrointestinal tract.

Chloropropanols in Food

MCPD and DCP in HVP

In 1990, the UK Ministry of Agriculture, Fisheries, and Food conducted surveys of the levels of 3-MCPD and 1,3-DCP in acid-HVP and again in 1992, which showed a decline in levels over this period. In the 1990 survey, 23 out of 39 samples tested contained 3-MCPD above 10 mg kg^{-1} but only a single sample contained 1,3-DCP, this at 0.05 mg kg^{-1}. Of the 34 acid-HVP samples surveyed in 1992, 7 contained 3-MCPD in excess of 10 mg kg^{-1} and a further 8 between 1 and 10 mg kg^{-1}. No samples contained 1,3-DCP. In April 1998, a further survey of 3-MCPD in 50 samples of acid-HVP using a more sensitive analytical method found that 21 samples did not contain detectable 3-MCPD. Of the remaining samples, 28 contained between 0.01 mg kg^{-1} and 0.1 mg kg^{-1}, and one that was possibly made with acid-HVP from older stock contained 2 mg kg^{-1}. No further government surveys were carried out, and the food industry has in general replaced acid-HVP by safer flavor enhancers.

MCPD and DCP in Soy Sauces

Surveys in continental Europe indicated that some soy sauces in Denmark, Germany, Sweden, and the Netherlands contained 3-MCPD at levels up to 124 mg kg^{-1}. Government surveys were made of approximately 100 sauces sold in the UK in 2000 and again in 2002. In 2000, 3-MCPD was quantified, after normalization to 40% dry matter, at or above 0.02 mg kg^{-1} in 25 of the samples and over 1 mg kg^{-1} in 16 samples, the highest containing 82.8 mg kg^{-1}. 2-MCPD was found in 26 samples at up to 17.6 mg kg^{-1}. 1,3-DCP was detected in 17 samples between 0.006 and 0.345 mg kg^{-1}, and 2,3-DCP in 11 samples at levels of 0.006–0.043 mg kg^{-1}.

Action was taken to remove the contaminated brands from the market in the UK and in several other countries, and the publicity this entailed produced a short-lived food scare in the UK (Figure 2).

Premium soy sauces are usually labeled as 'traditionally brewed' and are made by a fermentation process in which enzyme action hydrolyzes the proteins. These sauces should not contain materials made by the modern chemical hydrolysis. Government inspections in China revealed that many sauces were made in small factories without adequate controls and these were closed down. The soy sauce industry also found incidences of counterfeiting in which acid hydrolyzed products were labeled as traditionally brewed and sold with forged labels imitating those of premium sauces.

Two years later, a survey targeted at the same brands of soy sauce found 3-MCPD in only 8 of 99 samples, 2-MCPD in 3 samples, and 1,3-DCP in only 1 sample. The highest level of 3-MCPD was 35.9 mg kg^{-1}.

In surveys carried out in China between 2002 and 2004, 37 traditionally brewed soy sauce samples contained 3-MCPD below 0.02 mg kg^{-1}. All of 629 retail soy sauce samples contained 3-MCPD ranging between 0.005 and 189 mg kg^{-1}, and over 12% had levels exceeding the Chinese limit of 1 mg kg^{-1} for acid-HVP.

2-MCPD (maximum 20.3 mg kg^{-1}), 1,3-DCP (maximum 8.3 mg kg^{-1}), and 2,3-DCP (maximum 0.5 mg kg^{-1}) were detected in 48%, 19%, and 4% of the soy sauces, respectively.

Figure 2 Press reaction to finding chloropropanols in soy sauces.

Table 1 Frequency of detection of 3-MCPD in selected foods

Product category	*2007*				*2008*				*2009*			
	N	*Mean*	*Minimum*	*Maximum*	*N*	*Mean*	*Minimum*	*Maximum*	*N*	*Mean*	*Minimum*	*Maximum*
Bread	30	11	<3	32	34	17	5	90	30	11		36
Brown	2	8	5	10	1	11	11	11				
Other	14	12	3	26	12	20	5	90	1	8	–	–
White	8	9	<3	24	13	14	6	33	12	11	5	21
Wholemeal	6	15	4	32	8	16	10	23	6	17	9	36
Breakfast cereals	10	2	<3	<10	10	5	<3	9	10	3		6
Biscuits/crackers	15	27	<3	83	15	24	6	50	15	25	5	71
Coffee	10	3	<3	9	10	4	<3	10	10	15	9	21
Baby cereal foods	10	2	<3	3	4							
Soy sauces	3	6	5	6	3	<3	<3	<3	3	2		–

Source: Adapted from data published by the UK Food Standards Agency (http://www.food.gov.uk/) on surveys of process contaminants in UK retail foods.

In all, 80% of acid-HVP samples contained 3-MCPD in excess of 1 mg kg^{-1}. Relatively high levels of 3-MCPD were also found in soy sauce powder, oyster sauce, beef products, instant noodle spices, and health foods.

MCPD in Other Foods

Several surveys have been carried out to determine the levels of 3-MCPD in foods. These have invariably been targeted at foods known or thought to be at risk, such as malts and other cereal products. Only soy sauces and similar products are now monitored for the presence of 1,3-DCP, which is rarely encountered on account of the improvements in acid-HVP manufacture, and where it has been often found to be associated with small-scale fraudulent imitation of reputable brands.

In three surveys that were carried out in the UK during 2007–09 of foods of types thought likely to contain 3-MCPD, including bread, breakfast cereals, biscuits, soy sauces, and roasted coffee. Approximately 80 food samples were analyzed each year. The results are given in Table 1. The types of sample analyzed changed slightly over the years according to previous results, but there was not much variation. In all years, biscuits and crackers had the highest levels of 3-MCPD.

MCPD and Glycidol Esters

Only limited surveys have been carried out in the presence of MCPD esters in foods, and these have so far been largely restricted to vegetable oils. Further surveys can be expected when the analytical methods have been fully validated. Table 2

Table 2 Levels of 3-MCPD esters in selected foods

Food	*Range (mg kg^{-1})*
Biscuits	0.3–0.7
Bread	<0.01–0.04
Bread toasted	0.06–0.16
Cereal	<0.01–1.40
Crackers	0.1–1.14
Crispbread	0.42–0.58
Crisps	0.05–1.19
French fries	0.04–0.40
Chicken grilled	0.26–0.74
Ham	n.d.–2.64
Salami	0.88–6.41
Coffee	<0.1–0.39
Malt and beer	0.01–0.65
Frying oils (fresh and used)	<0.15–16.2
Margarine (fat portion)	<0.15–7.7
Refined coconut oil	1.42–1.69
Refined palm kernel oil	0.85–1.40
Refined palm oil	1.39–4.17
Refined vegetable oils	<0.15–18.8
Unrefined vegetable oils	<0.15–0.31
Coffee creamer	0.13–0.73
Cream (aerosol)	0.05–0.73
Infant formula	<0.08–0.59
Infant formula (fat portion)	0.57–4.10
Milk, growing-up	0.06–0.29
Human breast milk	<0.011–0.076

Source: Adapted from reports published by the International Life Sciences Institute (www.ilsi.org/).

shows levels of 3-MCPD esters measured in foods selected as being likely to contain them.

Refined vegetable oils usually contain 3-MCPD esters at about 15 mg kg^{-1} but levels of up to 20 mg kg^{-1} have been encountered. 3-MCPD esters have also been reported in cooked potato products such as crisps and fries (0.021.2 mg kg^{-1}), infant formulae (0.10.6 mg kg^{-1}), and toasted cereals (0.041.4 mg kg^{-1}).

Occurrence of 2-MCPD Esters

2-MCPD esters have been determined only in 60 vegetable oils where they were usually present at 0.5 mg kg^{-1} in refined oils and from 0.2 to 6 mg kg^{-1} in refined palm oils; however, some refined oils contained up to 11 mg kg^{-1}.

Occurrence of Glycidyl Esters

Glycidyl esters have similarly only been found in refined vegetable oils and in products containing them. Table 3 provides a summary of some data for the levels of glycidyl esters in some refined and unrefined oils.

Refined oils usually contained up to 5 mg kg^{-1}, but some had up to 30 mg kg^{-1} and palm oils 0.5–10 mg kg^{-1} glycidyl esters with some identified frying oils containing almost 30 mg kg^{-1}. Infant formulae made with refined oils as ingredients contained up to approximately 3 mg kg^{-1}.

Table 3 Levels of glycidol esters in selected oils and fats

Food	*Number*	*Range (mg kg^{-1})*
Infant formula (fat portion)	56	<0.15–3.0
Animal fats, unrefined	25	<0.1
Frying oils (fresh and used)	51	<0.15–28
Mayonnaise (fat portion)	17	<0.15–0.33
Palm shortening/olein	6	0.4–15.6
Refined palm oil	20	0.30–10
Refined seed oils	15	<0.10–0.60
Refined vegetable oils (not frying)	153	<0.15–4.1
Unrefined vegetable oils	122	<0.10
Vegetable oils	10	<0.2–3.7
Margarine (fat portion)	22	<0.15–5.0

Source: Adapted from reports published by the International Life Sciences Institute (www.ilsi.org/).

Food Safety Issues

The UK Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment and the Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment declared in 2001 that it was prudent to assume that 1,3-DCP is a genotoxic carcinogen and that exposures to 1,3-DCP should be reduced to as low a level as technologically feasible and also it was prudent to assume that 1,3-DCP may possess genotoxic activity *in vivo*.

The consensus of the expert committees was that the genotoxic activity of 3-MCPD seen *in vitro* was not expressed *in vivo*. Based on these evaluations, a provisional maximum tolerable daily intake (PMTDI) of 2 μg kg^{-1} bodyweight (bw) has been established. The EC has recently set a regulatory limit of 0.02 mg kg^{-1} for 3-MCPD in HVP and soy sauce. The UK Food Advisory Committee (FAC) has advised industry that 'they should continue to take all steps necessary to reduce concentrations of 3-MCPD in foods and food ingredients to the lowest technologically achievable level'. The toxicity of 2-MCPD has not been evaluated in such depth but its occurrence is much lower.

There is considerable concern about MCPD esters if completely hydrolyzed in the intestine they can provide 3-MCPD in excess of the recommended maximum intake. Toxicity testing has shown that excretion rates of 3-MCPD are lower from the esters than free 3-MCPD. The toxic effect of 3-MCPD and its esters (nephrotoxicity, normochromic anemia, and testicular toxicity) were similar but milder for esters (dipalmitate) than for free 3-MCPD.

In 2006, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluated 3-MPCD and 1,3-DCP. The committee decided to maintain the PMTDI 2 μg kg^{-1} bw for 3-MCPD that it originally established in 2001. However, establishment of tolerable intake for 1,3-DCP was considered to be inappropriate because of the nature of the toxicity (tumorigenic in various organs in rats and the contaminant can interact with chromosomes and/or DNA). However, as 3-MCPD is a precursor for 1,3-DCP, the reduction in 3-MCPD levels would also reduce 1,3-DCP levels. Based on this risk assessment, the Codex Alimentarius Commission adopted a Code of Practice for the reduction of 3-MCPD during the

production of acid-HVPs and products that contain acid-HVPs (CAC/RCP 64–2008). Codex also adopted a maximum limit for 3-MCPD of 0.4 mg kg^{-1} in liquid condiments containing acid-HVPs, excluding naturally fermented soy sauce.

Exposure

The adult exposure to 3-MCPD from its esters (assuming complete cleavage in the gut) has been estimated as from 1 to 9.8 μg kg^{-1} bodyweight 3-MCPD per day. This is 0.55 times the PMTDI of 3-MCPD. There is more concern for infants whose diet is limited to infant formula, which usually includes a component of refined oils. The estimated exposure for such infants was 7.325 μg kg^{-1} bodyweight 3-MCPD per day, 3.67.7 times the PMTDI.

Comparison of consumption data and levels of 3-MCPD in food in 2001 showed that for average UK consumers the dietary intake of 3-MCPD was 0.10 μg kg^{-1} bw^{-1} per day for adults, 0.18 μg kg^{-1} bw day for young people aged 4–18 years and 0.28 μg kg^{-1} bw per day for toddlers aged 1.5–4.5 years. These figures were approximately doubled in high level consumers, but all were well below the PMTDI of 2 μg kg^{-1} bw per day recommended by JECFA.

Effects of Domestic Cooking

Domestic cooking modeled in the laboratory for a limited number of selected foods has shown that the level of 3-MCPD increases in most depending on the cooking method. Grilling and toasting increased the 3-MCPD content of bread substantially, forming up to 0.3 mg kg^{-1}. The 3-MCPD content of most cheeses also increased, resulting in levels of up to approximately 0.1 mg kg^{-1}. Microwave cooking elevated 3-MCPD levels in some cheeses to a lesser degree. Frying batters increased 3-MCPD levels to approximately 0.1 mg kg^{-1}. No studies have yet been carried out on the effects of cooking on MCPD esters.

Formation

The formation of chloropropanols is affected not only by the glyceride precursor and chloride concentration but also the temperature and water content. The major precursors are triacylglycerols, with a smaller contribution from other glycerides such as phospholipids and glycerol that are present in foods at lower levels. Glycerol is a better precursor than acylglycerols in products such as baked goods with a low water content. The optimum sodium chloride concentration is between 5% and 10%. Monoacylglycerols are better precursors of 3-MCPD than the di- and triacylglycerols. 3-MCPD production increases with increasing temperature above 160 °C.

MCPD esters have been shown to be intermediates in the formation of free MCPD in food. In refined vegetable oils, where the water content is low, they are present at much higher levels than the free forms. The esters are formed from the reaction of chloride with glycerides at the high temperature of the deodorizer during the refining process. It is believed that the chlorine source is mostly derived from chloride taken up by the plant where it is converted to a number of oil-soluble organochlorine compounds that subsequently break down in the deodorizer.

Glycidol esters do not need the chlorine source and are formed mainly from diacylglycerols during oil deodorization at temperatures above approximately 230 °C.

Analysis

Free Chloropropanols

Several methods exist for the analysis of foods for chloropropanols, all of which have the sensitivity required to give good indications of exposure and to check compliance with regulations. The original methods for measuring 3-MCPD were developed for the analysis of liquid acid-HVP and soy sauce samples. The sample is saturated with salt and the solution held on a column of diatomaceous earth. The chloropropanols are extracted into an organic solvent (ethyl acetate, diethyl ether, or diethyl ether–hexane mixtures) by passing the solvent through the column. The diatomaceous earth prevents the formation of emulsions associated with classical liquid–liquid extraction. The added salt encourages the transfer of organic compounds into the organic solvent and has been shown not to produce additional chloropropanols.

In early procedures, a first elution with diethyl ether–hexane mixture extracted the dichloropropanols and a second elution with diethyl ether alone gave the monochloropropanediols. Portions of these solutions were combined and analyzed by gas chromatography with detection by mass spectrometry (GC–MS). The presence of the polar hydroxyl groups on the chloropropanol molecules meant that derivatization was required to block the activity of these groups. The derivatization reagent that found favor was heptafluorobutyryl imidazole (HFBI). This is a very strong reagent that was able to volatilize coextracted compounds as well as the chloropropanols, and the presence of fluorine in the derivatized molecule meant that electron capture detection could also be used. The mass spectra of the derivatized chloropropanols not only had molecular and fragment ions of quite low intensity but also had ions suitable for confirmation of the identity.

Diol Derivatization

Alternative methods developed and favored in continental Europe involved reaction of the diol group of MCPD with a small range of reagents. Organic derivatives of boronic acid bind across adjacent hydroxyl groups, and the reagent phenylboronic acid (benzeneboronic acid (PBA)) can simultaneously derivatize and extract chloropropanediols when an organic solvent solution of PBA is heated and shaken with the aqueous solution of the sample. The diol groups can also be derivatized with other reagents, including acetone (forming acetonides). The acetonide and phenylboronate derivatives have intense ions in their mass spectra, giving methods with high sensitivity (Figure 3).

Dichloropropanols

As the manufacturing methods for acid-HVP improved, methods for the determination of 1,3-DCP were less in

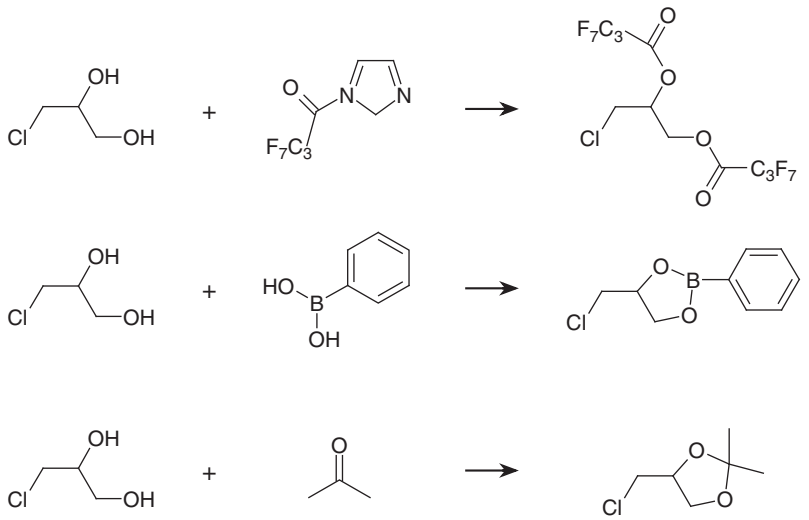

Figure 3 Derivatization of 3-MCPD. Top: with HFBI; center: with PBA; and bottom: with acetone.

demand, and the initial extraction of this compound was dropped except for some applications to the analysis of soy sauces. It also became clear that 1,3-DCP was only present where high levels of 3-MCPD were found, and so for many samples its determination was unnecessary.

As 1,3-DCP is considerably more volatile than the MCPDs it has also often been determined in soy sauces by headspace analysis, in which the sample is heated in a septum-capped sealed vial which caused partitioning of the 1,3-DCP between the sample and the gas phase. The headspace gas is sampled by a heated syringe, or by a sampling loop or the solid phase microextraction technique and the 1,3-DCP determined by GC–MS.

MCPD Esters

Esters of MCPD can be determined by two methods. In the first, the esters are transesterified or hydrolyzed to release free MCPD which is determined by any of the methods described above, and in the second method, the intact esters are determined by high performance liquid chromatography with MS (LCMS).

For direct determination the oil sample is dissolved in a solvent and either injected into an LCMS system, or a preliminary cleanup is carried out using mini columns of silica or columns of silica and C18 material arranged in tandem. Direct determination is made difficult by the fact that oils are not easily soluble in the relatively polar solvents required for LC, and the fact that the esters do not ionize easily. As individual esters are determined a large number of reference standards are required, these are now available commercially. LCMS cannot distinguish between positional isomers of MCPD esters without their prior separation by offline chromatographic techniques.

During indirect determination of MCPD esters all of the individual MCPD fatty acid esters are converted to 2- or 3-MCPD. The free 2- and 3-MCPD can be released from their esters by hydrolysis methods based on enzyme cleavage, or by acid- or alkaline methanolysis. The released 3-MCPD is measured in both the whole food sample and in the hydrolyzed fat, and the total levels of the free compounds calculated. These indirect methods of measuring MCPD esters are more complex than might appear. Acid hydrolysis is relatively slow and has concerns regarding the possible formation of MCPD during the reaction. Alkaline hydrolysis is rapid but requires care in application as the MCPD formed is rapidly dechlorinated in alkaline conditions. Alkaline hydrolysis is also not specific to MCPD esters as glycidol esters are also converted to 2- and 3-MCPD. This can be overcome by first removing the glycidol esters by pretreatment with sulfuric acid.

There is interest in separating monoesters of MCPD from diesters as their intestinal hydrolysis rates are likely to differ, but methods to achieve this separation are not yet readily available.

Glycidol Esters

Glycidol esters can be determined by adapting the indirect alkaline hydrolysis method used for MCPD esters. This entails conversion of the released glycidol to free MCPD and calculating the difference between the MCPD derived from MCPD esters and that derived from the MCPD esters and the glycidol esters together.

For the determination of glycidol esters alone, LCMS following removal of the triacylglycerols by gel permeation chromatography cleanup is also a popular technique.

MCPD and glycidol esters can be measured directly and simultaneously by injection into LC–MS systems.

See also: Food Safety Assurance Systems: Labeling and Information for Consumers. Foodborne Diseases: Overview of Chemical, Physical, and Other Significant Hazards. Risk Analysis: Risk Assessment: Chemical Hazards

Further Reading

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Furan

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Furan

Furan (Figure 1) is a five-membered unsaturated cyclic ether (C_4H_4O). It is a very volatile colorless liquid with a boiling point of 31.4 °C. It is only poorly soluble in water, but dissolves in organic solvents. Furan is produced commercially for use as a chemical intermediate and as a solvent. It has long been known a component of cigarette smoke. The name is also applied as an abbreviation of polychlorinated dibenzofurans, which are associated with the environmental contaminants known as polychlorinated dioxins, these should not be confused with the monocyclic compound.

Occurrence

Furan in Food

Furan was reported as present in a wide range of foods during the 1960s. The foods included canned cooked meat, hydrolyzed proteins, bread, and coffee. These findings were not followed up to a significant degree partly because furan was not expected to be a widespread food contaminant and partly because its volatility was so high that many analytical methods aimed at screening foods for volatile components simply missed its presence. Interest in furan as a food contaminant was revitalized in 1996 when it was found during tests of the effects of ionizing irradiation used for preservation of apple juice.

The first major survey of the levels of furan in food was conducted by the US Food and Drug Administration (FDA) in 2004. The FDA published an analytical method that was used to measure furan in a range of particular foods. The survey targeted foods that were sold in cans and jars as furan formed during manufacture would not have escaped through evaporation, and included baby foods, infant formulae, canned vegetables, packaged fruits, and sauce mixtures. The highest levels were found in roasted coffee and in starchy foods such as beans that had been heated in jars or cans.

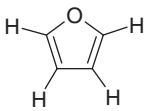

Figure 1 Structure of furan.

Furan levels of over 100 μg kg^{-1} have been found in three major food groups sampled in the US and Europe: coffee, baby food, and sauces and soups. Furan was detected in 262 of the 273 baby food samples reported by FDA (11) and European Food Safety Authority (EFSA) (10) with an average level of 28 μg kg^{-1}, and in 70 of 71 infant foods at similar levels.

Food Safety Issues

Toxicity

Furan is readily absorbed from the lung or intestine. After ingestion it primarily affects the liver where it is metabolized by the P-450 enzyme system with the formation of *cis*-2-butene-1,4-dial. This highly reactive compound is considered to be the genotoxic principle (causing damage to deoxyribonucleic acid, DNA) as it can bind to cellular nucleophiles including proteins and nucleosides. In the liver furan depletes adenosine triphosphate (ATP) irreversibly and causes double-strand breaks in DNA, leading ultimately to cell death.

Some studies of the toxicity and carcinogenicity of furan have been conducted by the US National Toxicology Program (NTP) using rats and mice, but only limited data are available, and there are no human studies. In NTP studies furan administrated by gavage in corn oil induced liver tumors in mice and cholangiocarcinomas, hepatocellular tumors, and mononuclear cell leukemia in rats. The mechanism of this carcinogenicity in rodents has not been fully clarified yet, but both genotoxic and nongenotoxic mechanisms have been proposed.

Carcinogenicity

Furan administered to rats and mice daily at between 2 and 8 mg kg^{-1} body weight per day (rats), and 8–15 mg kg^{-1} body weight per day to mice caused lesions of the liver and increased hepatocellular adenomas and carcinomas.

In a 2-year carcinogenicity study in rats and mice given repeated doses over 16 days or 13 weeks by gavage in corn oil increased mortality was seen with doses at or over 80 mg per kg body weight per day. Dose-related liver lesions were seen at high dose rats (60 mg kg^{-1}) with mice being slightly less affected. Hepatocyte proliferation and apoptosis were seen in a 3-week exposure of female animals at levels of up to 15 mg kg^{-1} body weight per day.

With lower doses of furan (up to 8 mg kg^{-1} body weight per day over 3 weeks) mice exposed to 1 mg kg^{-1} body weight per day or more had increased liver weight and incidence of

hepatic cytotoxicity. Mice exposed to 8 mg kg^{-1} body weight per day had hepatocyte proliferation.

Detailed studies of the mechanisms of tumor induction by furan compared the range of gene mutations with those of controls and concluded that the hepatocarcinogenicity might be partially attributable to genotoxicity, but the evidence is so far inconclusive. Epidemiological comparisons of coffee consumption with human cancers do not show a correlation.

Genotoxicity

Three *in vitro* studies into the genotoxicity of furan had inconclusive results. Furan has been shown not to be mutagenic but its metabolite *cis*-2-butene-1,4-dial is directly mutagenic at nontoxic concentrations in sensitive strains of *Salmonella typhimurium*. The metabolite forms adducts with DNA and proteins.

Accordingly, the International Agency for Research on Cancer has concluded that furan is reasonably anticipated to be a human carcinogen based on sufficient evidence of malignant tumor formation at multiple tissue sites in multiple species of experimental animals.

Furan is carcinogenic to rats and mice, with a dose-dependent increase in liver adenomas and carcinomas. EFSA have concluded that the weight of evidence indicates that furan is a carcinogen and is probably genotoxic.

Exposure

Previous to the discovery of the widespread presence of furan in food the major source of human intake was thought to be commercial use in the workplace. Furan has also been detected in the exhaled breath of smokers, and in the indoor air of homes. Another possible important source is in the air of kitchens and other places where food is heated.

Some typical ranges of furan levels determined in a number of foods are provided in Table 1. The data are taken mainly from surveys reported by the FDA and EFSA. It is of concern that possible human exposure is close to the equivalent doses that produce carcinogenic effects in experimental animals. The two food items that are the cause of most concern regarding exposure to furan are canned and jarred baby foods and coffee.

Table 1 Mean and maximum levels of furan (μg kg^{-1}) typically found in foods

Product	*Mean*	*Maximum*
Coffee, instant	500	2000
Coffee, roast bean	2000	5000
Coffee, roast ground	1000	6000
Baby food	25	200
Infant formula	20	50
Baked beans	25	120
Beer	5	30
Fruit juice	5	420
Meat products	20	120
Milk products	15	80
Sauces	10	120
Soups	25	220
Soy sauce	25	80
Vegetable juice	5	20
Vegetables	10	75

Coffee

Furanic compounds are important contributors to the flavor of coffee. The unroasted (green) beans are virtually free of furan but very high levels ($\sim$4 mg kg^{-1}) are formed when the beans are roasted. Handling processes including grinding lead to a reduction of furan levels but coffee powder still contains approximately 2 mg kg^{-1} furan. Other processes, such as the manufacture of instant coffee, cause a further reduction to 1 mg kg^{-1} or less.

Brewing coffee for consumption decreases the furan level much more significantly depending on the process. Espresso coffees have a high concentration on account of their small volume, and the doses from these and percolator machines are higher than that from cafetiere and instant coffee powder (Table 2).

Baby Food

A very high proportion of baby foods sold in can and jars contain significant levels of furan, typical 5–20 μg kg^{-1}, this being due to the industrial cooking, sterilization, and pasteurization processes and the presence of precursors in many of the food formulations. The highest concentrations (15–35 μg kg^{-1}) are found in canned or jarred baby foods based on pasta, meat, or vegetables, with lower levels (10 μg kg^{-1} or less) in canned or jarred infant beverages, cereals, and fruits.

Other Foods

Of the widely consumed canned and jarred foods surveyed by the FDA and EFSA canned starchy foods such as beans and soups contained the most furan, typically 100 μg kg^{-1}. Some other foods (malted products, gravies, and caramels) contained higher levels. Limited surveys have shown that many foods that are not cooked in closed containers also contain relatively high levels of furan. These are mainly crispbreads and toasted cereal products such as toasted bread.

Effects of Domestic Cooking

Although the topic has received little study it appears that domestic cooking procedures invariably reduce the furan content of foods. Although some furan is almost certainly formed during, for example, the reheating of canned soup, the

Table 2 Furan (μg kg^{-1}) typically found in coffee powders and brewed coffee

	Powder	*Brew*
Regular	1800	24
Decaffeinated	2000	16
Espresso	1700	44
Instant	400	8

procedure leads to an overall loss of furan, presumably to the atmosphere. Actions such as stirring and dispensing food on to plates, and leaving food and beverages to stand causes loss of furan. Mild domestic cooking methods such as reheating prepacked ready meals or soups in a microwave oven do not increase the furan content. There is some evidence that furan driven out of food during domestic cooking can be inhaled from the kitchen air during preparation.

Dietary Exposure

Dietary exposure to furan can be estimated from knowledge of the furan content and intake of foods. These estimates are necessarily based on the food types tested, which have tended to be those likely to contain relatively high furan levels. For adults the most important source is brewed coffee beverages. The intake from this source will obviously vary with the choice of brewing procedure and level of consumption, with cultural differences being significant. The intake for an infrequent consumer of instant coffee, for example, will be much lower than that of the typical Mediterranean heavy consumer of espresso coffees. EFSA has estimated the daily adult intake from coffee to be 2.4–116 μg per person, compared to 1.1–23 μg per person from canned or jarred vegetables and 1.3–50 μg per person from beer.

From the FDA data the adult intake mainly from brewed coffee, was approximately 0.15 μg kg^{-1} body weight per day. Exposure calculations for Germany made by the Federal Institute for Risk Assessment (BfR) were similar to the FDA. From EFSA data collected within the EU the furan intake estimates based on coffee were higher at approximately 0.5–0.7 μg kg^{-1} body weight per day, probably on account of different coffee consumption data.

There is concern regarding the exposure of babies to furan not only because its levels are relatively high in canned and jarred baby foods but also because canned and jarred baby foods may form a high proportion of their diet as well as the larger amounts (relative to body weight) of certain foods.

From the FDA data a mean intake for infants of approximately 0.4 μg furan per kg body weight was calculated for infant foods, and again, similar results were calculated by the BfR for Germany and for the EU by EFSA, which estimated the exposure of babies to furan at <0.2–26 μg furan per day or <0.03–3.5 μg kg^{-1} body weight (bw) per day for a 6-month-old baby weighing 7.5 kg consuming food exclusively from glass jars.

Estimates of exposure indicate a small margin (a factor of approximately 2000) between human exposure doses and those causing liver tumors in experimental animals.

Formation

Several chemical routes of furan formation in foods have been identified. The principal ones being the thermal oxidative degradation of ascorbic acid and unsaturated fatty acids, with degradation of sugars and carotenoids also being important. These reactions are complex but have been studied in some detail through experiments on model food systems comprising only a few reactants. It has become clear; however, that they fall short of representing what happens in whole foods. The furan formation routes shown in Figures 2–4 have been simplified considerably.

When food is heated, sugar and amino acids undergo a series of reactions known as the Maillard reactions, to form products having the distinctive brown color. Large numbers of compounds having a furan ring are formed along with furan itself. Many of these compounds are important flavor constituents and are nontoxic.

In many mechanisms furan is ultimately formed by the decarboxylation of 2-furoic acid or the breakdown of 2-furfural.

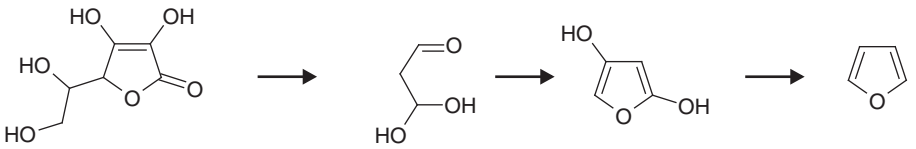

Figure 2 Simplified furan formation route from ascorbic acid.

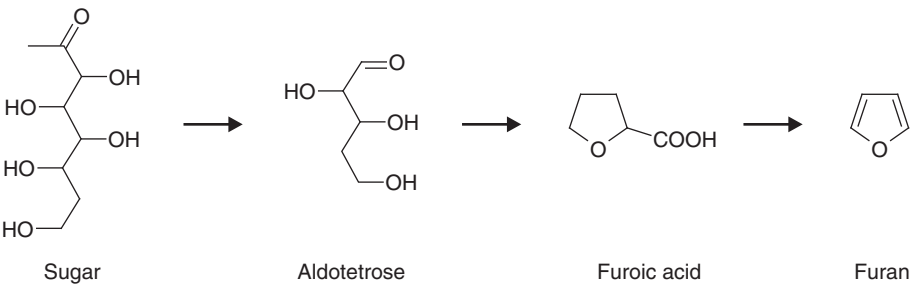

Figure 3 Simplified furan formation route from sugars.

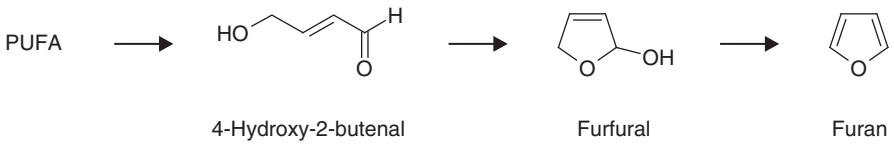

Figure 4 Simplified furan formation route from polyunsaturated fats.

Formation from Ascorbic Acid

Heat treatment of ascorbic acid readily forms furan but the degree of formation is reduced significantly in the presence of other compounds, even some that are also known to be furan precursors. It is strongly affected by the pH and the presence of sugars.

On heating ascorbic acid behaves in similar manner to reducing sugars. In the presence of air it is oxidized to dehydroascorbic acid which is then hydrolyzed to 2,3-diketogulonic acid, decarboxylated, and ultimately dehydrated to 2-furoic acid, 3-deoxypentosulose, and furfural.

In anaerobic conditions ascorbic acid is not oxidized but undergoes hydrolysis, elimination, and decarboxylation to form 2-deoxyaldotetrose and then furan.

Ascorbic acid can act as an oxidizing agent suspected of promoting oxidative degradation of lipids to form furan in some foods that are high in polyunsaturated fatty acids.

Formation from Carbohydrates

Several amino acids and carbohydrates react on heating to form aldotetrose derivatives that undergo cyclization to form furan. Glucose can be dehydrated and subjected to retro-aldol cleavage forming 2-deoxy-3-ketoaldotetrose and ultimately 3-furanone and furan. The final stages of furan formation from sugars is probably the reduction of 3-furanone by formic acid.

In amino acids heating produces acetaldehyde and a glycolaldehyde that undergo aldol condensation to form furan. Not all amino acids form glycolaldehyde and need another source such as reducing sugars for furan formation.

Formation from Fatty Acids

Furan is formed from mono- and diunsaturated fatty acids on heating, whether they are free or esterified in triacylglycerols. The mechanism is one of the free radical autoxidation, which is enhanced by lipid oxidation catalysts such as metal ions, and probably followed by cyclization and dehydration of 4-hydroxy-2-butenal.

Analysis

Early methods for the determination of volatile compounds in food were based on types of distillation with trapping using cold traps or solvent. These methods were not really appropriate for compounds as volatile as furan because evaporation of solvents used to elute analytes from traps also caused loss of furan. Modern methods are virtually all based on headspace gas chromatography with mass spectrometric detection. In this the food sample is placed in a vial sealed with a septum and warmed to partition the furan between the food and the air above it, known as the headspace.

Furan in the air of headspace vial is transferred to a gas chromatograph (GC) with a heated syringe (Figure 5), with a transfer tube or with a solid phase microextraction (SPME) device, which is an absorbent phase on a retractable needle held in the headspace. Detection is invariably by mass spectrometry operated in the selected ion monitoring mode.

The analysis requires considerable care at various stages. The food sample must be homogenized in order to obtain a representative sample. This necessitates stirring, with the concomitant potential for loss of furan, therefore a brief stirring procedure at low temperature is required. The sample is heated for approximately 20 min to equilibrate the furan between the sample and the headspace. At this stage the temperature must be low enough to avoid furan formation, typically 50 °C. A sample of the headspace (approximately 1 ml) is transferred to the GC by means of a heated syringe or a transfer line. Alternatively, SPME may be used in which furan is absorbed on to a coated needle and carried to the GC. All of these methods are easily automated.

GC separation of volatiles including furan can often best be achieved using a porous layer open tube column. This is a bonded porous-polymer phase based on polystyrene and divinylbenzene that is relatively inert and stable to water.

Mass spectrometry is used universally as the furan detector for GC. The mass spectrum of furan has a relatively intense molecular ion at m/z 68 and a fragment ion at m/z 39. Furan can be quantified by using a calibration graph prepared by standards or by adding a range of known levels of furan to samples (standard addition procedure).

Correction for the loss of furan in the later stages of the analysis can be made by adding deuterium labeled furan to the vial. The deuterated furan has all four hydrogen atoms replaced by deuterium. It mimics the behavior of the native furan through the analysis but can be measured independently by the mass spectrometer as it has a molecular ion at m/z 72. The labeled furan is unfortunately too expensive to be added to the food before homogenization unless only a small sample is taken, but this then runs the risk of getting an unrepresentative sample if the food is not homogeneous.

Recoveries from foods are generally better than 90% with limits of detection usually better than 0.3 $\mu g\,kg^{-1}$.

Control

There is so far only a limited and incomplete picture of the levels of furan in foods. There is no evidence so far to suggest

Figure 5 Headspace sampling.

that consumers should alter an infant or child's diet or eating habits to avoid exposure to furan. Also, on account of their complexity, information is lacking with regard to the formation mechanisms.

However, as furan is believed to be carcinogenic some efforts will undoubtedly be made to reduce consumer exposure, and various approaches are available.

Reduction of furan formation in food is challenging due to the large number of precursors in many foods, the requirement to heat process foods to give microbiological safety, and the necessity to heat food in sealed containers to maintain that safety during storage before consumption.

In terms of industrial processing there is limited scope for action. Particular heating regimes are necessary to reduce or prevent the microbiological growth. It must also be considered that changes to the heating regime might make the product unacceptable in terms of taste and appearance and/or promote the formation of other food processing contaminants such as acrylamide and chloropropanols.

As many of the formation mechanisms involve oxidation steps, a promising approach to mitigation is to reduce oxygen by the use of modified atmospheres during heating. Certain additives might also reduce oxidation, for example, by scavenging free radicals. Sulfite added to many foods reduces furan formation significantly.

Furan formation cannot viably be reduced by altering the formulation of some foods because some precursors and catalysts such as ascorbic acid, polyunsaturated fatty acid, and iron are beneficial to health and therefore are desirable food components.

See also: Foodborne Diseases: Overview of Chemical, Physical, and Other Significant Hazards. Risk Analysis: Risk Assessment: Chemical Hazards

Further Reading

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Hydroxymethylfurfural

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Glossary

Biomarker A biomarker, or biological marker, is in general a substance used as an indicator of a biological state. It is a characteristic that is objectively measured and evaluated as an indicator of normal biological processes.
Caramelization Caramelization is the browning of sugar, a process used extensively in cooking for the resulting nutty flavor and brown color.
Chromatography Chromatography is the collective term for a set of laboratory techniques for the separation of mixtures. The mixture is dissolved in a fluid called the 'mobile phase,' which carries it through a structure holding another material called the 'stationary phase.'
Dietary exposure Dietary exposure assessments combine food consumption data with data on the concentration of chemicals in food. The resulting dietary exposure estimate is then compared with the relevant toxicological or nutritional reference value for the food chemical of concern.
Hydroxymethylfurfural Hydroxymethylfurfural (HMF), also 5-(hydroxymethyl)furfural, is an organic compound derived from the dehydration of certain sugars during caramelization and the Maillard reaction.
Maillard reaction The Maillard reaction is a form of nonenzymatic browning that results from a chemical reaction between an amino acid and a reducing sugar during heating.
Mitigation Mitigation is action to decrease the intensity of undesired substances in foods such as thermal process contaminants in order to reduce their potential effects on consumers' health.
Nucleophile A nucleophile is a species that donates an electron pair to an electrophile to form a chemical bond in a reaction.
Xenobiotic A xenobiotic is a chemical that is found in an organism but that is not normally produced or expected to be present in it.

Description

Hydroxymethylfurfural (HMF) is generated by both the Maillard reaction and sugar dehydration, so it is widespread in processed foods. HMF is highly soluble in water, methanol, ethanol, and ethyl acetate and presents a very low solubility in petroleum ether. For its identification in foods, HMF presents a maximum of absorbance at 284 nm (18 000 M absorptivity) and a secondary band at 230 nm. Also, HMF is a multifunctional molecule containing a furan ring, a carbonyl group, and a hydroxymethyl group (Figure 1).

The hydroxymethyl group can undergo (1) halogen substitution with chloride and bromine to form 5-halomethylfurfurals, (2) oxidation to form 2,5-furandialdehyde, or (3) the Michael addition reaction. The aldehyde group is also reactive and can suffer (1) reduction, (2) condensation reactions, and (3) oxidation to a carboxylic group to form respective furoic acids. Cleavage of the furan ring can occur under acidic conditions to form formic acid and levulinic acid.

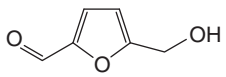

Figure 1 Chemical structure of HMF.

Finally, both hydroxymethyl and aldehyde groups can participate in polymerization reactions with HMF or other molecules. In living tissues, these reactive sites influence the biological activity and fate of HMF.

Health Concerns

Thermal processing and storage of foods cause a complex network of reactions leading to compounds that may impact on human health. Although HMF is a well-known substance in food and chemical industry, the relationship between HMF and its metabolites, and human health has not been studied in detail so far.

Recently, the presence of HMF in foods has raised toxicological concerns because its derivatives (5-chloromethyl- and 5-sulfidemethylfurfural) have been shown to be cytotoxic and genotoxic and to have tumoral effect. But *in vitro* studies have shown that HMF molecule has a weak direct genotoxic and mutagenic potential. Preliminary studies have shown that radiolabeled HMF is rapidly absorbed in the gastrointestinal tract of rodents and also rapidly cleared from all major tissues, with no evidence of significant accumulation, being finally excreted via urine (approximately 90%) and feces

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(approximately 10%). However, gastrointestinal absorption of HMF can be modulated by other constituents of the diet, such as the fiber content, which seems to reduce HMF transportation and uptake in caco-2 cell lines. Later investigations have demonstrated some covalent binding in the liver, bladder, and kidney and in the gastrointestinal tract after 24 h of exposure. Recently, a reliable method to measure aldehyde adducts to N-terminal valine in hemoglobin from HMF has been reported, which is a step forward for its use as a biomarker of HMF exposure.

There is evidence for the potentially harmful activity of HMF from *in vitro* test systems of different complexities and experiments with laboratory animals. However, the results are still controversial when extrapolated to human level. HMF is bioactivated *in vitro* to 5-sulfooxymethylfurfural (SMF), which has been demonstrated to induce genotoxic and mutagenic effects to bacterial and mammalian cells. The subsequent interaction of this reactive intermediate with critical cellular nucleophiles (DNA, RNA, and proteins) may result in structural damage to these macromolecules. In rodents, HMF has been identified as an initiator and promoter of colon cancer and nephrotoxicity, or chromosomal aberrations. The acute oral toxicity of the pure HMF compound is relatively low and the LD 50 was shown to be 3.1 g/kg of body weight (bw) in rats. The modified theoretical added maximum daily intake (mTAMDI) value was 1600 μg/person/day, which is above the threshold of concern of 540 μg/person/day derived from a large database containing data on subchronic and chronic animal studies.

Living bodies have a chemopreventive machinery to metabolize external substances (xenobiotics) and small endogenous compounds. Apart from the formation of a glycine conjugate or direct oxidation to 2,5-furandialdehyde, HMF is biotransformed by sulfonation. Sulfotransferases (SULTs) are a family of five types of detoxifying enzymes present in the liver, placenta, adrenal gland, endometrium, colon, brain, leukocytes, and other different human tissues. SULTs transfer a sulfomoiety from the cofactor 5′-phosphoadenosine-3′-phosphosulfate (PAPS) to the substrate, which is transformed to a sulfate derivative, and the process is called sulfonation. Sulfoconjuagtes are more water soluble due to better ionization of the sulfate group and decrease the passive absorption over membranes. However, the sulfonation can also result in the bioactivation of some HMF into reactive SMF toward biomolecules. In detail, the family of SULT1A1 is directly involved in the sulfonation of HMF. But extrapolation to humans could be more dramatic because humans express SULT in extrahepatic tissues more extensively than rats and may, therefore, be more sensitive to HMF.

Considering the levels of HMF in certain foods such as coffee, dried fruits, and biscuits, it is important to identify the most important sources of human exposure to HMF. Several investigators started to estimate the dietary exposure to HMF but this is a challenging issue. Coffee was identified as the most important source of HMF intake, representing 63% from the Norwegian population with a daily intake of 5.56 mg day^{-1}. A dietary exposure to HMF from coffee of 8.57 mg day^{-1} for the Spanish population and a total HMF intake of 10 mg $day^{-1}$are estimated. All in all, data from other countries and dietary habits are lacking and are necessary for an appropriate risk evaluation. However, there is a rough estimation showing that humans may ingest from 30 to 150 mg HMF day^{-1} (2.5 mg/bw/day). This value is several orders of magnitude higher than those of other processing contaminants like acrylamide or furan.

Mechanism of Formation

There are two main ways for HMF formation in food, which are (1) sugar degradation and caramelization and (2) the Maillard reaction. HMF is an intermediate in the Maillard reaction, which occurs when reducing hexose moieties are heated in the presence of amino acids or proteins. An alternative source of HMF involves direct thermal dehydration of fructose, sucrose, and, to a lesser extent, glucose. This reaction does not require the presence of amino groups. It is significantly accelerated under acid conditions.

Basically, the Maillard reaction implies condensation between amino groups of amino acids or proteins, typically the epsilon-amino group of lysine residues, and the reactive carbonyl groups of reduced sugars. The complexity of the reaction network is increased when a lipid is present, because lipid peroxidation products with a carbonyl group may take the place of sugar in the reaction. In the first step of this complex cascade of reactions, a stable Amadori product (1-amino-1-deoxy-2-ketone) is formed. The reaction is stopped at this step if reaction conditions are not appropriate or heat input is not severe enough. The Maillard reaction is definitively affected by the temperature/time of the process, reactants, pH, and moisture of the reaction media. The Amadori product is able to yield approximately 10 times more HMF than the respective sugar and amino acid under the same conditions. If thermal conditions and reaction media are appropriate, the colorless Amadori compounds will degrade to the Maillard reaction products, which are involved in aroma generation, color, and formation of melanoidins. At moderate and low pH, the Amadori product form 1,2-eneaminol, in which a hydroxyl group is lost at C-3 followed by deamination at C-1 and hydration to form an intermediary compound called 3-deoxy-2-hexulose, which gets further dehydrated to form HMF. This route is named as 1,2-enolization. The formation of HMF during the Maillard reaction is shown in Figure 2 in a simplified form.

However, HMF is also formed from sugar dehydration under acidic conditions. Caramelization requires higher energy of activation and subsequently higher temperatures than the Maillard reaction, although a similar intermediary is formed, 3-deoxy-2-hexulose, from hexoses degradation. Figure 3 shows the formation of HMF by dehydration of sugars during heating.

During caramelization, reducing sugars directly undergo 1,2-enolization, dehydration, and cyclization reactions to form HMF. Ketohexoses are known to produce HMF more efficiently and selectively than aldohexoses, and this may be due to the fact that aldohexoses enolize to a much lower degree than ketohexoses. In the case of sucrose, a nonreducing sugar, in the Maillard reaction first it is necessary to hydrolyze it into free glucose and fructose to start the reaction. Recently, Perez-Locas and Yaylavan (2008) demonstrated that sucrose degrades into glucose and a very reactive fructofuranosyl cation under reduced moisture conditions and this cation can be effectively converted into HMF. The authors concluded that the amount of HMF formed from sucrose is expected therefore

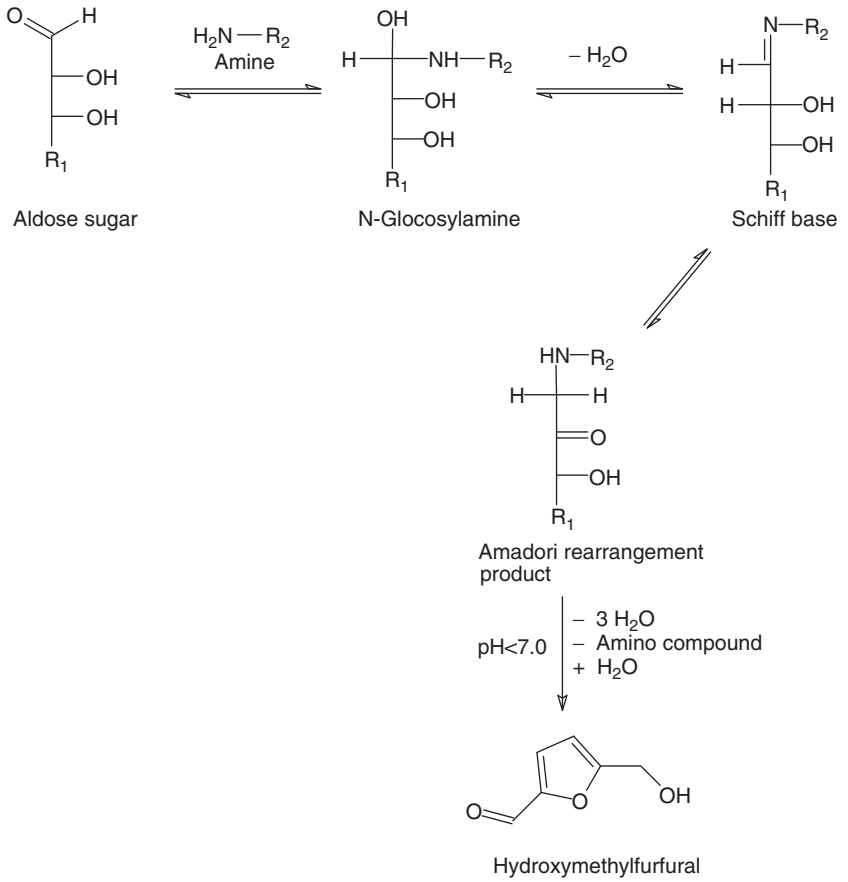

Figure 2 Formation of HMF as a consequence of the Maillard reaction.

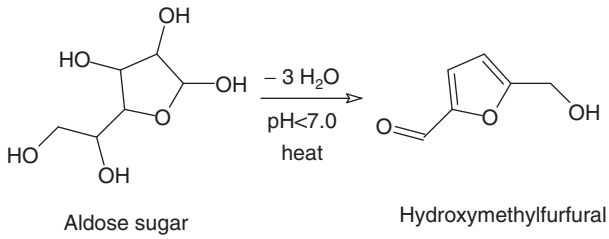

Figure 3 Formation of HMF during heating by dehydration of aldose sugars.

to be higher than that of respective fructose or glucose at higher temperatures due to the more efficient conversion pathway of fructofuranosyl cation into HMF relative to the less efficient classical 3-deoxyglucosone pathway that glucose and fructose follow at lower temperatures under dilute aqueous conditions. Another possible pathway is via cleavage of alpha-dicarbonyl compounds and recombination of methylglyoxal with glyceraldehyde. However, HMF is not a final product of the reaction and it is not necessarily accumulated in the thermally processed food. As a volatile, HMF takes part of the characteristic aroma profile of different heated foods, preferably under roasting. Under more severe heating conditions HMF can react and decompose or polymerize into high molecular-weight food structures, like melanoidins. In summary, the measurement of HMF in foodstuffs is a balance between formation and degradation reactions, transformation or evaporative losses. It is known that HMF can react further by decarboxylation, oxidation, dehydration, and polycondensation reactions and numerous key substances are formed such as furfural, 2.5-furfuraldehyde, 5-hydroxymethylfuroic acid, 5-methylfurfural, levulinic acid, and formic acid.

Food Sources

Although HMF is not present in fresh foods, it rapidly accumulates during thermal processing and storage of carbohydrate-rich products. HMF is widely recognized as a marker of quality deterioration, generated as a result of excessive heating or inadequate storage conditions of foods containing carbohydrates. It is commonly used as a marker of the extent of heat processing in foods where the Maillard reaction and/or caramelization is the main reaction taking place.

The presence of HMF in processed fruits such as fruit juices and concentrates, dried fruits, jams and jellies, alcoholic beverages such as beer and wine, bakery products such as biscuit and bread, breakfast cereals, heated dairy products, roasted nuts, and seeds has been widely reported. The level of HMF largely varies in processed foods depending on the processing and/or storage conditions.

HMF level has been used for measuring honey quality for years. Honey processing frequently requires heating both to

reduce viscosity, and to prevent crystallization or fermentation. Honey heating is usually carried out in two different ways: in air-ventilated chambers at 45–50°C for 4–7 days or by immersion of honey drums in hot water. It is well known that heating of honey results in HMF formation due to acid-catalyzed dehydration of hexoses. The presence of simple sugars (glucose and fructose) and organic acids presents a favorable condition for the production of HMF in honey. The factors affecting the formation of HMF in honey are temperature and time of heating, storage conditions, use of metallic containers, and chemical properties of honey that are related to the floral source from which the honey has been extracted.

HMF may form in low ppm quantities in many processed foods under mild heating conditions. HMF is usually present in low amounts in fruit juices, but concentration and storage conditions may significantly increase its level due to low moisture and high acid conditions. Bakery products contain low and moderate amounts of HMF. Even though the baking conditions are favorable for HMF formation, neutral or slightly basic recipes make bakery products less susceptible to form HMF during baking. HMF may accumulate in large quantities up to a few thousands ppm levels in roasted foods such as coffee, malt, and caramel products due to extremely high temperatures applied during processing. Drying and long-term storage conditions of dried fruits make them susceptible to form large amounts of HMF. High acid conditions are also known to stimulate HMF formation in large quantities in certain foods, including balsamic vinegar.

Mitigation

It has been well described that HMF forms during the Maillard and the caramelization reactions. Considering the possible mechanisms of formation, the factors affecting HMF concentration in foods are shown below:

Factors	*Correlation*
Precursors (sugars, amino acids)	Positive
Temperature	Positive
Time	Positive
pH	Negative
Moisture	Negative

Hexose sugars and amino acids appear as the major precursors for HMF formation in foods. High carbohydrate foods are, therefore, the most susceptible items in terms of HMF formation. Fruit juice concentrate, honey, jams, and jellies are among the foods containing high concentrations of precursors leading to HMF during processing and/or storage. Increasing the temperature during processing and/or storage also increases the rate of HMF formation. So, decreasing the thermal load during processing and storage at lower temperatures are considered as simple ways to mitigate HMF formation in foods. Lower temperature vacuum evaporation and cooking techniques are known to reduce HMF formation in juice concentrates and jams, respectively.

Lowering the pH strongly enhances HMF formation in foods. Processing and storage temperature of acid foods should be kept as low as possible to prevent an excessive formation of HMF. In general, increasing the storage time also increases the amount of HMF formed in foods. Low and moderate moisture conditions are usually associated with increased formation of HMF due to the accelerated Maillard reaction of sugar dehydration.

Analytical Methods

Colorimetric, spectroscopic, and chromatographic methods can be used for the determination of HMF in foods. However, problems have been reported for colorimetric and spectroscopic methods. These include the use of carcinogenic reagents, and the fact that the methods are nonspecific and, therefore, measure components in food that do not lead to HMF leading, to overestimate the HMF content. Several high-performance liquid chromatography methods have been reported for the determination of HMF in various foods. These techniques utilize ultraviolet (UV) detection because of the strong absorption of furfurals at 285 nm. However, many compounds naturally present or formed in foods during processing also absorb at this wavelength. Poor chromatographic resolution of these compounds may adversely affect the quantification of HMF during UV detection.

The usual approach for the extraction of free furfurals from solid food matrices entails extraction with water followed by clarification using the Carrez I and II reagents. This kind of sample treatment can extract almost all HMF present in the sample. Because UV detection is not specific, an accurate detection of HMF will be possible only if a good baseline separation is achieved. However, mass spectrometry detection coupled to liquid chromatographic separation offers more selectivity and accuracy, bringing a significant improvement to the analytical methodology of HMF. The presence of interferences may be problematic, particularly during the UV detection after liquid chromatography separation, when low concentrations of HMF are measured in processed foods. In such a case, (1) a rapid separation of HMF from the matrix coextractives in a narrow-bore column, (2) an efficient cleanup of the extract using solid phase extraction (SPE), and (3) a selective detection of HMF using MS may be combined in an analytical method. If lower concentrations of HMF are required to be detected in foods, not only the sensitivity of instrument's detection capability, but also the success of sample preparation by eliminating the interfering coextractives is important. Even if HMF is soluble in water, it has a hydrophobic moiety that may help in designing a SPE based cleanup strategy. If passed through a cartridge packed with hydrophobic sorbent material, the cartridge retains HMF and other less polar coextractives whereas polar compounds are not. HMF is eluted from the cartridge by passing diethyl ether. By doing so, the extract is not only cleaned up, but also HMF is concentrated to increase the detection sensitivity.

Legislation

For the occurrence of HMF in foods, legislation has been mainly limited to honey worldwide. Codex Alimentarius

(Alinorm 01/25, 2000) established that the HMF content of honey after processing and/or blending must not be higher than 80 mg kg^{-1}. The European Union (EU Directive 110/2001) fixed a HMF limit in honey of 40 mg kg^{-1} with the following exceptions: 80 mg kg^{-1} for honey coming from countries or regions with tropical temperatures and 15 mg kg^{-1} for honey with low enzymatic level. Fruit juices and concentrates that are widely consumed are also common sources of HMF. As one of the quality parameters in many fruit juices, a maximum HMF level of 20 mg l^{-1} has been established for fruit juices according to the European Fruit Juice Association's (AIJN) code of practice (AIJN, 1996).

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N-Nitrosamines

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Introduction

N-nitrosamines are aliphatic or aromatic derivatives of secondary amines, which have a nitroso (–NO) group attached to nitrogen. They are formed by the reaction of nitrite with secondary amines. It should be noted that nitrates may also form *N*-nitrosamines through reduction to nitrites by saliva or enzymes in the intestinal tract. Many *N*-nitrosamines are strongly carcinogenic. They contaminate several foods, beverages, and water, at low levels. The lower molecular weight *N*-*N*-nitrosamines are derived from alkyl or monocyclic secondary amines and have been well characterized. They are known as volatile *N*-nitrosamines on account of their means of analysis. Many larger and more polar *N*-nitrosamines that can be extracted from foods and measured are known as nonvolatile *N*-nitrosamines. A third 'class' is the apparent total *N*-nitrosamine content (ATNC), which is a combined measure of the volatile and nonvolatile *N*-nitrosamines and includes unidentified molecules, such as nitrosated proteins that have not yet been isolated from foods. When the ATNC is measured, its value usually far exceeds the sum of the identified volatile and nonvolatile compounds.

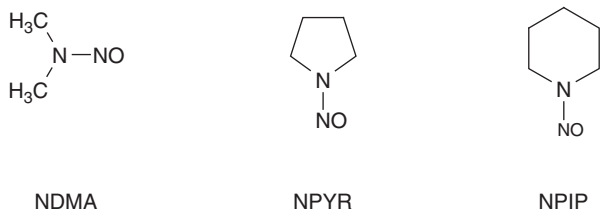
Structures of some important volatile *N*-nitrosamines are shown in Figure 1.

The *N*-nitrosamines of most importance and interest are compounds of relatively low molecular weight, in particular *N*-nitrosodimethylamine (dimethylnitrosamine (NDMA)). The other major *N*-nitrosamines formed from small secondary amines and most commonly encountered in food are *N*-nitrosodiethylamine (NDEA), *N*-nitrosomethylethylamine (NMEA), *N*-nitrosodipropylamine (NDPA), *N*-nitrosodibutylamine (NDBA), *N*-nitrosopyrrolidine (NPYR), and *N*-nitrosopiperidine (NPIP).

The major nonvolatile *N*-nitrosamines are the nitrosated amino acids and related compounds, but there are also important nitrosated products of industrial chemicals such as diethanolamine, that are associated with nonfood products such as cosmetics. The nitrosated amino acids include *N*-nitrosoproline, *N*-nitrososarcosine, *N*-nitrosothiazolidine-4-carboxylic acid, *N*-nitrosooxazolidine-4-carboxylic acid, and *N*-nitroso-2-methyl-thiazolidine-4-carboxylic acid. Structures of some important nonvolatile *N*-nitrosamines are shown in Figure 2.

The toxicity of *N*-nitrosamines came to light in the mid-1950s when it was found that mink fed with herring meal that had been treated with nitrite were dying from liver disease. It was discovered that secondary amines present naturally in the fish had reacted with the nitrite to form NDMA, which had induced cancers in the livers of the mink. This discovery led to the analysis of nitrite-treated foods for *N*-nitrosamines. Analysis progressed slowly until the availability of a very sensitive detector, a nitrosamine-specific nitrogen chemiluminescence detector first marketed as the thermal energy analyzer (TEA), which combined with the fast developing gas chromatographic systems, enabled the detection of very low levels of *N*-nitrosamines even in the 1970s.

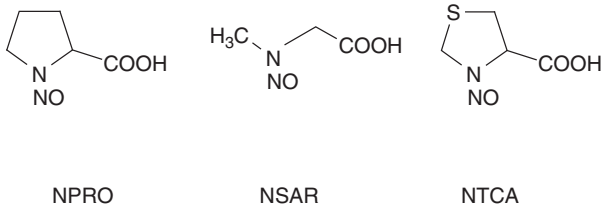
Products, such as tobacco, that contain secondary amines not usually found in foods, such as nicotine, invariably contain low levels of their nitrosation products, such as *N*-nitroso-*nor*-nicotine. *N*-nitrosamines are also associated with industrial nonfood products, especially those derived from rubber, and are produced in substantial amounts during the production of hydrazine-based rocket fuels, which has caused environmental pollution.



NDMA NPYR NPIP

NDMA = *N*-nitrosodimethylamine; NPYR = *N*-nitrosopyrrolidine; NPIP = *N*-nitrosopiperidine

Figure 1 Structures of some important volatile *N*-nitrosamines. NDMA, *N*-nitrosodimethylamine; NPYR, *N*-nitrosopyrrolidine; NPIP, *N*-nitrosopiperidine.



NPRO NSAR NTCA

NPRO = *N*-Nitrosoproline; NSAR = *N*-Nitrososarcosine; NTCA = *N*-Nitrosothiazolidine carboxylic acid

Figure 2 Structures of some important nonvolatile *N*-nitrosamines. NPRO, *N*-nitrosoproline; NSAR, *N*-nitrososarcosine; NTCA, *N*-nitrosothiazolidine carboxylic acid.

Table 1 Range of volatile *N*-nitrosamines measured in selected foods 1973–2001

Food	N-*nitrosamine* ($\mu g\ kg^{-1}$)
Cheese	Not detected–0.5
Milk	Not detected–0.45
Bacon (cooked)	Not detected–6.5
Ham	Not detected–0.1
Salami	Not detected–0.33
Fish (cooked)	Not detected–13.1
Oil	Not detected–0.38
Beer	Not detected–6.8

Occurrence in Food

A summary of typical levels of *N*-nitrosamines are given in Table 1 below:

The foods that are most frequently contaminated with *N*-nitrosamines are cured meats and cheeses, smoked fish and meat, foods dried by combustion gases (malt, milk products, and spices), and some pickled vegetables. Today the NDMA content of products in Western countries has in most cases been reduced to less than 1 $\mu g\ kg^{-1}$. In a large food survey carried out in France in the late 1980s *N*-nitrosamines were found in beer, cured meats, and smoked fish. NDMA was found in 89% of all samples with a maximum level of16 $\mu g\ kg^{-1}$. In beers, NDMA was present in all samples but the maximum level was less than 0.3 $\mu g\ l^{-1}$, very much lower than the 20 $\mu g\ l^{-1}$ encountered in the 1970s and 1980s. NDEA is not frequently encountered but was found in almost all of the distilled stone fruit spirits surveyed in France, at up to 12 $\mu g\ l^{-1}$. NDMA was found in 95% of almost 90 cured meat samples, but in 72% the levels were below 0.5 $\mu g\ kg^{-1}$.

Of the nonvolatile *N*-nitrosamines, *N*-nitrosoproline, and *N*-nitrosothiazolidine-4-carboxylic acid are most frequently found in foods, the other compounds being reported only sporadically. Of note, with the exception of *N*-nitrososarcosine, which is a relatively weak carcinogen, *N*-nitrosated amino acids are not mutagenic and not carcinogenic.

Malt, Beer, and Whiskey

Soon after the discovery of *N*-nitrosamines in fishmeal, they were found to be present in flame-kilned malt, and in beers and whiskies made from the malt. NDMA was found to be present in approximately 70% of all beers tested, with mean concentrations of 2–3 $\mu g\ kg^{-1}$ and maxima of approximately 70 $\mu g\ kg^{-1}$. The levels of NDMA were higher than those of other *N*-nitrosamines. The precursors of NDMA were found to be the secondary amines hordenine and gramine, natural constituents of barley. *N*-nitrosamine levels in malt were reduced by a switch to using indirect heating of the malt during the curing process, but some *N*-nitrosamine persisted in many beers. The source of this was eventually found to be nitrite that had been derived from the bacterial reduction of nitrate in the water used for brewing. This problem has been addressed by ensuring that the yeast used for brewing contains only very low levels of bacteria. NDMA was also present in whiskey made from malt, which continued to cause a problem after changes to the kilning operations as many whiskies are kept to mature for many years before sale.

In a survey of approximately 140 beers purchased in 2003, approximately 80% contained no NDMA subject to a detection limit of 0.1 $\mu g\ l^{-1}$ and only three samples contained over 0.5 $\mu g\ l^{-1}$. The NDMA content was not related to beer strength or type or geographical origin.

Dairy Products

Milk products, mainly dried milk powder and cheese contain comparatively low concentrations of NDMA, again usually less than 1 $\mu g\ kg^{-1}$. NDMA can be formed in milk if direct flame drying procedures are used.

Meats

Many meats, principally the pork products including bacon and hams, are cured by treatment with nitrite in the form of sodium and/or potassium salts. Nitrite is effective in preventing germination and growth of bacteria and in particular, *Clostridium botulinum*, which produces a highly toxic metabolite. Pork contains the secondary amines dimethylamine, proline, piperidine, and pyrrolidine, and it is NDMA, *N*-nitrosoproline, *N*-nitrosopiperidine, and *N*-nitrosopyrrolidine that are most often found in cured meat products. *N*-nitrosopyrrolidine is mainly formed by heat-induced decarboxylation of nitrosoproline or by the direct nitrosation of pyrrolidine, which is a natural component of meat products.

Relatively high amounts of peptide-bound *N*-nitrosoproline can be released by enzymatic degradation or chemical hydrolysis, and this could occur in the digestive system. Nonvolatile *N*-nitroso compounds have been found in nitrite-treated or smoked meat products in much higher concentrations, for example, *N*-nitrosoproline in concentrations up to 360 $\mu g\ kg^{-1}$ in cured meat. Smoked meats contain *N*-nitrosothiazolidine-4-carboxylic acid at levels of up to approximately 1.5 $mg\ kg^{-1}$. The *N*-nitrosothiazolidine-4-carboxylic acid content of bacon has been shown to increase on frying. Smoked meats also contain the noncarcinogenic *N*-nitrosothiazolidine, a nitrosamine that has been found in smoke-treated products in concentrations up to 10 $\mu g\ kg^{-1}$.articleid

Other Foods

N-nitrosamines are not usually detected in surveys of vegetables, fruits, cereals, and cereal products or nonalcoholic beverages. However, the use of artificial fertilizers has increased the levels of nitrates in these foods, particularly leafy greens. Nitrates may be precursors for nitrites *in vivo*.

Water can be a source of NDMA in foods, alcoholic beverages, and other drinks. Here it is formed by the action of certain disinfectant chemicals and from secondary amines present in ion-exchange resins.

Cooking

N-nitrosamines are formed during some forms of cooking, through reaction of amines naturally present with nitric oxide

(NO) in flame gases, or through acceleration of reactions of amines with NO present in the food on account of the elevated temperatures. Formation rates and levels formed depend on the amine and nitrite composition of the food and also the cooking method. Frying produces more *N*-nitrosamines in meat than grilling or baking.

Barbeque cooking produces the highest levels of *N*-nitrosamines on account of the high temperature and the use of naked hot gases. The widespread use of barbeque style grills to cook seafood in the Far East probably contributes to higher levels of *N*-nitrosamine in the food there and high incidence of certain cancers.

Microwave cooking of bacon gives significantly lower levels of *N*-nitrosamines than frying, and cooking of foods by indirect heating procedures, such as microwaving and steaming, produce much less NDMA than direct heating on a barbeque or charcoal grill.

Food Safety Issues

Toxicity

Over 300 *N*-nitroso compounds have been tested for carcinogenicity in animals, and most *N*-nitrosamines have been found positive. Many are also mutagenic and teratogenic. Both NDMA and NDEA are classified as probably carcinogenic to humans (group 2A) by the International Agency for Research on Cancer. Rats and mice exposed continuously to low levels of NDMA for several months had a significantly increased incidence of lung, liver, and kidney tumors including adenomas, carcinomas, and sarcomas in the lung, liver, and kidneys, and hemangiomas in the liver.

N-nitrosamines are readily absorbed from the gastrointestinal tract but do not accumulate in body tissues. They require metabolic activation of their mutagenic and carcinogenic properties. This is catalyzed mainly in the liver by the body's cytochrome CYP2E1 function oxidase system, which hydroxylates and cleaves the *N*-nitrosamine molecule forming a diazonium ion that alkylates nucleophilic sites including deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), which in turn initiates cancer formation. *N*-nitrosamines are carcinogenic in all mammalian species tested and it is assumed that they are similarly human carcinogens. They produce tumors in several but often specific organs depending on the nitrosamine and the animal species. The major organs affected are the liver, lung, kidney, bladder, pancreas, esophagus, and tongue.

Analysis of data collected over several years that compared dietary intake of NDMA with cancer has shown *N*-nitrosamines to be associated with a higher incidence of rectal cancer in both men and women.

Exposure

Humans are rarely exposed to large doses of *N*-nitrosamines but to very low levels in several items of diet that are consumed regularly. This is of concern because *N*-nitrosamines have been shown to have a stronger carcinogenic effect when given repeatedly in low doses than as a single high dose. The total exposure of humans to *N*-nitrosamines has been estimated to be approximately 1 μmol day^{-1}. Diet is the major source, providing over 70% of the total. *N*-nitrosamine intake and excretion studies suggest that *in vivo* formation contributes considerably to the human *N*-nitrosamine exposure.

The US Environmental Protection Agency considers NDMA to be a probable human carcinogen, with an estimated 10^{-6} lifetime exposure cancer risk at 0.7 ng l^{-1} in drinking water. Only a few countries have imposed limits on the *N*-nitrosamine content of foods and these have been restricted to certain commodities. The *N*-nitrosamine level in foods is reasonably well controlled by limits on the quantity of nitrite added to meat and regulations and guidelines regarding the use of direct heating. Maximum admissible concentrations in water have been set at 7 ng l^{-1} (NDMA), 20 ng l^{-1} (NMEA), and 2 ng l^{-1} (NDEA). Although the US regulations limit the *N*-nitrosamine content of beer and bacon to 5 μg kg^{-1}, Italy, Switzerland, and Germany have a limit of 0.5 μg kg^{-1} *N*-nitrosamine in beer.

Although NDMA amounts in beer have been markedly reduced, consumption of beer still is a major factor with respect to the total dietary exposure to *N*-nitrosamines. Approximately 30% of the exposure via food results from the consumption of beer, contributing to approximately 0.1 μg of NDMA per person per day. The daily uptake of NDMA resulting from the consumption of beer in France and Spain has been estimated to be 0.02–0.03 μg per person.

More Asian foods than Western tend to contain *N*-nitrosamines, and these at higher level, possibly due to the higher content of seafood in the diet and the popularity of barbeque cooking. Red meat has been associated with gastric cancer, and higher levels of *N*-nitrosocompounds have been found in the feces of red meat eaters than in people consuming white meat, but there is no direct epidemiological evidence that *N*-nitrosamine are responsible. *N*-nitrosamines can be formed endogenously from secondary amines and nitrite, particularly from fish and vegetables. A meal rich in fish and nitrate-rich vegetables could contribute approximately 3 μg NDMA to the diet. Adult intakes of ingested *N*-nitrosamine have been estimated at approximately 0.1–0.3 μg per person per day, with men consuming slightly more than women, mainly because of beer consumption. The *N*-nitrosamine intake of children from food has been calculated to range from 0.007 to 0.025 μg per person per day.

The contribution to *N*-nitrosamine intake from passive smoking is much higher than intake through food items. In confined spaces tobacco smoke has been estimated to provide up to 3 μg *N*-nitrosamine per adult person per day.

Human exposure to nonvolatile *N*-nitroso compounds has been estimated to be between 10 and 100 μg per person per day; however, research suggests that most nonvolatile *N*-nitroso compounds in food are not carcinogenic.

Formation

Chemically, *N*-nitrosamine formation is thought to proceed via the oxidation of NO to NO_2 in acid conditions and then to N_2O_3 and N_2O_4, wherein each reacts with secondary amines to form *N*-nitrosamines. The nitrosation of secondary amines takes place at acidic pH. It is catalyzed by nucleophilic anions,

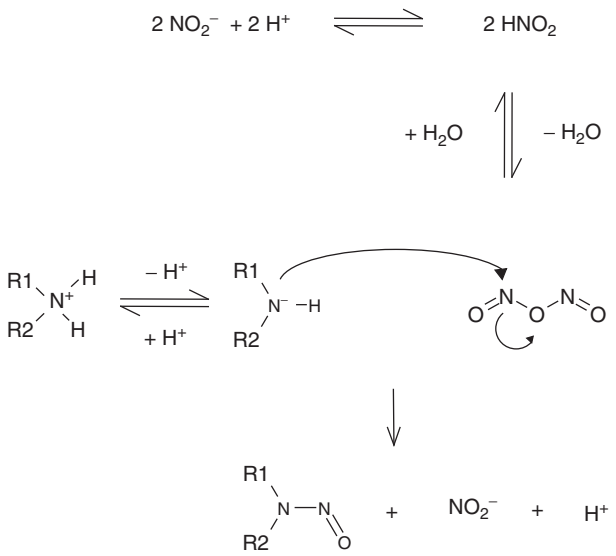


Figure 3 Mechanism of formation of *N*-nitrosamines from secondary amines. Reproduced from Stadler RH and Lineback DR (2009) *Process-Induced Food Toxicants: Occurrence, Formation, Mitigation, and Health Risks*. Hoboken, NJ: John Wiley & Sons, Inc.

such as thiocyanate, bromide, and chloride, and inhibited by ascorbic acid, α-tocopherol, and sulfamic acid and its salts.

The formation mechanism is shown in Figure 3.

Nitrosation of primary amines ultimately leads to alcohols, but tertiary amines can be nitrosated slowly after dealkylation.

Other nitroso compounds can be formed by nitrosation of thio compounds or phenols to give *S*-nitroso and *C*-nitroso compounds, respectively. These do not have carcinogenicity associated with *N*-nitrosamines. *N*-nitrosamides, *N*-nitrosoureas, *N*-nitrosocarbamates, and *N*-nitrosoguanidines are also formed by nitrosation reactions but are thought to be too reactive to exist in foods in stable forms.

In malt, NDMA is formed from nitrosation of dimethylamine released from the naturally occurring tertiary amines hordenine, gramine, or existing freely in the cereal.

N-nitrosamines are formed in some other foods, such as milk powder and some cheeses, during smoking and drying processes as described earlier. They are also formed in water by the action of disinfectant chemicals and from secondary amines present in ion-exchange resins, and water can be contaminated from environmental sources including fires and industrial activities. This contaminated water can be used for direct consumption or for food manufacture.

Several compounds inhibit or prevent *N*-nitrosamine formation, and fortunately most of these (ascorbic and erythorbic acids, butylated hydroxyanisole, butylated hydroxytoluene, and tocopherols) are themselves of low toxicity.

Analysis

Analytical methods for *N*-nitrosamines in food can be divided into those that determine either volatile, nonvolatile, or the apparent total *N*-nitrosamine content (ATNC). These methods involve gas chromatography (GC), liquid chromatography (LC), and no chromatography, respectively. The end determination (detection and measurement) can be made with the nitrosamine-specific TEA in all cases or with mass spectrometry for the chromatographic procedures.

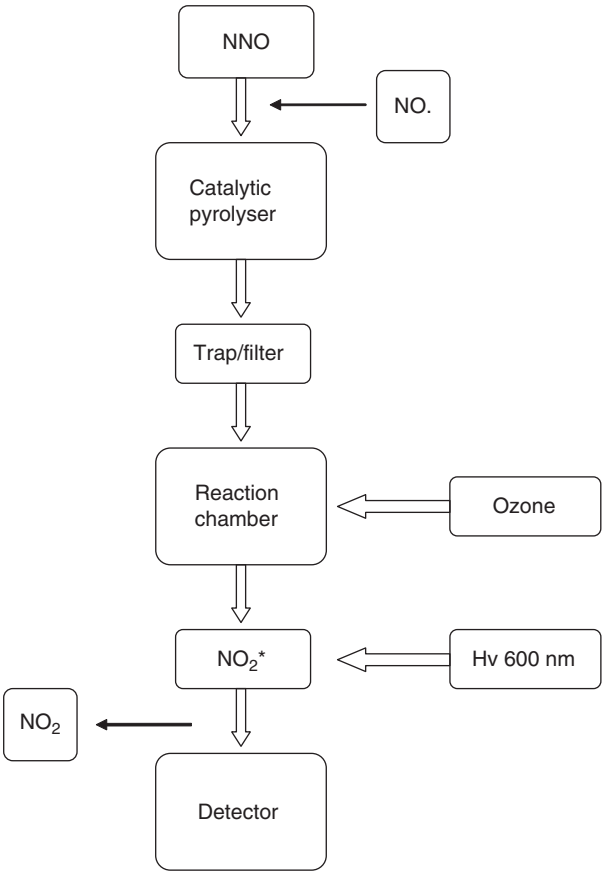


Figure 4 Schematic representation of the operation of nitrogen chemiluminescence detector.

The Thermal Energy Analyzer

The detection of *N*-nitrosamines was revolutionised in the 1970s by the introduction of the TEA, a chemiluminescence detector that was extremely sensitive and specific in detecting *N*-nitrosamines. Today different versions of the detector are available. In certain configurations it can be used to detect non-nitroso nitrogen as well as *N*-nitrosamines, and the generic name 'nitrogen chemiluminescence detector' may be preferred (Figure 4).

In this detector, the sample, in the form of a hot gaseous eluent from a GC, passes through a catalytic pyrolyzer under vacuum at a temperature of 350–500 °C. The temperature is set to be high enough to cleave a nitrosyl radical (NO) from the *N*-nitrosamine, leaving the other organic material to be pyrolyzed to carbon dioxide. The reaction products pass from the pyrolyzer through a filter and the nitrosyl radical is reacted with ozone to produce the electronically excited species (NO_2 radical). This decays to its ground state, emitting light in the near-infrared region, which is converted into an electrical signal by a

sensitive photomultiplier. The lower limit of detection for the instrument is 10 pg at a signal to noise ratio of 3:1.

Limited confirmation that compounds suspected to be *N*-nitrosamines by TEA detection are so, may be made by decomposing them by irradiating their solutions with ultraviolet (UV) light. Then reanalysis with the TEA would show that the response disappears for *N*-nitroso compounds.

Volatile *N*-Nitrosamines

The volatile *N*-nitrosamines including NDMA, NDEA, NMEA, NDPA, NDBA, NPIP, NPYR, and a few minor compounds are isolated from foods by solvent extraction or by distillation. It is somewhat surprising that the more modern headspace sampling methods of volatiles analysis, such as solid phase microextraction (SPME), have had only limited application to *N*-nitrosamine analysis.

Certain procedures are common to most methods. These are the inclusion of a nitrosation suppressant, an artefactual nitrosation indicator, and an internal standard. The nitrosation suppressants most commonly used are sulfamic acid or ammonium sulfamate. Artefactual nitrosation indicators are secondary amines known to be absent from the food matrix but which form *N*-nitrosamines, which are also known to be absent. As dipropylamines and piperidine are not commonly encountered in foods, these have been used the most often. Their use is not pertinent to ATNC determination, where there is no chromatographic separation.

Isotopically labeled internal standards are available and are ideal for gas chromatography–mass spectrometry (GC–MS) and liquid chromatography–mass spectrometry (LC–MS) methods, but cannot be used for TEA detection as the instrument cannot distinguish between the isotopes.

For solid food samples, distillation methods are normally used. The sample is placed in a heated round-bottomed flask in water or in mineral oil containing a nitrosation suppressant, an artefactual nitrosation indicator, and an internal standard. An antifoaming reagent may also be required. The flask is connected to a condenser or more usually in traps sitting a succession of cooling baths of decreasing temperature cooled with ice, dry ice, or liquid nitrogen. Distillation is achieved by passing a slow stream of nitrogen through the heated sample and collecting the distillate in the traps.

For liquid samples, such as beer or water, liquid–liquid extraction methods are used. The sample can be shaken in a separatory funnel with a nonmiscible volatile organic solvent, such as dichloromethane (DCM) or diethyl ether, but it is more convenient and efficient to use solid phase extraction (SPE) methods in which the liquid is adsorbed onto commercially available short-packed columns of an inert support, such as Celite® or a diatomaceous earth. No significant chromatographic separation occurs. The packing material holds the liquid sample and the nitrosamines are extracted from it by passage of DCM. The solid nature of the support prevents emulsion formation.

For both distillation and SPE the solvent is dried and carefully concentrated. A low-boiling solvent is required for successful removal from more volatile *N*-nitrosamines such as NDMA. Despite the concern about the use of chlorinated solvents, DCM meets the requirements most closely and is the solvent mostly often used.

To avoid losses of NDMA and NDEA the DCM must be evaporated very slowly and this is achieved by using a Kuderna–Danish concentrator. This apparatus comprises a round-bottomed flask of 50–250 ml volume with a cone joint at the base to which is attached a tube of 5–10 cm in length with a tapered tip. The top of the flask has a socket joint fitted with a vertically mounted Snyder fractional distillation column. The column is filled with glass materials suitable for fractional distillation. The basal tube is partly immersed in a water bath at approximately 50 °C. The DCM is slowly evaporated until none remains in the main body of the flask, and the collection tube is removed. The DCM is evaporated from the tube under a stream of nitrogen to a volume of approximately 0.5 ml. A low volume of a higher boiling solvent may be added to prevent complete evaporation and potential loss of nitrosamines. The method has been widely used and has been adopted as an official method in several countries.

Solid food samples can be extracted in a similar manner by mixing them with the column material but they are more often analyzed by distillation methods. Clean-up methods involving small chromatographic SPE columns of Florisil®, cyano, silica gel, aminopropyl, and alumina have been applied.

Volatile *N*-nitrosamines can be extracted from beer, water, and certain foods by using SPME. In this technique, a solid needle tipped with a fiber coated with a bonded absorbent organic phase, such as polydimethylsiloxane–divinylbenzene or divinylbenzene–carboxen–polydimethylsiloxane, is immersed into the liquid sample or into the headspace above a solid sample (which may be heated). Over a short period of time the *N*-nitrosamines are absorbed into the fiber, which is placed into a GC–MS or GC–TEA injector port and the *N*-nitrosamines desorbed by rapid heating in the injector of a GC. However, the technique is not reliably repeatable and many common *N*-nitrosamines are insufficiently volatile to produce the sensitivity required.

Supercritical fluid extraction (SFE) in conjunction with a silica SPE cartridge clean-up has been used occasionally to extract volatile *N*-nitrosamines from meat.

Nonvolatile *N*-Nitrosamines

Nonvolatile *N*-nitrosamines can be extracted from foods with polar organic solvents but considerable postextraction clean-up is required. The most popular methods for extracting nonvolatile *N*-nitrosamines from foods, such as meat, are based on dispersal of the sample into an adsorbent diatomaceous earth such as Celite, which is packed into a column and the *N*-nitrosamines eluted with polar solvent.

Alternatively the nonvolatile *N*-nitrosamines are extracted by homogenization of the minced sample with a polar solvent, such as water, acetonitrile, methanol, or mixtures of water with acetonitrile or methanol. The extract is filtered through Celite and concentrated using a rotary evaporator. The concentrate is washed with a nonpolar solvent to remove fats and extracted with a moderately polar solvent, such as ethyl formate or ethyl acetate.

The extract derived from these procedures require considerable clean up, which can take the form of column

chromatography on a cellulose/basic alumina medium with polar organic solvent elution, or use of a strongly basic anion-exchange resin.

The nonvolatile *N*-nitrosamines, typically *N*-nitrosoaminoacids are separated by gas chromatography or by liquid chromatography as described below.

N-nitroso-hydroxypiperidine has been used as an internal standard and artefactual nitrosation prevented by the addition of nitrosating inhibitors, such as sulfamic acid or ascorbic acid.

Chromatography and Detection

Volatile *N*-nitrosamines can be determined by GC on a variety of packed or capillary columns. The relatively high volatility gives short retention times and moderately polar stationary phases are required for high sensitivity. Chemical derivatization is not necessary.

The GC column can be interfaced easily with the TEA, but for mass spectrometry the low molecular weight volatile *N*-nitrosamines, such as NDMA, have poor mass spectra in that the molecular ion is small and of low mass, therefore subject to interference from coeluting compounds. Chemical ionization modes give a strong signal for the protonated molecular ion and are used in the official US methods for water analysis along with large volume injection. The technique has, however, not been applied to foods, where the need for large sample sizes and extensive concentration and clean-up is not compatible with the complex matrices.

Volatile *N*-nitrosamines are quantified by the use of calibration graphs with internal standards being used to correct for losses during the analysis. Selection of an internal standard is limited for the TEA as stable isotopes cannot be distinguished from the native compound. Therefore, an *N*-nitrosamine not present in the food sample must be used. *N*-nitrosodi-isopropylamine is often being used for this purpose.

The limit of detection for solid samples, such as meats and malt, using the distillation method or beers with SPE, both with GC–TEA detection, limits were approximately 0.1 mg kg^{-1}.

Most nonvolatile *N*-nitrosamines can be measured by GC after derivatization to reduce polarity and increase volatility. *N*-nitrosoaminoacids can be methylated at the carboxylic acid function or reacted with trimethylsilyl donors to give trimethylsilyl esters and simultaneously trimethylsilyl ethers of any hydroxyl groups.

High-performance liquid chromatography (HPLC) has equally been applied to nonvolatile *N*-nitrosamine analysis. Linking HPLC systems to the TEA detector is rather difficult in that the solvent evaporates to a high-volume gas in the TEA pyrolyser that has to be removed to maintain a vacuum in the reaction chamber. This can be achieved by a series of cold traps of decreasing temperature, the last containing a slurry of isopentane and liquid nitrogen which condenses all solvent components while allowing the NO to remain gaseous. A special pyrolyser furnace is also required.

Alternatively, nonvolatile *N*-nitrosamines in the eluent from the HPLC column can be collected and denitrosated by hydrobromic acid to produce secondary amines which can be measured by fluorescence detection after conversion to dansyl derivatives. The measurement is made before and after denitrosation and the nonvolatile *N*-nitrosamine content calculated by difference.

In practice the fact that the majority of nonvolatile *N*-nitrosamines have been shown not to be carcinogenic has led to a decline in interest in the determination of these compounds in recent years.

Apparent Total *N*-Nitroso Content

Denitrosation reactions can be used to cleave all of the nitroso bonds in a food sample, releasing nitric oxide that can be detected with the TEA. This technique gives a measure of what has become known as the ATNC.

No chromatographic separation is used. Various reagents have been studied for their ability to cleave NO from *N*-nitrosamines, and the reaction has been much studied to optimize the conditions so that the NO derived from *N*-nitrosamines can be distinguished from the signals from nitrite, *C*- and *S*-nitroso compounds.

Hydrogen bromide is the favored denitrosation reagent. In the procedure most often used, liquid samples, such as beer, are injected into a refluxing mixture of hydrobromic acid and acetic acid in ethyl acetate. The released NO is swept in a stream of argon through a series of cold traps to remove reagent vapor and through alkali traps to remove the acids and passed into the TEA. Before the NO cleavage, inorganic nitrite, *C*- and *S*-nitroso compounds are decomposed by adding acetic acid to the sample. Solid samples can be measured by placing them in the empty apparatus and adding the denitrosation reagent later.

All of the *N*-nitroso compounds in the food sample are converted quantitatively to NO, and a quantitative measure of the ATNC can be made by injection of standards of a nitrosamine. Sequential injections are made of reagent blank, duplicated samples, and standards. The procedure requires great care in operation as the response is rather dependent on the sample water content, gas flow rates, and trap conditions. It is also necessary to use very pure reagents and to clean the apparatus frequently to avoid false positive results.

NDMA is measured in water in the US Environmental Protection Agency method by extraction of a large volume of water using a small charcoal trap, elution of NDMA with a small volume of DCM, and GC–MS in chemical ionization (CI) mode with a large volume injection. The use of GC–MS enables incorporation of isotopically labeled internal standards.

Control

Contamination of food by *N*-nitrosamines has been reduced very substantially since NDMA was found in food products in the 1960s. The food industry reacted quickly to the problem helped by research by government and industry association laboratories. The formation processes were studied and as a result manufacturing processes were revised considerably. Continued efforts have reduced consumer exposure dramatically, particularly in the brewing industry. However, it is believed that further reduction of nitrite and nitrate concentrations in cured meats, or further lowering of processing temperatures would increase the

microbial risks by *C. botulinum* and other bacteria. In many countries, consumers have been advised to reduce the consumption of processed meats.

See also: Foodborne Diseases: Overview of Chemical, Physical, and Other Significant Hazards. Risk Analysis: Risk Assessment: Chemical Hazards

Further Reading

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Relevant Websites

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http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_121.pdf
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World Health Organization.

Polycyclic Aromatic Hydrocarbons (PAHs)

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Glossary

Biomarker A substance that can be used as an indicator of a change in physiology, pathophysiology, or disease state.
Carcinogen A substance that can cause cancer.
Chronic exposure Exposure to a chemical for 365 days or more, as specified in the Agency for Toxic Substances and Disease Registry Toxicological Profiles.
Cocarcinogen A substance that is not carcinogenic by itself but will increase the carcinogenicity of a known carcinogen.
Diels–Alder reaction A cycloaddition reaction between a conjugated diene and alkene. In this chemistry the result is a six-membered carbon (cyclohexene) ring.
Diol epoxide A chemical compound containing two hydroxyl groups and a cyclic ether with three ring atoms.
Mutagen A substance that causes mutations. A mutation is a change in the genetic code or deoxyribonucleic acid sequence that is inherited. Mutations can lead to birth defects, miscarriages, or cancer.
***o*-Quinone** A chemical compound with two carbonyl groups occupying adjacent positions on an aromatic ring.
Radical cation A free radical species that carries a positive charge.
Teratogen A chemical that causes birth defects.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a large group of diverse organic compounds that contain two or more fused aromatic rings ranging from the two-ring naphthalene and naphthalene derivates to complex ring structures containing up to 10 rings. PAHs with up to six fused aromatic rings are often known as 'small' PAHs, whereas those containing more than six aromatic rings are called 'large' PAHs. PAHs have also been classified into alternant and nonalternant compounds. Alternant PAHs are those compounds composed solely of fused six-member benzene rings, whereas nonalternant PAHs contain both six-member benzene and five-member carbon rings. Common structural features of PAH compounds are illustrated in Figure 1. Differences in the configuration of rings may lead to differences in properties. In their purest form, PAHs are solids with low volatility at room temperature and range in appearance from colorless to white or pale yellow–green. They are relatively insoluble in water and most can be photooxidized and degraded to simpler substances.

PAHs are formed by condensation of smaller organic compounds by pyrolysis or pyrosynthesis. Smaller organic compounds are pyrolyzed at a high temperature and the free radicals produced join together as aggregate large PAH molecules (pyrosynthesis). Diels–Alder-type rearrangements are commonly involved in the formation of the PAHs. Temperature is an important factor to affect both the structure and diversity of the PAHs formed. Large PAHs are formed at lower levels than small PAHs due to the kinetic limitation in their production through addition of successive rings. In addition, with many more isomers possible for larger PAHs, the occurrence of specific structures is much lower.

PAHs are ubiquitous environmental pollutants. They are not only found naturally in the environment but they can also be man made. PAHs are formed as a result of incomplete combustion of carbon-containing materials, such as wood, coal, oil, gas, or biomass. They are also created in car and diesel exhaust, smoked or charbroiled food, and are present in cigarette smoke condensate, and tobacco products. According to their origins, PAHs are classified into pyrogenic PAHs (Figure 2) arising from fossil fuel combustion and petrogenic PAHs (Figure 3) that are unique to crude oil and contaminate water after an oil spill. Petrogenic PAHs differ in structure to pyrogenic PAHs in that they are either extensively alkylated or oxygenated to yield PAH quinones.

PAHs are complex mixtures of hundreds of chemicals, including derivatives of PAHs, such as nitro- and hydroxy-PAHs, as well as heterocyclic PAHs. In the 1970s, the United States Environmental Protection Agency (US EPA) listed 16 PAHs as priority pollutants. These priority PAHs include naphthalene, acenaphthylene, acenaphthene, fluorene, anthracene, phenanthrene, fluoranthene, pyrene, chrysene, benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene (B[*a*]P), indeno[1,2,3-*cd*]pyrene, benzo[*g*,*h*,*i*]perylene, and dibenz[*a*,*h*]anthracene (Figure 2). Among these priority PAHs, B[*a*]P, a known human carcinogen, is commonly used as an indicator for PAH exposure.

PAHs are present in air, soil, water, and food, and routes of exposure include inhalation, dermal contact, and ingestion. Some exposures may involve more than one route simultaneously affecting the total absorbed dose (such as dermal and

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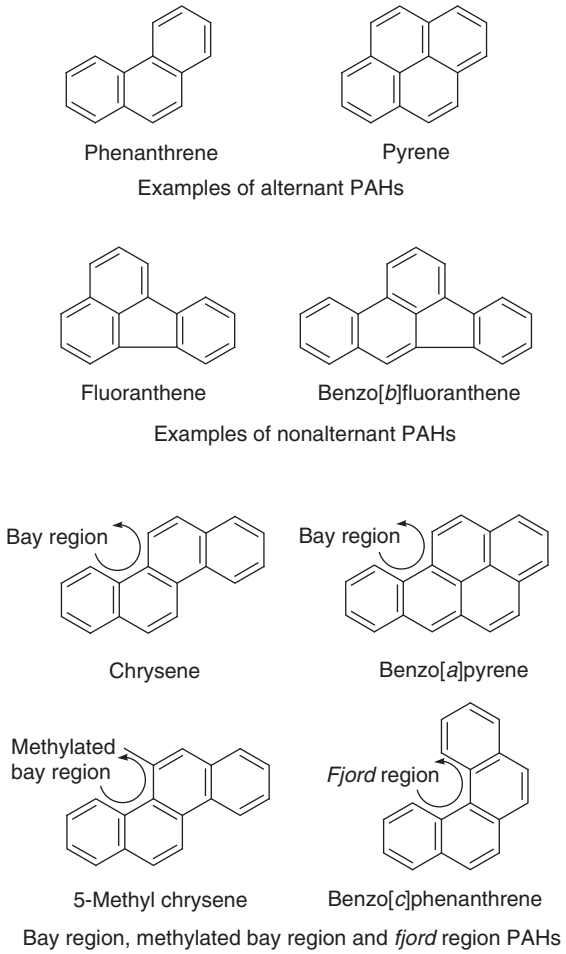

Figure 1 Common structural features of PAH compounds.

inhalation exposures from contaminated air). Occupational sources of exposure mainly involve workers in coal gasification plants, municipal incinerators, smoke houses, and aluminum production facilities. Nonoccupational sources of exposure mainly involve diet, smoking, and burning of coal and wood. In most cases, intake of food is the major involuntary exposure route of PAHs. Therefore, this article will only discuss the occurrence of PAHs in food, health concerns, analysis, mitigation and biomonitoring, and guidelines to reduce PAHs in food.

Occurrence in Food

The food groups with the highest concentration of PAHs include meat and meat products, fish and marine products, dairy products (cheese, butter, cream, and milk), vegetables, cereals, beverages (roasted coffee and tea), and vegetable and animal fats and oils. The occurrence of PAHs in food is mainly due to food preparation and processing techniques such as smoking, roasting, grilling (which includes barbequing), frying, drying, and steaming. The growth of crops and plants in contaminated soils or the presence of marine life or fish in contaminated water provides a portal for PAHs to gain entry into the food chain. In some cases, the presence of PAHs in food products occurs through the use of contaminated packaging material.

Food Preparation and Processing Techniques

Food preparation and processing techniques that increase the amount of PAHs in foods include smoking, roasting, grilling, frying, drying, and steaming, etc. Among them, smoking, roasting, and grilling are the major sources of PAHs in food.

Smoking is often used to preserve and flavor meat, fish, and cheese, etc. The amount of PAHs generated from smoking is influenced by many factors such as temperature of smoke generation, type of wood fuel, oxygen concentration during smoke generation, and type of smoker. The lowest concentration of PAHs was detected in the smoke from the combustion of tree heather wood in the drum, whereas the highest concentration of PAHs was reached when rock rosewood was used in the kiln. Formation of PAHs generated from roasting and grilling (broiling) is due to the pyrolysis of edible oil or fat on the surface of foods, or the pyrolysis of food contacting with flames at high temperature (150–400 °C). Grilling over hot coals (barbequing) is a source of PAHs when meat is cooked in close contact with the hot flame and the meat has not been trimmed of fat or skin. It also occurs when fat drips onto the hot coals. The preparation of coffee beans by roasting is typically performed at high temperatures (180–300 °C) as well. Direct-fired roasting can lead to a higher amount of PAHs in coffee due to the direct contact of the coffee beans with the burner flame. Indirect-fired roasting leads to a lower amount of PAHs in coffee though the combustion gases from the burner do make a contact with the coffee beans.

The process of frying is usually much faster than roasting or grilling (broiling) and thus results in a lower amount of PAHs. If vegetable oil or fat is overheated, the PAHs formed in the cooking fume or oils can be transferred to the kitchen air or fried foods. The process of drying applied to meats, fruits, cereal, grains, oilseeds, and tea can cause the contamination by PAHs from the fine particulate matter smaller than 2.5 μm (PM2.5). In some cases, tea leaves are dried using combustion gas from burning wood, oil, or coal, where combustion products may come into contact with tea leaves and the tea product can absorb the PAHs formed. Steaming has an advantage over other cooking methods because it can minimize or avoid pyrolysis of oil or fat during the cooking process, and thus steaming is considered a healthy cooking technique.

Growth of Crops and Plants in Contaminated Soil

Most PAHs are lipophilic in nature and are absorbed on and concentrated in PM2.5, commonly known as soot. Some atmospheric fine particulate matter containing PAHs contaminate the soil after rain. Consequently, crops such as cereals, grain, fruits, and vegetables grown in contaminated soil will absorb the PAHs. The waxy surface of vegetables and fruits can concentrate PAHs through surface adsorption, and thus the concentration of PAHs is generally greater on plant surfaces (peel and outer leaves) than on internal tissue. In addition, when plants used for fodder are grown in contaminated soil and eaten by grazing herds, PAHs can unintentionally end up in dairy products.

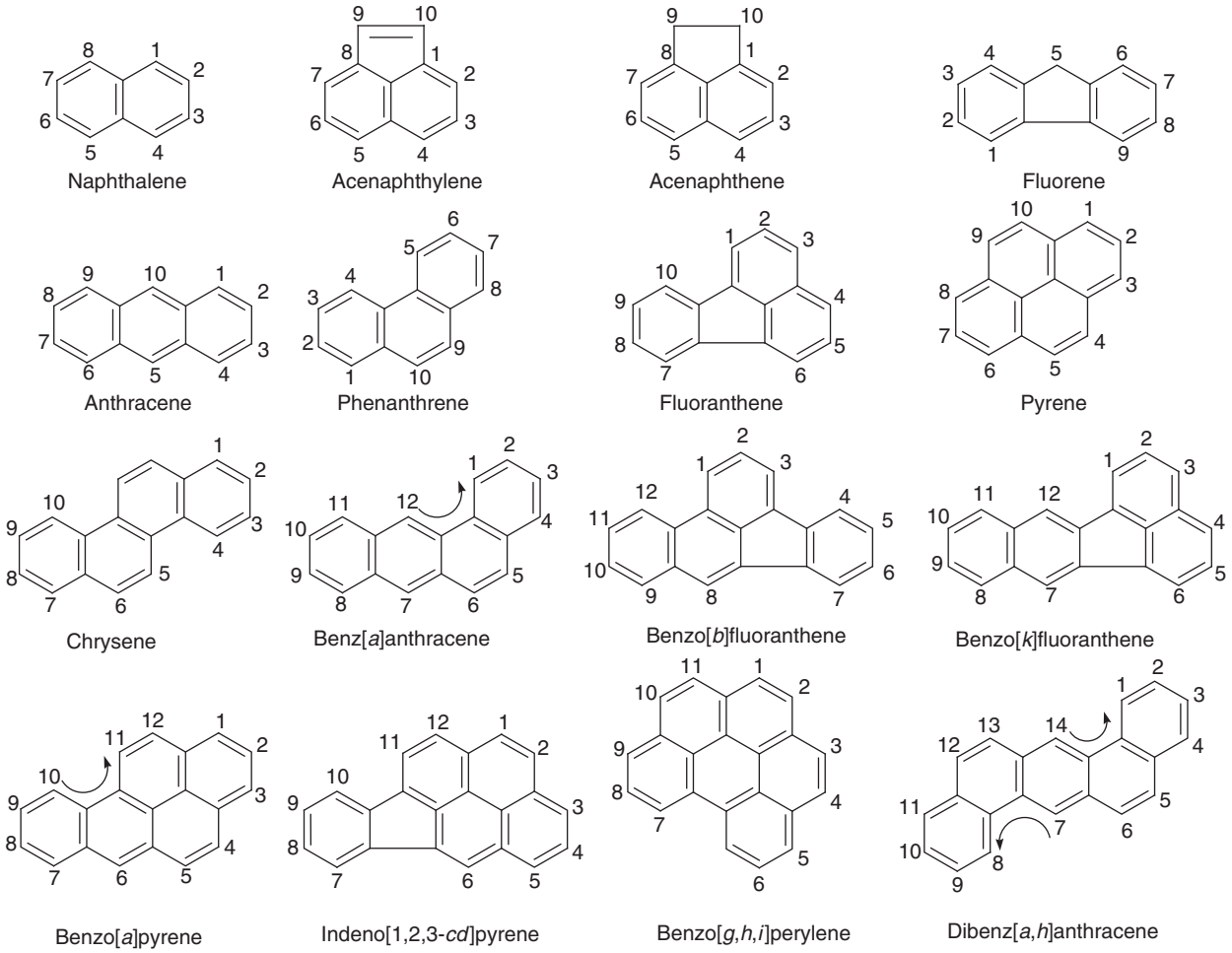

Figure 2 The 16 priority pyrogenic PAHs listed by the United States Environmental Protection Agency (US EPA).

Seafood and Fish Contamination

Oceans and waterways contaminated with crude or engine oil due to either oil tanker disasters and oil spills, for example, Deepwater Horizon, or industrial waste will create sediments more heavily contaminated with both petrogenic and pyrogenic PAHs. Filter-feeding bivalves such as mussels, clams, and oysters near contaminated sediments are the first invertebrate targets for exposure to PAHs as they filter large amounts of water and have a very poor metabolic clearance for PAHs. PAHs can then move through the food chain through predation. The teleosts fish can rapidly metabolize PAHs. In some instances, the metabolites of PAHs can be more harmful than the parent PAHs. Thus both shellfish and finfish are affected.

Use of Contaminated Packaging Materials

Owing to the permeable nature of plastic packaging materials, for example, polyethylene, PAHs can impregnate the material and can be retained on recycling. PAHs present in recycled plastic bags can then diffuse into the food source. Jute bags used for transport and storage of hazel nuts, coffee, cocoa beans, etc., can be contaminated with PAHs, as they are present in the batching oil used to soften the jute fabric.

Health Concerns

Cancer risk has been widely accepted as the most significant health concern associated with PAH-contaminated food. PAHs comprise the largest group of chemical compounds known to cause cancer. However, some PAHs are not carcinogenic but may act as cocarcinogens. PAHs have a low acute toxicity to humans. Based on animal studies the most relevant sites of tumor formation following PAH ingestion are the gastrointestinal tract and digestive system.

Exposure of humans to a single PAH is rare because PAHs always exist in complex mixtures. The mixture is not always of constant composition and thus makes the assessment of health risk difficult. Nevertheless, epidemiological studies support an association between exposure to individual PAH and occurrence of human cancer. Such individual PAHs serve as markers for exposure to the entire PAH mixture. In general, PAHs are carcinogenic when they contain four or more fused

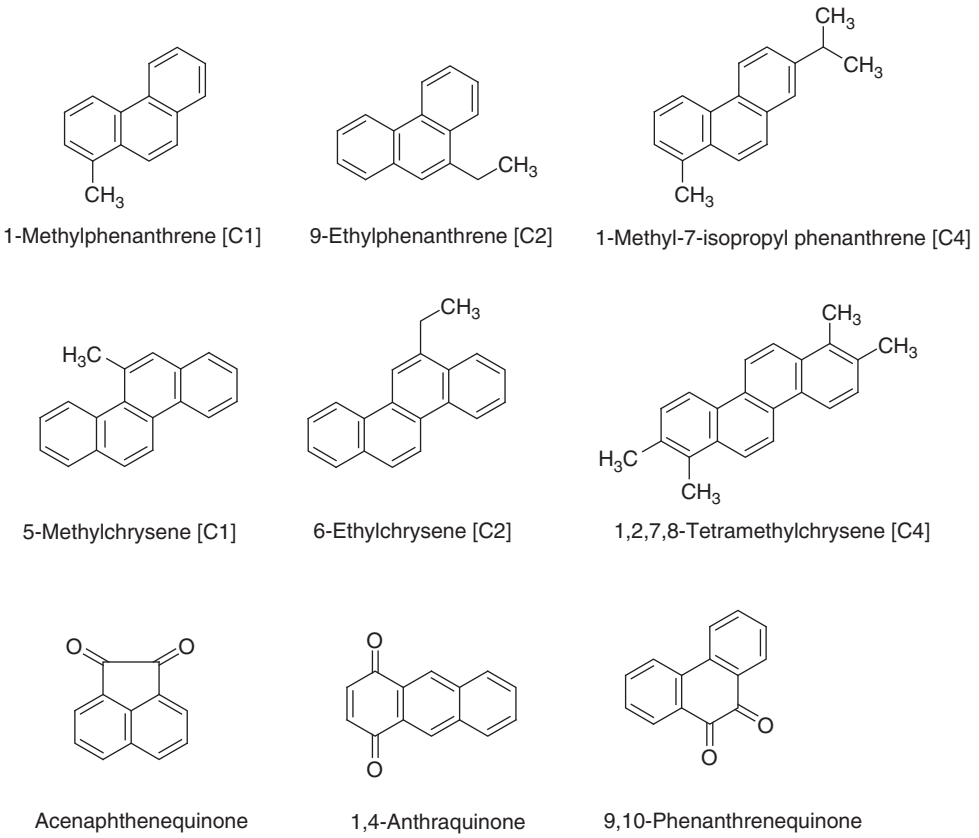

Figure 3 Representative petrogenic PAHs.

rings and a terminal benzene ring, which creates a bay region in the structure (Figure 1). Introduction of a methyl group in the bay region or formation of a *fjord* region generally increases the carcinogenicity of the PAH. Four priority PAHs listed by the US EPA contain a bay region and are strongly carcinogenic in experimental animals. These four carcinogenic priority PAHs are chrysene, benz[*a*]anthracene, B[*a*]P, and dibenz[*a*,*h*]anthracene.

PAHs by themselves are biologically inert and to exert their carcinogenic, mutagenic, and teratogenic effects they must be metabolically activated to biologically reactive intermediates, which covalently modify deoxyribonucleic acid (DNA) to form DNA adducts that may result in mutation. Three pathways of metabolic activation of B[*a*]P, a representative PAH, have been proposed in the literature (Figure 4). These three pathways involve the formation of the following reactive intermediates: radical cations (formed by P-450 peroxidases); the *anti*-diolepoxides (formed by P450 1A1/1B1 monooxygenases and epoxide hydrolase); and the reactive and redox-active PAH *o*-quinones (formed by the combined action of P-450 isozymes, epoxide hydrolase, and aldo-keto reductases (AKRs)).

In addition to the carcinogenicity of PAHs, data from animal studies show that certain PAHs can produce mutagenic/genotoxic, reproductive (both male and female offspring) and developmental, immunotoxic, cardiovascular, and neurologic effects. The mutagenicity/genotoxicity of PAHs shows considerable overlap with carcinogenicity, in agreement with the mechanistic link between PAH–DNA adduct formation, mutations, and cancer outcome following PAH exposure. The reproductive and developmental toxicity of PAHs occurs because their lipophilic character enables them to cross the placenta into the developing embryo and fetus. The immunotoxic effect following exposure to PAHs is most often reported as immunosuppression, which is associated with an increased susceptibility of exposed individuals to the development of cancers or infectious diseases. However, the critical endpoint for the health risk evaluation is the well-documented carcinogenicity of several PAHs.

Analysis

Foods are analyzed for PAHs to ensure their levels are below the legislative limits of concern estimated by government agencies, and to detect fraud and compliance with labeling. Much of the analysis is focused on the detection of pyrogenic PAHs and thus measurements of petrogenic PAHs are not routinely made. Because PAHs are present as mixtures, levels of different pyrogenic PAHs are quantified and summed against the toxicity equivalent quotient (TEQ) for B[*a*]P which has a TEQ equal to 1.0. For example, as chrysene is 1000-fold less carcinogenic than B[*a*]P, its concentration level would be multiplied by 0.001. The analysis of PAHs in food samples presents challenges due to the presence of high amounts of interfering compounds present in complex food matrices.

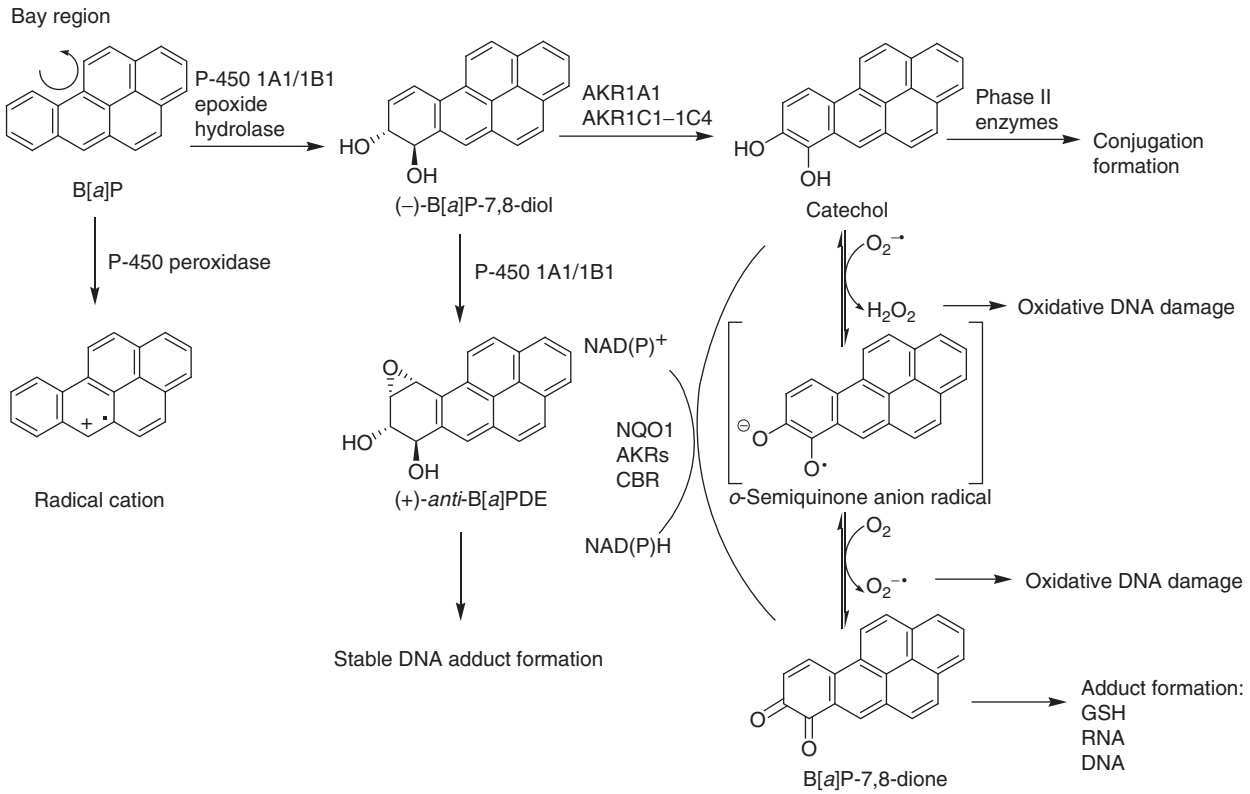

Figure 4 Pathways of PAH metabolic activation using B[*a*]P as an example. Abbreviations: AKR, aldo–keto reductase; (+)-*anti*-B[*a*]PDE, (+)-*anti*-7*beta*,8*alpha*-dihydroxy-7,8,9,10-tetrahydro-9*a*,10*a*-oxo-benzo[*a*]*pyrene*; CBR, carbonyl reductase; GSH, glutathione; NADP, nicotinamide adenine dinucleotide phosphate; NQO1, NADPH: quinone oxidoreductase; P-450, cytochrome P-450; RNA, ribonucleic acid.

Thus proper extraction and clean-up procedures are needed to minimize this effect.

Direct measurement of PAHs in food is conducted using high-performance liquid chromatography (HPLC) coupled with ultraviolet–visible (UV-Vis) or fluorescence detection, and gas chromatography coupled with mass spectrometry (GC–MS). The first official method for the analysis of B[*a*]P in food was based on the ultraviolet absorption of an extract of the food purified by thin layer chromatography in the 1970s. The International Organization for Standardization (ISO) established the analysis for the determination of an enlarged set of PAHs in edible fats and oils by HPLC with fluorescence detection. The sensitivity of a fluorescence detector is generally 20–30 times than that of a UV-Vis detector. In many instances, a fluorescence and UV-Vis detector are placed in tandem for PAH analysis to provide more information on analyte identity. GC–MS typically can achieve a better separation of complex mixtures of PAHs from food extracts. Its combination with isotopically labeled internal standards can accomplish exact quantitation and unambiguous structural identification of the individual PAHs in the mixture.

In addition to direct measurement of PAHs in food sources, it is also possible to measure PAH metabolites as an indicator of past exposure in biological tissues and fluids including blood, urine, and feces, following ingestion of PAH-contaminated foods. Liquid chromatography coupled with mass spectrometry (LC–MS) methods have been developed for PAH metabolites. The softest ionization technique, electrospray ionization, is suitable for the analysis of PAH metabolites, whereas the atmospheric pressure chemical ionization technique can be used to measure parent PAHs due to their better ionization properties under these conditions. The most exact quantitation of PAH metabolites is by LC–MS when this is coupled to the use of isotopically labeled internal standards for each analyte. In most cases, HPLC coupled with UV or fluorescence detection can be used together with LC–MS to simultaneously detect both parent PAHs and their metabolites.

There is considerable evidence that PAHs are enzymatically converted to highly reactive metabolites that bind covalently to macromolecules such as DNA. Thus PAH–DNA adducts may be used as an indicator of exposure in research settings and can be measured in a variety of biological samples of humans and animals. Sensitive methods available to detect PAH–DNA adducts include immunoassays, i.e., enzyme-linked immunosorbent assays (ELISA), radioimmunoassay (RIA), dissociation-enhanced lanthanide fluoroimmunoassay, and ultrasensitive enzyme RIA, [^{32}P]- and [^{35}S]-postlabeling with scintillation counting, surface-enhanced Raman spectroscopy, and synchronous luminescence spectroscopy.

Mitigation and Biomonitoring

It is difficult to completely eliminate PAHs from food to reduce health risks due to their ubiquitous nature. However, the

levels of PAHs in food can be reduced by educating manufacturers and consumers, and by reducing emissions of PAHs to the environment and reducing contamination of the water supply. For instance, the use of PAH-producing commercial smoking and drying processes of food should be avoided. Appropriate advice should be given to consumers who prepare smoked, roasted, grilled, and barbequed food to reduce intake levels of PAHs. Cereals, grain, fruits, and vegetables grown in contaminated soil should be thoroughly washed or peeled before consumption. Seafood and fish from contaminated oceans and waterways should only be consumed if the levels of pyrogenic and petrogenic PAHs are below the limits of concern. Appropriate food packaging such as cellulose-based wrapping can be used to minimize the contamination with PAHs.

Individual risk assessment could be conducted by measuring biomarkers of exposure and effect. Historically, biomarker studies have been conducted in occupationally exposed individuals and smokers. PAHs and their metabolites would be biomarkers of exposure, whereas fingerprints of the exposure long after the metabolites have been cleared would be biomarkers of effect. 1-Hydroxypyrene, a metabolite of pyrene, has been widely used as a urinary biomarker of PAH exposure. In contrast to other PAH metabolites, which are excreted mainly in feces, 1-hydroxypyrene is excreted in urine. The advantage of measuring 1-hydroxypyrene is the relatively high concentrations (2–10%) of pyrene in all PAH mixtures, whereas the disadvantage is that the exposure to the more potent carcinogenic PAHs such as B[*a*]P is not measured. Measurement of 3-hydroxy-B[*a*]P, a metabolite of B[*a*]P, has been historically useful for detecting occupational exposures to PAHs, but lacks sufficient sensitivity to detect nonoccupational exposures to PAHs in food. Another drawback of measuring 3-hydroxy-B[*a*]P is that it is not a metabolite derived from a biological intermediate that might be related to the carcinogenic process. Recently, progress has been made in measuring B[*a*]P-tetraols in the urine of smokers that are derived from the hydrolysis of the diol epoxides and are considered ultimate carcinogens.

As an alternative, PAH–DNA adducts formed from reactive metabolites (mainly diol epoxides) of B[*a*]P and other carcinogenic PAHs have been detected in human bio-specimens following intake of charbroiled meat, exposure to tobacco smoke, or by living in polluted areas. As covalent binding of electrophilic PAH metabolites to DNA is thought to be a key step in the initiation of cancer, measurement of PAH–DNA adducts could be an intermediate biomarker of effect and also of the dose of the ultimate reactive metabolite. In addition, adducts of PAHs with proteins such as albumin and hemoglobin could be promising biomarkers of effect.

Guidelines to Reduce PAHs in Food

World Health Organization (WHO)

The Food and Agriculture Organization of the United Nations and the WHO Joint Expert Committee on Food Additives (JECFA) stated that it is impossible to assume that a safe threshold of PAHs exposure exists, as the adverse health effects of PAHs are due to their toxic metabolites. JECFA evaluated 13 genotoxic and carcinogenic PAHs, namely benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, B[*a*]P, chrysene, dibenz[*a,h*]anthracene, dibenzo[*a,e*]pyrene, dibenzo[*a,h*]pyrene, dibenzo[*a,i*]pyrene, dibenzo[*a,l*]pyrene, indeno[1,2,3-*cd*]pyrene, and 5-methylchrysene. Most of these 13 PAHs are in common with the 16 priority PAHs listed by the US EPA except dibenzopyrenes and 5-methylchrysene. JECFA recommended that B[*a*]P can be used as a surrogate marker of exposure to all these PAHs. In 2009, the Codex Alimentarius Commission (CAC) adopted the first guideline for reducing PAHs intake from smoked or dried food. This guideline emphasized that the combustion of fuel both in smoking and direct drying processes involved in food preparation leads to the formation of PAHs that are possible human carcinogens. In view of the wide use of smoking and direct drying in both food industry and private households, this guideline can also be used as an instructional and education tool for manufacturers and consumers.

Europe

In 2002, the Scientific Committee on Food (SCF) of the European Union (EU) assessed 33 PAHs in food and identified 15 priority PAHs that possess both genotoxic and carcinogenic properties. These 15 EU priority PAHs are benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, benzo[*g,h,i*]perylene, chrysene, cyclopenta[*cd*]pyrene, dibenz[*a,h*]anthracene, dibenzo[*a,e*]pyrene, dibenzo[*a,h*]pyrene, dibenzo[*a,i*]pyrene, dibenzo[*a,l*]pyrene, indeno[1,2,3-*cd*]pyrene, 5-methylchrysene, and B[*a*]P. As recommended by SCF, B[*a*]P was set as a marker for the occurrence and effect of carcinogenic PAHs in food. Because other PAHs are less carcinogenic than B[*a*]P, the total exposure to PAHs would have to exceed that seen from B[*a*]P alone. It is also stated by SCF that the estimated maximum daily intake of B[*a*]P from food is approximately 420 ng B[*a*]P per person, which is approximately 5–6 orders of magnitude lower than the daily doses observed to induce tumors in experimental animals.

In 2005, the European Commission (EC) Regulation 208/2005 was introduced in response to food-contamination problems and 15 EU priority PAHs plus benzo[*c*]fluorene were selected for further investigation by the EC to enable the assessment of the risk of long-term adverse health effects following dietary intake of PAHs. This EU legislation set maximum allowed concentrations for B[*a*]P in various food products in the range 1–10 $\mu g\,kg^{-1}$, and for B[*a*]P and benz[*a*]anthracene in liquid smoke flavoring products of 10 and 2 $\mu g\,kg^{-1}$, respectively. The maximum levels of B[*a*]P in different types of food that are allowed are listed in Table 1. The EC also adopted the 2005/10/EC directive, which describes methods of sampling, sample preparation, and criteria for methods of analysis for B[*a*]P in food.

In 2011, the EC published Regulation 835/2011, an amendment to Regulation 1881/2006 based on the level of B[*a*]P in seafood. The Regulation 835/2011 sets the new maximum permitted levels of four PAHs (B[*a*]P, benz[*a*]anthracene, benzo[*b*]fluoranthene, and chrysene) in seafood. The new levels

Table 1 The limits of maximum level of benzo[*a*]pyrene in different types of food (Commission Regulation of EU, No. 208/2005)

Product	*Maximum level (μg kg^{-1} wet weight)*
Oil and fat	2.0
Foods for infants and young children	1.0
Smoked meats and smoked meat products	5.0
Muscle meat of smoked fish and smoked fishery products, excluding bivalve mollusks	5.0
Muscle meat of fish, other than smoked fish	2.0
Crustaceans and cephalopods, other than smoked	5.0
Bivalve mollusks	10.0

will be introduced on 1 September 2012 and a new set of lower levels will become effective from 1 September 2014. There will be no maximum limit for fish and fishery products that have not been smoked, other than fresh, chilled, or frozen bivalve mollusks. Interestingly, these limits are not based on levels of petrogenic PAH whose TEQ relative to B[*a*]P is unknown.

North America

In the United States, the presence of carcinogens in food is covered by the 'Delaney Clause' of the Federal Food, Drug and Cosmetic Act enforced by the Federal Food and Drug Administration (FDA). Before the 1958 amendment, a substance added to food was presumed safe until it was proven otherwise. The amendment prohibits the FDA approving the use of a food additive found to cause cancer in animals or humans. It has been criticized as being too restrictive by setting a zero level of risk. It applies to only 400 of the 2700 substances that were added to food before the amendment as information on the remaining additives is lacking.

The food preparation and processing techniques described in this article are still permitted to take place. For smoke flavoring agents, the United States have allowed the use of these aromas, provided that B[*a*]P residue does not exceed 0.03 μg kg^{-1} in the final products. The Committee on Diet Nutrition and Cancer appointed by the National Research Council of the United States stated that of the PAHs that commonly occur in the American diet, only three (B[*a*]P, benz[*a*]anthracene, and dibenz[*a*,*h*]anthracene) have been found to be carcinogenic in animals following oral administration.

In Canada, exposure from media other than air has not been taken into account. Only five PAHs (B[*a*]P, benzo[*b*]-fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, and indeno[1,2,3-*cd*] pyrene) are considered to be entering the environment in a quantity or concentration or under conditions that may constitute a danger to human life or health as defined under the Canadian Environmental Protection Act. The estimated intake (based on B[*a*]P equivalents) of three of the selected PAHs (i.e., B[*a*]P, benzo[*b*]fluoranthene, and benzo[*k*]fluoranthene) for an adult is approximately twofold greater than that inhaled; however, it is likely that there are considerable variations in toxicokinetics and potency by ingestion versus inhalation.

Asia

In China, maximum amounts of B[*a*]P in foods have been set within the regulation GB7104-1994 by the Ministry of Health since 1994. This regulation limits the content of B[*a*]P to 5 μg kg^{-1} in smoked meat, smoked fish products, and cereals. The content of B[*a*]P in edible vegetable oils is set to 10 μg kg^{-1}.

In Singapore, the practice for the reduction of contamination of food with PAHs from smoking and direct drying processes is required by the Agri-Food and Veterinary Authority to comply with the standards recommended by CAC.

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See also: Analytical Methods: Overview of Methods of Analysis for Chemical Hazards. Disciplines Associated with Food Safety: Food Safety Toxicology. Foodborne Diseases: Overview of Chemical, Physical, and Other Significant Hazards. Institutions Involved in Food Safety: FAO/WHO Codex Alimentarius Commission (CAC). Public Health Measures: Fundamentals of Food Legislation; Health Education, Information, and Risk Communication; Monitoring of Contaminants. Safety of Food and Beverages: Fruits and Vegetables; Milk and Dairy Products; Seafood

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HAZARDS OF FOOD CONTACT MATERIAL

Contents

Bisphenol A and Endocrine Disruption

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Glossary

Developmental Origins of Health and Disease (DOHaD) hypothesis Postulates that adult diseases, including obesity, diabetes and cancer, can originate due to poor environmental conditions experienced during early development, including gestation.

Endocrine disruptor An exogenous chemical, or mixture of chemicals, that can interfere with any aspect of hormone action.

Low dose Doses in the range of human exposures to endocrine disruptors, which are at doses below those used for traditional toxicological studies.

'Low dose' hypothesis The proposal that endocrine-disrupting chemicals have effects, especially on reproduction and development, at very low doses; the effects observed in exposed animals will occur at similar doses in humans; and humans are being affected by environmental exposures to endocrine disruptors.

Xenoestrogen A synthetic or natural compound, not produced by the body, that mimics the actions of estrogen.

Introduction

There are approximately 80 000 chemicals currently in commerce, and 2000 new chemicals are thought to be added to this list each year. The US Food and Drug Administration (FDA) indicates that at least 1200 of these chemicals have properties that can interfere with the endocrine system by mimicking or blocking the actions of hormones, altering hormone synthesis or secretion, or altering the way that cells respond to hormones. In total, this class of chemicals is referred to as 'endocrine disruptors' or 'endocrine-disrupting chemicals' (EDCs).

A large number of these chemicals come into contact with food. This article will focus on reviewing the science of endocrine disruption and how these chemicals enter the food supply. It will provide an overview of the kinds of chemicals that are known to contaminate food, and then focus on one example: bisphenol A (BPA), an EDC that has been extremely well studied by academic and government scientists and regulatory agencies. This article will cover the controversies associated with BPA and consider whether the lessons learned from this chemical have wider implications for other EDCs found in food.

Overview of EDCs

In the 1950s and 1960s, Rachel Carson's work defined the environmental movement. With the publication of her book, 'Silent Spring,' the public became aware that exposures to chemicals in the environment could affect the health of wild animals and could be a harbinger for devastating effects on human populations as well. Although Carson did not identify environmental contaminants as 'endocrine disruptors' per se, she did note that many of these chemicals were altering the metabolic machinery and nervous systems of exposed animals. She also was one of the first to note that many of these chemicals were bioconcentrating in the food chain, and this bioconcentration was responsible for at least some of the observed toxicities. Chemicals applied to trees to kill insects were accumulating in those insects' bodies and were then consumed by birds. As the birds ate more and more insects, as well as other nontarget organisms (i.e., worms and berries), the chemicals accumulated at higher levels in their bodies and produced adverse effects including egg shell thinning, neuromuscular diseases, wasting, and death. Remarkably, Carson noted that the effects of these chemicals were most profound on the young hatchling birds rather than their parents.

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Throughout the 1980s and 1990s, Carson's work was expanded on by a number of wildlife biologists including Theo Colborn. Colborn also noticed that many environmental chemicals were affecting the health of wildlife, yet her detailed work was the first to connect the effects of many of these chemicals to alterations in the endocrine system. The connection she drew was that animals were being affected in a variety of ways: diminished reproduction, thyroid problems, altered behavior, and metabolism changes including wasting; each of these factors is controlled by the endocrine system. In 1991, Colborn summoned a small group of scientists from a diversity of backgrounds including ecology, endocrinology, medicine, law, reproductive physiology, toxicology, wildlife management, and cancer biology to discuss their own findings on the effects of environmental chemicals on gene imprinting, sexual differentiation and reproductive function, neurobehavioral development, and autoimmune diseases in mammals and fish. By the end of the meeting, the scientists had agreed on the term 'endocrine disruptor,' writing: "We are certain of the following: A large number of man-made chemicals that have been released into the environment, as well as a few natural ones, have the potential to disrupt the endocrine system of animals, including humans."

Since that time, a number of definitions for 'endocrine disruptor' have been produced. The US Environmental Protection Agency (EPA) defines an endocrine disruptor as "an exogenous agent that interferes with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development and/or behavior." The World Health Organization (WHO) has produced a similar definition, which states that an EDC is "an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations." Importantly, this definition hinges on the ability to define an 'adverse effect,' which itself is strongly debated in academic and risk assessment communities. Finally, in 2012, representatives from the Endocrine Society, the world's oldest and largest organization devoted to research on hormones, discussed how a simplification of these definitions would provide clarity to the research field. In a rare public health statement, scientists from the Endocrine Society defined an EDC as "an exogenous chemical, or mixture of chemicals, that can interfere with any aspect of hormone action."

Xenoestrogen Hypothesis

In the process of studying EDCs, a group of scientists have also proposed the Xenoestrogen Hypothesis, which theorizes that exposure to hormonally active agents – and in particular those chemicals that mimic the actions of natural hormones – could alter the development of the fetus. These scientists have suggested that *in utero* exposure to EDCs may contribute to endocrine-related diseases including testicular cancer and breast cancer. In fact, numerous epidemiology studies suggest that these and other endocrine-related diseases have increased in incidence in European and the US populations over the past 50 years. Furthermore, studies in developing countries indicate that chronic diseases including diabetes, cardiovascular disease, and even cancers are increasing in incidence, and there has been considerable speculation that chemical exposures, including EDCs, could play a significant role.

'Low Dose' Hypothesis

A related scientific hypothesis that has been proposed is the 'Low Dose' Hypothesis, which suggests that EDCs have effects on exposed animals at doses that are thought to be safe for humans, and humans are also affected by these same environmentally relevant doses. A number of scientists have argued that this concept should not be considered a hypothesis any longer because a significant body of evidence from epidemiology and laboratory animal studies indicates that EDCs affect health endpoints in the range of human exposures. Others, including many risk assessment agencies, maintain that these data are not convincing and point out that epidemiology studies are often not controlled in a way that can conclusively link exposures to particular chemicals to diseases.

The 'Low Dose' Hypothesis, and the data that supports this scientific paradigm, challenges the way the current chemical risk assessments are performed. In traditional toxicology studies, high doses are tested for overt health effects, and then these results are extrapolated using 'uncertainty' factors to calculate a so-called 'tolerable' dose for human exposures, which is assumed to pose no appreciable risk. This dose is rarely tested. Endocrinologists point out that (1) hormones act at extremely low doses in the body, and therefore chemicals that mimic these signaling molecules are also expected to have effects at very low doses; (2) hormones have effects at high doses that are quite different from their effects at low doses. For example, at high doses, estrogen is overtly toxic, but at low doses it has hormonal effects via specific interactions with receptors; (3) the endpoints that are used in traditional toxicology tests may be insufficient to capture the kinds of endpoints that are affected by EDCs.

Developmental Origins of Health and Disease

A final concept that requires attention when considering EDCs is the Developmental Origins of Health and Disease (DOHaD) theory, which proposes that many adult diseases (cancer, obesity, and type 2 diabetes) originate much earlier in life. Some of the earliest evidence for DOHaD came from the exposure of human fetuses to diethylstilbestrol (DES), a potent synthetic estrogen prescribed to pregnant women in the 1940–70s to prevent miscarriage. Not only did this drug have no effect on the ability to maintain pregnancy, but also it was later linked to reproductive tract malformations and cancers in the offspring exposed in the womb. The lesson from DES was two-fold: First, this was an important example of the adverse effect of exogenous hormone exposures during early development, which was not well appreciated at the time, and second, it demonstrated that exposures that occur *in utero* do not necessarily produce congenital birth defects – overt signs of toxicity – but can disrupt organ function in a way that does not manifest until puberty or adulthood.

Endocrine Disruptors in Food

Food and food packaging is an important source of chemical exposures. Some of these compounds are found naturally in foods, whereas others are introduced during the growing, processing, or packaging of food. Importantly, food as consumed should be considered as a source of chemical mixtures that can have the same mechanism of toxicity, or unpredictable interactions can increase toxicity. Thus, although many chemicals are studied as single compounds in *in vitro* and *in vivo* laboratory studies, a number of mixture studies of EDCs have suggested that EDCs with similar modes of action can have additive or even synergistic effects when applied in combination. This means that the toxicity information available from the study of single chemicals must be considered carefully when real-world exposures are concerned.

Phytoestrogens

One source of EDCs is in fact natural, i.e., plant-based (phyto)estrogens. A number of plants contain these plant-based estrogens, and evolutionary biologists have considered the production of these hormone-mimicking agents to be a reactionary response by the plant to protect against herbivory; animals that consume large quantities of plants that contain estrogens are likely to experience infertility. Thus, future generations of the herbivore are less likely to eat future generations of the plant.

Although many plants consumed by humans contain phytoestrogens, one of the best studied examples is soy. Many studies have been dedicated to understand how soy, which contains several individual estrogenic compounds as well as the individual compounds themselves, alters health endpoints in humans. A number of studies have noted that populations with diets high in soy have lower rates of cancer, leading some to conclude that soy – and its phytoestrogens – is protective against cancers. Yet, other studies from laboratory animals indicate that the timing of exposure to these agents is important in considering the health outcomes of consumption. Neonatal exposures to genistein and other phytoestrogens produce alterations in mammary gland development, development of sexually dimorphic regions of the brain, and other estrogen-sensitive endpoints. Thus, even though phytoestrogens are considered 'natural' products, their hormonal activity must be considered in the context of the developmental period of exposure.

Pesticide Residues

A number of EDCs are used as herbicides, insecticides, and fungicides on food crops, and low doses of these chemicals are retained as residues on both fresh and processed plants. Additionally, as mentioned above, many of these chemicals can contaminate the food chain when chemicals sprayed on crops are fed to farm animals. This is not a new problem. In the 1960s, Rachel Carson noted that dichlorodiphenyltrichloroethane (DDT) could be measured in the bodies of the vast majority of Americans, writing, "Among the general population with no known gross exposures to insecticides it may be assumed that much of the DDT stored in fat deposits has entered the body in food. To test this assumption, a scientific team from the US Public Health Service sampled restaurant and institutional meals. *Every meal sampled contained DDT.* From this, the investigators concluded, reasonably enough, that 'few if any foods can be relied upon to be entirely free of DDT.' Analysis of prison meals disclosed such items as stewed dried fruit containing 69.6 ppm and bread containing 100.9 ppm of DDT!" Although DDT has not been used as an insecticide in the US for more than four decades, it (and its metabolites) continues to be measured in human bodies as well as in food.

Industrial Chemicals and Byproducts

A number of industrial chemicals and byproducts are some of the most potent disruptors of the endocrine system. Among these are the polychlorinated biphenyls (PCBs), which had a number of industrial applications. Because of accidents and improper disposal, these chemicals were introduced into the environment, where they persist for long periods of time because of their stability. Another group of EDCs is the dioxins, which consists of a large number of related compounds including dibenzofurans. These are produced as unwanted byproducts in the production of chemical reactions as well as certain natural processes such as forest fires. As environmental pollutants, these so-called 'persistent organic pollutants' (POPs) accumulate in various biota and bioaccumulate in the food chain. POPs include a number of organochlorine pesticides that are no longer used but continue to persist. Monitoring of human milk for POPs undertaken in response to the Stockholm Convention has indicated that women in all countries have been exposure to these exogenous chemicals, although the distribution of POPs differs greatly between industrialized and developing countries. For example, women in developing countries have higher levels of DDT and other pesticides, whereas women in industrialized countries have higher levels of PCBs and dioxins.

Food Packaging

A final source of EDC exposures from food is the packaging itself. A large number of epoxy resins, plastics, and other food-contact materials contain chemicals with endocrine activity, and these chemicals can leach from these food-contact materials into the food itself. Although, like pesticide residues, these chemicals are likely to be found only in small doses, even these small doses are of concern because of their ability to interfere with the endocrine system. As mentioned above, hormones act at extremely low doses, in the part-per-trillion and part-per-billion range. Thus, even small amounts of compounds leaching from food-contact materials are a public health concern. The remainder of this article will focus on one chemical, BPA that is used in a large number of food-contact applications.

Overview of BPA

BPA was first synthesized in 1891 via the combination of two equivalents of phenol with one equivalent of acetone in the

presence of an acid catalyst. It was tested in the 1930s for hormonal activities and was found to have estrogenic properties, although it was never developed for pharmaceutical use. In the 1950s and 1960s, BPA began to be used in the production of plastics and epoxy resins, which eventually would contribute to numerous consumer products. At that time, it was not fully tested for toxicity, and even though it was known to be an estrogen, its hormonal activities were ignored for decades. Today, BPA is one of the highest volume chemicals produced worldwide with approximately 6 billion pounds produced per year and approximately 100 t inadvertently released into the atmosphere during the production process.

Our understanding of BPA's hormonal activities and other toxicities continues to grow as assays examining additional receptor-mediated events are developed. Both *in vitro* and *in vivo* assays demonstrate that this chemical is an estrogen. For years it was considered a 'weak' estrogen because it binds to the nuclear estrogen receptors (ER) with a much lower affinity than 17β-estradiol, a natural estrogen produced in the body. Yet, recent studies indicate that BPA can be equally potent as estradiol in specific biological contexts and binds to membrane ERs and G protein-coupled receptor 30 (GPR30), a type of membrane-bound receptor, with the same affinity as estradiol. Even though BPA is classified as a xenoestrogen, additional studies indicate that it has other endocrine-disrupting properties including the ability to bind estrogen-related receptor γ and the aryl hydrocarbon receptor. BPA also alters thyroid hormone signaling and weakly binds the androgen receptor. Thus, the effects of BPA in a biological context are unlikely to exactly mimic the effects of endogenous estrogens because this chemical can activate multiple pathways.

BPA in Food and Food Packaging

One of the best studied aspects of BPA is its use in food-contact materials. BPA is used as a component of the epoxy resins that line food and beverage cans to prevent interactions between the food materials and the metallic can. When these resins are applied to the surface of the can, some percentage of free BPA remains incompletely polymerized, and this BPA comes in contact with the food; this occurs even when the canning process is optimally conducted. Additionally, the high heat and pressure associated with the sterilization process promotes the migration of BPA out of the resin, and the heat and length of time of storage can also influence this process.

A relatively large number of studies, including several market surveys intended to replicate 'average' daily diets, have demonstrated that the majority of canned foods available in the US and Canada contain measurable levels of BPA. In total, every kind of food (i.e., soups, vegetables, meats, juices, convenience foods, and even pet foods) contains BPA, typically in the range of tens or hundreds of ng g^{-1} food. Additionally, these studies indicate that a single food type (i.e., canned green beans) can have variable concentrations of BPA from can to can, suggesting that human exposure levels cannot be directly calculated by knowing what foods are consumed. Furthermore, even different cans of the exact same food from the same manufacturer can have highly variable concentrations of BPA, indicating that additional unidentified factors can influence the migration of BPA from can linings.

A small number of studies have measured BPA in fresh foods including vegetables, fruits, and meats. The concentrations found in fresh foods, when detected, are very low (typically less than 1 ng g^{-1} food), providing additional evidence that the canning process introduces significant amounts of BPA into the human diet. The source of BPA in fresh foods has not been determined, but because BPA is found in air, dust, and water samples, these are plausible contributing factors.

BPA is also an essential component of polycarbonate plastics, and BPA molecules that are not completely polymerized during the production process can leach from these plastics under normal conditions of use. BPA is also released when these materials are heated, brushed, or exposed to materials with specific pH ranges. For years, polycarbonate plastics were used in the production of the majority of baby bottles, although consumer pressure and recent regulatory actions in several countries, including parts of the EU, Canada, and the US, have shifted toward the use of other types of plastic. Polycarbonate is used in a large number of other consumer products including plastics that come in contact with foods and beverages, and studies using liquid simulants have demonstrated that BPA migration often takes place in the low ng ml^{-1} range.

Finally, a small number of studies have measured the BPA content of food-contact materials including take-out cardboard containers and other paper products (i.e., napkins and paper towels) used in the kitchen. BPA is measured in these products typically in the $\mu g\, g^{-1}$ range, although few studies have determined what concentrations leach into foods. There is some evidence that cardboards and papers made from recycled paper have higher concentrations of BPA; this is likely due to the use of printing dyes containing BPA and the contamination of recycled papers with thermal papers containing BPA, which will be discussed in more detail below.

Nondietary Sources of BPA

The WHO and regulatory agencies around the world have indicated that they believe the majority of human exposures to BPA occur via dietary sources, with leaching into canned foods the highest contributor. However, the WHO acknowledges that their estimates are based on limited data. In contrast to these estimates, a few studies have shown that thermal papers could be a significant source of human exposures. BPA is applied to the surface of these papers, where it changes color when heat is applied, allowing them to be used for receipts, travel tickets, and other applications. BPA readily transfers from these materials to skin and it is absorbed dermally into the bloodstream.

The list of consumer products containing BPA is quite extensive, and the relative contributions from these various sources remain unknown. Cigarette filters and some types of medical equipment, including implantable medical devices, contain BPA. Therefore, some populations (i.e., smokers,

chronically ill patients, infants in the neonatal intensive care unit, etc.) are thought to have higher exposures from these sources. BPA is also found in children's toys, sports equipment, eyeglass lenses, CDs and DVDs, personal care products, dental sealants, paints, plastic dishware and utensils, and household detergents.

Controversies about BPA

Although several important sources of human BPA exposures are known, all sources remain to be elucidated, and the relative contributions of these various sources – as well as their relevant routes of exposure – remain unknown. One major question in the field surrounds actual human exposure levels; a large number of biomonitoring studies clearly indicate that the vast majority of individuals in developed countries have BPA or BPA metabolites in their bodies at all times. The question remains whether the levels that are found in human tissues and fluids are high enough to produce toxicity; the mere presence of a chemical is not considered sufficient evidence that it causes harm. More than two dozen studies have reported free BPA concentrations in the low ng ml^{-1} range in human blood samples from nonoccupationally exposed individuals. Yet, in spite of the consistency reported in numerous studies, the concentration of BPA circulating in the bloodstream remains a major point of controversy. Several scientists have proposed that BPA measured in blood samples does not originate from the blood but is instead a product of contamination. Because BPA is found in many laboratory plastics and reagents as well as air, dust, and water, contamination must be ruled out. However, several studies have made significant and convincing attempts to control and rule out contamination, but this controversy persists.

There is also a large amount of controversy surrounding whether BPA produces effects in laboratory animals, particularly at low doses that are relevant to human exposures. Of course, this question itself has a scientific problem in that 'doses that are relevant to human exposures' are not completely known, considering the large number of consumer products that contain this chemical and the unknown contributions to total exposure concentrations from these various sources. In spite of the difficulties setting a low dose cut-off, several systematic reviews have concluded that BPA does have effects at doses that have been predicted to be safe for humans. In 2007, an expert panel of scientists working on BPA concluded that developmental exposures to BPA (i.e., those that occur during prenatal or perinatal development) alter development of the brain and male reproductive tract, alter enzyme activity, growth and metabolism, and influence behaviors. The panel also concluded that adult exposures alter the male reproductive tract, i.e., adversely affect testicular function. In 2008, the US National Toxicology Program concluded that there was 'some concern' for the effects of BPA on the development of the prostate and brain and on neurobehaviors. In 2010, the US FDA agreed with this assessment, although they maintained that BPA was safe in its current uses in consumer products.

Continuing to feed into this same controversy, there are a small number of studies that have shown no effects of BPA at low doses. Many of these 'no effect' studies are large guideline studies examining validated endpoints and utilizing 'good laboratory practices.' Thus, one major question is why some (often small, academic) studies show effects at low doses and other (often large, industry funded) studies show no effects at low doses. An obvious concern that has been addressed in this field and in other studies of EDCs is whether there is a funder effect, i.e., whether the source of funding has a causative, rather than a correlative relationship with the ability to detect effects of environmental chemicals. There are other issues that need to be considered as well: (1) the large studies examining 'validated' endpoints typically look at insensitive measures of endocrine function, such as the number of pups born to a pregnant female, rather than more sensitive measures like alterations to sex-typical behaviors; (2) the large studies often do not use positive controls to confirm that the endpoints examined respond to low doses of hormones; (3) the large studies often utilize rodent strains that are insensitive to hormones; and (4) the large studies often cannot rule out contamination of the negative control group with hormones or other EDCs. These problems highlight the differences between toxicological approaches and endocrine approaches to studying EDCs.

Finally, there remains controversy regarding whether environmental exposures to BPA are safe for the general human population. A number of epidemiology studies have examined the effects of BPA on endpoints such as obesity, cardiovascular disease, neurobehavioral abnormalities in children, circulating concentrations of endogenous hormones, and others. Unlike epidemiology studies of prescribed pharmaceuticals, where an unexposed population can be compared with individuals administered the compound in question, for BPA comparisons are typically made between individuals with higher exposures and those with lower exposures. However, exposure parameters are determined based on a single, or occasionally a few, urine samples, which may not be the best indicator of typical or previous exposures. Even considering this limitation, a number of epidemiology studies have proposed links between human exposures and diseases/dysfunctions. These studies have not reached the point where they can indicate causation, but they do provide evidence that human exposures to BPA do not have negligible effects.

Can Information Gained from Studying BPA Be Applied to Other EDCs?

BPA is one of the best studied chemicals on earth and is often considered a 'model' for other EDCs. But can the information learned from BPA studies be applied to other chemicals with hormonal activities? This is a fundamental question on several levels: First, it asks whether risk agencies must know every detail about a chemical's actions before taking regulatory action against it. Second, it asks whether there are 'conserved properties' expected for chemicals that interfere with the endocrine system, and whether our knowledge about the actions of natural hormones is sufficient to predict the effects of EDCs. Finally, this question points to the extra-scientific factors involved in the use of chemicals including the benefits derived from those chemicals, the potential risks from using those chemicals, and the role of industry profits in these

discussions. All of these remain open questions, and although BPA may provide lessons that should be applied to other EDCs, this is not the current standard practice in the many fields that contribute to chemical safety assessments.

Conclusions

Food and food packaging serve as significant sources of human exposures to EDCs. Some of these chemicals are natural components found in plant-based foods, but others are hormones added to farm animals to influence body growth and pesticides applied to crops that can bioaccumulate and therefore are also found in meat, eggs, and dairy products. Some are environmental pollutants and some are chemicals used in the resins and plastics used in the food packaging process. Human exposures to single chemicals are likely to be low, but the effects of combinations of these chemicals are largely unstudied and chemical mixtures could have unexpected synergistic effects.

There are more than a thousand studies examining various aspects of BPA use and safety. A few hundred of these studies have examined the effects of BPA on laboratory animals, and more than 100 studies have examined doses below the US EPA 'safe' dose for humans (50 $\mu g\,kg^{-1}\,day^{-1}$). Greater than 90% of these studies have indicated the potential for some type of harm from exposures, with changes to the development of hormone-sensitive organs including the male and female reproductive tracts, the mammary gland, the immune system, metabolic machinery, and the brain. Epidemiology studies hint at similar plausible effects in people exposed via environmental sources (i.e., those that are not occupationally exposed). Yet, BPA continues to be used in a large number of consumer products including numerous food-contact materials, and additional uses continue to be developed.

Ultimately, there are a large number of controversies surrounding BPA and EDCs in general. Countries that use the precautionary principle have limited or banned uses of BPA, indicating that they believe there is sufficient evidence to take action to protect the public from this chemical. Consumer pressures have also influenced the use of BPA in specific products such as baby bottles, but additional studies are needed to determine whether the replacement products also have endocrine-disrupting activities. In sum, the issue of whether the valid uses for BPA – and many other EDCs – can outweigh the costs of their use remains a question that will need to be addressed in the future and continually reevaluated.

See also: Environmental Contaminants: Dioxins, Furans and Dioxin-like Polychlorinated Biphenyls; Environmental Estrogens – Hazard Characterization. Food Technologies: Packaging. Hazards of Food Contact Material: Food Packaging Contaminants; Phthalates

Further Reading

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Relevant Website

www.ourstolenfuture.com
Our Stolen Future book.

HAZARDS OF FOOD CONTACT MATERIAL

Food Packaging Contaminants

J Muncke, Food Packaging Forum, Zurich, Switzerland

Glossary

Flavor scalping Adsorption or absorption of chemical substances from the foodstuff to the food contact material. For example, lipophilic substances that are used as flavoring agents in foods can partition to the packaging material.

Food contact material Packaging, processing, and storage equipment that come into direct contact with food.

Food contact substance Chemical that is used or unintentionally present in food contact materials.

Food simulants Solvents, oil, or polymeric resin that are used to mimic chemical properties of foodstuffs but that lack their chemical complexity and thus simplify chemical analysis of migrants from food contact materials.

Migrant Food contact substance that partitions from food contact material into foodstuff or food simulant.

Migration Process of chemical partitioning from the food contact material to the food.

Release Chemical migration of monomers due to polymer degradation.

Definition

Food packaging contaminants are organic or inorganic chemicals that originate from the food packaging. They are intentionally added substances with a technical function, manufacturing byproducts, impurities of starting materials, or contaminants that are present due to packaging or material recycling (Table 1).

Food packaging contaminants can originate either from the direct food contact material itself or from adhesives, printing inks, and secondary packaging. In the United States of America (USA), food packaging contaminants are also known as indirect food additives and as such not considered to be food contaminants.

Types of Food Packaging Materials and Market Share

Chemical contamination of food from food packaging material largely depends on the type of material in contact with the packaged food. Polymers are the most important food packaging materials (Figure 1), with the most abundant types constituting aproximately 70% of the market share, namely: low-density polyethylene, polypropylene, polyethylene terephthalate (PET), high-density polyethylene, polystyrene (PS) and expanded PS, and polyvinylchloride.

Table 1 Type and example of food packaging contaminants

Type of food packaging contaminants	*Example*
Manufacturing chemicals with technical function	Monomers, additives, catalysts, and printing ink
Nonintentionally added substances (NIAS)	Polymerization byproducts (oligomers), break down products, and impurities of starting materials
Recycling contaminants	Flavor substances, mineral oil, heavy metals, and persistent organic chemicals

Relevance of Food Packaging as Food Contaminant Source

One of the challenges for control of food packaging contaminants is the large amount of different contaminants

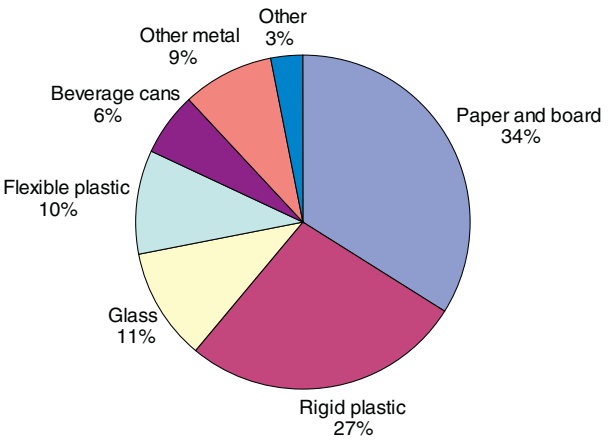

Figure 1 Food packaging materials market share (by value). Reproduced from Rexam (2012) Packaging unwrapped. *Consumer Packaging Report 2011/12.* http://www.rexam.com/files/pdf/packaging_unwrapped_2011.pdf.

 doi:10.1016/B978-0-12-378612-8.00218-3

originating from packaging that can be present in foodstuffs. For food safety control, more than 3000 substances of varying origin (packaging, prefilling, storage, and processing) are considered relevant. This is due to various compounds commonly used for manufacturing plastics, in coatings, and inks or adhesives. Furthermore, synthetic polymers always contain unknown byproducts (nonintentionally added substances (NIAS)) due to the complexity of the chemical manufacturing process. And the environmentally beneficial practice of material recycling can also be a source of inadvertent chemical contamination.

When compared to other types of chemical food contaminants, like pesticides, food packaging is highly relevant (Table 2) with varying levels of contamination for different substances and packaging materials. In fact, food packaging contaminants with their very diverse chemistry are the largest single source of chemical food contaminants. Quantification of food contaminants relies on specific analytical methods for measuring a specific chemical in a given (and usually complex) food matrix. For each contaminant a new analytical method needs to be developed and this can be very time consuming and expensive. However, quantification is only possible for substances with known chemical identity. For unknown chemicals, detection and specific quantification is not possible.

Migration

The process of chemical partitioning from the packaging into food is called migration. Migration is relevant for smaller size molecules and ions (below 1000 Da). The extent to which a substance migrates depends on the physicochemical properties of the migrant, the packaging material, and the food (e.g., fat content) as well as the temperature and duration of storage (Figure 2). Foods that are high in fat content will tend to be more contaminated with lipophilic substances than aqueous foods. The size of packaging in proportion to the amount of food is also relevant, as smaller size packaging has a larger surface to volume ratio, implying a proportionally higher contamination of the food.

Chemical Migrants

The types of chemicals that can migrate from packaging into food are highly diverse and depend on the type of packaging material. Several different types of packaging materials are commonly used (Table 3). For inert materials like stainless steel, ceramic, or glass, only chemicals that are present on the inside surface, directly in contact with the foodstuff, can partition into food. For example, heavy metals that are used in ceramic glazing, or are naturally present in glass raw materials, can transfer from the inner surface to the food by surface

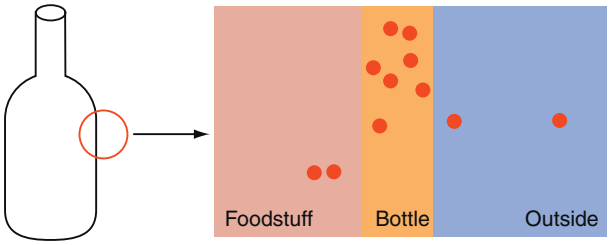

Figure 2 Migration of small size molecules (shown as red dots) from food packaging into food and from environment into food through food packaging.

Table 2 Comparison of potentially hazardous chemicals in food from crop protection to food packaging

Chemical food contaminant	*Crop protection*	*Food packaging*
Legal limits	0.002–50 mg kg^{-1} Maximum residue limits (MRLs) based on compliance with good agricultural practice (GAP)	0.0015–60 mg kg^{-1} Maximum limits based on migration studies and toxicological data
Detected levels	0.001–1 mg kg^{-1} Mean levels with rare examples of levels that cause acute illnesses	Not routinely monitored, but instances of acute illness rarely reported
Frequency of detection	Generally, far less than 1% of fruits and vegetables are analyzed and of these, approximately 1.5% of imported and 1% of domestic samples contain residues above their MRLs or other violation, i.e., pesticide not approved.	Not routinely monitored except for special studies, such as bisphenol A
Control	Minor exceedences of the MRL are not considered to be of public health significance and are handled as technical violations of GAP. As such regulatory warning letters are issued and in the case of repeated violations, future shipments are detained and sampled	Infrequent and perhaps impossible for large number of migrants and unknown substances
Hazard characterization	Premarket assessment of well-defined chemistry and toxicology leading to an acceptable daily intake	Premarket assessment of possible known migrants, but often limited chemistry and toxicology data

Source: Modified from Grob K, Biederman M, Scherbaum E, *et al.* (2006) Food contamination with organic materials in perspective: Packaging materials as the largest and least controlled source? A view focusing on the European situation. *Critical Reviews in Food Science and Nutrition* 46: 529–535.

Table 3 Food packaging materials by type with examples for typical contaminants

Food packaging type	*Materials*	*Typical contaminants*
Plastic	Polyethylene terephthalate	Formaldehyde Acetaldehyde Antimony Ultraviolet (UV) stabilizers
Plastic	Polyethylene	Polyolefin oligomeric saturated hydrocarbons (POSH) Nonylphenol
Plastic	Polyvinylchloride	Vinyl chloride Phthalates Epoxidised soybean oil (ESBO; glass jar closures) Organotins
Plastic	Polystyrene	Styrene Styrene trimers
Plastic	Polypropylene	POSH Erucamide, oleamide Butylated hydroxytoluene
Plastic	Polycarbonate	Bisphenol A
Plastic	Cellulose	Triacetin
Metal	Tin, steel or aluminum with coating	Bisphenol A diglycidyl ether Bisphenol A Ortho-phenylphenol Tin Aluminum
Paper	Nonstick coating	Perfluorinated compounds
Carton (for liquid foods)	Paperboard, aluminum, and polyethylene	POSH Isopropyl thioxanthone Benzophenones
Carton (for dry foods)		Mineral oils Benzophenones Phthalates
Carton (secondary packaging)	Card board	Mineral oils
Glass	Glass container, closure with gasket	Phthalates ESBO Lead
Glass	Glass container, plastic closure	UV stabilizers
Ceramic	Glazed ceramic	Heavy metals

exchange. For these materials neither chemical diffusion from within the packaging material is possible, nor can compounds from the outside (printing inks and adhesives) migrate through the packaging into the food. This inertness is due to the chemical structure of glass, an amorphous noncrystal solid with pore sizes that are too small to allow molecules or single atoms to pass through.

However, chemical contamination of glass-packaged oily foods is observed when plasticizers, like epoxidized soybean oil or phthalates, migrate from the closure's gasket. Careful manufacturing, as well as specially developed low migration closures address this issue.

For noninert materials, like plastics, elastomers, and paper and board, migration can occur either from the packaging material itself or from the outside of the packaging. For example, mineral oils used in printing inks have been found to migrate through carton board packaging into dry foods. Paper-based materials have a large pore size and this permits migration of smaller molecules from the outside to the food inside. To reduce or prevent food contamination, barrier materials can be used. For example, this can be a carton with an inner bag made of aluminum foil. Another form of chemical contamination may be caused by offset migration, when the outside layer of a food contact material comes into contact with its inside layer and thereby transfers chemicals like printing ink components to the direct food contact side. This can be the case for beverage cartons or paper cups that are stacked on top of each other.

Release

Another source of chemical contaminants is from the degrading polymer, when small-sized monomers are released and can migrate into the food. Chemical contamination by release is relevant mainly for reusable food contact materials, like plastic baby bottles or plastic kitchenware (Table 8). Under normal conditions of use, the polymer should not degrade; however, under highly alkaline or acidic conditions some types of polymer will degrade and subsequently release monomers. For example, a polycarbonate container that is made with bisphenol A (BPA) can degrade under alkaline conditions and, as a consequence, continuously release BPA into the contents. Release also becomes relevant over time, when a plastic ages and starts to become brittle. Additives, like ultraviolet stabilizers, are added to plastics to prevent photodegradation.

Scalping

Partitioning of small molecules from foodstuff into packaging material is also possible. This process is called scalping. It can be an indirect source of food packaging contaminants when plastic packaging materials are reused or recycled. Limonene, a flavor substance typically present in fruit juices and sugary soft drinks, partitions into plastic packaging. For refillable plastic bottles this is an issue because limonene is not sufficiently removed by washing. For this reason the refilling of plastic bottles has been deemed unsuitable in certain countries (Switzerland and Japan). Scalping is also of concern when plastic packaging was misused to store nonfood items, such as hazardous chemicals, before recycling. Before it is turned into new food packaging, postconsumer plastic is, therefore, subject to a recycling process that is designed to eliminate chemical contamination. The effectiveness of this process is assessed by challenge tests. In Europe, each food-grade plastic recycling facility must undergo independent review by the European Food Safety Agency (EFSA). In addition, both Europe and the USA restrict the amount of nonfood packaging plastic that is permitted for recycling into food packaging.

Detection of Chemical Contaminants from Food Packaging

Study Design

Identifying the presence of a chemical in food does not render information about the chemical's source. Chemical contamination of food can occur at various stages during food production, processing, filling, and storage. To identify packaging as the source of food contamination a study requires a specific protocol (Table 4). In some cases, a dynamic study design is chosen, where several samples are taken at different times under controlled storage conditions (i.e., temperature and light). Alternatively, for solid foods, samples can be taken from different layers within the packaged food. If there is a difference in contaminant concentrations between the

Table 4 Study designs for identification of food packaging migrants

Time frame	*Sample source*	*Sample type*	*Specifics*
Dynamic: several subsequent time points for measurement in the same sample	Retail: marketed food in final packaging (with or without secondary packaging)	Food	Evidence for migration from food packaging, but no evidence for actual origin of contaminant (i.e., technical agent, NIAS, environmental contaminant, secondary packaging, etc.); no control over time and temperature, i.e., how long has food been in contact with the packaging since filling and what were storage conditions
		Food simulant	If food simulant is used, the originally packaged foodstuff is discarded and the initial migration is not assessed
	Supplier: prefilling packaging before actual food contact	Food	Control over time and temperature from the time food was put in contact with the packaging; may lack final packaging context (printing ink, adhesive, and secondary packaging)
		Food simulant	Common design for premarket food contact material testing; usually lacks final packaging context (inks, adhesives, and secondary packaging)
Static: single time point measurement	Retail: marketed food in final packaging (with or without secondary packaging)	Food	Suitable for dry foods only, where there is no mixing within the packaging Evidence for migration from the packaging is derived by comparing migrant levels in the outer food layer in direct contact with the packaging to the inner food layer.
	Supplier: prefilling packaging before actual food contact	Food simulant	Only makes sense in the context of a larger sampling campaign, where samples are taken throughout food processing, filling, and storage

inner layer compared to the outer layer, with the outer layer showing higher contaminant levels, then migration from the packaging can be suspected as the main source of the chemical contamination.

For certain contaminants, like phthalates that are ubiquitous in the environment, identifying the contamination source can be challenging. Contaminants may migrate from food packaging even though they were not added to the packaging (in contrast to the NIAS that are byproducts of packaging material manufacture). In this case the packaging may have absorbed chemicals from secondary packaging or the environment that can then pass through the packaging into the food.

The third option is to analyze the packaging material before it comes into contact with food. The food contact material can either be extracted or the residual level of a chemical of interest can be assessed, which would allow modeling the maximum possible migration into food. Alternatively, the virgin food contact material can be subjected to an actual migration experiment using food simulants and subsequent chemical quantification.

Food Simulants

Migration of chemicals from packaging into food is usually assessed using food simulants. Food simulants are substitutes for foods that are used to enable chemical analysis. This is useful because quantification requires a distinct and sensitive analytical method for each specific chemical and type of foodstuff. Food simulants represent different groups of food in terms of their chemical properties (**Tables 5** and **6**): hydrophilic, lipophilic, or amphiphilic. Several different types of food simulants are commonly used. For example, migration into oily foods is measured using vegetable oils as the food simulant. For water-based foods, solutions of 10% ethanol or 3% acetic acid are used. Foods that have properties of emulsions (water-in-oil) are simulated by 50% ethanol solutions. Migration of volatile substances into dry foods is approximated using a solid food simulant based on a synthetic polymer with defined pore size (known under the trade name 'Tenax' and in the European Union (EU) as food simulant E).

In general, migration into food simulants is expected to exceed migration into actual foods. Therefore, food simulants generally are thought to overestimate the actual migration into foods. There are, however, exceptions; for example, the migration of perfluorinated compounds into butter. This group of chemicals partitions to a smaller extent into vegetable oil than into an emulsion like butter. A more realistic food simulant for butter, therefore, is a 50% ethanol solution.

For overall migration testing (i.e., an assessment of the mixture of chemicals that can migrate from the entire packaging into food), all food simulants can be used. However, as this analysis is unspecific, the most commonly used food simulant for this purpose is distilled water.

Test Conditions

The extent of chemical migration from food packaging into food and food simulant is highly correlated with temperature and time. This means that appropriate test conditions must be chosen to best reflect the reality of food processing, filling into the packaging, transport, distribution, storage and, if applicable, preparation (i.e., for foods that are cooked or heated in their packaging) (**Table 7**). For food contact materials that are intended for repeated use (like bowls, cooking tools, etc.) migration needs to be assessed in three consecutive experiments under relevant testing conditions, whereby the third experiment needs to comply with the specific migration limits

Table 5 Food type and corresponding food simulant (US Food and Drug Administration guidance)

Food type	*Food simulant*
Aqueous and acidic foods, beverages	10% Ethanol; water (distilled); and 3% acetic acid
Low- and high-alcoholic foods, beverages	50% Ethanol
Fatty foods	Food oil (e.g., corn oil), HB307, and Miglyol 812 (HB307 is a mixture of synthetic triglycerides, primarily C_{10}, C_{12}, and C_{14}. Miglyol 812 is derived from coconut oil)

Table 6 EU list of food simulants. Details on which specific foods are to be tested with which food simulants are given in the relevant regulation (European Commission, EC 10/2011)

Food Simulant	*Abbreviation*	*Use*
10% Ethanol	Food simulant A	Aqueous foods
3% Acetic acid	Food simulant B	Aqueous and/or acidic (pH $<$ 4.5),
20% Ethanol	Food simulant C	Aqueous, alcoholic ($\leq$20% ethanol), and/or fatty food
50% Ethanol	Food simulant D1	Fatty, alcoholic ($>$20% ethanol), and/or emulsions (oil-in-water)
Vegetable oil	Food simulant D2	Fatty, with free fats contacting the food contact material surface
Tenax (poly(2,6-diphenyl-p-phenylene oxide), particle size 60–80 mesh, and pore size 200 nm	Food simulant E	Dry foods (for specific migration testing)
Distilled water	–	Overall migration testing

Table 7 Test conditions reflecting specific filling, transport/storage, and use conditions

Test conditions		*Reality of use*	*Example*
Time	*Temperature*		
240 h (10 days)	40 °C	Filling, storage, and/or use at room temperature	PET bottle; midterm storage (>1 month)
48 h (2 days)	121 °C	After filling sterilization by autoclave	Glass jar with twist-off cap; PP pouch
24 h	40 °C	One day storage at room temperature	Sandwich pack
2 h	70 °C	Consumption of hot contents (single use)	PS cup, paper cup
1 h	175 °C	Home preparation of food in original packaging by oven cooking	PET tray; fatty food simulant
4 h	Boiling point of food simulant (reflux)		PET tray; aqueous food simulant
0.5 h (30 min)	40 or 20 °C	Short exposure at room temperature	PP reusable juice cup
0.5 h (30 min)	175 °C	Mixing and serving hot food	PA cooking spatula

Abbreviations: PA, Polyamide; PET, polyethylene terephthalate; PP, polypropylene; PS, polystyrene.
Source: Adapted from Cooper I (2007) Plastics and chemical migration into food. In: Barnes KA, Sinclair CR, and Watson DH (eds.) *Chemical Migration and Food Contact Materials*, pp. 228–250. Cambridge: CRC Press/Woodhead Publishing Limited.

(EU). This approach assesses migration of additives, residual monomer, and byproducts. Single-use food packaging articles, like PET soft drink bottles or PS ice cream containers, must comply with their intended-use conditions. If consumers reuse these items, legal compliance is not required.

Assessment of Overall Migration

In Europe, overall migration to any of the food simulants should not exceed 10 mg dm^{-2} for plastics and multilayered multimaterials such as beverage cartons consisting of several layers of different materials. The measurement of overall migration for nonvolatile substances from packaging is an assessment of packaging quality and measured in food simulants. A defined piece of packaging material is weighed and then incubated with the food simulant under the appropriate testing conditions (depending on the intended use). Unspecific migration is then assessed by evaporating nonoily food simulants and determining the residue's mass. For oily food simulants the mass loss of the plastic food contact material is assessed. Because plastics can absorb oil this assessment requires an initial extraction or cleaning phase, followed by subsequent weighing.

Chemical Quantification

Quantification of specific migrants from food packaging requires a dedicated analytical method for each migrant of interest. The different steps that are necessary for compound quantification depend on the physicochemical properties of the compound (vapor pressure, lipophilicity, molecular weight, etc.), on the properties of the food contact material (lipophilicity, pore size, etc.) and on the nature of the foodstuff into which migration should be assessed (fatty/aqueous/emulsion; liquid/dry).

Migration is usually assessed experimentally using a migration cell, where a defined surface of the packaging material is brought into contact with a food simulant under controlled conditions (temperature, pressure, and time). Subsequently the migrant of interest is analyzed in the food simulant. Analyses are usually carried out using liquid or gas chromatography for quantification with subsequent mass spectrometry for identification/confirmation. Analytical methods may vary and essentially depend on the migrant of interest (molecular weight, state of matter, chemical stability, etc). Migrants can be analyzed directly in the food or in an appropriate food simulant, whereby the concentration in food is legally specified (i.e., must not exceed the legal limit).

Biological Assessment of Overall Migration

Food packaging can transfer many different compounds into foods, including unknown substances that are present due to manufacturing side reactions. The current risk assessment approach manages single substances and assumes that relevant effects are only caused at higher concentrations. This approach has been criticized lately, because recent toxicological research has highlighted that additional aspects of toxicity can be relevant. In particular, mixtures of chemicals can act additively and cause an effect, even though individual compounds are present at low doses. Furthermore, there is an ongoing debate about effects of chemicals at low doses that are missed by conventional testing at higher concentrations. Exposure to chemicals during sensitive windows of development (pre- and neonatal) can impact health throughout life. Endocrine disrupting chemicals are of specific concern. Therefore it has been suggested to additionally use effect-directed analysis of overall migration, rather than only relying on substance-by-substance chemical safety testing. So far only academic studies have used this approach and it is not commonly required for safety testing of food packaging materials by regulators.

Migration Modeling

It is possible using appropriate mathematical models to assess the migration of a chemical into food. The concentration of the substance of interest in the packaging needs to be known.

Table 8 Nonpackaging food contact materials and typical migrants

Food contact material	*Article type*	*Migrant*
Polyamide (nylon)	Cooking and serving spoon	Primary aromatic amines
Melamine–formaldehyde resin	Children's tableware	Formaldehyde
Polycarbonate	Refillable water bottle	Bisphenol A

The extent that a compound can migrate will depend on the material properties (pore size, thickness, shape, and surface area); concentration of the compound in the material; thermodynamic properties of the migrant; chemistry of the foodstuff and on other aspects, like temperature at filling and during storage, and storage time. The most commonly used models are based on Fickian diffusion and have been validated for different food contact plastics under various experimental conditions. Migration models have been found to give fairly accurate predictions of actual migration for several migrants, but under certain conditions and for certain migrants, exceptions have been noted.

Food Contact Materials Other than Packaging

During the processing, filling, and preparation of foods several materials come into contact with foodstuffs (storage containers, conveyor belts, gloves, chopping boards, surfaces, cookware, etc.). These items can also be sources of chemical migration into food. Therefore, such types of food contact materials are included in the US and EU regulation of food contact materials. Examples for reusable articles and typical migrants are given in Table 8.

Migrants Reacting with Foods

Migrants that react with food ingredients and thereby form new chemical species, present a special challenge. For example, BPA diglycidyl ether (BADGE) can form chlorinated or hydroxylated species with food compounds. Therefore, when only BADGE contamination is assessed in food, these species are missed. The result is an underestimation of BADGE migration. Another example is benzoic acid (migrating from packaging material), which may react with ascorbic acid present in the food to form benzene. In such cases, it can be a challenge to identify the source of food contamination. Furthermore, the use of food simulants will miss such unwanted crossreactions in many cases.

Risk Assessment and Management in the EU, Japan, and USA

The underlying principle of regulation is that there are levels of chemical food contamination which are considered not dangerous to human health for the entire population. This threshold-based risk assessment principle is universal for all legislatures; however, the risk management varies in different countries.

In Europe, risk management is based on migration from food packaging into food. In this approach, single substances are permitted to contaminate foods at levels below the tolerable daily intake (TDI), derived from toxicological data. In this approach, a worst-case human exposure is assumed. However, such a practise may also lack an incentive for packaging producers to reduce migration to technically feasible levels. Industry maintains that the use of chemicals beyond what is technically necessary is not economic and therefore would not be common practice.

In the USA, risk management includes an estimate of human exposure to food packaging contaminants through the diet. In particular, the Food and Drug Administration (FDA) provides Consumption Factors and food-type distribution factors that allow an estimation of actual consumer exposure. This approach is more realistic in terms of actual human exposure to food packaging contaminants; however, it is also not simple to validate the correctness of the estimation factors that are used, leaving room for potential systematic error. It can be assumed that permitted levels of food contact substances in food packaging would tend to be higher with this estimated exposure approach compared to a regulation based on migration.

EU

In Europe, food packaging contaminants are regulated by the European Commission, Directorate General Health and Consumers under the Food Packaging Framework Regulation (Food Packaging Framework Regulation (EC 1935/2004), Article 3: "Materials and articles, including active and intelligent materials and articles, shall be manufactured in compliance with good manufacturing practice so that, under normal or foreseeable conditions of use, they do not transfer their constituents to food in quantities which could: (a) endanger human health;[…]"). EFSA provides scientific advice to the Commission on food packaging contaminants. Food packaging contaminants are managed based on toxicological testing of single substances. For plastic packaging and plastics layers in multimaterial packaging these levels are specified as specific migration limits (SML) and based on a substance's TDI. For a substance that lacks an SML, the overall migration limit of 10 mg dm^{-2} of food contact surface or 60 mg kg^{-1} food (for baby foods) applies. The overall migration limit applies to nonvolatile substances.

The European food contact materials regulation has two notable provisions:

1. The Fat Reduction Factor (FRF) can be applied when assessing the SML for lipophilic migrants from plastics into fatty foods. The underlying rationale for the FRF is that lipophilic foods will contain higher levels of lipohilic contaminants; however, consumers only consume a limited amount of highly fatty foods per day (i.e., less than the risk assessment assumption of 1 kg of food per person per day). The FRF does not apply to packaging for infant foods.
2. The Functional Barrier concept permits the use of not explicitly authorized substances in food packaging plastic

layers behind barrier materials that reduce their migration into food. These substances may not exceed the migration threshold of 10 μg kg^{-1} food. Exempted from this rule are nanoparticles and compounds that are carcinogenic, mutagenic, or toxic to reproduction.

Japan

In Japan, acceptable levels of food packaging contaminants are self-regulated by the food packaging industry and similar to regulations in Europe and the US. An overall migration limit is set at 30 mg/kg food, and for select substances, specific migration limits exist.

USA

In the USA, regulation of food contact materials (Code of Federal Regulations Part 21) is the responsibility of the FDA. Food contact substances are chemicals that are present in food packaging and must be shown to be safe before they can be marketed. They are authorized either by the Food Additive Petition or by the Food Contact Notification Program. The latter requires less toxicological testing; however, exposure levels must not exceed 1 mg per person per day. If exposures are above this level, a Food Additive Petition is required. A third option for authorization is the Threshold of Regulation: If the estimated daily exposure to a food contact substance lies below 1.5 μg per person per day, toxicological data is not required at all, if the substance's chemical structure is without toxicity alerts. Furthermore, chemicals may also be used in food packaging if they are generally recognized as safe (GRAS). Not all substances that are used under GRAS status are listed by FDA. For the other food contact substances, inventories are available online. Substances that were authorized under the Food Contact Notification Program can only be used by the notifying company.

See also: Food Technologies: Packaging. Foodborne Diseases: Overview of Chemical, Physical, and Other Significant Hazards. Hazards of Food Contact Material: Bisphenol A and Endocrine Disruption; Phthalates. Risk Analysis: Risk Assessment: Chemical Hazards. Safety of Food and Beverages: Packaging Material and Auxiliary Items

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Relevant Websites

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HAZARDS OF FOOD CONTACT MATERIAL

Phthalates

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Glossary

Hazard index The sum of the hazard quotients for a series of chemicals of interest.

Hazard quotient The human exposure to a chemical of interest (mg kg^{-1}-day^{-1}) divided by its reference dose (RfD) (mg kg^{-1}-day^{-1}).

RfD An estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. It can be derived from a no adverse effect level, lowest observed adverse effect level, or benchmark dose, with uncertainty factors generally applied to reflect limitations of the data used.

Uncertainty factor One of several, generally 10-fold, default factors used in operationally deriving the reference dose from experimental data

Chemical Name and Structure

The phthalate esters that are the subject of this article are derivatives of phthalic acid, in which the acid groups are in the ortho position. The two alcohol groups can be the same or different and straight or branched chain. Phthalates are manufactured by reacting phthalic anhydride with alcohols (Figure 1).

Uses

Phthalate esters are used to impart flexibility to plastic products. Many consumer and food packaging products contain phthalate esters. The major uses of the selected phthalate esters are summarized in (Table 1). The phthalate esters are not covalently bound in the products in which they are used and can leach into the surrounding environment.

Hazard Characterization

Extensive data have been published since 2000, showing that phthalate esters with side chains of 3–10 carbon units are developmental toxicants. The phthalate diester is rapidly converted to the phthalate monoester in rats and humans.

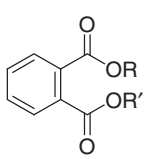

Figure 1 General structure of phthalate esters.

This metabolism occurs by nonspecific esterases in the digestive tract before absorption and in tissues after absorption. For those phthalate esters that cause developmental effects, the phthalate monoester metabolite is believed to be the toxic chemical.

Matsumoto *et al.* summarized the studies available in human populations. A number of studies show an association between effects (sperm and semen parameters in adults; anogenital distance in newborn males; and serum hormone levels in newborn infants) and environmental exposure to phthalates. After a thorough review of these studies, Matsumoto *et al.* concluded: "some of the findings in human populations are consistent with animal data suggesting that PAEs (phthalic acid esters) and their metabolites produce toxic effects in the reproductive system. However, it is not yet possible to conclude whether phthalate exposure is harmful for human populations."

Based on the extensive data available from laboratory animals, there is concern that environmental exposure to phthalate esters could cause adverse effects on the developing human male reproductive system. Additional concern is raised by the demonstration that phthalate monoesters known to cause developmental toxicity in rats are detected in a large percentage of human amniotic fluid samples.

Benson conducted a literature review for the phthalate esters that have the potential to be developmental toxicants based on structure–activity relationships. The focus was on the phthalate esters with a side chain containing 4–10 carbon units in the ortho position. The shorter chain phthalate esters (dimethyl phthalate and diethyl phthalate) were not included because these chemicals were not found to be developmental toxicants in laboratory animal studies. Relevant toxicological data following *in utero* exposure was located for dibutyl phthalate (DBP), diisobutyl phthalate (DiBP), butyl benzyl

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Table 1 Phthalate diester, its corresponding monoester metabolite, and uses

Phthalate diester	*Monoester metabolite*	*Major uses*
Dibutyl phthalate CAS # 84-74-2 Molecular weight: 278	Monobutyl phthalate (MBP)	Plastic products containing nitrocellulose, polyvinyl acetate, or polyvinyl chloride; pharmaceutical coatings; lubricant for aerosol valves; antifoaming agent; skin emollient; nail polishes; fingernail elongators; and hair spray
Diisobutyl phthalate CAS # 84-69-5 Molecular weight: 278	Monoisobutyl phthalate	Same as dibutyl phthalate but less commonly used
Butylbenzyl phthalate CAS # 85-68-7 Molecular weight: 312	MBP and monobenzyl phthalate	Polyvinyl chloride products including vinyl tile, food conveyer belts, carpet tile, artificial leather, tarps, automotive trim, weather strippers, traffic cones, and vinyl gloves
Diethylhexyl phthalate CAS # 117-81-7 Molecular weight: 390	Monoethylhexyl phthalate	Polyvinyl chloride products including building products, car products, clothing, food packaging, children's products, and in medical devices (storage containers, bags, and flexible tubing)
Dipentyl phthalate CAS # 131-18-0 Molecular weight: 306	Monopentyl phthalate	Not found
Diisononyl phthalate CAS # 68515-48-0 and 28553-12-0 Molecular weight: 419	Monoisononyl phthalate	Flexible polyvinyl chloride products including children's toys, flooring, gloves, food packaging material, drinking straws, and garden hoses

phthalate (BBP), diethylhexyl phthalate (DEHP), dipentyl phthalate (DPP), and diisononyl phthalate (DiNP). Benson focused on the most sensitive effect in the most sensitive life stage, the reproductive tract of the developing male fetus, to develop the reference dose (RfD) for each of the phthalate esters.

The development of the male reproductive tract is dependent on the presence of testosterone and the androgen receptor. Any chemical that reduces the concentration of the androgen receptor–testosterone complex during the critical developmental window has the potential of causing irreversible malformations of the male reproductive tract. The mechanism of action in laboratory rats for each of the phthalate esters that are the subject of this article is the decrease in the concentration of fetal testosterone in Leydig cells during the critical developmental window in the rat. The decrease in testosterone concentration is triggered by a decrease in gene expression for the proteins involved in the rate-limiting steps of testosterone synthesis. These steps include transport of cholesterol into the mitochondria facilitated by steroidogenic acute regulatory protein (STAR) and the conversion of cholesterol to pregnenolone by the enzyme CYP11A1 (also known as P450 side-chain cleavage enzyme).

There is experimental evidence that the decrease in fetal testosterone caused by cumulative exposure to these six phthalate esters in rats follows a dose additional model (Howdeshell *et al.*). Therefore, it is appropriate to conduct a cumulative risk assessment for simultaneous exposure to these phthalate esters using a dose addition model. This article provides examples of a cumulative risk assessment using exposure information from a US and German population.

Calculation of the Reference Dose

The standard US Environmental Protection Agency (EPA) approach was used to derive the RfD for the selected phthalate esters. The uncertainty factors included a factor of 10 for intraspecies variability, a factor of 10 for interspecies variability, and, if needed, a factor for extrapolation from a lowest observed adverse effect level (LOAEL) to a no adverse effect level (NOAEL).

For these phthalate esters the most vulnerable target for adverse health effects is the reproductive tract of the developing male fetus. Therefore, the RfD is based on exposure–response data from studies following *in utero* exposure. This RfD will protect humans from other adverse effects that may occur at a higher exposure during neonatal or adult life.

Benson derived RfDs for DBP, DiBP, BBP, DEHP, DPP, and DiNP using the NOAEL or LOAEL values for each phthalate ester from the published literature. If appropriate, benchmark dose analysis was conducted using EPA software. These values are summarized in **Table 2**.

These RfDs are based on the assumption that the adverse health effect observed at the lowest exposure in laboratory rats (decrease in testosterone concentration in fetal Leydig cells) is relevant to humans. Some recent toxicological studies provide fairly convincing evidence that the decrease in fetal testosterone concentration is a species-specific response. Gaido *et al.* first demonstrated that adverse developmental effects occurred in mice in the absence of a decrease in testosterone concentration and an absence in a decrease in gene expression for the proteins involved in the rate-limiting steps in cholesterol transport into the mitochondria and formation of pregnenolone. A 250- or 500-mg DBP per kg-day exposure to the mouse from gestational day 16–18 significantly increased the seminiferous cord diameter, number of multinucleated gonocytes per cord, and number of nuclei per multinucleated gonocytes. The exposure of 250 mg kg^{-1}-day^{-1} was the lowest exposure tested in this study.

Two research groups have investigated the possibility of a species-dependent response using xenografts of testicular tissue from different species implanted into rodent hosts.

Table 2 Reference dose for selected phthalate esters

Chemical	*Critical effect and reference*	*POD/UF*	*RfD*
DBP	Decreased fetal testosterone NOAEL = 30 mg kg^{-1}-day^{-1} Lehmann *et al.* (2004)	NOAEL/100	0.3 mg kg^{-1}-day^{-1} or 0.00108 mmol kg^{-1}-day^{-1}
DiBP	Decreased fetal testosterone production $BMDL_{1SD}$ = 80 mg kg^{-1}-day^{-1} Howdeshell *et al.* (2008)	$BMDL_{1SD}$/100	0.8 mg kg^{-1}-day^{-1} or 0.00288 mmol kg^{-1}-day^{-1}
BBP	Decreased fetal testosterone production $BMDL_{1SD}$ = 102 mg kg^{-1}-day^{-1} Howdeshell *et al.* (2008)	$BMDL_{1SD}$/100	1 mg kg^{-1}-day^{-1} or 0.00327 mmol kg^{-1}-day^{-1}
DEHP	Small or absent male reproductive organs $BMDL_{10}$ = 27 mg kg^{-1}-day^{-1} NTP 2005	$BMDL_{10}$/100	0.3 mg kg^{-1}-day^{-1} or 0.000692 mmol kg^{-1}-day^{-1}
DPP	Decreased fetal testosterone production $BMDL_{1SD}$ = 17 mg kg^{-1}-day^{-1} Howdeshell *et al.* 2008	$BMDL_{1SD}$/100	0.2 mg kg^{-1}-day^{-1} or 0.000548 mmol kg^{-1}-day^{-1}
DiNP	Decreased fetal testosterone LOAEL = 750 mg kg^{-1}-day^{-1} Borch *et al.* (2004)	LOAEL/1000	0.8 mg kg^{-1}-day^{-1} or 0.00179 mmol kg^{-1}-day^{-1}

Abbreviations: BBP, butyl benzyl phthalate; DBP, dibutyl phthalate; DEHP, diethylhexyl phthalate; DiBP, diisobutyl phthalate; DiNP, diisononyl phthalate; DPP, dipentyl phthalate; LOAEL, lowest observed adverse effect level; RfD, reference dose.

POD is the point of departure and UF is the total uncertainty factor.

$BMDL_{1SD}$ = lower 95% confidence limit of the exposure necessary to give a 1 standard deviation decrease in testosterone versus control.

$BMDL_{10}$ = lower 95% confidence limit of the exposure necessary to give a 10% increase in adverse effect versus control.

RfD (mmol kg^{-1}-day^{-1}) = Rfd (mg kg^{-1}-day^{-1})/molecular weight (mg $mmol^{-1}$).

Mitchell *et al*. compared the effect in human fetal tissue versus rat fetal tissue xenografts in host mice. Human fetal tissue xenografts in mice showed no significant decrease in testosterone concentration following exposure to DBP or monobutyl phthalate. In contrast, rat fetal tissue xenografts showed decreases in testosterone concentration and decreased expression of rat testis CYP11A1 and STAR mRNA as found in intact rats.

Heger *et al*. found comparable results. These researchers implanted fetal rat, fetal mouse, or fetal human testis tissue into immunodeficient rat and mouse hosts. Host animals were treated by gavage with a range of doses of DBP. Consistent with the *in utero* response, phthalate exposure induced mononuclear gonocytes formation in rat and mouse xenografts, but only the rat xenograft exhibited suppressed expression of steroidogenesis genes. Across the range of doses tested, human fetal testis xenografts exhibited mononuclear gonocyte induction at all exposures (100, 250, and 500 mg kg^{-1}-day^{-1}) but were resistant to suppression of steroidogenic genes at all exposures.

It is not known whether transport of the toxic metabolite to the testicular xenografts is the same as that in intact animals. In the studies of Heger *et al*., an exposure of 100 mg kg^{-1}-day^{-1} of DBP in the host animal is an adverse effect level in the human testicular xenograft. In intact rats, Lehmann *et al*. found a no effect level for a decrease in testosterone concentration at 30 mg kg^{-1}-day^{-1} and an effect level at 50 mg kg^{-1}-day^{-1}. This comparison would suggest that a hazard quotient for DBP using a decrease in testosterone concentration or using induction of mononuclear gonocytes as the end point would be in the same range. However, there are no comparable data available for the other phthalate esters.

Exposure Assessment

Because the phthalate esters are used in such a wide variety of consumer products and are not covalently bound in the products in which they are used, human exposure to the phthalate esters is widespread. Some common examples are listed below.

- Inhalation of emissions from manufacturing facilities
- Dermal uptake from use in vinyl gloves and cosmetics
- Ingestion of dust from emissions from floor and carpet tile and products used in automotive interiors
- Ingestion from plastic coatings on pharmaceuticals
- Ingestion from uses in children's chew toys
- Ingestion from uses in food packaging
- Intravenous exposure from uses in medical storage bags and tubing.

Phthalate esters have no direct uses in food. Atmospheric deposition on agricultural products from nearby manufacturing facilities is possible. However, the occurrence of a phthalate ester in food is more likely to be the result of contamination by transfer of the phthalate ester from materials in contact with the food during processing, handling, or transportation. Examples of materials that can contain a phthalate ester capable of transfer to the food include plastic bottles and containers, flexible plastic tubing, food conveyer belts, and various food-packaging materials.

Limited data on the estimated exposure of humans to DBP, BBP, DEHP, and DiNP from food are available from a dietary study conducted in Denmark and from measurement of these chemicals in selected fatty foods in the United Kingdom.

Mean exposure to DBP is 0.2–1.8 μg per kg of body weight-day; BBP is 0.1–0.4 μg per kg of body weight-day; and DEHP is 2–3 μg per kg of body weight-day. DiNP was below the detection limit in the foods surveyed. Migration is more extensive to fatty foods because the octanol–water partition coefficient (log Kow) exceeds 1.

Data on human exposure to phthalate esters are available for the US population from the National Health and Nutrition Examination Survey (NHANES) surveys in 1999–2000, 2001–2002, 2003–2004, 2005–2006, and 2007–2008. These studies measured the concentration of the monoester metabolites in the urine of a cross section of the US population aged 6 years and above. As the monoester metabolites and additional oxidation products are rapidly excreted in urine (that is, they do not bioaccumulate), measurements of metabolites in urine can be used to estimate total exposure to phthalate esters in the individual.

Kohn *et al*. used an earlier data set from 289 individuals and a linear two-compartment model to estimate the exposures (mg kg^{-1}-day^{-1}) in the general population. No study has used the same method as Kohn *et al*. to update these exposure estimates, taking into account the additional data sets available from the NHANES surveys. However, these subsequent NHANES data sets show values that are comparable to or somewhat lower than those used in the original analysis of Kohn *et al*. Therefore, large differences in the exposure estimates are not anticipated.

Data on human exposure to phthalate esters are also available from a German population (Wittassek and Angerer). The authors calculated the oral exposure from the urinary levels of the metabolites and urinary excretion factors determined from human metabolism studies. These data for both populations are summarized in Table 3 and Table 4. Data on exposure from food only are not available for the US or German populations. However, comparison of the results presented above implies that food is a significant contributor to the total exposure to phthalate esters.

Because of the widespread nature of human exposure to phthalate esters, it is more informative to consider total exposure, rather than focus on specific sources of exposure.

Table 3 Exposure to selected phthalate esters for a US population in mg kg^{-1}-day^{-1}

Chemical	*Median*	*95th Percentile*	*Maximum*
DBP	0.0013	0.0061	0.094
DiBP	0.0002	0.0011	0.016
BBP	0.00088	0.0040	0.029
DEHP	0.00071	0.0036	0.046
DPP	Not available	Not available	Not available
DiNP	<LOD	0.0017	0.022

Abbreviations: BBP, butyl benzyl phthalate; DBP, dibutyl phthalate; DEHP, diethylhexyl phthalate; DiBP, diisobutyl phthalate; DiNP, diisononyl phthalate; DPP, dipentyl phthalate; LOD, limit of detection.

Exposure to BBP, DEHP, and DiNP is directly reproduced from Kohn M, Parham F, Masten S, *et al.* (2000) Human exposure estimates for phthalates. *Environmental Health Perspectives* 108: A440–A442, Table 2. The original data used reported the urinary excretion of DBP and DiBP as a single summed value. Subsequent data collected by NHANES show that urinary excretion of DiBP (creatinine corrected) is approximately 15% of the excretion of DBP (creatinine corrected). Accordingly, the DBP values have been reduced by 15% and the remainder is attributed to DiBP.

Table 4 Exposure to selected phthalate esters for a German population in mg kg^{-1}-day^{-1}

Chemical	*Median*	*95th Percentile*	*Maximum*
DBP	0.0021	Not provided	0.230
DiBP	0.0015	Not provided	0.0273
BBP	0.0003	Not provided	0.0022
DEHP	0.0027	Not provided	0.0422
DPP	Not available	Not available	Not available
DiNP	0.0006	Not provided	0.0368

Abbreviations: BBP, butyl benzyl phthalate; DBP, dibutyl phthalate; DEHP, diethylhexyl phthalate; DiBP, diisobutyl phthalate; DiNP, diisononyl phthalate; DPP, dipentyl phthalate.

Data are reproduced from Wittassek M and Angerer J (2008) Phthalates: Metabolism and exposure. *International Journal of Andrology* 31: 131–138, for 102 individuals.

Risk Assessment

DBP, DiBP, BBP, DEHP, DPP, and DiNP cause a similar spectrum of adverse health effects in laboratory rats and operate by the same mechanism of action: reduction of the testosterone concentration in the fetal Leydig cells. The reduced concentration of the testosterone–androgen receptor concentration causes a failure in subsequent signaling pathways that are required for the development of the male reproductive organs. Therefore, it is appropriate to use a dose addition model in cumulative risk assessment for these phthalate esters. A response addition model is not appropriate.

The simplest way of doing a dose addition approach is to calculate the hazard quotient for each chemical and then calculate the hazard index as the sum of the individual hazard quotients. The step-by-step procedure is described below.

1. The hazard quotient is calculated for the median exposure to each phthalate ester by dividing the median exposure in mg kg^{-1}-day^{-1} (Table 3 or 4) by the RfD in mg kg^{-1}-day^{-1} for the respective phthalate ester (Table 2).
2. The hazard quotients are added for the median exposure to get the hazard index for the median exposure.
3. Step 1 and 2 are repeated for the 95th percentile exposure and maximum exposure.
4. All values are rounded to one significant digit.
5. The calculated Hazard Quotients and Hazard Indices for the US and German populations are summarized in Table 5 and 6, respectively.

Risk Characterization

A hazard index of less than 1 means that there is little probability that an adverse effect might be observed from the combined exposure to the chemicals. A hazard index of 1–100 means that there is a potential that an adverse effect might be observed from the combined exposure to the chemicals. A hazard index of 100 or more indicates that the estimated exposure is comparable to the NOAEL observed in laboratory animals and indicates a high potential that an adverse effect might be observed in humans

Table 5 Hazard quotient and hazard index calculation for a US population

Chemical	*Median exposure*	*95th Percentile exposure*	*Maximum exposure*
	Hazard quotient	*Hazard quotient*	*Hazard quotient*
DBP	0.004	0.02	0.3
DiBP	0.0003	0.001	0.02
BBP	0.0009	0.004	0.03
DEHP	0.002	0.01	0.2
DPP	Not calculated	Not calculated	Not calculated
DiNP	Not calculated	0.002	0.03
	Median exposure	95th Percentile exposure	Maximum exposure
	Hazard index	Hazard index	Hazard index
	0.007	0.04	0.6

Abbreviations: BBP, butyl benzyl phthalate; DBP, dibutyl phthalate; DEHP, diethylhexyl phthalate; DiBP, diisobutyl phthalate; DiNP, diisononyl phthalate; DPP, dipentyl phthalate.

Table 6 Hazard quotient and hazard index calculation for a German population

Chemical	*Median exposure*	*95th Percentile exposure*	*Maximum exposure*
	Hazard quotient	*Hazard quotient*	*Hazard quotient*
DBP	0.007	Not calculated	0.8
DiBP	0.002	Not calculated	0.003
BBP	0.0003	Not calculated	0.002
DEHP	0.002	Not calculated	0.01
DPP	Not calculated	Not calculated	Not calculated
DiNP	0.0008	Not calculated	0.05
	Median exposure	95th Percentile exposure	Maximum exposure
	Hazard index	Hazard index	Hazard index
	0.01	Not calculated	0.9

Abbreviations: BBP, butyl benzyl phthalate; DBP, dibutyl phthalate; DEHP, diethylhexyl phthalate; DiBP, diisobutyl phthalate; DiNP, diisononyl phthalate; DPP, dipentyl phthalate.

from the combined exposure to these chemicals. As can be seen from the results summarized in Tables 5 and 6, the hazard index for the median, 95th percentile, and maximum exposed individuals are less than 1. Thus, it is unlikely that humans are experiencing an adverse developmental effect from current environmental exposure to these phthalate esters.

Management Interventions

The United States and the European Union have limited the quantity of BBP, DBP, and DEHP in children's toys that can be taken into the mouth to 0.1% by mass of the product. The European Union has also restricted the use of BBP, DBP, and DEHP to food contact surfaces only for nonfatty foods up to 0.1%, 0.05%, and 0.1%, respectively, in the final product. These restrictions also established Specific migration limits of 30, 0.3, and 1.5 mg kg^{-1} food for BBP, DBP, and DEHP, respectively. There are no comparable restrictions in the US on the use of BBP, DBP, and DEHP in products with contact to food.

Current Status

The European Union is continuing to investigate the safety of the uses of BBP, DBP, and DEHP under the Registration, Evaluation, Authorization and Restriction of Chemicals program.

See also: Foodborne Diseases: Overview of Chemical, Physical, and Other Significant Hazards. Hazards of Food Contact Material: Food Packaging Contaminants

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HAZARDS OF FOOD CONTACT MATERIAL

Nanotechnologies and Nanomaterials

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Glossary

Agglomeration The tendency of nanoparticles to aggregate or clump together.
Biopersistent Resistant to solubilization or normal processes of tissue clearance or elimination.
Engineered nanoparticles Particulate material, generally of low solubility, engineered or manufactured to exploit functionality or properties different from those of particles of the same chemical composition with a larger particle size. The use of the term 'engineered' attempts to differentiate manufactured nanoparticles from those likely to be found in the environment from anthropomorphic or natural sources (e.g., ultrafine air pollutants).
Nanomaterials Materials engineered at the nanoscale to have novel functionality or properties. Such properties will typically, but not exclusively, be demonstrated in the size range 1–100 nm, but this size range should be considered approximate.
Nanoscale The size range from approximately 1–100 nm.

Description and Definitions

In recent years, a range of technologies derived from almost all scientific disciplines, across chemistry, physics, and biology, have advanced substantially in their ability to manipulate materials at the nanometer scale. Although frequently referred to as nanotechnology in the singular, in reality there is no one unifying technology that covers the wide range of nanomaterials being developed. The most common definitions for nanomaterials use as their basis size or structure in at least one dimension of between 1 and 100 nm. Thus a nanoparticle has three dimensions between 1 and 100 nm, a nanorod or nanotube has two dimensions in this range, and a nanosheet or nanomembrane has one dimension in this range.

These size boundaries are arbitrary and do not reflect in any way a point or range necessarily related to novelty or hazard. Properties of interest and relevance to safety assessment extend above the 100 nm cutoff without inflexion or discontinuity related to the arbitrary 100 nm cutoff. Also, particulate matter may have a size distribution where a certain proportion is in the nanoparticle range. There is no consensus on what proportion of the nanoscale component of a powder would result in a departure from its normal functional and toxicological properties. Current definitions of nanotechnology are beginning to reflect the significance of functional novelty as a characteristic more important than size.

This article relates to general background information on engineered nanoparticles (ENPs) as they pertain to food manufacture. The article neither covers potential ENP use in medical, veterinary or agricultural preparations nor nanotechnology applications in food packaging or other industrial uses. Although there may be potential for industrial or cosmetic ENPs being released into the environment, at this time it is hypothetical that they may enter the human food chain. A significant issue is whether nanomaterials released into the environment will retain the unique characteristics that determine their novel functionality, and whether analytical techniques are sufficiently sensitive and specific to characterize these properties.

Historical Context

Although there is a general perception that nano-structured materials in food is a very recent, or even future, issue, much of what is currently presented as nanotechnology utilizes long-standing principles and technologies previously known by other names. Certainly, the sophistication of current nanotechnologies has grown dramatically, but in many cases the advance relates to achieving high levels of precision and uniformity in the size and other characteristics of previously available nano-dimensioned materials.

Colloidal metals, earths, and other particulates, in the nanometer range have been known and studied for the better part of a century. Colloidal silver, with a long history of medical use, is today called nano-silver. Gold leaf with a thickness less than 100 nm has been used as an edible food decoration for hundreds of years. Engineered nanostructures in food, such as ice cream and other food emulsions, have been a routine aspect of food processing for centuries.

Surfactant micelles are dynamic self-assembling nanostructures with dimensions inclusive of the low nanometer scale. Nanoparticles, or more accurately aggregates and agglomerates of nanoparticles such as the fumed silicon dioxides, also have a long history of use in food, cosmetics, and

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pharmaceuticals. Indeed food, whether fresh or manufactured, is naturally structured at the nanometer scale. Ribosomes, the protein synthetic structure of cells, for example, have a size of approximately 30 nm, and other cellular structures are of similar size. The first food a newborn child consumes at its mothers breast is a nano-food, as is all milk. The milk protein casein is a micelle of approximately 20 nm diameter. The organoleptic properties of ice cream depend on its nanostructure of fat globules, stabilized by the nano-membranes of 10–20 nm casein micelles. Thus nanostructures and nanomaterials in food are neither new nor novel. Nonetheless, the sophistication of the nanotechnologies is increasing and new, unfamiliar materials will eventually be developed for food applications, including those associated with food packaging and processing materials. As is true for any other new material for food use, careful consideration of their safety is essential before their introduction.

Safety Concerns

Safety concerns about nanomaterials are driven mainly by the accumulated knowledge of the health effects of inhaled nanoparticles; for example, the increase in cardiovascular, airways, and pulmonary diseases, and mortality associated with ultrafine air pollutants. Human exposure to ultrafine air pollutants has been occurring for millennia, with significant increases since the industrial revolution. With the development of nanofibers and nanorods, such as single- and multiwalled carbon nanotubes, concerns have also been raised about the potential for inhaled nanoparticles to mimic the adverse health effects of pathogenic fibers, such as asbestos and silicates. Although these concerns may be valid in some applications, there is no indication that materials of this type are under development for food-related applications. Nor are health effects observed with inhaled nanomaterials necessarily relevant for food.

The international research effort on the safety of nanomaterials is primarily directed at occupational exposure because this is where the highest exposure potential exists. The nanotoxicology literature is virtually exploding, with hundreds of papers being published each year, with many reviews periodically summarizing and consolidating the information.

Current knowledge indicates the potential for nanoparticles to produce adverse health effects essentially relies on their ability to:

- mobilize tissue macrophages to elicit sustained local inflammatory reactions, generally mediated by macrophage-generated reactive oxygen species (ROS) and inflammatory cytokines,
- cross membrane barriers preventing entry of larger particles to the systemic circulation, or
- in some cases, penetrate cell membranes and initiate ROS formation, impair cellular functional pathways, or to initiate apoptosis.

Many of these factors are influenced by nanoparticle size, surface characteristics, manufacturing impurities and chemicals entrained within/on the nanoparticle, or the ability of the nanoparticle to dissolve, releasing its chemical constituents. Nanoparticle surfaces modified by chemical functionalization or adherent coatings can significantly alter intrinsic toxicity.

Mechanistic aspects and comparative toxicity of different types of nanoparticles have been studied in a range of *in vitro* experimental systems, such as cell cultures. However, caution must be exercised in extrapolating from such studies to assess human health risks. Cell cultures often lack critical metabolic and transport functions that can modify toxic responses. There is substantial uncertainty regarding the relevance of concentrations eliciting effects *in vitro* to those that may be in tissues after whole animal or human exposure. At this time the latter are not well defined. At this time, concentrations that nanomaterials may achieve in tissues *in vivo* are not well defined, although pharmacokinetic modelling may address some of these uncertainties. Furthermore, many such studies fail to adequately characterize the nanoparticles or the effects of the dispersing media on their aggregation and agglomeration. It has been suggested the term 'nanoparticles' is used indiscriminately in many articles. Because of the above, a misleading impression may be gained of potential *in vivo* toxicity by relying only on *in vitro* information. Furthermore proteins and other macromolecules adhere to the surface of nanoparticles to create a 'corona' that largely determines how cells 'see' and react with nanoparticles. The corona may well be different *in vitro* from that *in vivo*, and could change as the nanoparticle moves from extracellular fluid into the cell.

Perhaps the most important potential property of a nanoparticle influencing its longer term toxicity is its biopersistence – that is, the ability to penetrate into body tissues and resist normal clearance mechanisms (macrophage engulfment or renal/fecal excretion). If, and to what degree, a nanoparticle is biopersistent is dependent on both the composition of the particle and the surface area to volume ratio. Although bulk silica is essentially insoluble in aqueous media, silica nanospheres used as intravenous drug delivery vehicles slowly dissolve in biological fluids and are eliminated as the dissolved silica. An example of the importance of biopersistence, however, is seen with pathogenic fibers such as asbestos, and probably carbon nanotubes. With these materials, aspect ratio (diameter to length) is a critical factor determining potential for adverse health effects after inhalation.

Current data do not suggest that long term accumulation of nanoparticles, where it occurs, is in itself sufficient to cause toxicity. Titanium dioxide, for example, administered over a lifetime to rats and mice at very high doses (50,000 ppm in the diet) produces no ill effects. Nonetheless, the long term fate of nonsoluble bio-stable nanoparticles is not generally well understood.

Novel Characteristics of Nanomaterials

Nanomaterials are developed on the premise that their characteristics, when compared to those of the bulk-phase materials from which they are constituted, confer novel functional properties. In the case of nanomaterials for food use, these can include:

- Packaging material biosensors that detect food spoilage.
- Materials that alter oxygen permeability and thereby limit food spoilage.

- Encapsulation of food components to regulate their systemic absorption.
- Modification of food texture to enhance taste or other characteristics.
- Materials that sense the environment within a food package and release preservatives or other food additives only as required to protect the food within the package.

Pharmacokinetics as a Source of Novelty

Pharmacokinetics is the study of the absorption, distribution, metabolism, and excretion of materials in living organisms. A difference in pharmacokinetics between soluble small organic molecules and insoluble bio-stable nanoparticles is at least partially predictable on the basis of existing data and on anatomical and physiological considerations. Of greatest potential concern is the finding that certain bio-stable nanomaterials exhibit long tissue retention times, in particular in the liver, spleen, and lymph nodes. In this regard however, very small nano-nanoparticles (less than 10 nm) with very high surface areas and very low mass, composed of even very slightly soluble materials such as silica, have the potential to be slowly biodegraded through slow dissolution or other processes. For example, zinc oxide would not normally be considered as a soluble material, but it is slowly solubilized in biological fluids.

Altered pharmacokinetic behavior is one of the major potential sources of novelty for nanomaterials, and nanoparticles especially. Although the use of nanoparticles is expected to be limited in food compared to other industry sectors, their pharmacokinetic characteristics, particularly tissue retention and poor excretion, should be considered early in product development of poorly soluble nanoscale and microscale particulates. This work should include classical pharmacokinetic studies following oral ingestion, using techniques that differentiate between dissolved and particulate material in biological matrices, as well as investigations of the fate of insoluble materials in long-term studies. It is appreciated, however, that measurement of nanoparticles in tissue can be a significant challenge.

Absorption

To be absorbed from the gastrointestinal tract (GIT), a particulate in a food (whether nanoscale or microscale) must first resist dissolution and degradation in the stomach and intestine and be able to diffuse across the gastric mucosa to the surface of the epithelia where it can be absorbed. Because both particle dissolution kinetics and solubility are size dependent, nanoscale particulate materials can dissolve more quickly, and theoretically, to a greater extent than macroscopic particles of the same material. Nanomaterials which dissolve in the water or lipid phases in the food, or GIT, cease to be nanoparticles. Similarly nanomaterials that are susceptible to digestion or other forms of degradation in the gut resulting in soluble materials, also cease to be nanomaterials and their subsequent pharmacokinetics will be that of conventional materials.

Intestinal absorption and tissue disposition of particulates is, compared with small soluble molecules, significantly restricted by the large number of physiological barriers that exist to defend the body from particulate invasion. Nevertheless, evidence has accumulated demonstrating some absorption of certain nanoscale and microscale particulates across the intestinal mucosa, via M cells (which favor particles around one micrometer in size) to underlying lymphoid follicles, and also through normal columnar epithelia. The absorption is, however, very limited, to just a few percent of the administered dose.

A number of interrelated factors influence particle uptake from the gut. Size is but one, and not necessarily the most important aspect to be considered; composition, hydrophobicity, charge, and surface modifications also influence absorption. Thus, although reducing particle size from microscale to nanoscale may increase absorption, this is not universally true, and it appears to be material dependent. For example, 2.5 nm diameter dendrimers of polystyrene are not absorbed to a significantly greater extent than 50 nm polystyrene spheres.

Distribution

Unlike soluble chemicals, nanoparticles appear to be predominately absorbed from the intestine via the lymphoid system and distributed to local lymph nodes. There is little data to support significant uptake of nanoparticles through intestinal capillaries directly into portal blood and thence to the liver. Tissue disposition studies have typically reported low levels (up to a few percent of dose), mainly distributed to the mesenteric lymph nodes, the liver, and the spleen, due to uptake by the phagocytic cells embedded within these tissues.

Bio-stable particulates including polystyrene spheres and carbon black nanoparticles have been reported to be retained for significant periods in Peyer's patches and mesenteric lymph nodes, but importantly, with no associated short-term pathology. Long-term studies have not yet been conducted.

Studies of intravenously administered particulates have shown that under normal circumstances, they rapidly adsorb protein to form a protein corona. This promotes recognition and rapid clearance from the blood by the mononuclear phagocytic system (MPS) (previously known as the reticuloendothelial system) with particles predominately recovered in the Kupffer cells of the liver and in splenic macrophages, in lymph nodes, and in some cases in the bone marrow. The protein coronas are complex and variable, and of the approximately 3700 proteins in the plasma proteome, approximately 50 have been identified in contact with various nanoparticles. Opsonins, which may form part of the protein corona, may enhance uptake of the coated material by cells of the mononuclear MPS. Their presence creates a molecular signature that is recognized by immune and other cells, and which determines the biodistribution.

Whether nanoparticles bind proteins at all depends mainly on their surface characteristics, primarily hydrophobicity and charge. Other factors including core constituents, size, shape, and curvature influence the amount of protein bound, but not protein identity. In pharmaceutical applications, nanomaterials have been engineered to reduce this clearance, for example, through binding of polyethylene glycol (PEG) molecules to the surface to achieve usable blood circulation times. The PEG molecules prevent protein binding and reduce

recognition and uptake by the MPS leading to longer half lives of the nanoparticle.

Retention of bio-stable particulates can be very prolonged. For example, after intravenous (IV) administration of 13 nm quantum dots, 100% of the dose was recovered in the bodies of mice at 28 days, mainly in the muscle, skin, bone, and liver.

Elimination

The renal elimination of particulates from the blood is relatively well understood. For particulates, glomerular filtration is strictly dependent on the hydrodynamic diameter (HD) of the particle. For example, inulin which has a HD of approximately 3 nm is completely filtered whereas for IgG protein, which has a HD of approximately 11.0 nm, glomerular filtration is negligible. For bio-stable or slowly biodegraded materials, a HD below 5.5 nm is likely to be needed to permit complete elimination in urine. Similar findings have been reported for hydrophilic macromolecules such as dendrimer-based MRI contrast agents.

The hepatobiliary system is the primary route of excretion for particles not excreted in urine. The weight of evidence to date suggests elimination of particles in the bile and feces may occur at low to moderate levels, but this is a relatively slow and inefficient process with only modest percentages of the administered dose eliminated.

Regulation

Current size-based definitions of nanotechnologies and materials do not provide a sound foundation for a consideration of a safety assessment approach, as they do not capture any concept of functional or biological novelty. Although many countries have developed programs to promote nanotechnology development, none has developed separate safety assessment programs for nanomaterials. The current regulatory systems rely on safety evaluation protocols that are well established for the regulation of chemicals.

An underlying concern for the food industry and other proponents of nanotechnology is that the development of nanotechnology for food processing and packaging may be on a similar trajectory to technologies based on genetically modified (GM) organisms. Often community resistance to technological development is based on uncertainties and concerns about safety evaluation.

In the broader sense, all materials have structure at the nanometer scale. All polymers, gels, emulsions, clays, colloids, and larger organic molecules are nanomaterials, many of them engineered. Living cells contain functional nanostructures and our environment contains a wide range of natural and anthropogenic nanoparticles. From a regulatory perspective, it is novelty, not size, which raises concern. Thus, if sugar were fabricated into nanotubes (nano fairy floss/cotton candy) this material may well have very interesting novel properties in some applications but its addition to food would result only in dissolved sugar and not therefore present any novelty from a risk perspective.

Similarly, as most food additives are present in foods in a dissolved form, either in lipids or water, their preparation as nanoscaled powders to facilitate dispersion and dissolution presents neither novelty nor additional regulatory concern. Conversely, where a substance remains particulate in the final food as consumed, nano size has potential to alter characteristics relevant to safety assessment and therefore requires greater scrutiny.

In responding to the increased sophistication of the nanotechnologies, the primary focus for both regulators and the food industry is thus on materials likely to exhibit physicochemical and biological novelty.

The FAO/WHO reviewed the safety assessment and regulation of nanomaterials in food and agriculture in 2009. This report concludes that current risk assessment methodologies employed by the FAO/WHO and Codex are suitable for engineered nanoparticles. However, the report emphasized that the impacts of any novel features of nanomaterials need to be addressed. Furthermore, interaction of nanomaterials with other components of food (e.g., proteins, lipids, carbohydrates, and nucleic acids) must be taken into consideration. A similar position has emerged from ongoing reviews of the applications of nanotechnology in food by national regulatory agencies, such as Food Standards Australia New Zealand (FSANZ), the US Food & Drug Authority (FDA) and the European Food Safety Authority (EFSA).

See also: Disciplines Associated with Food Safety: Food Safety Toxicology. Food Technologies: Nanotechnology and Food Safety. Public Health Measures: Assessment of Novel Foods and Ingredients. Risk Analysis: Risk Assessment: Chemical Hazards; Risk Communication: Novel Foods and Novel Technologies. Safety of Food and Beverages: Safety Consideration in Developing Functional Foods

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US Food and Drug Administration (FDA).

FOOD ADDITIVES

Contents

Food Additives – General

LD Tomaska, Tharwa, ACT, Australia
S Brooke-Taylor, Bright, VIC, Australia

Glossary

Acceptable daily intake (ADI) The amount of a substance that may be consumed every day throughout an entire lifetime without appreciable risk. Expressed as mg per kg bodyweight per day, the ADI is normally calculated by dividing the no observed (adverse) effect level, by a safety/uncertainty factor of 100 in order to address the uncertainties associated with the available scientific data.

ADI 'not specified' A term used by the Joint FAO/WHO Expert Committee on Food Additives to signify that no adverse effects have been identified from exposure to the food additive, even at levels many times higher than those necessary for its technological function. Consequently, there is no appreciable risk from exposure arising from its use as a food additive under 'good manufacturing practices.'

Food additive Natural or synthetic chemically defined substances added to food to preserve flavor or enhance its taste and appearance or for other technological functions.

International Numbering System (INS) The INS for food additives developed and maintained by the Codex Alimentarius.

No observed adverse effect level (NOAEL) A level of chemical that produces no adverse effect. Expressed usually in mg per kg bodyweight per day.

Food Additives and History of Use

The use of food additives is not a modern invention. The earliest food additives, used as long ago as 5000 years, included vinegar to pickle vegetables; salting and smoking to help preserve meat and fish; herbs to improve flavor; and vegetable coloring to improve appearance of food. Today, food additives are added to food for these and a very wide variety of other purposes including uses to regulate acidity, prevent food from adhering to surfaces, reduce foaming, enhance texture, improve food's baking quality or color, and so on. Food additives can increase both the shelf life and the safety of food by preventing growth of spoilage and disease-causing organisms. They also offer the benefit of readily available and lower-priced food to consumers. This may also serve to reduce wastage. The use of food additives make possible the amazing choices of processed foods that millions of people enjoy on a daily basis, and reduce the time that they need to spend on food preparation.

Food additives can be naturally derived, for example, ascorbic acid (vitamin C) in citrus fruit and lecithin in eggs and soybeans, or can be synthetic. Some food additives have more than one functional use, for example, chitinase, used as a thickener, is also thought to have some antibacterial properties. Whether natural or synthetic, they are used in food in small quantities.

The first half of the twentieth century saw a significant increase in the use of food additives. Initially, their use was not controlled, and a number of these were subsequently shown to pose safety concerns. For example, in the early 1900s, borax (or boric acid) was widely used as a food preservative. Following administration to human volunteers, borax was shown to be too toxic for use in food. More recently, borax has been shown to be a potential reproductive toxin that posed an

Table 1 Food additive functional classes

The food additive functional classes are based on the Codex Class Names and the International Numbering System for Food Additives (CAC/GL 36-1989).

Acidity regulator
A food additive that controls the acidity or alkalinity of a food:

Acid
Acidifier
Acidity regulator
Alkali
Base
Buffer
Buffering agent
pH Adjusting agent

Anticaking agent
Reduces the tendency of particles of food to adhere to one another:

Anticaking agent
Antistick agent
Drying agent
Dusting agent

Antifoaming agent
A food additive that prevents or reduces foaming:

Antifoaming agent
Defoaming agent

Antioxidant
A food additive that prolongs the shelf life of foods by protecting against deterioration caused by oxidation:

Antibrowning agent
Antioxidant
Antioxidant synergist

Bleaching agent
A food additive (nonflour use) used to decolorize food. Bleaching agents do not include pigments:

Bleaching agent

Bulking agent
A food additive that contributes to the bulk of a food without contributing significantly to its available energy value:

Bulking agent
Filler

Carbonating agent
A food additive used to provide carbonation in a food:

Carbonating agent

Carrier
A food additive used to dissolve, dilute, disperse, or otherwise physically modify a food additive or nutrient without altering its function (and without exerting any technological effect itself) in order to facilitate its handling, application or use of the food additive or nutrient:

Carrier
Carrier solvent
Diluent for other food additives
Encapsulating agent
Nutrient carrier

(*Continued*)

Table 1 Continued

Color
A food additive that adds or restores color in a food:

Color
Decorative pigment
Surface colorant

Color retention agent
A food additive that stabilizes, retains or intensifies the color of a food:

Color adjunct
Color fixative
Color retention agent
Color stabilizer

Emulsifier
A food additive that forms or maintains a uniform emulsion of two or more phases in a food:

Clouding agent
Crystallization inhibitor
Density adjustment agent (flavoring oils in beverages)
Dispersing agent
Emulsifier
Plasticizer
Surface active agent
Suspension agent

Emulsifying salt
A food additive that, in the manufacture of processed food, rearranges proteins in order to prevent fat separation:

Emulsifying salt
Melding salt

Firming agent
A food additive that makes or keeps tissues of fruit or vegetables firm and crisp, or interacts with gelling agents to produce or strengthen a gel:

Firming agent

Flavor enhancer
A food additive that enhances the existing taste and/or odor of a food:

Flavor enhancer
Flavor synergist

Flour treatment agent
A food additive that is added to flour or dough to improve its baking quality or color:

Dough conditioner
Dough strengthening agent
Flour bleaching agent
Flour improver
Flour treatment agent

Foaming agent
A food additive that makes it possible to form or maintain a uniform dispersion of a gaseous phase in a liquid or solid food:

Aerating agent
Foaming agent
Whipping agent

Gelling agent
A food additive that gives a food texture through formation of a gel:

Gelling agent

(*Continued*)

Table 1 Continued

Glazing agent
A food additive that when applied to the external surface of a food, imparts a shiny appearance or provides a protective coating:
Coating agent
Film forming agent
Glazing agent
Polishing agent
Sealing agent
Surface-finishing agent
Humectant
A food additive that prevents food from drying out by counteracting the effect of a dry atmosphere:
Humectant
Moisture/water retention agent
Wetting agent
Packaging gas
A food additive gas that is introduced into a container before, during or after filling with food with the intention to protect the food, for example, from oxidation or spoilage:
Packaging gas
Preservative
A food additive that prolongs the shelf life of a food by protecting against deterioration caused by microorganisms:
Antimicrobial preservative
Antimicrobial synergist
Antimold and antirope agent
Antimycotic agent
Bacteriophage control agent
Fungistatic agent
Preservative
Propellant
A food additive gas that expels a food from a container:
Propellant
Raising agent
A food additive or a combination of food additives that liberate(s) gas and thereby increase(s) the volume of a dough or batter:
Raising agent
Sequestrant
A food additive that controls the availability of a cation:
Sequestrant
Stabilizer
A food additive that makes it possible to maintain a uniform dispersion of two or more components:
Colloidal stabilizer
Emulsion stabilizer
Foam stabilizer
Stabilizer
Sweetener
A food additive (other than a mono- or disaccharide sugar) that imparts a sweet taste to a food:
Bulk sweetener
Intense sweetener
Sweetener

(*Continued*)

Table 1 Continued

Thickener
A food additive that increases the viscosity of a food:
Binder
Bodying agent
Texturizing agent
Thickener

unacceptable risk to human health. As a result, borax is neither permitted for use in food manufacturing in a majority of countries nor is it traded internationally for food purposes. However, it is still used illegally in some countries to lengthen the shelf life of certain foods, such as rice noodles and dumplings, and therefore continues to pose a health risk to consumers.

Chemical Characterization

The Codex Alimentarius defines food additives as: "any substance not normally consumed as a food by itself and not normally used as a typical ingredient of the food, whether or not it has nutritive value, the intentional addition of which to food for a technological (including organoleptic) purpose in the manufacture, processing, preparation, treatment, packing, packaging, transport or holding of such food results, or may be reasonably expected to result, (directly or indirectly) in it or its by-products becoming a component of or otherwise affecting the characteristics of such foods. The term does not include contaminants, or substances added to food for maintaining or improving nutritional qualities, or sodium chloride."

Food additives as a group consist of a plethora of different chemicals numbering in several thousands; they cannot be described here individually; a number of food additives are discussed in more detail in other articles of this encyclopedia. The Codex Alimentarius Commission, an international body made up of some 190 countries, currently groups food additives into 27 classes, based on their functional use (**Table 1**).

Every food additive is assigned a unique number in the International Numbering System (INS), adopted and extended by the Codex Alimentarius. Most countries use these numbers in one form or another, to uniquely identify a food additive in legislation and for labeling purposes. The INS assigns a unique three- or four-digit reference number to each additive (e.g., ascorbic acid is 300), providing a shorthand way of labeling the ingredients of prepackaged foods and preventing confusion potentially caused by different nomenclature, spelling, and language differences. Number ranges have been preassigned to food additive classifications, so also gives information on the primary purpose of the additive, even without knowing the name (e.g., 600–699 series are flavor enhancers).

Control of Food Additives

Although it is true that all chemicals pose a potential risk to human health at some level of exposure (the Paracelsus

Principle), food additives as a class of chemicals are generally low in toxicity to human. The specific regulation of food additives to protect human health began in the mid-twentieth century when legislation, such as the 1958 Food Additives Amendment and 1960 Color Additives Amendment of the US Food and Drug Administration, required manufacturers to prove the safety of new additives before use. The testing requirements for food additives have increased steadily over the years and additives with suspected structures are required to undergo additional testing. For example, potential carcinogens need to be tested in two lifetime feeding studies in animals, usually the rat and mouse.

Today, the use of food additives is regulated by national legislation in most countries, and in international trade, as based on the recommendations of the Codex Alimentarius Commission. The overriding principle in controlling the use of these chemicals in food is that only additives which are shown to be safe can be used. This requirement is based on the fact that a choice can be made about the use of food additives; in other words, it is a discretionary activity. This is in contrast to naturally occurring contaminants and the presence of many microbial organisms in food, which are not deliberately added and, consequently, their presence in food must be controlled through other means.

Today, all new food additives undergo a safety assessment to minimize any potential adverse effects of food additive to human health. An extensive range of animal and other tests have been devised to assess health risk to humans. These tests assess the potential for acute and chronic toxicity, genetic damage, or cancer and any possible effect on development and reproduction. It is argued that these tests do not address the entire spectrum of toxicities that can be caused by chemicals, especially issues such as behavioral changes and that these issues need to be addressed through new tests.

A formal process exists for analyzing the test data on food additives. This safety evaluation is carried out at a number of different levels. At a global level it is performed by a scientific advisory body to the Food and Agriculture Organization (FAO) and World Health Organization (WHO), namely the Joint FAO/WHO Expert Committee on Food Additives (JECFA). In addition to countries, JECFA advises the Codex Alimentarius Commission, which has international responsibility for managing food safety and developing food standards. JECFA has been meeting annually to conduct safety evaluations of additives since 1956. The members of JECFA are selected on the basis of their scientific credentials. If supported by the safety data, JECFA will establish an acceptable daily intake (ADI) for a food additive as part of its evaluation. JECFA will also conduct an exposure assessment to determine if the proposed uses and maximum levels (MLs) in food are within the ADI. Their findings are published and are otherwise made public on the Internet.

Therefore, food additives are regulated as a positive list, which means that only food additives specifically listed in the regulations of a country can legally be added to food products, and the MLs in food must not be exceeded. This is in contrast to chemical and microbiological contaminants which are regulated as a negative list: Only those contaminants that are of concern to human health are subject to regulatory control with specified maximum limits in food.

A significant number of food additives currently in use have been assigned 'ADI not specified,' based on a documented history of safe use or extensive testing indicating a low order of toxicity compared with any possible exposure. These additives are permitted for use under conditions of good manufacturing practice. Substances approved this way in the USA and other countries are classified as 'generally recognized as safe'. More recently, Codex Alimentarius has assigned numerical use limits for many of these additives as part of their inclusion in the General Standard for Food Additives.

Legislative discrepancies exist between different countries in the definition of 'food additive.' For example, in the USA the term 'food additive' is not only limited to substances added for a technological purpose but also includes ingredients indirectly added for other purposes, such as boiler water additives and monomers from plastic food contact materials, and consequently some 3000 substances are approved for use as 'food additives.' In contrast, Canadian regulations recognize only approximately 400 'technical' food additives. Under international agreement, the Codex Alimentarius standards, which underpin international trade through their reference in the World Trade Organization (WTO) Sanitary and Phytosanitary (SPS) Agreement, are binding on national governments unless the country can demonstrate, through the use of a sound scientific risk assessment that the standard is insufficient to protect the health of their population. Where Codex is silent, countries are free to adopt their own standards subject to certain disciplines, for example, nondiscriminatory. National differences may often be ascribed to political and societal expectations of each country and may be challenged under WTO SPS Agreement.

Possibly the principal concern currently expressed by consumers in relation to food additives, both natural and synthetic, is the belief that their consumption may be associated with unwanted behavioral effects such as hyperactivity, especially in children. Another concern is that they may be linked to food allergies and insensitivities. The implicated food additives include food dyes and colorings (such as tartrazine, quinoline yellow, sunset yellow, and ponceau 4R), antioxidants (such as butylated hydroxy anisole (BHA) and butylated hydroxy toluene (BHT)), emulsifiers and stabilizers (such as gums and lecithin), flavorings and taste enhancers (such as monosodium glutamate (MSG), spices, and artificial sweeteners), and preservatives (such as benzoates, nitrates, and sulfites). The majority of these and other food additives have undergone reevaluation based on their human health risk assessments.

The majority of these as well as other food have undergone reevaluations of their human health risk assessments as a part of a periodic review. Current credible scientific evidence does not support a link between exposure to many of these food additives and adverse behavioral effects such as hypersensitivity. It is argued that the conventional testing of food additives is insufficiently sensitive to detect effects that are specific to humans and occur at a relatively low incidence in a population. A UK 'Southampton' study attempted to test the link between food additive coloring and changes in behavior. In this study, children received two mixtures of four synthetic colors plus sodium benzoate preservative in the diet and their behavior was observed. The study did not find a link.

Consequently, human health risks, such as behavioral changes or sensitivities, that may be caused by food additives be managed by labeling of prepacked food, to enable sensitive individuals to identify and avoid the specific chemical of concern. Policies covering unpackaged food and meals eaten out are highly variable. Education of consumers to avoid such additives is currently one of the approaches taken to manage these risks. There is also a shift by manufacturers to substitute the food additives with 'natural/from nature' foods that have the desired properties such as juice concentrates in place of colorings.

Human health continues to be at risk from the illegal use of chemicals that are not approved for use as food additives in the manufacture of food. For example, in developing countries, street-food vendors have been reported to continue to misuse of boric acid and Sudan Red.

Hazard Identification and Characterization

Most countries now require that all new food additives undergo a safety assessment before they are permitted for use in food manufacture. Also, new data on the toxicity of, or exposure to, an already approved food additive will trigger the need to reevaluate its risk to human health. Some countries regularly schedule periodic reviews of approved food additives in the recognition that testing requirements have increased, based on new knowledge about how chemicals can cause adverse effects in humans. Reviews can also be triggered by new information in the scientific or medical literature, ensuring that approved food additives continue to be safe to humans.

The purpose of performing a human health risk assessment for any given food additive is to determine a safe level of intake. Such an assessment determines whether the food additive can be allowed for use in food and in what amounts. The safe level of intake is defined as ADI, and is the level of intake by a human of a food additive, per day, for which no appreciable adverse effects are expected to occur when consumed over a lifetime. The ADI is usually expressed as milligrams of the chemical ingested per kilogram of bodyweight. The concept of ADI is based on the premise that, for compounds other than carcinogens and certain other toxins, there exists a discrete threshold of exposure below which adverse effects do not occur. Any chemical that is assessed to be both genotoxic and a potential carcinogen is not permitted for use as a food additive.

The safety assessment includes an evaluation of toxicological studies that identify the toxicity profile of a chemical, such as adverse effects and target organs of toxicity, and identification of the threshold level of chemicals that are associated to no toxic effects. Such a safety assessment identifies levels of chemicals that produce no observed adverse effect level (NOAEL), which is used to derive the ADI. It is customary to apply a 100-fold safety/uncertainty factor to the NOAEL to derive the ADI.

Many of the food additives in use today have been assessed by JECFA as well as by various regional bodies, such as the European Food Safety Authority and countries such as the USA, Canada, Japan, and Australia and are regarded as safe, when used as specified.

Exposure Assessment

An additional consideration of a safety assessment is the premise that the permitted use of food additives must not exceed the ADI. For example, although a specific color may be used in many foods up to a specified ML, the combined daily exposure to that coloring agent from all of these foods must not exceed the ADI established for that chemical. This is determined by an exposure assessment, which considers daily consumption of foods or food groups and concentration of the food additives in those foods or food groups. Often, the maximum permitted levels in each food group are used in these calculations, which tend to overestimate exposure for most people, and add a significant margin of safety to the assessment.

The methodology used for exposure assessment varies between countries due to different dietary patterns and the availability and quality food consumption data. JECFA undertakes exposure assessment for food additives using data drawn from the WHO Global Environment Monitoring System (GEMS)/Food Contamination Monitoring and Assessment Program (WHO GEMS food) as well as considering assessments made available to it by individual member countries. When feasible, JECFA will use the GEMS/Food Consumption Cluster Diets as the food consumption component of the exposure assessment.

Risk Characterization

Risk characterization considers the assessment of exposure to a chemical in context of its ADI. It determines whether the ADI may be exceeded and what the potential health consequences, i.e., severity and target population, may be, taking into consideration uncertainties and assumptions in the information.

For a new food additive, the risk characterization is relatively straightforward. If the ADI is not exceeded by the possible exposure, the additive is recommended for approval. However, if the ADI is exceeded, the additive is not recommended for approval. In such cases, the sponsor may either submit additional data to support an increase in the ADI, reduce the use levels in food, or eliminate some uses entirely. As the variety of processed foods increases, and the use of food additives widens, the possibility of exceeding the ADI increasingly comes under closer scrutiny for many established food additives.

One of the main issues debated is whether exceeding an ADI is a human health risk. Clearly, the ADI is the health standard for a food additive, so legislatively it should not be exceeded. For a scientist, the considerations include the fact that the ADI usually incorporates a 100-fold 'safety/uncertainty factor' that sets the ADI for a chemical 100 times below the level that caused no effect in the most sensitive animal species in experimental studies. In addition, exposure estimates often overestimate the level of a food additive in the diet, thereby adding an additional margin of safety. So, while exceeding the ADI for a food additive needs to be redressed, it should rarely be a cause for immediate public health action and concern.

Specifications and Methods of Analysis

The past misuse of food additives to adulterate food has, to some extent, driven the development of analytical methods. These are used today for regulatory purposes to ensure that food additives do not exceed their MLs or are not being used for unapproved uses or in unapproved foods. For some chemicals, rapid field tests have been developed to allow inspectors to quickly identify foods that have been adulterated. Analytical methods need to have large throughput capacity to handle large number of samples.

During the meetings of JECFA, the WHO experts conduct the safety assessment and the FAO experts focus on developing specifications and methods of analysis for those specifications. The specifications for the food additive is based on the specific chemical that is used in the toxicity testing of the food additive as that is the material being evaluated and approved. Slight variations in the manufacturing process or in the case of natural products, the extraction and purification process can alter the specifications of a food additive and possibly introduce a potential harmful contaminant. Consequently, all of the food additives have a defined method of analysis, identified under their unique identifier within the INS. These can be found in a number of internationally published sources of monographs:

- Combined Compendium of Food Additive Specifications, FAO JECFA.
- Food Chemicals Codex.
- European Union (EU) directives on food additives specification.
- The Specifications and Standards for Food Additives, Ministry of Health and Welfare (Japan).

Conclusion

Food additives are among the safest chemicals present in food because of the rigorous testing that is required before they may legally be used in food. Although certain safety issues associated with some food additives continue to be debated, the periodic review of food additives ensures that such concerns are addressed, using the most advanced testing protocols.

See also: Food Additives: Antioxidants. Colorants; Flavors and Flavor Enhancers. Preservatives. Foodborne Diseases: Overview of Chemical, Physical, and Other Significant Hazards. Other Significant Hazards: Physical Hazards in Foods. Safety of Food and Beverages: Risks of Food Adulteration

Further Reading

WHO (2009) *Principles and Methods for the Risk Assessment of Chemicals in Food*. Environmental Health Criteria (EHC) 240. ISBN 9789241572408.

Redbook (2000) Guidance for industry and other stakeholders toxicological principles for the safety assessment of food ingredients, July 2000, Revised July 2007. Available at http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/FoodIngredientsandPackaging/Redbook/default.htm (accessed February 2013).

Relevant Website

http://www.who.int/foodsafety/chem/gems/en/index1.html
WHO – Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme.

FOOD ADDITIVES

Antioxidants

C Schyvens, Food Standards Australia New Zealand, Canberra, ACT, Australia

Glossary

Clastogen An agent (certain chemicals, X-rays, ultraviolet light) capable of causing breaks in chromosomes.

Forestomach A nonglandular stomach that exists in certain animal species and is located between the esophagus and the primary glandular stomach, but is not present in humans.

Hyperplasia An increase in the number of normal cells in an organ or tissue. The presence of hyperplasia may be a sign of abnormal or precancerous changes.

Reactive oxygen species (ROS) Chemically reactive molecules containing oxygen, which can result in cellular damage if overproduced.

Two-generation study A study that investigates the effect of a test substance on the integrity and performance of the male and female reproductive systems, e.g., gonadal function, estrous cycle, mating behavior, conception, gestation, parturition, lactation, and weaning in the parents and progeny (F1 offspring), and the growth and development of the two generations of offspring (F1 and F2).

Introduction

Antioxidants are a group of food preservatives that delay or prevent the deterioration of foods by oxidative mechanisms. They include butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tertiary butylhydroquinone (TBHQ), tocopherols, ascorbic acid, citric acid, and sulfites, the latter of which also has antimicrobial properties. These molecules act by scavenging free radicals or oxygen, or by inhibiting enzymes that facilitate oxidation.

Oxidation of food is a common problem of both fresh and processed food. In many cases, reducing exposure to air by effective food packaging or by using modified atmosphere can help reduce oxidation. However, total exclusion of oxygen is often not feasible. In many foods, specific enzymes may also be involved in the oxidation of food molecules, leading to quality changes in the food. One type of oxidation reaction involves enzymes (phenolases) that oxidize amino acids, such as tyrosine in fruits including bananas and apples, causing browning. Antioxidants that inhibit enzyme-catalyzed oxidation include molecules that bind free oxygen, i.e., reducing agents such as ascorbic acid (vitamin C) and molecules that inactivate the enzymes, such as citric acid and sulfites.

Food can also undergo autooxidation; for example, the autooxidation of unsaturated fatty acids involves the reaction between carbon–carbon bonds and oxygen in the same or adjacent molecule. The products of autooxidation, called free radicals, are very reactive, producing compounds that cause off-odors and off-flavors characteristic of rancidity. Antioxidants, such as BHA, BHT, and TBHQ, react with free radicals to slow down the rate of autooxidation. Some of these antioxidants continue to attract public controversy regarding their safety to human health. This article outlines the risk assessment findings for BHA and BHT.

History of BHA and BHT

BHA and BHT are two antioxidants that are used to prolong the shelf-life of foods by protecting them against deterioration caused by oxidation, such as fat rancidity and color changes. BHA is an effective antioxidant in animal fats used in baked products. It is stable at high temperatures. BHT has characteristics similar to BHA and is also an effective antioxidant in animal fats, although it has limited use in oils.

Both antioxidants are also used in industries other than food. Since 1947, BHA has been used as an antioxidant and preservative in food packaging, animal feed, and cosmetics. BHT was patented in the US in 1947 and received approval for use as a food additive and preservative by the US Food and Drug Administration in 1954. Since 1959, BHT has been generally recognized as safe for use in foods.

Chemical Characterization

BHA

BHA (3-tertiary-butyl-4-hydroxyanisole) is a mixture of the two chemical isomers, 2(3)-tertiary-butyl-4-hydroxyanisole (3-BHA) and 2-tertiary-butyl-4-hydroxyanisol (2-BHA). Food grade BHA contains more than 85% 3-BHA and less than 15% 2-BHA.

BHA is a white or slightly yellow waxy solid that has an aromatic odor and a slightly bitter burning taste. It degrades following prolonged exposure to light. It is often produced in tablet form to prevent caking. BHA is insoluble in water, but is

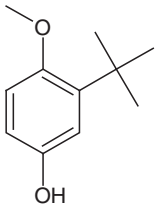

Figure 1 Structural formula of BHA (2-BHA isomer).

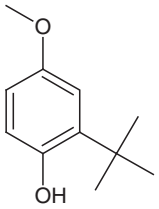

Figure 2 Structural formula of BHA (3-BHA isomer).

freely soluble in fats and oils. It has a melting point of 48–55 °C and boiling point of 264–270 °C (Figures 1 and 2).

BHT

BHT (2,6-ditertiary-butyl-*p*-cresol; 4-methyl-2,6-ditertiary-butyl-phenol) is a white crystalline solid. It is insoluble in water but readily soluble in fats and oils, exhibiting greater solubility in fats and oils than BHA or TBHQ. It has a melting point of 70 °C and a boiling point of 265 °C (Figure 3).

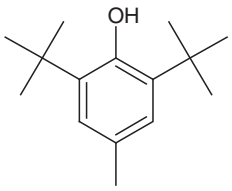

Figure 3 Structural formula of BHT.

Hazard Identification and Characterization

BHA

BHA is rapidly absorbed from the gastrointestinal tract (GIT), metabolized and excreted in the urine and feces, mainly in the form of metabolites. The acute toxicity of BHA in rats and mice has been reported as low, with oral LD_{50} values >2000 mg kg^{-1} bodyweight.

A large number of long-term toxicity and carcinogenicity studies have been performed with BHA, demonstrating proliferative changes in the forestomach. The studies have been undertaken using species with a forestomach (mice, rats, and hamsters) and without (guinea-pigs, dogs, pigs, and monkeys). Gastric epithelial hyperplasias, papillomas, and carcinomas linked to BHA exposure were only seen in species with forestomachs.

BHA is not genotoxic. Some clastogenic activity exerted *in vitro* by BHA and its metabolite, TBHQ, is concluded to be secondary to the formation of reactive oxygen species via a nongenotoxic threshold mechanism involving prooxidant species.

The National Toxicological Program (NTP) has listed BHA as 'reasonably anticipated to be a human carcinogen.' This has been based on studies in experimental animals demonstrating that dietary exposure to BHA caused benign and malignant tumors of the forestomach (papilloma and squamous-cell carcinoma) in rats of both sexes and in male mice and hamsters. There are no data available on the carcinogenicity of BHA in humans. NTP classification notwithstanding, the current consensus opinion is that these findings are not relevant to humans.

The Joint Food and Agriculture Organization (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) and the European Union Scientific Committee on Food (SCF) have considered the toxicity data on BHA on several occasions. A no observed adverse effect level (NOAEL) was established in 1989 by JECFA and SCF of 0.1% BHA in the diet, equivalent to 50.0 mg kg^{-1} bodyweight per day from chronic toxicity studies in rats. This was based on data indicating that 0.125% BHA but not 0.1% BHA in the diet induced mild hyperplasia in the rat forestomach. Subsequent consideration by the WHO and others have highlighted the limitations of rodent forestomach tumors for predicting human carcinogenicity due to mode-of-action and exposure conditions, concluding that these findings were not relevant to humans.

In 2011 the European Food Safety Authority (EFSA, SCF's contemporary) established a NOAEL at 100 mg kg^{-1} bodyweight per day, noting that, as humans do not have a forestomach, forestomach hyperplasia in rodents may no longer be considered relevant for human risk assessment. This value was based on a NOAEL for reproductive and developmental toxicity of 0.125% BHA in the diet (equivalent to at least 100 mg kg^{-1} bodyweight per day) that demonstrated rat pups showing growth retardation, increased mortality, and behavioral effects at higher dose levels.

In 1989, the JECFA and the SCF established an acceptable daily intake (ADI) for BHA of 0–0.5 mg kg^{-1} bodyweight per day based on the rat NOAEL of 50.0 mg kg^{-1} bodyweight per day using an uncertainty factor of 100. In 2011, the EFSA established an ADI for BHA of 1.0 mg kg^{-1} bodyweight per day based on the rat NOAEL of 100 mg kg^{-1} bodyweight per day due to adverse developmental effects in rat pups, and using an uncertainty factor of 100.

BHT

BHT is rapidly absorbed from the GIT. On absorption, BHT is distributed to the liver and body fat. Excretion is primarily via urine and to a smaller extent in the feces. There are significant species differences in the metabolism of BHT. In particular, biliary excretion of BHT seems not to be as significant in humans as it is in rats, rabbits, and dogs.

The acute toxicity of BHT in rats, mice, cats, and rabbits is low with oral LD_{50} values of >2000 mg kg^{-1} bodyweight and $>10\,000$ mg kg^{-1} bodyweight in guinea-pigs.

Short-term or subchronic exposure to BHT induces histopathological changes in the liver, lungs, and/or kidneys of mice, rats, and chickens. Increases in relative thyroid and

adrenal weights in rats were also identified. In mice and rats, exposure to high levels of BHT may also cause hemorrhage as a consequence of vitamin K antagonism.

In long-term toxicity studies, BHT at high doses can exert liver and lung tumor-promoting effects in some animal models. JECFA noted that in view of the probable involvement of hepatic enzyme induction in the development of hepatocellular damage associated with exposure to repeated doses of BHT, a well-defined threshold was demonstrated at 100 mg kg^{-1} bodyweight per day in rats. A NOAEL of 25 mg kg^{-1} bodyweight per day for BHT was based on effects in the reproductive studies (litter, size, sex ratio, and pup body weight gain during lactation) and hepatic enzyme induction seen in two separate two-generation studies in rats. BHT is not genotoxic.

In 1989, the SCF established an ADI of 0–0.05 mg kg^{-1} bodyweight per day for BHT based on the rat NOAEL of 5 mg kg^{-1} bodyweight per day and using an uncertainty factor of 100. An ADI for BHT was later established by JECFA in 1996 at 0–0.3 mg kg^{-1} bodyweight per day based on the rat NOAEL of 25 mg kg^{-1} bodyweight per day and using an equivalent uncertainty factor of 100. In 2012, EFSA reviewed the data for BHT and noted the outcomes from the two-generation rat studies. Consequently, the ADI was revised using the rat NOAEL of 25 mg kg^{-1} bodyweight per day and using an uncertainty factor of 100, to derive a value of 0.25 mg kg^{-1} bodyweight per day.

Exposure

BHA

Routes of exposure to BHA are ingestion, inhalation, and dermal contact. However, exposure to the latter two routes is minimal and only ingestion will be considered here.

The 1999 JECFA review of BHA dietary intake noted that estimates of national mean intake by consumers of BHA were lower than the ADI of 0–0.5 mg kg^{-1} bodyweight, ranging from 1% of the ADI for Japan to 80% of the ADI for Australia/New Zealand, and the US. The JECFA estimate assumes that all foods contain the maximum permitted levels of the food additives, often resulting in significant overestimation of exposure, given that foods in general contain much lower amounts of food additive than the maximum permitted levels and that many foods do not contain the additive at all.

Estimates of the intake of high consumers of BHA, based on food additive levels in national standards, exceeded the ADI in some cases, but ranged from 30% of the ADI for France to 260% of the ADI for Australia/New Zealand. However, the available data were insufficient to estimate the number of high consumers or the magnitude or duration of intake at levels above the ADI over a lifetime

The EFSA review of BHA dietary intake concluded that when considering the maximum permitted limits (MPLs), estimates reported for the UK adult population give a mean dietary exposure of 0.1 mg kg^{-1} bodyweight per day and 0.14 mg kg^{-1} bodyweight per day for high-level consumers, which are within the ADI. The main contributors to the total anticipated mean exposure to BHA (greater than 10%) were soups (14%), sauces and seasonings (13%), fine bakery wares (33%), and breakfast cereals (20%).

The mean dietary exposure of European children (aged 1–14 years) ranged from 0.1 to 0.3 mg kg^{-1} bodyweight per day and from 0.2 mg kg^{-1} bodyweight per day at the 95th percentile, which are within the ADI. The main contributors to the total anticipated mean exposure to BHA (>10% in all countries, contributions differed by country) were fine bakery wares (18–69%), snacks (10–19%), processed potato products (14–71%), and breakfast cereals (15–23%). These 2011 EFSA estimates are based on combining national data on food consumption with the maximum permitted usage levels for the additive, which can result in an overestimation of exposure.

BHT

The JECFA review of BHT intake noted that estimates of mean intake based on model diets or individual consumption data combined with the General Standard for Food Additives levels of use, consistently exceeded the ADI. Using this method, the mean intake was estimated to be between 0.70 and 0.99 mg kg^{-1} bodyweight (230% and 240% of the ADI for China and the US, respectively), and the intakes of high consumers were estimated to be from 2.0 to 6.0 mg kg^{-1} bodyweight. This method assumes that all foods contain the MPLs of the food additives, often resulting in significant overestimation of exposure, given that foods in general contain much lower amounts of food additives than the MPLs.

In contrast, intake estimates based on national levels of use were relatively consistent, ranging from 0 to 0.11 mg kg^{-1} bodyweight per day (0–30% of ADI) on the basis of poundage, 0.502 to 0.1 mg kg^{-1} bodyweight per day (20–40% of the ADI) on the basis of household surveys or sales data, 0.02 to 0.09 mg kg^{-1} per day (10–30% of the ADI) on the basis of model diets and nation levels of use (except the USA), and 0.02 to 0.1 mg kg^{-1} bodyweight per day (0.1–30% of the ADI) on the basis of individual consumption data.

JECFA also recognized that BHT is likely to be used in conjunction with other antioxidants, such as BHA and TBHQ, which act synergistically with BHT. Consequently, the amount of BHT used in practice will be lower and it will be used in fewer foods than assumed in the estimates. This also indicates that JECFA figures are an overestimate .

The 2012 EFSA review of BHT dietary intake concluded that when using a worst-case scenario of combined exposure to BHT from the food categories where its use is authorized, the EFSA Panel on Food Additives and Nutrient Sources Added to Food (ANS) estimated the potential exposure for adults to be on average 0.01–0.03 mg kg^{-1} bodyweight per day and 0.03–0.17 mg kg^{-1} bodyweight per day at the 95th percentile. For children, the ANS estimated the potential exposure in the range of 0.01–0.09 mg kg^{-1} per day at the mean and in the range of 0.05–0.30 mg kg^{-1} bodyweight per day at the 95th percentile. Given the assumptions, this is likely to be an overestimate of the actual exposure.

The ANS Panel noted that at the average exposure of adults to BHT is unlikely to exceed the 2012 ADI of 0.25 mg kg^{-1} bodyweight per day and at the 95th percentile. However, for children, while the ADI would unlikely be exceeded at the mean, it did exceed it for high consumers (assume 95th percentile) in some European countries, for example, Finland and the Netherlands.

Risk Characterization

Long-term toxicity and carcinogenicity studies involving the oral consumption of BHA or BHT in laboratory animals have indicated some species-specific toxicological effects. Given the extensive and long history of use of these synthetic antioxidants in food, together with animal studies, the weight of scientific evidence indicates that these chemicals are safe for use as food additives, with a clearly defined ADI. Although some dietary exposure estimates indicate limited exceedance of ADI for BHA and for BHT by average and higher consumers, these are based on the assumption that all foods contain the maximum permitted levels of BHA/BHT and as such tend to significantly overestimate actual exposure. On the basis of this information, it is concluded that there are presently no public health and safety concerns from the consumption of BHA or BHT in food at the MPLs. Increasing use of chemicals such as BHA and BHA in foods and other goods, however, needs to be monitored to ensure that the exposure remains within the allowed limits.

Methods of Analysis

A number of methods of analysis are available for the detection of BHA and BHT and are described in the scientific literature. For BHA, high-performance liquid chromatography (HPLC) and spectrofluorometric methods have been described. For BHT, HPLC, gas chromatography (GC) coupled with mass spectrometry (MS), gas chromatography with a flame ionization detector (FID) and direct Fourier transform infrared (FITR) spectroscopic methods are available.

Specifications have been prepared for BHA at the 33rd JECFA (1988), published in *FNP* 38 (1988) and *FNP* 52 (1992). Specifications for BHT have been prepared at the 37th JECFA (1990), published in *FNP* 52 (1992) superseding specifications prepared at the 30th JECFA (1986), published in *FNP* 37 (1986). Metals and arsenic specifications have been revised at the 61st JECFA (2003).

Acknowledgment

Chemical structures were produced using ACD/ChemSketch (Freeware), Advanced Chemistry Development, Inc., Canada.

See also: Food Additives: Food Additives – General; Natural Preservatives. Foodborne Diseases: Overview of Chemical, Physical, and Other Significant Hazards. Safety of Food and Beverages: Oils and Fats

Further Reading

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FOOD ADDITIVES

Colorants

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Glossary

Acceptable daily intake (ADI) The amount of a substance that may be consumed every day throughout an entire lifetime without appreciable risk. Expressed as mg per kg bodyweight per day, the ADI is normally calculated by dividing the no observed (adverse) effect level, by a safety/uncertainty factor of 100 in order to address the uncertainties associated with the available scientific data.

Acceptable daily intake 'not specified' A term used by the Joint FAO/WHO Expert Committee on Food Additives to signify that no adverse effects have been identified from exposure to the food additive, even at levels many times higher than those necessary for its technological function. Consequently, there is no appreciable risk from exposure arising from its use as a food additive under 'good manufacturing practices.'

Food additive Natural or synthetic chemically defined substances added to food to preserve flavor or enhance its taste and appearance or for other technological functions.

International Numbering System (INS) The INS for food additives developed and maintained by the Codex Alimentarius.

No observed adverse effect level (NOAEL) A level of chemical that produces no adverse effect. Expressed usually in mg per kg bodyweight per day.

Overview

Food manufacturing processes can cause degradation or loss of the natural pigments in the raw food and production of many processed foods, such as ice cream and snack foods, require the addition of color. Given raw materials often vary in color intensity, colorants may be added to ensure the processed food is uniform in color.

Colorants are used as food additives at relatively low levels to achieve the technological function; they may be natural or synthetic in origin. Most natural colors are derived from plant tissues and include:

- anthocyanins giving red and blue color, which are obtained from strawberries and grapes;
- carotenoids giving yellow, orange, and red color, which are obtained from annatto, saffron, paprika, carrot, and mushrooms; and
- phenolics with orange/yellow color, which are obtained from turmeric.

Many carotenoids used as food colorants are also synthesized chemically. Synthetic colorants are used, as there are often problems in terms of food processing with the intensity variation and water solubility associated with the natural colorants.

In general, colorants are water soluble, and their stability may be affected by light, heat, pH, and reducing and oxidizing agents. A number of food dyes have been chemically synthesized and approved for use in various countries, although different countries still vary in the spectrum of food colorants permitted. The Codex Alimentarius Commission (Codex) recommends standards that aim to harmonize the use of food additives, including colorants, and better facilitate trade in food, while ensuring human health is protected. All synthetic colorants have undergone extensive toxicological analysis before they can be used. Such tests are carried out in animals and require interpretation as to their relevance to humans, i.e., safety assessment.

The use of synthetic colors in foods has been an area of debate in recent years due to a proposed linked between behavioral disturbances, especially hyperactivity, in children and dietary exposure to colors in food. A study published in 2007 by McCann *et al.* contributed to this debate, with the authors concluding that their research demonstrated the effect of food color mixtures (which included Ponceau 4R, Sunset Yellow, and Quinoline Yellow) and preservatives (sodium benzoate) on increased hyperactivity in sensitive children. Subsequent assessments of this study by many independent scientific bodies concluded that the study provided limited evidence of small effects on children's behavior, but that these effects were not consistent and the study did not give cause to amend current safety limits for these food additives.

Therefore, although a causative link between exposure to colorants in food and hypersensitivity in children has not been demonstrated to date in animal studies, it is argued that animal studies may not be able to pick up these effects; however, food safety experts maintain that long-term studies, as well as multigeneration studies in animals do have the

capacity to identify neurotoxic effects. Epidemiological studies may be helpful, but they present challenges that include the design and size of the study and adequate exclusion of confounding factors.

Allergy and sensitivity reactions affect a small proportion of the population, with estimates ranging between 1% and 5% of the population with greater numbers reported for children than adults. In many countries, concerned individuals manage potential risks from food additives through avoidance of processed foods that contain the suspected agent, including those that are suspected to be caused by colors. The regulatory approach is to provide consumers with the relevant information on the label of foods to allow consumers to make an informed choice. However, this information is not consistently applied among unpackaged foods, for example, bulk food and foods eaten outside of home.

Some colorants are no longer approved for use in food, but are still used in some countries illegally, and may pose health risks. One of these is Sudan Red, which is a suspected carcinogen. The illegal use of this color in chili peppers has caused international concern, because many products were contaminated; however, in this instance, the levels of Sudan Red found in these foods were too low to pose a significant risk to human health.

The remainder of this article examines in detail the risk assessment information relevant to some colors of recent public interest: Ponceau 4R, Quinoline Yellow, and Sunset Yellow.

Ponceau 4R

History

Ponceau 4R (which is known by more than 100 synonyms including C.I. Food Red 7, Cochineal Red A, and New Coccine) is a synthetic red azo dye used in a range of alcoholic and nonalcoholic beverages, and a variety of foodstuffs including confectionary, desserts, cheeses, meats, preserved fruits, and sauces. The Codex General Standard for Food Additives specifies maximum limits for Ponceau 4R of 50–500 mg kg^{-1} in a range of foods and beverages. In Europe, the maximum permitted levels (MPLs) in food and beverages range up to 500 and 200 mg kg^{-1}, respectively. In Australia and New Zealand, the MPLs are up to 70 mg kg^{-1} in beverages and 290 mg kg^{-1} in foods. Ponceau 4R is not an approved food additive in the US or Canada due to a rationalization of the number of food additives.

The main health issue that attracts public interest in relation to the dietary consumption of Ponceau 4R (and a number of other synthetic food colors) is the possible effects on children's behavior, especially hyperactivity. The United Kingdom's Food Safety Authority (UKFSA) commissioned the 'Southampton study,' which investigated the effect of mixtures of six synthetic food colors (one of which was Ponceau 4R) and a preservative (sodium benzoate) on the behavior of children. The authors of this study suggested there was a link between consumption of these additive mixtures and hyperactivity in children. The UK Committee on Toxicity, Consumer Products and the Environment and European Food Safety Authority (EFSA) expert panels reviewed the results of this study and concluded that, although it provided limited evidence of small effects on children's behavior, these effects were not consistent and that the study did not give cause to amend current safety limits for these food additives.

Subsequently, the UKFSA Board decided there should be voluntary action by manufacturers in the UK to remove these colors by 2009. On 9 July 2008, the European Parliament ruled that food products containing specific food colors (tartrazine, Quinoline Yellow, Sunset Yellow, carmoisine, Ponceau 4R, and Allura Red) would need to be labeled 'May have an adverse effect on activity and attention in children.' This came into effect in July 2010. The risk management decisions by the UKFSA and the EU Parliament in relation to those food colors are not supported by the scientific opinion of EFSA.

In March 2011, the United States Food and Drug Administration (US FDA) held a public hearing, and sought the advice of its Food Advisory Committee on a possible linkage between dietary exposure to food colors and hyperactivity in children. In preparation for the hearing, the US FDA reviewed the available evidence, including the Southampton study and concluded that it did not warrant further action.

It is recognized that some people prefer to avoid certain food additives and therefore Ponceau 4R, like other food additives, is required to be identified by its class name (e.g., color) and by an individual name or code number (i.e., E124).

Chemical Characterization

Ponceau 4R exists principally as trisodium-2-hydroxy-1-(4-sulfonato-1-naphthylazo)-6,8-naphthalenedisulfonate (chemical abstracts service (CAS) No. 2611-82-7; International Numbering System No. 124) (Figure 1), with subsidiary coloring matters and sodium chloride or sodium sulfate.

From a food technology perspective, Ponceau 4R is a strawberry red color shade and is stable to light, heat, and acid but fades in the presence of ascorbic acid and SO_2.

Hazard Identification and Characterization

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has evaluated the toxicity of Ponceau 4R on several occasions, most recently in 2011. The acceptable daily intake (ADI) of 0–4 mg per kg bodyweight per day was established in 1983 and confirmed in 2011 based on the no observed adverse

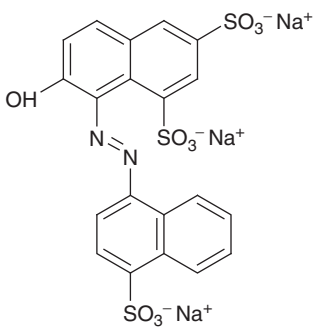

Figure 1 Structure of Ponceau 4R (trisodium-2-hydroxy-1-(4-sulfonato-1-naphthylazo)-6,8-naphthalenedisulfonate).

effect level (NOAEL) of 375 mg per kg bodyweight per day for renal effects in a long-term mouse study and using a 100-fold safety factor. In laboratory animal studies, Ponceau 4R was poorly absorbed from the digestive tract, where it is anaerobically reduced by microflora, with small levels of the resulting metabolites systemically absorbed. Ponceau 4R does not accumulate in any tissue. JECFA concluded that Ponceau 4R was not carcinogenic, genotoxic, neurotoxic, or caused reproductive or developmental toxicity. JECFA reviewed human data in relation to alleged allergic reactions to Ponceau 4R (urticarial and vasculitic reactions) but was unable to draw any conclusions due to limitations in the study design. Although Ponceau 4R was one of the six colors tested in the study by McCann *et al.* in 2007, dosing as a mixture did not enable the possible effect of Ponceau 4R to be elucidated.

The EU Scientific Committee on Food (SCF) evaluated Ponceau 4R in 1984, establishing the same ADI as JECFA. EFSA reconsidered Ponceau 4R, noting the absence of genotoxic and carcinogenic potential, and reaffirming its previous view that the study by McCann *et al.* provided no basis to amend the ADI. However, EFSA did revise the European ADI for Ponceau 4R from 4 to 0.7 mg per kg bodyweight per day without reevaluating the original laboratory animal studies that underpinned the ADI established by JECFA. Based on the JECFA summary report, EFSA concluded that the NOAEL for glomerulonephrosis in the pivotal, long-term mouse study was 70 mg per kg bodyweight per day. EFSA noted that sensitivity (i.e., mainly skin-related) reactions to Ponceau 4R (within mixtures of other colors) had been reported in some individuals within clinic populations. However, these reactions would be rare in the general population. On this basis, EFSA concluded that such sensitive individuals may react at doses of Ponceau lower than the ADI.

Exposure

The dietary exposure of Ponceau 4R for different population groups was examined by EFSA, Food Standards Australia New Zealand (FSANZ), and JECFA, and are summarized in Table 1. The relatively large difference in dietary exposure estimates between Europe and Australia are attributable to the use of maximum permitted, or reported use levels, and mean analyzed levels, respectively. JECFA considered that the use of the latter gives a more realistic exposure estimate.

Table 1 Dietary intakes of Ponceau 4R in different regions

Country (years)	*Mean intake (mg per kg bodyweight)*	*High-level intake*[a] *(mg per kg bodyweight)*
Europe		
Children, 1–10	0.3–2.5	0.7–6.7
UK		
Children, 1.5–4.5	1.4	3.5
Adults	0.5	1.1
Australia		
2–5	0.01	0.02
6–12	0.01	0.02
13–18	<0.01	0.01
19–24	<0.01	0.01
>25	<0.01	0.01
>2	<0.01	0.01

[a]95th or 97.5th percentile.

Risk Characterization

Conservative intake calculations performed by EFSA and using MPLs of Ponceau 4R in foods found an exceedance of the ADI of 0.7 mg per kg bodyweight per day for high percentile (97.5th) adults and 1–10-year-old children (95th/97.5th). In contrast, the use of analytical levels measured in foods to generate a more realistic Australian exposure estimate determined that all groups of consumers were well below the ADI (maximum of approximately 0.5% of the ADI in high-level consumers). The Australian exposure estimates are also below the EFSA ADI (approximately 35% of the ADI in high-level consumers). Given that the exposure estimates such as those of EFSA are recognized to significantly overestimate exposures, it is concluded that the current intakes of Ponceau 4R are not likely to pose a risk to human health.

Methods of Analysis

Methods of analysis for Ponceau 4R are described in the detailed product specifications prepared by the 74th JECFA in 2012 and EFSA in 2009.

Summary

Ponceau 4R is a red food color permitted to be added to a variety of beverages and solid foodstuffs in Europe and Australia. Health issues that are, at times, attributed to artificial colors per se include possible intolerance reactions in sensitive individuals and adverse effects on children's behavior. However, available data provide no compelling scientific case that Ponceau 4R can do either. The toxicity of Ponceau 4R has been evaluated by JECFA and EFSA. The compound is poorly absorbed from the digestive tract and is of relatively low toxicity, with no evidence of carcinogenicity, genotoxicity, neurotoxicity, or reproductive and developmental toxicity. Conservative dietary exposure calculations using maximum permitted European levels determined that exposures from diets were well within the European ADI of 0.7 mg per kg bodyweight per day and the JECFA ADI of 4 mg per kg bodyweight per day. In contrast, the use of analytical concentration data from Australia that reflect actual exposures rather than maximum levels permitted in foods indicated that the dietary exposure for the whole population was less than 2% of the JECFA ADI of 4 mg per kg bodyweight per day. In recognition that some people prefer to avoid this colorant, many countries mandate the labeling of prepackaged food to facilitate consumer choice.

Quinoline Yellow

History

Quinoline Yellow (also known as Acid Yellow, Food Yellow 13, C.I. 47005, and C.I.) is a synthetic colorant, classified into the

quinophthalone class of dyes. Quinoline Yellow is commonly used in confectionery, soft drinks, as well as other food and beverages. It is also used in the medical and cosmetic industries. Some sensitivity to Quinoline Yellow has been reported following exposure; however, a direct causative relationship has not been directly demonstrated.

Quinoline Yellow is a permitted food additive in certain countries. For example, in the EU, Quinoline Yellow is permitted in beverages, with a maximum level of 200 mg l^{-1} for alcoholic and 100 mg l^{-1} in nonalcoholic beverages. It is also used in a variety of foods, with MPLs ranging from 50 mg kg^{-1} in complete weight control formulas to 500 mg kg^{-1} in sauces, decorations, and coatings. In Australia, the MPLs for Quinoline Yellow in beverages is 70 mg l^{-1} and in other foods at 290 mg kg^{-1}.

In contrast, Quinoline Yellow is not listed as a permitted food color in Canada or the US, but is permitted in medicines and cosmetics. At present, there are no provisions for Quinoline Yellow by Codex.

Following the release of the McCann *et al.* study and suggested link between food colors and behavior alterations in children, there was considerable focus on the use of colors in food by food regulatory agencies across the world. Independent scientific review of this study (see Ponceau 4R above) concluded that, although it provided limited evidence of small effects on children's behavior, these effects were not consistent and that the study did not give cause to amend current safety limits for these food additives.

In 2011, US FDA Food advisory committee meeting also considered the outcomes of the McCann *et al.* study and concluded that the link between the dietary exposure to color additives in food and behavioral problems (e.g., hyperactivity) in children could not be established.

In 2009, the EFSA Panel on Food Additives and Nutrient Sources Added to Food (ANS) reevaluated Quinoline Yellow as part of their on-going review of food additives. The ANS findings concurred with those of the Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC), in relation to the McCann *et al.* study. As part of the broader review on Quinoline Yellow, a study investigating the long-term effects of chronic exposure to Quinoline Yellow and the reproductive and developmental toxicity of exposure was considered. In 2009, EFSA evaluated the toxicological database of Quinoline Yellow and established an ADI of 0–0.5 mg kg^{-1} bodyweight.

Chemical Characterization

Quinoline Yellow (CAS No. 8004-92-0; Molecular weight 477.38 g $mole^{-1}$) is a water soluble dye used to color food yellow/yellow–green and is stable under standard conditions. It can exist in three forms, usually as a mixture of monosulfonate ($C_{18}H_9NNaO_5S$), disulfonate ($C_{18}H_9NNa_2O_8S_2$), or trisulfonate ($C_{18}H_9NNa_3O_{11}S_3$). The principal component, however, is the disulfonate form, otherwise known as disodium 2-(1,3-dioxoindan-2-yl) quinoline-6,8-disulfonate (Figure 2). For purity purposes, the disulfonate form must be greater than or equal to 70%. Uncolored compounds such a sodium chloride and sodium sulfate may also be present in this mixture. Quinoline Yellow can be in a methylated or unmethylated state, and toxicological data of either form is used in safety assessments.

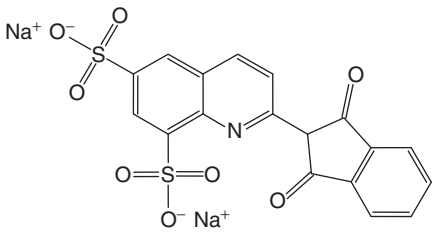

Figure 2 The chemical structure of the principal form of Quinoline Yellow (disodium 2-(1,3-dioxoindan-2-yl) quinoline-6,8-disulfonate).

Hazard Identification and Characterization

The toxicological evaluation of Quinoline Yellow as a food additive has been reviewed a number of times by the JECFA, with its first consideration by the committee in 1964 at its 8th meeting. A summary of JECFA evaluations of Quinoline Yellow from 1964 to 2011 are shown in Table 2. The most recent evaluation was conducted by JECFA at the request of Codex.

At this meeting, no new data was available to the committee for consideration; however, unpublished long-term generational reproductive toxicity studies not previously

Table 2 A summary of Joint FAO/WHO Expert Committee on Food Additives (JECFA) considerations for Quinoline Yellow

JECFA meeting	*Year*	*Outcome*	*References*
8th	1964	Acceptable daily intake (ADI) not established due to lack of toxicological data	WHO (1965)
13th	1969	Temporary ADI established of 0–1 mg kg^{-1} bodyweight	WHO (1970)
18th	1974	Temporary ADI of 0–0.5 mg kg^{-1} bodyweight	WHO (1974)
22nd	1978	Temporary ADI upheld	WHO (1978)
26th	1982	Extension of temporary ADI	WHO (1982)
28th	1984	ADI 0–10 mg kg^{-1} bodyweight	WHO (1984)
74th	2011	ADI established at the 28th meeting was withdrawn and a temporary ADI of 0–5 mg kg^{-1} bodyweight was established	WHO (2011)

Reports referenced can be found in the section Relevant Websites.

considered were included. The EFSA's redefinition of the ADI for Quinoline Yellow in 2009 was also taken into account.

Experimental feeding studies in rats and dogs has shown that the absorption rate of Quinoline Yellow is low (approximately 3–4%), with a large proportion of the absorbed color excreted in the urine. Unabsorbed Quinoline Yellow is excreted via the feces. Toxicological assessments over several evaluations has indicated that Quinoline Yellow is not carcinogenic or genotoxic. Generational studies in animals have indicated no adverse effect on reproduction.

Following deliberations of the JECFA in 2011, a temporary ADI of 0–5 mg kg^{-1} bodyweight was established, pending receipt of an unpublished toxicological study by 2013.

Exposure

The dietary exposure to Quinoline Yellow in a variety of population groups has been estimated in Australia, Europe, and the UK (Table 3). Major food contributors to this exposure were found to be soft drink, bakery products, and desserts.

Dietary exposure estimates to Quinoline Yellow in Australia were lower than those in the UK or Europe. JECFA considered this large difference to be a result of the approach taken in estimating the dietary exposures for the different region. For example, the dietary exposures for Australia were based on actual analytical concentrations determined in food, whereas the Europe and UK dietary exposure estimates used the assumption that all foods to which this color is added contained it at the maximum permitted use level, resulting in a significant overestimate. Dietary exposure estimates derived from the actual concentration data in foods was considered to provide a more accurate estimate of the dietary exposure to Quinoline Yellow.

Table 3 Estimated dietary exposures to Quinoline Yellow in three regions at the mean- and high-level intake

Country (years)	*Mean intake (mg per kg bodyweight day)*	*High-level intake (mg per kg bodyweight per day)*
Australia[b]		
2–5	<0.01 (0.002)	0.01 (0.005)
6–12	<0.01 (0.001)	0.01 (0.003)
13–18	<0.01 (0.001)[a]	<0.01 (0.002)[a]
19–24	<0.01	0.01
>25	<0.01	0.01
>2	<0.01	0.01
Europe[c]		
1–10	0.8–3.5	1.8–9.6
UK[d]		
1.5–4.5	3.1	7.3
Adults	0.9	2.1

[a]Age groups is for 13–16 years.
[b]Mean concentrations derived from food analysis; high-level intake refers to the 90th percentile consumers. Exposures shown in square brackets are the revised exposures for Australia for children only.
[c]Maximum concentrations based on permitted use levels was used; high-level intake refers to the 95th percentile consumers.
[d]Maximum concentrations based on permitted use levels was used; high-level intake refers to the 97.5th percentile consumers.

Risk Characterization

Quinoline Yellow has not been associated with any significant long-term toxicity, is not genotoxic or carcinogenic and there is no evidence of adverse effects on reproduction or development based on studies in animals. A recent study suggested, but could not establish a link between the dietary exposure to multiple color additives including Quinoline Yellow in food and behavioral problems such as hyperactivity in children. A temporary ADI of 0–5 mg kg^{-1} bodyweight was set by JECFA in 2011. The exposure estimates indicate that dietary exposure to this colorant is below the temporary ADI of 0–5 mg per kg bodyweight per day. Given the low toxicity of Quinoline Yellow, the conservative method of establishing the ADI and the fact that the exposure is well within the ADI, dietary exposure to Quinoline Yellow is considered to be safe to human health.

Methods of Analysis

The tentative specifications and methods of analysis for Quinoline Yellow are described by EFSA and JECFA.

Summary

Quinoline Yellow is a synthetic dye that is used in the food as a coloring agent in confectionery, soft drinks, and other food and beverages. It is permitted for use in food in Australia and Europe, however, it is not permitted in the US or Canada. The use of Quinoline Yellow in foods has been an area of debate recently due to the McCann study that purported a link between hyperactivity in children and dietary exposure to a mixture of colors including Quinoline Yellow. However, reviews of this study by various independent scientific authorities concluded that the study did not give cause to amend current safety limits for these food additives. Recent reviews conducted by EFSA and JECFA have not found any new evidence to indicate a concern over the safety of Quinoline Yellow. A temporary ADI of Quinoline Yellow was set recently by JECFA at 0–5 mg per kg bodyweight per day, pending receipt of an unpublished toxicological study. The estimates for Quinoline Yellow intake from food, estimated in several countries and regions indicate that dietary exposure is within the limits of the JECFA temporary ADI and therefore is safe to human health.

Sunset Yellow FCF

History

Sunset Yellow FCF is a synthetic azo dye with a long history of use as a coloring for beverages and variety of foods, including confectionary, desserts, soups, cheeses, savory snacks, sauces, and preserved fruits. The Codex GFSA includes provisions for Sunset Yellow FCF in a wide range of foods and beverages with maximum levels ranging from 50 to 400 mg kg^{-1}. In Europe, the MPLs in food and beverages range from 50 to 500 mg kg^{-1} with a similar range in Australia and New Zealand. In Canada and the US, Sunset Yellow FCF is permitted to be added to foods with no maximum levels specified.

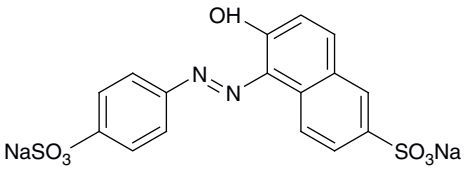

Figure 3 Structural formula for the principal component of Sunset Yellow FCF.

As for several other synthetic food colors, Sunset Yellow FCF has attracted attention because of a claimed effect on children's behavior. The UKFSA commissioned the 'Southampton study,' which investigated the effect of mixtures of six synthetic food colors (one of which was Sunset Yellow FCF) and also contained a preservative (sodium benzoate) on the behavior of children. This study, and the resulting risk management actions in the UK, EU, and the US, are described in the section above, Ponceau 4R and Quinoline Yellow.

Chemical Characterization

The major chemical component of Sunset Yellow FCF is disodium 6-hydroxy-5-(4-sulfonatophenylazo)-2-naphthalenesulfonate (CAS No. 2783-94-0; Figure 3). Sunset Yellow FCF is an orange color shade and possesses good light, heat, and acid stability, but fades in the presence of ascorbic acid and SO_2.

Hazard Identification and Characterization

The JECFA has evaluated the toxicity of Sunset Yellow FCF on several occasions, most recently in 2011. JECFA initially established on ADI of 0–2.5 mg kg^{-1} bodyweight. The current JECFA ADI of 0–4 mg kg^{-1} bodyweight was established based on the NOAEL of 375 mg per kg bodyweight per day for reduced bodyweight in the offspring of rats in a long-term study and using a 100-fold safety/uncertainty factor. In laboratory animal studies, Sunset Yellow FCF is poorly absorbed from the digestive tract where it is metabolized by bacteria to yield sulfanilic acid and 1-amino-2-naphthol-6-sulfonic acid, which are absorbed and metabolized to various *N*-acetylated forms. Sunset Yellow FCF has not shown potential for carcinogenicity, genotoxicity, or developmental toxicity. JECFA considered reports suggesting that asthma or chronic idiopathic urticaria/angioedema in humans may be induced by oral exposure to Sunset Yellow FCF and noted that most of these reports are characterized by poorly controlled challenge procedures and that no conclusions could be made from the available evidence.

The EU SCF evaluated Sunset Yellow FCF in 1984, establishing an ADI of 0–2.5 mg kg^{-1} bodyweight. EFSA reevaluated Sunset Yellow FCF based on the existing database in addition to the supplementary studies published in the scientific literature. EFSA noted the absence of genotoxic and carcinogenic potential. It also reaffirmed a previous view that the 2007 McCann study on a link between hyperactivity in children and exposure to some food additives including colors, provided no basis to amend the ADI. However, EFSA concluded that findings for the testes in a supplementary 90-day rat study gave reason to reduce the ADI, by an extra safety/uncertainty factor of 2.5, to 0–1 mg kg^{-1} bodyweight and made the ADI temporary. Both JECFA and EFSA noted that the test material for this study was obtained from a local market in India and its purity was not specified. Despite a lack of treatment-related effects on the testes in other rodent studies, including 2-year studies in mice and rats at higher dose levels, EFSA recommended that clarification of the effects of Sunset Yellow FCF on the testis, sperm morphology, and sperm mobility should be provided, based on a 28-day study performed according to Organisation for Economic Co-operation and Development test guideline 407.

With regard to intolerance reactions in humans, EFSA concluded that there was only limited scientific evidence to suggest such a response. However, EFSA concluded that sensitive individuals may react at doses that are within the ADI. This final statement, however, did not appear to be supported by any scientific evidence.

Table 4 Dietary intakes of Sunset Yellow FCF in different regions

Region (years)	*Mean intake (mg per kg bodyweight per day)*	*High-level intake[a] (mg per kg bodyweight per day)*
Europe		
Children, 1–10	0.3–2.5	0.7–6.7
UK		
Children, 1.5–4.5	1.4	3.5
Adults	0.5	1.1
Australia		
2–5	0.05	0.12
6–12	0.04	0.12
13–18	0.03	0.09
19–24	0.03	0.08
>25	0.01	0.04
>2	0.02	0.06

[a]90th, 95th, or 97.5th percentile.

Exposure

The dietary intake of Sunset Yellow FCF for different population groups were examined by EFSA, Food Standards Australia New Zealand (FSANZ), and JECFA, and are summarized in Table 4. The relatively large difference in dietary exposure estimates between Europe and Australia are attributable to the use of maximum permitted or reported use levels, and mean analyzed levels, respectively. JECFA considered that the use of the latter gives a more realistic exposure estimate.

Risk Characterization

The toxicity of Sunset Yellow FCF has been evaluated recently, indicating no concern over its carcinogenic, genotoxic, or developmental potential. Some questions remain over effects on testes, but this is currently not supported by other studies on testicular effects. Intake calculations performed by EFSA using MPLs of Sunset Yellow FCF in food found exceedances of the ADI of 0–1 mg kg^{-1} bodyweight for high percentile (97.5th) adults and 1–10-year-old children (95th/97.5th), but such methodology generally produces significant overestimates. In

contrast, the use of levels from the analytical survey in Australia to generate a data-based exposure estimate determined that all groups of consumers were well below the ADI of 0–4 mg kg^{-1} bodyweight (maximum of approximately 3% of the ADI in high-level consumers). The Australian exposure estimates are also below the EFSA ADI (approximately12% of the ADI in high-level consumers). Current dietary intakes of Sunset Yellow FCF are therefore not considered to present any human health concerns.

Methods of Analysis

Methods for the analysis of Sunset Yellow FCF in food are described by EFSA.

Summary

Sunset Yellow FCF (also known as Orange Yellow S, C.I. Food Yellow 3, FD&C Yellow #6) is a synthetic azo dye permitted to be added to a variety of beverages and foods. The toxicology of Sunset Yellow FCF has been recently evaluated by the JECFA and EFSA. Systemic absorption of the compound is minimal following oral ingestion. The toxicological properties of Sunset Yellow FCF are well characterized and there is no evidence of carcinogenicity, genotoxicity, or developmental toxicity. ADIs of 0–1 and 0–4 mg kg^{-1} bodyweight have been established by EFSA and JECFA, respectively. EFSA established a lower ADI because of concerns regarding potential effects on the testes observed in a 90-day rat study. However, both EFSA and JECFA noted that the test article in this study was of unspecified purity. Treatment-related effects on the testes have not been observed in other rodent studies of longer duration using higher dose levels of Sunset Yellow FCF. Dietary exposure assessments using modeling assumptions that produce a very conservative estimate in Europe determined that there were exceedances of the EFSA ADI of 0–1 mg kg^{-1} bodyweight. In contrast, the use of analytical concentration data from Australia to generate a more realistic exposure estimate indicated that intakes were well below the JECFA ADI of 0–4 mg kg^{-1} bodyweight and also below the lower ADI established by EFSA. Exposure estimates based on analytical data are more reliable and therefore, the conclusion is reached that the current use of Sunset Yellow FCF is safe.

As for several other synthetic colors, it has been claimed that Sunset Yellow FCF causes intolerance reactions in sensitive individuals and adverse effects on children's behavior. However, there is little robust scientific evidence that Sunset Yellow FCF can cause such effects. Labeling of processed prepackaged foods provides a tool for consumers wishing to avoid this colorant.

Acknowledgment

Chemical structures produced using ACD/ChemSketch (Freeware). Advanced Chemistry Development, Inc., Canada.

See also: Food Additives: Food Additives – General. Foodborne Diseases: Overview of Emerging Food Technologies

Further Reading

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FOOD ADDITIVES

Flavors and Flavor Enhancers

TE Kaitano, Food Standards Australia New Zealand, Wellington, New Zealand

Glossary

Acceptable daily intake (ADI) An estimate of the amount of a substance that can be ingested daily over a lifetime without appreciable risk to health.

Adverse reaction With respect to food, an unintended and undesirable response by the body to food. Examples include food allergies that involve an immune response to specific components of food, and food intolerances that include metabolic food disorders such as phenylketonuria, and idiosyncratic reactions that occur through unknown mechanisms.

Blood–brain barrier A barrier that separates the brain and spinal cord from the blood. The barrier protects the central nervous system from unwanted and/or potentially harmful substances.

ED_{50} The dose causing 50% of subjects in an experiment to show a specified treatment-related effect following administration of a test substance.

Median lethal dose (LD_{50}) The dose of a test substance causing 50% mortality in experimental animals.

Parenteral Administration of a substance into the body via a route other than oral, e.g., by intravenous means.

Umami It is the savory taste, which is one of the five basic tastes (others are salty, sour, sweet, and bitter).

Flavorings are substances used to impart taste and/or smell to food, and/or to intensify the existing flavors of products. Flavor enhancers activate receptors for the umami or savory taste (the four other tastes are sweet, salt, sour, and bitter) and, thus, introduce a new taste to products. Thus, flavor enhancers are actually themselves a type of flavor. Flavorings have a long history of safe use in a wide variety of foods, from confectionery and soft drinks to cereals, cakes, and yogurts. Compared with flavors, the number of flavor enhancers in use is far fewer, but consumer concern for their safety has made them the subject of much public debate.

Flavoring agents are composed of large and divergent groups of materials, including:

- artificial substances unlikely to occur naturally in food;
- natural materials not normally consumed as food, their derived products, and the equivalent nature-identical flavorings
- herbs and spices, their derived products, and the equivalent nature-identical flavorings; and
- natural flavoring substances obtained from vegetable and animal products normally consumed as food, whether processed or not, and their synthetic equivalents.

The safety evaluation of flavoring agents presents a special challenge. Flavoring substances are generally consumed in low amounts, but there are several thousand individual flavoring substances in commercial use worldwide.

All of the existing individual flavoring substances can be arranged into approximately 40 groups comprising substances with related chemical structures and similar known or predicted metabolic fates. A general method for the safety evaluation of flavoring agents takes the approach that in most cases, dietary exposure to these substances is low and self-limiting, and the majority of flavors are metabolized rapidly to innocuous end products. This fact limits the need for toxicological testing of many flavoring agents, and therefore the safety evaluation of flavors is done by combining data on intake, metabolic fate, and basic toxicity to perform assessments of flavorings in related structural groups.

The current Joint Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Committee on Food Additives (JECFA) Procedure for the Safety Evaluation of Flavoring Agents is outlined in Environmental Health Criteria (EHC) 240 of the International Programme on Chemical Safety (IPCS). The approach incorporates a series of criteria designed to provide a method to evaluate the large number of flavorings. The criteria take account of available information on dietary exposure from current uses, structure–activity relationships, and known or predicted metabolism, plus any available toxicity data on the compound or structurally related compounds. The use of these criteria provides a means of sorting flavoring substances in terms of presence or absence of potential safety concerns and provides guidance on the nature and extent of data required to perform a safety evaluation.

The criteria take advantage of the fact that some flavoring agents occur as normal constituents of mammalian tissues or are metabolized to form such constituents and are then completely metabolized to innocuous end products, such as

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carbon dioxide and water. Flavoring agents with these characteristics are considered to be safe for consumption if dietary exposure is below the threshold of concern for the structural class, but are evaluated on the basis of toxicity data if dietary exposure is above the threshold of concern for the structural class. This safety evaluation may involve the use of toxicity data on the individual substance concerned or may rely, at least in part, on toxicity data on substances of closely related structure.

For flavoring agents that are not known or predicted to be metabolized to innocuous end products, the safety evaluation must be based on toxicity data, even if estimated dietary exposure is low. In such cases, there must be an adequate margin of safety between dietary exposure to flavoring agent and the no observed effect level or no observed adverse effect level (NOEL/NOAEL) for the substance or the NOEL/NOAEL for a substance of closely related structure on which the safety evaluation relies.

Flavoring agents currently in use for which no toxicity or metabolic data exist, and for which estimated dietary exposure is extremely low, less than 1.5 μg day^{-1}, are considered not to present a safety concern provided they do not contain structural alerts for genotoxicity.

The safety evaluation procedure is not intended to be applied to flavoring agents with existing unresolved problems of toxicity. Alternative approaches are used when data on specific flavoring agents are considered to warrant such action. Where acceptable daily intakes (ADIs) had previously been established for some flavoring agents or groups of flavoring agents, they are retained, as the information on which they are based is considered to be relevant for an evaluation of their safety and, in addition, they may have other uses as food additive.

Arguably, the most contentious flavor enhancer added to food from the perspective of a safety to human health is monosodium L-glutamate (MSG). Public perception continues to raise this as an issue of interest to the health authorities of many countries. An overview on its safety is presented in this article.

Monosodium L-glutamate

Overview

L-Glutamate occurs naturally in many foods and has a characteristic savory taste, sometimes called umami. Its salts including monosodium L-glutamate (MSG) have been used as flavor enhancers for over a century.

Since the 1960s, MSG has been reported to trigger minor adverse reactions now called the 'MSG symptom complex.' However, studies, such as blind challenge studies, to date have failed to prove an unequivocal link between MSG and the reported symptoms.

MSG has been associated with brain lesions in animals at plasma levels that can only be attained by parenteral injection or a large oral bolus. Such high plasma levels cannot be reached in humans when MSG is used as a flavor enhancer because the maximum palatable level of glutamate in food is limited to approximately 60 mg kg^{-1} bodyweight; ingestion with food delays and significantly dampens the elevation of plasma glutamate levels; and glutamate is extensively metabolized by enterocytes. Humans are therefore not at risk of adverse neurological effects from exposure to dietary MSG.

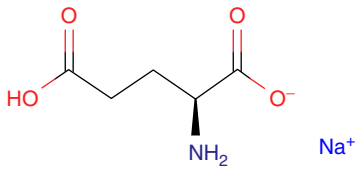

Figure 1 Chemical structure of monosodium L-glutamate, CAS No. 142-47-2. Reproduced from United States National Library of Medicine (2012) Sodium glutamate. Available at http://chem.sis.nlm.nih.gov/chemidplus/.

The conclusion from the assessment of a plethora of studies is that glutamate is safe for use by the general population as a flavor enhancer. Many countries regulate the labeling of food with information about additives such as MSG to inform consumer choice and provide a tool for individuals who prefer to avoid food additives, including MSG.

History

In 1908, Kikunae Ikeda, a Japanese scientist, discovered that MSG was responsible for the characteristic meaty or savory taste of the broth of dried bonito and Japanese seaweed (*Laminaria japonica*), and called this taste 'umami'. He patented an MSG production method involving hydrolysis of plant and animal proteins using strong acids. MSG is now produced via fermentation processes using starch hydrolysates and molasses from sugarcane and sugar beets and is used in a wide variety of processed and restaurant food as a flavor enhancer. Potassium, ammonium, and magnesium salts of glutamic acid are also used for the same purpose.

MSG has been the subject of much public controversy owing to its claimed association with what was initially termed 'Chinese restaurant syndrome,' but is now generally referred to as 'MSG symptom complex.'

Chemical Characterization

Pure MSG is a water-soluble white powder. Glutamates dissociate in aqueous solution to give the glutamate anion and the corresponding cation, for example, Na^+, K^+, and Mg^{2+}. Therefore, there is no difference between glutamate added as MSG, and that occurring naturally in food. The chemical structure of MSG is shown in Figure 1.

Some cations such as Na^+, K^+, and Mg^{2+} enhance the umami taste. L-Glutamate bound in protein molecules and D-glutamate do not possess this taste. All references to MSG and glutamate in this article refer to the L-isomer.

Hazard Identification and Characterization

Self-reported reactions in humans include headaches, general weakness, palpitations, and pain at the back of the neck starting about an hour after eating food containing MSG, and lasting for 2 h with no apparent long-term effect. It was hypothesized in the late 1960s that MSG, along with salt and

alcohol, could be responsible for the MSG syndrome complex. Since then, numerous 'case reports' and studies have appeared in the literature attempting to either prove or disprove this link.

MSG is now considered to be one of the most intensively scrutinized food additives. It has been reviewed twice by the JECFA, last in 1987. The European Commission published an opinion of its Scientific Committee for Food (SCF) in 1991, whereas the United States Food and Drug Administration commissioned the Federation of American Societies for Experimental Biology (FASEB) to compile a report on MSG, which was published in 1995. In Australasia, Food Standards Australia New Zealand (FSANZ) published a safety assessment of MSG in 2003. Academics have also reviewed MSG, one of the most notable being a consensus conference at the University of Hohenheim in 1997. A recent review focused on whether MSG triggers asthma in asthmatic individuals.

The list of adverse reactions claimed to be associated with MSG has grown significantly since 1968. It now includes burning sensations in the back of the neck, forearms and chest; facial pressure/tightness; chest pain; headache; nausea; palpitation; numbness in the back of the neck, radiating to arms and back; tingling, warmth, weakness in the face, temples, upper back, neck and arms; bronchospasm (observed in asthmatics only); drowsiness; and weakness.

The JECFA Review

JECFA reviewed hundreds of studies covering acute, subchronic, and chronic toxicity tests in animals and studies in human volunteers. The oral median lethal dose (LD_{50}) for MSG in the mouse and rat is very high, between 10 and 20 g kg^{-1} bodyweight. In this context, the largest palatable dose of MSG for humans is approximately 60 mg kg^{-1} bodyweight, so acute toxicity in humans is highly unlikely. Subchronic and chronic toxicity studies in rats and mice at dietary levels of up to 4% the diet did not reveal any adverse effects in either species. In dogs, no treatment-related effects were seen in 2-year feeding studies at dietary levels of up to 10%.

JECFA specifically reviewed the potential neurotoxicity of MSG because brain lesions (focal necrosis of the hypothalamus) had been reported in rodents and rabbits after intravenous or subcutaneous administration of glutamate or after very high bolus doses by gavage. In all, JECFA reviewed 59 different studies in mice, rats, hamsters, dogs, rabbits, guinea pigs, duck, and primates.

Mice were the most sensitive species for the brain lesions findings. Blood levels associated with neuronal damage were 100–300 μmol dl^{-1} in neonates, 380 μmol dl^{-1} in weanlings, and >630 μmol dl^{-1} in adults. In humans, plasma levels of this magnitude have not been attained even after bolus doses of 150 mg kg^{-1} bodyweight (approximately 10 g for a 70 kg adult). The oral effective dose $(ED)_{50}$ for production of brain lesions in the neonatal mouse is approximately 500 mg kg^{-1} bodyweight by gavage, whereas the largest palatable level for humans is approximately 60 mg kg^{-1} bodyweight with higher doses causing nausea. It was thus concluded that voluntary ingestion would not exceed this level and therefore could not reach threshold levels associated with this toxicity finding.

In humans, extensive studies in volunteers have failed to link MSG to the range of symptoms seen in the MSG symptom complex. In studies with lactating mothers, oral administration of 6 g MSG in water or liquid diet did not affect normal glutamate levels in milk. Studies in humans have also shown that oral administration of palatable doses of MSG does not result in an appreciable increase in blood glutamate levels.

On the basis of the available evidence, JECFA concluded that the total dietary intake of glutamates from all sources, i.e., glutamates naturally present in food and those added at levels necessary to achieve the desired flavor-enhancing effect, do not represent a health hazard. Consequently, JECFA concluded it was not necessary to set an ADI for L-glutamic acid and its monosodium, potassium, calcium, and ammonium salts.

European Commission Review

The European Commission's SCF reviewed acute, subchronic, and chronic toxicity studies in mice, rats, and dogs and found no MSG-related adverse effects. MSG neither affected any reproductive nor developmental parameters in mice, rats, rabbits, and monkeys. The SCF noted that no brain lesions have occurred in mice, rats, or hamsters exposed to very high doses of MSG in the diet.

In terms of idiosyncratic reactions to MSG in humans, the SCF noted that some of the reactions have also been observed with glutamate-free foods. Considering the large normal dietary intake of glutamates and data from animal and human studies, the SCF concluded it was not necessary to establish an ADI for glutamates.

FASEB Review

FASEB concluded that there was no evidence suggesting that orally administered MSG can affect the central nervous system in humans. Based on testimonial reports and a review of the literature at the time, FASEB concluded there was a subpopulation of presumably healthy individuals who react with the MSG symptom complex within 1 h of ingesting a bolus of ≥ 3 g MSG given without food. They also concluded that there appeared to be a subpopulation of individuals with severe unstable asthma who may experience bronchospasms after ingesting large doses of MSG (1.5–2.5 g) without food.

However, a more recent review concluded that there was no evidence to suggest MSG is associated with asthma attack in chronic asthmatics. Another study using mouse models of asthma concluded MSG was not involved in the development of asthma or in acute asthmatic responses.

The FSANZ Review

FSANZ's review of eight studies conducted between 1987 and 1999 investigating the putative link between MSG and asthma attacks concluded that the evidence for MSG-induced asthma attacks is inconclusive. The weight of evidence from more recent studies suggests MSG is not a significant trigger factor for asthma.

Regarding the link between dietary exposure to MSG and the MSG symptom complex, FSANZ concurred with FASEB that a small subpopulation may experience adverse reactions on consuming large amounts (>3 g) of MSG in the absence of food. These reactions are neither serious nor long lasting and, more significantly, are expected to be attenuated when MSG is consumed with food, particularly carbohydrate-rich food.

The Hohenheim Consensus Meeting on MSG

Hohenheim consensus conferences are meetings convened by the University of Hohenheim bringing together global experts. One of these was convened to discuss MSG in 1997, with a subsequent update in 2007.

The conference concluded that glutamate has not been shown to trigger bronchospasms or to exert adverse effects on the lung. Studies involving known asthmatics with self-reported MSG sensitivity have failed to establish such a link. The conference also concluded that there are no clear criteria that could be used to define MSG-sensitive individuals.

It was also concluded there is no evidence that dietary glutamate (natural and when added as a flavor enhancer) causes neurological effects in humans. Plasma levels of glutamate do not rise even when a meal containing added glutamate is consumed because up to 95% of glutamate in food is used as an energy source by enterocytes. The blood–brain barrier prevents influx of plasma glutamate into the central nervous system. Even a defective blood–brain barrier is unlikely to increase the risk of glutamate-mediated neurotoxicity since dietary intake of glutamate does not cause an increase in plasma levels.

In considering the potential effects of dietary glutamate on the fetus, it was concluded that the placenta metabolizes glutamate to the extent that it is an effective barrier. Studies have shown that fetal levels of glutamate remained the same even when maternally high plasma levels were attained. Therefore, orally administered glutamate is not expected to affect fetal development.

Exposure

Humans are exposed to dietary glutamate from birth, even when exclusively breastfed, because human milk also contains glutamate. It is estimated that a 4 kg infant consuming 600 ml milk per day would ingest approximately 76 mg free glutamate and 1.3–1.5 g bound glutamate per day.

Global consumption of MSG used as a food additive increased by 2.5 times between 1995 and 2007. The amount of bound and free glutamate naturally occurring in food is shown in Table 1.

Table 1 Natural glutamate content in foods

Food type	*Bound glutamate (mg 100 g^{-1})*	*Free glutamate (mg 100 g^{-1})*
Cow's milk	819	2
Human milk	229	22
Parmesan cheese	9 847	1200
Eggs	1 583	23
Chicken	3 309	44
Duck	3 636	69
Beef	2 846	33
Pork	2 325	23
Cod	2 101	9
Mackerel	22 382	36
Salmon	2 216	20
Peas	5 583	200
Corn	1 765	130
Carrots	218	33
Spinach	289	39
Tomatoes	238	140
Potatoes	280	180
Concentrated extracts (e.g., marmite and oyster sauce)	–	900–1960

Source: Reproduced from Yoshida Y (1998) Umami taste and traditional seasonings. *Food Reviews International* 14: 213–246 and Nicholas and Jones (1991).

Risk Characterization

The only toxic effects observed in studies with MSG were brain lesions induced in laboratory animals at very high plasma glutamate levels following very high bolus doses without food. Such high plasma levels cannot be reached in humans when MSG is used as a flavor enhancer because food delays and significantly dampens the elevation of plasma glutamate levels; glutamate is extensively metabolized by enterocytes and the maximum palatable level of glutamate in food is limited to approximately 60 mg kg^{-1} bodyweight. Humans are therefore not at risk of adverse neurological effects from exposure to dietary MSG.

Assessment of many studies involving individuals with self-reported sensitivity to glutamate have failed to prove an unequivocal link between glutamate ingestion and the 'MSG symptom complex,' particularly those symptoms that can be objectively measured, for example, bronchospasms. There are no traits or phenotypes that are uniquely associated with these individuals.

In conclusion, available evidence confirms the use of MSG at levels sufficient to attain a flavor-enhancing effect does not present a risk to the general population. The dose levels associated with adverse effects in experimental animals far exceed the levels necessary to achieve the desired technological effect in food. Moreover, the quantity of MSG that can be added to food is limited because large amounts of MSG make food unpalatable.

Reviews by several regulatory and expert bodies have all reached the same conclusion, and MSG continues to be used globally. Individuals who prefer to avoid this food additive can do so, as many countries provide labeling information for processed food. However, it is more difficult to do this with unpackaged food or meals eaten outside the home. It is worth noting that avoiding MSG altogether is difficult because glutamate is naturally present in a wide variety of foods as shown in Table 1.

See also: Foodborne Diseases: Overview of Chemical, Physical, and Other Significant Hazards

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FOOD ADDITIVES

Preservatives

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Introduction

The safety of certain preservatives, as with other food additives, has been the subject of debate among the consumer groups, academics, and food regulators. As a case study, this article looks at the safety issues associated with the preservatives sodium nitrate and nitrite. Nitrate and nitrite ions are ubiquitous in the environment and occur naturally in fruits and vegetables as a part of the nitrogen cycle. Nitrate and nitrite, as their sodium or potassium salts, have been used as food additives in cured meats for many years primarily to prevent growth and toxin production by *Clostridium botulinum*. The toxicology of nitrate and nitrite has been comprehensively reviewed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), which established acceptable daily intakes (ADIs) of 0–3.7 mg kg^{-1} bodyweight for nitrate and 0–0.07 mg kg^{-1} bodyweight for nitrite.

These reviews also considered concerns that the presence of nitrite and *N*-nitrosatable compounds may give rise to the endogenous formation of potentially carcinogenic *N*-nitroso compounds. Exposure to nitrate in the diet occurs mainly through ingestion of fruits and vegetables, particularly leafy green vegetables. Conversely, exposure to nitrite occurs mainly through the endogenous conversion of ingested nitrate to nitrite in human saliva, and this exposure pathway is generally considered to significantly exceed the exposure to nitrite through direct addition to food. The European Food Safety Authority (EFSA) concluded that exposure to nitrate and nitrite in fruits and vegetables is not likely to be associated with appreciable health risks, and as such, the recognized health benefits from consuming these foods should prevail. When used as food additives, EFSA has advised that levels of nitrate and nitrite added to meat should be kept to the minimum level to achieve their technological function, in order to ensure the microbiological safety of meat, and potential exposure to preformed *N*-nitroso compounds is kept to minimum achievable levels.

History of Nitrate and Nitrite

Nitrate and nitrite, as their sodium or potassium salts, have been used as food additives for many years to prevent growth of and toxin production by pathogenic bacteria, in particular *C. botulinum*, and as a color retention agent. Addition of nitrite or nitrate improves the microbiological safety of these foods and extends their safe shelf life. This offers benefits to consumers in terms of the availability of a variety of different foods that are safe, convenient, and cost effective. Nitrite exerts a concentration-dependent antimicrobial effect in cured meat products, including against *C. botulinum*. Nitrate, by contrast, has no direct activity against *C. botulinum* but functions as a source of nitrite, generated by microbial activity in particular traditional products.

Although the use of nitrate and nitrite may improve the microbiological safety of meat, the use of nitrite to cure meat was also seriously questioned in the 1970s due to the potential for the presence of preformed *N*-nitrosamines, which may have a carcinogenic potential. These issues were considered in reports published by the National Academy of Sciences in the early 1980s, which to some extent alleviated public concern in relation to potential human health risks associated with cured meats.

Significant concentrations of nitrate are also found naturally in various fruits and vegetables. It has long been established that these levels are dependent on a number of factors including the use of fertilizer, location and soil type, carbon dioxide concentrations (in greenhouse vegetables), seasonal light intensity and duration of light exposure, and water availability. Nitrate concentrations in vegetables may also vary up to orders of magnitude dependent on the vegetable species and the part of the plant sampled. High concentrations of nitrate tend to accumulate in the leaves, roots, petioles, or stems of certain plants meaning that leafy vegetables including lettuce or spinach, and root crops such as beetroot, may accumulate high concentrations of nitrate. In contrast, levels of nitrate in vegetables such as carrots or onions are likely to be lower. Nitrite concentrations generally tend to be low in fresh undamaged vegetables; however, levels can increase rapidly in certain nitrate-rich vegetables, particularly if pureed and stored at room temperature. In addition to temperature, this increase is dependent on nitrate reductase activity in the plant and the level of bacterial contamination.

Chemical Characterization

Sodium nitrate ($NaNo_3$; 7631-99-4) consists of clear, colorless, odorless transparent crystals or white granules that are deliquescent in moist air. It is freely soluble in water and slightly soluble in ethanol. Sodium nitrite ($NaNo_2$; 7632-00-0) is a white or slightly yellow solid that is freely soluble in water and sparingly soluble in ethanol.

Hazard Identification and Characterization

JECFA has evaluated the toxicity of nitrate and nitrite on several occasions and most recently in 2002. An ADI of 0–3.7 mg kg^{-1} bodyweight was established for nitrate based on a no observed adverse effect level (NOAEL) of 370 mg per kg bodyweight per day in a 2-year rat study and applying a 100-fold safety factor. At higher doses, rats showed slightly depressed growth and inanition.

For nitrite, JECFA established an ADI of 0–0.07 mg kg^{-1} bodyweight based on a NOAEL of 6.7 mg per kg bodyweight per day in a 2-year rat study in which effects were seen in the heart and lungs at higher doses. Nitrite reacts with hemoglobin to form methemoglobin in the blood, which is the critical acute toxicological endpoint following nitrate and nitrite exposure. JECFA considered that as increased levels of methemoglobin can occur after a single dose, the acute toxicity of nitrite should be reviewed at a future meeting. The EU Scientific Committee for Food (SCF) also reviewed the toxicological effects of nitrate and nitrite and established an ADI of 0–3.7 mg kg^{-1} bodyweight for nitrate in 1990 and retained the ADI in 1995. The SCF derived an ADI of 0–0.06 mg kg^{-1} bodyweight for nitrite. In 2008, the EFSA confirmed the ADI for nitrate.

There have been a number of studies that have investigated the carcinogenic potential of nitrate and nitrite, and concerns have also been raised that the additives may give rise to the formation of potentially carcinogenic *N*-nitroso compounds. The results of carcinogenicity studies with nitrate in mice and rats were negative. In a 2-year National Toxicology Program study, there was equivocal evidence of an increased incidence of squamous cell papilloma and carcinoma in the forestomach in female mice given sodium nitrite at 165 mg per kg bodyweight per day. Nitrite was not carcinogenic in male mice or male and female rats.

At its 44th meeting, JECFA concluded that the epidemiological data provided no evidence for an association between exposure of humans to nitrate and nitrite and the risk for cancer. Additional epidemiological studies evaluated at the 50th meeting did not provide evidence that nitrate or nitrite was carcinogenic to humans. The Committee did note that several *N*-nitroso compounds were formed endogenously when nitrite and *N*-nitrosatable compounds were present together at high concentrations. However, it was concluded that as there was no quantitative evidence of endogenous formation of carcinogenic *N*-nitroso compounds at levels likely to be achieved in the diet, a quantitative risk assessment on the basis of endogenously formed *N*-nitroso compounds was not appropriate.

A recent epidemiological study in 2012 by An Pan *et al.*, which looked at a possible link between nitrates/nitrites and premature deaths, attracted some attention when it's authors stated, "this study provides clear evidence that regular consumption of red meat, especially processed meat, contributes substantially to premature death." The study claimed that when deaths were broken down into specific causes, eating any kind of red meat increased the chances of dying from heart disease by 16% and from cancer by 10%. Processed red meat raised the risk of heart disease death by 21% and cancer death by 16%. However, although this and several other studies associate processed-meat consumption with an increased risk of mortality, a causal link remains to be established.

Exposure

The EFSA Panel on Contaminants in the Food Chain investigated the amounts of nitrate found in vegetables consumed and any relevant considerations on the possible balance between risks and benefits. Using a conservative approach, the panel compared nitrate exposure estimates with the ADI for nitrate (equivalent to 222 mg day^{-1} for a 60 kg person). On that basis, it was estimated that a person eating 400 g of mixed vegetables would ingest approximately 157 mg of nitrate per day, which is below the ADI and the panel noted that for most people, actual nitrate intakes were likely to be substantially lower. For a small part of the population in some countries that eat mainly leafy vegetables, the ADI could be exceeded. However, it was concluded that the estimated exposures to nitrate from vegetables are unlikely to result in appreciable health risks, and as such, the recognized beneficial effects of consumption of vegetables should prevail.

Importantly, the report also recognized that exposure to nitrite occurs mainly through the endogenous metabolism of ingested nitrate to nitrite, and although fruits and vegetables contribute 11–41% of exogenous nitrite dietary intake, this amount is significantly exceeded by the endogenous reduction of secreted salivary nitrate. As such, the exposure assessment focused mainly on nitrate concentrations in vegetables and considered nitrite only in the context of dealing with the total body burden of nitrate and its metabolites. More recently, the EFSA Panel on Food Additives and Nutrient Sources Added to Food issued a statement on nitrites in meat products. The panel noted that in several European countries the mean exposure estimates for nitrite added to foods were above the ADI. The panel concluded that, consistent with recommendations made by the SCF in 1995, exposures to preformed nitrosamines in food should be minimized by technological practices including reducing the levels of nitrate and nitrite added to foods to ensure microbiological safety.

In a study conducted by the Australian regulatory body, Food Standards Australia New Zealand, the major sources of estimated nitrate dietary exposures across different population groups were vegetables (42–78%) and fruits (including juices) (11–30%). Highest concentrations of nitrate were generally found in leafy green vegetables such as spinach, consistent with other international findings. Vegetables (44–57%) and fruits (including juices) (20–38%) were also the major contributors to estimated dietary nitrite exposure across the population groups. Nitrite exposure from processed meats accounted for only a relatively small amount of total dietary nitrite exposure (5–7%). Estimated Australian dietary exposures to nitrate and nitrite were not considered to represent an appreciable health and safety risk. Rather, the health benefits of fruits and vegetables are widely accepted, including evidence of a protective effect of certain vegetables, legumes, and fruits against the development of a number of noncommunicable chronic diseases, among them cancer and cardiovascular disease.

Methods of Analysis

Methods of analysis for nitrates and nitrites are described in the detailed product specifications prepared by the 44th JECFA (1995), published in Food and Nutrition Paper Add 3 19 (1995).

Risk Characterization

The main concern associated with the addition of sodium nitrate/nitrite to food was its carcinogenic potential. Assessment of studies in laboratory animals found no evidence to support these concerns and epidemiological data provided no evidence for an association between exposure of humans to nitrate and nitrite and the risk for cancer. Significantly, exposure to nitrite occurs mainly through the endogenous metabolic conversion of ingested nitrate to nitrite, and although fruits and vegetables contribute between 11% and 41% of exogenous nitrite dietary intake, this amount is significantly exceeded by the body's reduction of secreted salivary nitrate.

Another concern associated with nitrates/nitrites centers around the concern that these food additives may give rise to the formation of potentially carcinogenic *N*-nitroso compounds. Assessments of this issue concluded that several *N*-nitroso compounds are formed endogenously when nitrite and *N*-nitrosatable compounds are present together at high concentrations, but there was no quantitative evidence of endogenous formation of carcinogenic *N*-nitroso compounds at levels likely to be achieved in the diet.

Estimates of the dietary exposure from added nitrates/nitrites suggest that it is likely to be within the ADI, albeit the mean exposure estimates for nitrite added to foods are above the ADI in some European countries. Given that nitrites are formed from natural as well as added nitrates by the body's metabolic processes, any risk to human health must be considered as a balance between the benefit that the food preservation confers on the safety of food and the health risk of exceeding the ADI. Preformed nitrosamines in food can and should be minimized by technological practices including reducing the levels of nitrate and nitrite added to foods to ensure microbiological safety.

See also: Bacteria: *Clostridium botulinum*. Environmental Contaminants: Nitrate and Nitrite. Food Additives: Antioxidants; Food Additives – General. Foodborne Diseases: Overview of Chemical, Physical, and Other Significant Hazards. Processing Contaminants: *N*-Nitrosamines

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FOOD ADDITIVES

Natural Preservatives

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Glossary

Antimicrobial A substance that fights microbial infection by killing microorganisms or inhibiting their replication or growth.

Antioxidant A substance that reduces damage due to oxygen, such as that caused by free radicals.

Functional group An atom or group of atoms, such as a carboxyl group, that replaces hydrogen in an organic compound and that defines the structure of a family of compounds and determines the properties of the family.

Shelf life The time length that foods, beverages, pharmaceutical drugs, chemicals, and many other perishable items are given before they are considered unsuitable for sale, use, or consumption. In some regions, a best before, use by or freshness date is required on packaged perishable foods.

Background to Food Preservatives

The efficiency of chemical preservatives depends primarily on the concentration of the preservative, the composition of food, and the type of microorganism or process to be inhibited. They are among the most effective means for maintaining food quality and are generally used for many preservation purposes. Examples of food preservatives that inhibit microbes include benzoates, nitrites, ascorbates, and sorbates. Preservatives that act as an antioxidants include butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). Some chemicals such as sulfites and sulfur dioxide can be used for both. For labeling purposes, preservatives currently authorized in the European Union are given numbers in the range from E200 to E297.

Sorbic acid and its more soluble potassium salt are among the most efficient and versatile food preservatives used today. Sorbate preservatives are effective inhibitors of most common microorganisms that can attack foods, without affecting the taste, color, or flavor. Sorbates are used at their optimum effectiveness below pH 6.0 and are relatively ineffective at pH 7.0.

Benzoic acid received its name from the gum benzoin, the plant from whose resin it was first derived. In the nineteenth century it was synthesized from coal tar. Today it is manufactured from toluene, a petroleum by-product. It is classified as a carboxylic acid, weakly acidic, with a pH of 2.8. In normal conditions it has a white, flaky appearance that actually consists of small, needlelike crystals. Both benzoic acid and sodium benzoate have inhibitory effects on the yeast growth. Like most food additives, the amount of benzoates that can be added to foods is carefully controlled. For example, the Codex Alimentarius Commission (Codex), an intergovernmental organization that makes recommendations on food safety standards, and limits the amount of benzoic acid and its salts to 0.05–0.1% by volume. Most foods are allowed no more than 1 mg kg^{-1}. Liquid egg products, diet foods, chewing gum, and processed vegetables are the foods with the highest amount of benzoate legally allowed.

Sulfites are used as an antioxidants to prevent the discoloration of light-colored fruits and vegetables, such as dried apples and dehydrated potatoes. They are also used in the winemaking process because they inhibit bacterial growth without interfering with the development of yeasts. The use of sulfites in foods that are important sources of thiamine (vitamin B_1) is prohibited because of their ability to destroy thiamine. In the USA, the Food and Drug Administration (FDA) estimated that more than 1 million asthmatics are sensitive or allergic to sulfites. In 1986 FDA ruled that the sulfites used specifically as preservatives must be listed on the label, regardless of the amount in the finished product. FDA also banned the use of sulfites on fresh fruits and vegetables intended to be sold as such. In other countries, sulfites used in food processing, but not serving as preservatives in the final food, must be listed on the label if they are present at levels of 10 ppm or higher, which is consistent with Codex labeling recommendations.

BHA and BHT are phenolic compounds added to foods to preserve fats. They also may have antiviral and antimicrobial activities. BHA is a mixture of the isomers 3-tert-butyl-4-hydroxyanisole and 2-tert-butyl-4-hydroxyanisole. Oxygen reacts preferentially with BHA or BHT rather than oxidizing the fats or oils, thereby protecting them from spoilage. BHA is generally used to keep fats from becoming rancid. It is also used as a yeast defoaming agent. BHA can be found in butter, meat, cereals, chewing gum, baked goods, snack foods, dehydrated potatoes, and beer. It is used to preserve food odor, color, and flavor. Many packaging materials incorporate BHT.

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Both antioxidants have undergone the safety evaluations by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and a number of national authorities. A transient elevation of plasmatic lipids and cholesterol can be observed with BHA. An acceptable daily intake (ADI) of 0–0.5 mg kg^{-1} bodyweight was established by JECFA for BHA in 1989. A lower ADI (0–0.05 mg kg^{-1} bodyweight) for BHT was established in 1989 on the basis of reproduction and thyroid and hematological effects observed in a 90 day feeding study in the rat by the Scientific Committee for Food of the European Commission. Further details on the toxicology of these compounds can be found in other article.

All food preservatives are submitted to a scientific risk assessment consisting of establishing a health-based reference value, such as the ADI and comparison of that value with the predicted or measured dietary exposure. The concentration limits for food additives are therefore established on the basis of their safety assessment consistent with their technical function. For over the past 10 years, systems to monitor the intake of these substances were established by many countries. Recommendations for similar procedures were also made by the Codex Committee on Food Additives. The possible adverse health effects of the additives include possible reactions to benzoates or sulfites in susceptible subjects suffering from asthma or urticarial and local gastric irritations and toxicity in subjects with reduced sulfite oxidase activity. The ADIs for benzoates and sulfites were established by JECFA at 0–5 and 0–0.7 mg kg^{-1} bodyweight, respectively. Further details on the benzoates are provided in other article.

Natural Preservatives

Owing to greater consumer concern for synthetic chemical additives, food preserved with natural preservatives has become more popular. Plant essential oils, as well as similar compounds, have been considered as promising natural antimicrobials. Their selection should be based on the sensory and chemical compatibility with the target food, on the efficiency against undesirable microorganisms, on the safety concern, and cost. Originally added to change or improve taste, spices and herbs can also be used to enhance the shelf life. Usually, compounds with phenolic groups are most effective. Among these, the oils of clove, oregano, rosemary, thyme, sage, and vanillin have been found to be the most consistently effective compounds. They are generally more inhibitory against Gram-positive than Gram-negative bacteria. Plant essential oils are usually a mixture of several components. The oils with high levels of eugenol (allspice, clove bud and leaf, bay, and cinnamon leaf), cinnamamic aldehyde (cinnamon bark, cassia oil), and citral are usually strong antimicrobials. Activity of sage and rosemary is due to borneol and other phenolics in the terpene fraction. The volatile terpenes carvacrol, p-cymene, and thymol are responsible for the antimicrobial activity of oregano, thyme, and savory. Spices (woody stemmed plants), which have strong antimicrobial activity include all spice, cinnamon, clove, mustard, and vanillin. As a general observation, spice extracts are less effective than the whole spice. The greater resistance of Gram-negative bacteria to essential oils is likely due in part to the greater complexity of the double membrane containing cell envelope of these organisms, in contrast with the single membrane structure of Gram-positive bacteria. The modulating influence of food composition on the antimicrobial effectiveness of essential oils is an important area of study. The presence of fat, carbohydrate, protein, salt, and pH generally influence the effectiveness of these agents in foods. Their antimicrobial strength is also reduced in foods with low water activity. By encapsulating antimicrobial essential oils, not only they can be protected from heat, but also be released at a controlled rate to deliver effective inhibitory concentrations over extended periods.

Biological preservation implies a novel scientifically based approach to improve the microbiological safety of foods. By definition, this concept refers to the use of antagonistic microorganisms or their metabolic products to inhibit or destroy undesired microorganisms in foods. Owing to their typical association with food fermentation and long tradition as food-grade bacteria, lactic acid bacteria (LAB) are generally recognized as safe. LAB can exert a biopreservative or inhibitory effect against other microorganisms as a result of competition for nutrients and/or of the production of bacteriocins and other antagonistic compounds, such as organic acids and hydrogen peroxide. Bacteriocins are extracellularly released peptides or protein molecules that are bactericidal for bacteria that are closely related to the producing microorganism. However, some bacteriocins do not kill susceptible bacteria but instead exhibit a bacteriostatic mode of action. The majority of LAB bacteriocins studied have been shown to be small peptides with a molecular weight below 10 000 Da. Their bactericidal activity appears to be limited to other Gram-positive bacteria; each producer bacterial strain has a mechanism of self-protection against its own bacteriocin. One group of these low molecular weight bacteriocins contains unusual amino acids, such as lanthionine and β-methyl-lanthionine. These bacteriocins are known as 'lantibiotics.' The most prominent member of this group is nisin, a bacteriocin produced by strains of *Lactococcus lactis* and it is the only one that has found practical applications in food technology. Unfortunately, Gram-negative bacteria are generally not sensitive to LAB bacteriocins unless the barrier function of their outer membrane is destroyed by the treatment with chelating agents. There are several possible strategies for the application of bacteriocins in the preservation of food, such as inoculation of the food with LAB that produce bacteriocins, addition of purified or semipurified bacteriocin to food, and the use of a product previously fermented with bacteriocin-producing strain. One hurdle that needs to be overcome before the commercial use of a new bacteriocin as a biopreservative is its legal acceptance as a food additive. To date, only nisin has been accepted as a food additive. Novel approaches are needed to increase the effectiveness of protective cultures in foods, such as the use of recombinant DNA technology for the amplification of bacteriocin genes, or the microencapsulation technology to protect the compounds.

See also: Environmental Contaminants: Nitrate and Nitrite. Food Additives: Antioxidants; Preservatives

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FOOD ADDITIVES

Sweeteners

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Glossary

Chromosomal aberrations These are the disruptions in the number or structure of chromosomes in a cell.

Clastogenicity It refers to breaks in chromosomes, leading to sections of the chromosome being deleted, added, or rearranged.

Hyperplasia It is an increase in the number of cells/proliferation of cells.

Transitional cell carcinoma A type of cancer that typically occurs in the urinary system: the kidney, urinary bladder, and accessory organs. The term arises from the transitional epithelium, a tissue lining the inner surface of these organs.

Introduction

Sweeteners are food additives used as substitutes for sugar, mainly sucrose and fructose (honey and high-fructose corn syrup). Those that are not natural are, in general, called intense sweeteners. They have a relative sweetness many times that of sugar, which means they can be used in much smaller amounts. Most of the sugar substitutes approved for use in food are artificially synthesized compounds. However, some bulk natural sugar substitutes are also used, including sorbitol and xylitol, which are produced by catalytic hydrogenation of the appropriate reducing sugar. Steviol glycosides are natural plant extracts and are approximately 300 times sweeter than sugar. Some nonsugar sweeteners are polyols, also known as 'sugar alcohols.' These are, in general, less sweet than sucrose but have similar bulk properties and can be used in a wide range of food products.

Many intense sweeteners were discovered by accident. In 1879, a lab worker spilled a chemical he was synthesizing onto his hands, he accidentally licked his fingers and saccharin was discovered. Almost 60 years later, a graduate student detected the sweet taste of a chemical that had accidentally contaminated the cigarette he was smoking, thus discovering cyclamate. In 1965, a chemist licked his fingers while concocting an antiulcer drug and brought aspartame into the world. Now more than 6000 different types of foods and drinks worldwide are sweetened by the use of these and other intense sweeteners. The most widely approved intensely sweet sugar substitutes are stevia, aspartame and its derivative neotame, sucralose, acesulfame, and saccharin.

Intense sweeteners may be used either individually or blended with other sweeteners. Blends are commonly used by the food industry because the sweetener flavors can be synergistic. This can reduce the total amount of individual sweeteners needed in food. A common blend is acesulfame potassium with aspartame, which is often used in diet soft drinks.

More than 100 years ago, the introduction of the artificial sweetener saccharin in the USA began as an ongoing controversy over its safety toward human health that was finally resolved in 2001. Intense sweeteners are regulated as food additives and undergo a comprehensive safety assessment before being permitted in the food supply. To date, the available scientific information does not support a change in conclusions about the safety of the currently approved intense sweeteners. However, community concerns continue to be raised about aspartame and saccharin. This article outlines the risk assessment data for these two intense sweeteners, as well as steviol glycosides.

Nonetheless, discrepancies in the way food additives are regulated and contribute to consumer perceptions about their safety. For example, the use of cyclamates has attracted public interest, possibly resulting from the fact that it is in use in many parts of the world, but not in use or restricted in some countries, such as the USA. The USA banned the use of cyclamates in 1970s based on studies in which high doses of cyclamates caused bladder cancer in rats, a tumor that is also very commonly found in untreated rats. In Canada, cyclamate is restricted for use as a tabletop sweetener; it is unrestricted in the UK, and Joint FAO/WHO Expert Committee on Food Additives (JECFA) has established an acceptable daily intake (ADI) of 11 mg kg^{-1} bodyweight (bw) day^{-1}. Codex Alimentarius Commission continues to work toward harmonization of standards, including those for intense sweeteners, as there are both safety and trade issues in question. As new information arises, reviews will continue to ensure that the ingestion of such sweeteners in food remains safe.

Aspartame

History

Aspartame or L-α-aspartyl-L-phenylalanine methyl ester (CAS No. 22839-47-0; INS No. 951) was inadvertently discovered in 1965. As a consequence of its relatively high sweetness (it is approximately 200 times sweeter than sugar), only small

amounts are needed to sweeten foods and beverages. Aspartame is one of the most widely used nonnutritive, intense sweeteners in the world, with food regulatory agencies independently approving it for use in a range of foods including desserts, carbonated soft drinks, yoghurt, weight-control products, and confectionary, as well as its use as tabletop sweeteners.

Aspartame was first approved by the US Food and Drug Administration (USFDA) in 1974 and marketed in 1981. In Europe, aspartame was authorized for use in foods and as a tabletop sweetener during the 1980s. During the postmarket surveillance period, a number of health effects were claimed to be associated with dietary exposure to aspartame including neurological and behavioral effects (such as seizures and hyperactivity), allergic-type reactions, and cancer (brain and breast). A range of other adverse effects (multiple sclerosis, lupus, Alzheimer's, etc.) were also claimed to be linked to exposure to aspartame, through nonscientific Internet communications, and these claims were fuelled by alleged biases in the original US approval process and concerns over the integrity of the studies used to support approval. Comprehensive toxicological reviews and specific studies investigating these claims found no evidence to support any link with aspartame exposure.

The only identified food safety issue in relation to the consumption of aspartame is for a very small proportion of the population that has the rare genetic disorder, phenylketonuria (PKU). This disorder is characterized by a deficiency in the enzyme, phenylalanine hydroxylase, which metabolizes the amino acid phenylalanine. The buildup of phenylalanine in blood can result in toxicity to the brain. PKU is managed by controlling phenylalanine intake in the diet or a combination of diet and medication. As many artificially sweetened foods and soft drinks contain aspartame (which breaks down to phenylalanine in the digestive tract), mandatory labeling was introduced to alert people with PKU that the product contained phenylalanine.

It is recognized that some people prefer to avoid certain food additives and therefore aspartame, like other food additives, is required to be identified by its class name (e.g., sweetener) and by an individual name or code number (e.g., E951 in the UK and Europe).

Chemical Characterization

Aspartame is a methyl ester of a dipeptide consisting of two amino acids, aspartic acid, and phenylalanine (Figure 1). In the digestive tract, aspartame is completely hydrolyzed to its constituent amino acids in addition to methanol. On this basis, there is no systemic exposure to the parent compound. Aspartic acid, phenylalanine, and methanol are all found naturally in food at levels of intake greatly overshadowing of that arising from the gastrointestinal tract hydrolysis of aspartame. For example, aspartic acid and phenylalanine derived from aspartame intake have been calculated to be approximately 2% and 3%, respectively, of the total dietary intake of these amino acids in adults and approximately 1% of the total dietary intake in children. In addition to these metabolites, aspartame may be converted to aspartylphenylalanine diketopiperazine (DKP) when food products containing aspartame are stored under conditions of high temperatures and high pH.

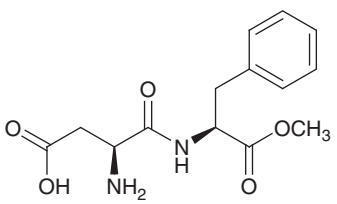

Figure 1 Structure of aspartame.

Hazard Identification and Characterization

The toxicological database for aspartame is extensive, consisting of studies conducted in both laboratory animals and humans. Aspartame, its metabolites, and breakdown product have been the subject of comprehensive reviews by the JECFA, the European Food Safety Authority (EFSA), and the USFDA in addition to a number of nongovernment expert panels. The consensus of these reviews is that the consumption of aspartame below the ADI is safe for all groups of consumers, with the exception of individuals with PKU.

JECFA established an ADI of 40 mg kg^{-1} bw day^{-1} for aspartame based on the no-observed adverse effect level (NOAEL) of 4000 mg kg^{-1} bw day^{-1}, the highest dose tested in a rat multigeneration study. This ADI is consistent with that set by the European Commission's (EC), Scientific Committee on Food (SCF) (1985), and the Food Directorate of Health Canada (1979) (formerly the Health Protection Branch of Health and Welfare Canada). The USFDA originally set an ADI of 20 mg kg^{-1} bw day^{-1} based on the NOAEL of 2000 mg kg^{-1} bw day^{-1} for reduced bodyweights in offspring in the same study at the next highest dose of 4000 mg kg^{-1} bw day^{-1}. Note that JECFA and others considered the reduced bodyweight to be an indirect effect resulting from decreased food consumption. Following the evaluation of additional human data, the USFDA set an ADI of 50 mg kg^{-1} bw day^{-1} for aspartame. The ADI for DKP set by JECFA and the SCF (1985) is 7.5 mg kg^{-1} bw day^{-1} based on the NOAEL of 7500 mg kg^{-1} bw day^{-1} for decreased bodyweight gain at greater than 1500 mg kg^{-1} bw day^{-1} in a chronic rat study. However, the USFDA considered the decreased bodyweight gain to be incidental in nature and therefore used the highest dose (3000 mg kg^{-1} bw day^{-1}) as the basis for its ADI of 30 mg kg^{-1} bw day^{-1}.

European legislation for harmonizing the use of aspartame was introduced in 1994 following evaluations by the SCF in 1984 and 1988, with supplementary reviews performed in 1997 and 2002. In its most recent evaluation, the SCF in 2002 concluded on the basis of its review of all data available that there was no need to revise its earlier risk assessment, which concluded that aspartame is safe. The SCF also confirmed the previously established ADI of 40 mg kg^{-1} bw day^{-1}.

An extensive review carried out by a panel of internationally recognized scientists, who evaluated more than 500 studies, articles, and reports conducted over the last 25 years on aspartame, including unpublished works submitted to the USFDA for the approval of aspartame. The panel concluded that aspartame is safe, in the amounts people currently consume in food. No credible evidence was found that aspartame could cause cancer, affect nervous system function, learning or behavior, or have any adverse effect on health, even when consumed in much greater amounts than the ADI.

During 2008 and 2009, the Advisory Forum of EFSA, made up of risk assessment experts from EU Member States, reviewed the available information and data on the safety of aspartame in view of ongoing public concern. An organizing team considered and reported on the amount of aspartame people were consuming and the information available on the effect of aspartame on brain function, appetite, allergies, immune system, metabolism, diabetes, and cancer (including cancer epidemiology and genotoxicity). The national experts did not identify any new evidence that suggested that EFSA should reconsider its previous opinion that aspartame is a safe food additive.

In 2007, a study by the European Ramazzini Foundation (ERF) suggested that aspartame can cause cancer in rats at levels close to ADI. EFSA reviewed this study and released an updated scientific opinion in March 2009. EFSA (2009) concluded that on the basis of all the evidence currently available, including the published ERF study, that aspartame did not produce cancer.

More recently, two new studies have been released; the first by Soffritti *et al*. in 2010 from the ERF, which shows that life expectancy in mice remains unchanged following a lifetime of daily exposure to aspartame. The report also claims that the incidence of some cancer types at death is slightly increased among mice consuming aspartame. The second is an epidemiological study by Halldorsson *et al*. in 2010, which examined an association between the consumption of sugar-sweetened and artificially sweetened soft drinks and the risk of preterm delivery in Danish pregnant women. EFSA have evaluated these new studies and in a statement dated 28 Feb 2011, EFSA concluded that these two recent publications did not give reason to reconsider previous safety assessments of aspartame or of other sweeteners currently authorized in the European Union.

At the request of the European Commission, EFSA is currently undertaking a full reevaluation of aspartame, including all unpublished data that were used to support the original European authorization.

Exposure

The dietary intake of aspartame for different populations groups in the USA, seven European countries, Brazil, and Canada was reviewed by Butchko *et al*. in 2002 and are summarized in Table 1. More recent data from the US indicated that mean and 95th percentile intakes were 4.9 and 13.3 mg kg^{-1} bw day^{-1}, respectively. In Australia and New Zealand, the highest consumption of aspartame was in adults 25–39 years of age (mean = 3.4 mg kg^{-1} bw day^{-1}, 95th percentile = 9.98 mg kg^{-1} bw day^{-1}).

Table 1 Dietary intakes of aspartame in different countries

Country	*Mean intake (mg kg^{-1} bw)*	*90th percentile intake (mg kg^{-1} bw)*
USA	–	2–3 (adults) 2.5–5 (children)
Finland	1.15 (diabetic children)	–
France	–	7.8 (97.5th percentile, diabetic children)
Germany		2.75 (whole population)
Italy	0.03 (teenagers)	
The Netherlands	2.4 (whole population)	7.5 (95th percentile)
Norway	0.9–3.4 (whole population)	–
UK	–	1.6 (whole population) 0.25 (35–64-year olds) 10.1 (97.5th percentile)
Brazil	1.02 (diabetics) 1.28 (dieters)	–
Canada		5.5 5.5–11.4 (children)

Abbreviation: bw, bodyweight.

Risk Characterization

Aspartame has been extensively studied, and evaluation of these studies has concluded that there are no remaining concerns over its toxicology, with a clear ADI established. As illustrated in the above section (Table 1), surveys conducted to date have determined that the consumption of aspartame is well below the ADI for all age groups and on this basis poses no safety issues for the health of consumers.

Methods of Analysis

Methods of analysis for aspartame are described in the detailed product specifications prepared by the 25th JECFA (1981), published in FNP 19 (1981) and in FNP 52 (1992).

Summary

Aspartame is a nonnutritive, intense sweetener used to replace sugar in food and beverages. It has been approved for use in more than one hundred countries and is an ingredient in several thousand different types of foods and drinks. Controversy about aspartame started during the premarket approval review and continued in the postmarket surveillance period in the early 1980s, it arose and focused mainly on fears of possible adverse neurological and gastrointestinal effects and a suspected link with cancer. Public concern over aspartame was further raised as these and other adverse effects were claimed on the Internet. Comprehensive scientific evaluations conducted independently by national food regulatory agencies and nongovernment panels of internationally recognized experts have consistently concluded that aspartame is a safe food

additive. Despite the weight of scientific evidence (aspartame is often described as one of the most studied food additives in history), public perception about possible negative health effects persist, posing periodic challenges for risk communicators.

Saccharin (Including Sodium and Calcium Saccharin)

History

Saccharin is approximately 300–500 times sweeter than sugar and is heat stable, allowing it to be used in cooking and baking, as well as a sweetener for foods and beverages. It is approved for use in more than 100 countries. It is generally used in combination with other sweeteners as it can have a slight bitter aftertaste when used on its own.

Although saccharin has been in use for more than a century, its safety has repeatedly been questioned. On the basis that sodium saccharin was carcinogenic in laboratory rats, in the 1970s saccharin was delisted as a food additive in Canada, although restricted, access as a tabletop sweetener was maintained. In 1977, a moratorium was placed on the use of saccharin in the USA until additional research on its safety could be conducted. The moratorium was withdrawn in 1991. In addition, some countries (e.g., the UK and USA) introduced the requirement that all foods containing saccharin had to be labeled with a warning statement alerting consumers that saccharin may be hazardous to health, by virtue of its carcinogenicity in laboratory animals. In Australia, the carcinogenic potential of saccharin for humans was not accepted, particularly at the low dose levels to which humans are exposed.

Currently in countries and regions, such as Australia, the USA and the EU, saccharin is approved for use as a sweetener in food in accordance with specific conditions.

Chemical Characterization

Saccharin(3-oxo-2,3-dihydrobenzo[d]isothiazol-1,1-dioxide) is a condensed heterocyclic *o*-sulfobenzimide, discovered in the late 1870s by chemists in the USA. It is commercially available in four forms: acid saccharin, sodium saccharin, potassium saccharin and calcium saccharin. Sodium saccharin is the most commonly used form because of its high solubility, stability, and low production costs. In the literature, the term 'saccharin' is sometimes used in a generic sense to encompass both saccharin and its salts. However, all four forms are manufactured to meet Food Chemicals Codex specifications (Figure 2).

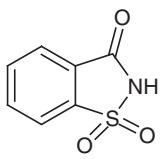

Figure 2 Structure of saccharin.

Hazard Identification and Characterization

Saccharin is excreted unchanged predominantly in the urine, and there is no evidence to suggest that it is metabolized in animals or humans.

Sodium saccharin has been administered to rats and mice in chronic feeding and multigenerational reproduction studies. Most studies, which were considered to be adequate for regulatory purposes, showed no adverse effects, but a statistically significant increase in bladder tumors (transitional cell carcinomas) was found in male rats at high dose levels ($\geq$4% in diet) in some reproduction studies. Similar effects were not observed in mice or female rats. The most plausible explanation of these effects in male rats at high dietary exposure of sodium saccharin is that the sodium ion causes the formation of a calcium–phosphate precipitate in urine which is cytotoxic to the urothelium, resulting in mild regenerative hyperplasia that leads to tumor formation in a small number of rats. A similar response in male rats is also observed following dietary exposure at high concentration to other sodium salts such as glutamate, aspartate, citrate, or chloride. At lower dose levels of sodium saccharin ($<$4%), there was neither tumor formation nor evidence of pathological changes in the bladder.

Although, saccharin did not induce gene mutations in the standard bacterial *in vitro* studies (Ames tests), there is a range of positive and negative findings, with many of the positive *in vitro* findings being attributed to increased osmolality (i.e., nonspecific ionic effects). It was also suggested that impurities or contaminants in the manufacture of saccharin might explain the positive results of the *in vivo* studies. However, the parent compound does not resemble an electrophilic chemical carcinogen that would bind to DNA, and the available evidence indicates no binding of sodium saccharin to DNA in rat bladder or liver. Overall, the results of tests for genotoxicity do not support a mechanism for the induction of bladder tumors in rats involving direct interaction of sodium saccharin with DNA.

In 1997, the International Agency for Research on Cancer (IARC) concluded that sodium saccharin produces bladder tumors in male rats by a non-DNA reactive mechanism that is not relevant to humans because of critical interspecies differences in urine composition (i.e., elevated pH and sodium levels).

The epidemiological studies on saccharin did not show any evidence that saccharin ingestion increases the incidence of bladder cancer in human population. The most recent reviews concluded that there is no detectable association between saccharin consumption and bladder cancer in humans.

The JECFA has considered the data on saccharin on several occasions. In 1982, at the 21st JECFA meeting the previous ADI of 5 mg kg^{-1} bw day^{-1} was changed to 2.5 mg kg^{-1} bw day^{-1} due to concerns over the carcinogenic potential (bladder tumors) in animals following high dietary doses of saccharin. JECFA's evaluation in 1993 considered that the 1% dietary level in a two-generation feeding study in rats (equivalent to 500 mg kg^{-1} bw day^{-1}) was the NOEL and dismissed the bladder cancer in male rates as unrelated to humans. Consequently, JECFA allocated a group an ADI of 5 mg kg^{-1} bw day^{-1} to saccharin and its calcium, potassium, and sodium salts based on a safety factor of 100.

Exposure

Most global surveys have indicated that there are no exceedences of the ADI for saccharin, including the most recent survey undertaken in Australia.

Risk Characterization

Studies in animals and humans indicate that, after absorption, saccharin is excreted unchanged in the urine. Excretion occurs relatively rapidly with no evidence of accumulation. Based primarily on mechanistic studies, there is convincing evidence that the saccharin-induced bladder cancer observed in male rats is a species-specific high-dose phenomenon. The evidence also suggests that it occurs through a nongenotoxic mode of action. As a consequence, it is unlikely to be relevant for humans because of critical interspecies differences in urine composition. Epidemiological studies have been unable to demonstrate an association between dietary exposure to saccharin and bladder cancer.

Based on an adequate range of studies measuring relevant toxicological endpoints for saccharin, a NOEL of 500 mg kg^{-1} bw day^{-1} was established in two-generation long-term rat study. After incorporating a 100-fold safety factor for intra- and interspecies extrapolation of the NOEL, an ADI of 5 mg kg^{-1} bw day^{-1} was allocated by JECFA to saccharin and its calcium, potassium, and sodium salts. Dietary intake of saccharin is well below the JECFA ADI for all age and gender groups.

Methods of Analysis

Several international pharmacopoeias specify colorimetry and infrared absorption spectrophotometry as the methods for identification, and titration with sodium hydroxide or perchloric acid as methods for assaying the purity of saccharin, sodium saccharin, and calcium saccharin.

Specifications have been prepared at the 24th JECFA (1980), published in FNP 17 (1980) and in FNP 52 (1992). Metals and arsenic specifications for saccharin were revised at the 57th JECFA (2001) (http://www.fao.org/ag/agn/jecfaadditives/results.htmladditiveName=saccharin&ins=&cas1=&cas2=&cas3=&techFunction=-1&searchBy=aname).

Summary

Saccharin has been the subject of extensive scientific research and is one of the oldest researched intense sweeteners. The term 'saccharin' is sometimes used in a generic sense to encompass both saccharin and its salts. Despite bladder tumors observed in male rats that were fed high dietary doses of saccharin, it is considered safe for human consumption based on an extensive body of scientific evidence. It was established that the tumors observed in male rats treated at high doses with saccharin were species specific and not relevant to humans consuming saccharin, because the bladder tumors observed in rats are due to a mechanism not relevant to humans. An internationally established ADI of 0–5 mg kg^{-1} bw day^{-1} has been set by the JECFA. Current surveys suggest that dietary exposure for average and high consumers is within this ADI. Therefore, despite the early literature linking saccharin consumption to an adverse health outcome in humans, the overall weight of scientific evidence does not support this conclusion, and it is considered to be a safe food additive for human consumption.

Steviol Glycosides

History

The term steviol glycosides refers to mixtures of compounds extracted from the leaves of the plant *Stevia rebaudiana*. Several of these glycosides have the property of intense sweetness. Two of the major steviol glycosides contributing to the sweet taste of stevia extracts, namely, stevioside and rebaudioside A are reported to be 100–400 times as sweet as sucrose.

Purified stevia extracts have a long history of use as sweeteners in some countries (e.g., Japan), whereas regulatory approval of steviol glycosides in Europe, the USA, Australia, and New Zealand has been relatively recent. Steviol glycosides were approved as food additives in the USA, Australia, and New Zealand in 2008 and in Europe in 2011. Steviol glycosides are permitted in a large variety of foods and beverages over a wide range of maximum permitted levels. For example, in Australia and New Zealand, maximum permitted levels range from 50 (in fruit and vegetable juices) to 1100 mg kg^{-1} (in sugar confectionary).

Chemical Characterization

The major sweet components of steviol glycoside preparations are stevioside (CAS No. 57817-89-7; Figure 1) and rebaudioside A (CAS No. 58543-16-1). Other steviol glycosides include rebaudioside B, rebaudioside C, rebaudioside D, rebaudioside F, dulcoside A, rubusoside, and steviolbioside, which are generally present at levels lower than stevioside or rebaudioside A. The structural formula of stevioside, one of the principal components of steviol glycoside preparations, is given in Figure 3.

Hazard Identification and Characterization

The JECFA has evaluated steviol glycosides on several occasions. In 2004, at the 63rd JECFA meeting noted that steviol glycosides are poorly absorbed and are metabolized by the intestinal microflora by successive hydrolytic removal of glucose units to steviol which is well absorbed. Steviol glycosides have not shown potential for genotoxicity, carcinogenicity, or reproductive/developmental toxicity. A temporary ADI of 2 mg kg^{-1} bw for steviol glycosides, expressed as steviol, was established on the basis of a NOAEL of 383 mg kg^{-1} bw day^{-1} in a 2-year feeding study in rats and applying a safety factor of 200. The safety factor of 200 incorporated an additional twofold factor because of uncertainty surrounding potential pharmacological effects of steviol glycosides in humans.

In 2008, at the 69th JECFA meeting new clinical studies were considered on the effects of steviol glycosides on blood pressure in healthy volunteers with normal or low-normal blood pressure and on glucose homeostasis in men and women with type 2 diabetes mellitus. JECFA concluded that

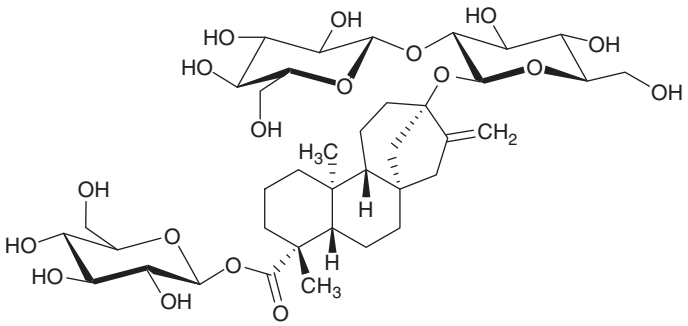

Figure 3 Structure of stevioside.

Table 2 Estimated dietary exposure to steviol glycosides[a]

Region	*Mean exposure (mg kg^{-1} bw day^{-1})*	*High-level exposure [b](mg kg^{-1} bw day^{-1})*	*References*
GEMS/Food cluster J[c]	0.9		
GEMS/Food clusters B and M[c]	5.0		WHO (2009)
USA	4.8–5.8		
Europe			
Children, 1–14 years	0.4–6.4	1.0–12.7	EFSA (2011a, b)
UK			
Adults	1.9–2.3	5.6–6.8	
Australia[d]			
2–6 years	1.5–2.2	2.5–4.4	FSANZ (2011)
7–16 years	0.9–1.6	1.6–3.4	
17 years and above	0.5–1.0	1.1–2.4	
New Zealand[d]			
5–14 years	1.0–1.8	1.9–4.0	
15 years and above	0.4–0.8	0.9–2.0	

[a]Expressed as steviol equivalents.
[b]90th, 95th, or 97.5th percentile.
[c]http://www.who.int/foodsafety/chem/gems/en/index1.html
[d]The lower end of the ranges for Australia and New Zealand were obtained assuming a 30% market uptake scenario (i.e., assumes steviol glycosides would account for 30% of total intense sweetener use). The upper end of the ranges corresponds to the highest values obtained from two 'brand loyal' exposure scenarios.
Source: Reproduced from FSANZ (2011) Application A1037-Food Standards Australia New Zealand. Available at: http://www.foodstandards.gov.au/foodstandards/applications/applicationa1037stev4605.cfm

the new studies showed no adverse effects at doses up to 4 mg kg^{-1} bw day^{-1}, expressed as steviol. The new data were considered sufficient evidence to justify removal of the temporary ADI designation and the additional twofold safety factor. A full ADI for steviol glycosides of 4 mg kg^{-1} bw, expressed as steviol, was therefore established.

EFSA and Food Standards Australia New Zealand (FSANZ) have also evaluated steviol glycosides, and both established an ADI of 4 mg kg^{-1} bw, expressed as steviol.

Exposure

Dietary exposure estimates to steviol glycosides have been made by JECFA, EFSA, and FSANZ and are summarized in Table 2. A major factor contributing to the differences in these exposure estimates is the differing assumptions regarding the degree of replacement of dietary sugars and other sweeteners in the diet by steviol glycosides. To better estimate actual exposure to steviol glycosides, the EU decided in late 2011 that it will request actual use levels of steviol glycosides in foods to enable EFSA to perform a refined dietary exposure assessment.

In the assessment conducted by JECFA, estimates were made for 13 grouped geographical regions worldwide, assuming that steviol glycosides would replace all dietary sugars at the lowest reported relative sweetness ratio for steviol glycosides and sucrose, i.e., 200:1. The resulting dietary exposure estimates ranged from 0.9–5.0 mg kg^{-1} bw day^{-1}, expressed as steviol, depending on the region. JECFA also evaluated an estimate of dietary exposure to steviol glycosides based on the replacement of all dietary sugars in the USA. Using data indicating a per capita intake in the USA of 176 g of caloric sweetener per day, dietary exposure to steviol

glycosides was estimated to be 4.8 mg kg^{-1} bw day^{-1} using the relative molecular mass of rebaudioside A or 5.8 mg kg^{-1} bw day^{-1} using the relative molecular mass of stevioside, to convert to steviol equivalents. JECFA noted that these estimates are conservative, in that it is very unlikely that all sweeteners in the diets would be replaced by steviol glycosides. It was estimated by JECFA that actual intakes would likely be 20–30% of these values.

The most recent dietary exposure assessment conducted by FSANZ considered a 30% market share scenario and two separate scenarios for 'brand loyal' consumers. These scenarios are also based on conservative assumptions that are likely to lead to considerable overestimates of dietary exposure.

Risk Characterization

Steviol glycosides have a low-toxicity profile, with no evidence of mutagenic or carcinogenic potential, or effects on fetal development. A clear ADI has been established at 4 mg kg^{-1} bw. Although some dietary exposure estimates suggest a potential to exceed the ADI, these are considered to be overconservative in their assumptions about the extent of the use of these sweeteners in the food supply. Assuming a more realistic 30% market uptake scenario, the highest predicted dietary exposure is expected to be approximately 60% of the ADI. No human health concerns are therefore expected from approved uses of steviol glycosides in food.

Methods of Analysis in Food

Analytical methods have been developed for the quantification of steviol glycosides in food and beverage matrices.

Summary

Steviol glycosides are intensely sweet plant-derived compounds permitted to be added to a wide variety of foods and beverages. An extensive toxicological database on steviol glycosides has been evaluated by several government authorities who concluded that there is no evidence of genotoxicity, carcinogenicity, or reproductive/developmental toxicity. An ADI of 0–4 mg kg^{-1} bw has been established from laboratory animal studies by the JECFA. Clinical trials in human volunteers have demonstrated the safety of steviol glycosides at levels corresponding to the upper end of the ADI range. Some dietary exposure assessment scenarios have resulted in predicted exposures that exceed the ADI; however, assuming a 30% market uptake scenario of steviol glycosides, the highest predicted exposure is within the ADI.

See also: Foodborne Diseases: Overview of Chemical, Physical, and Other Significant Hazards

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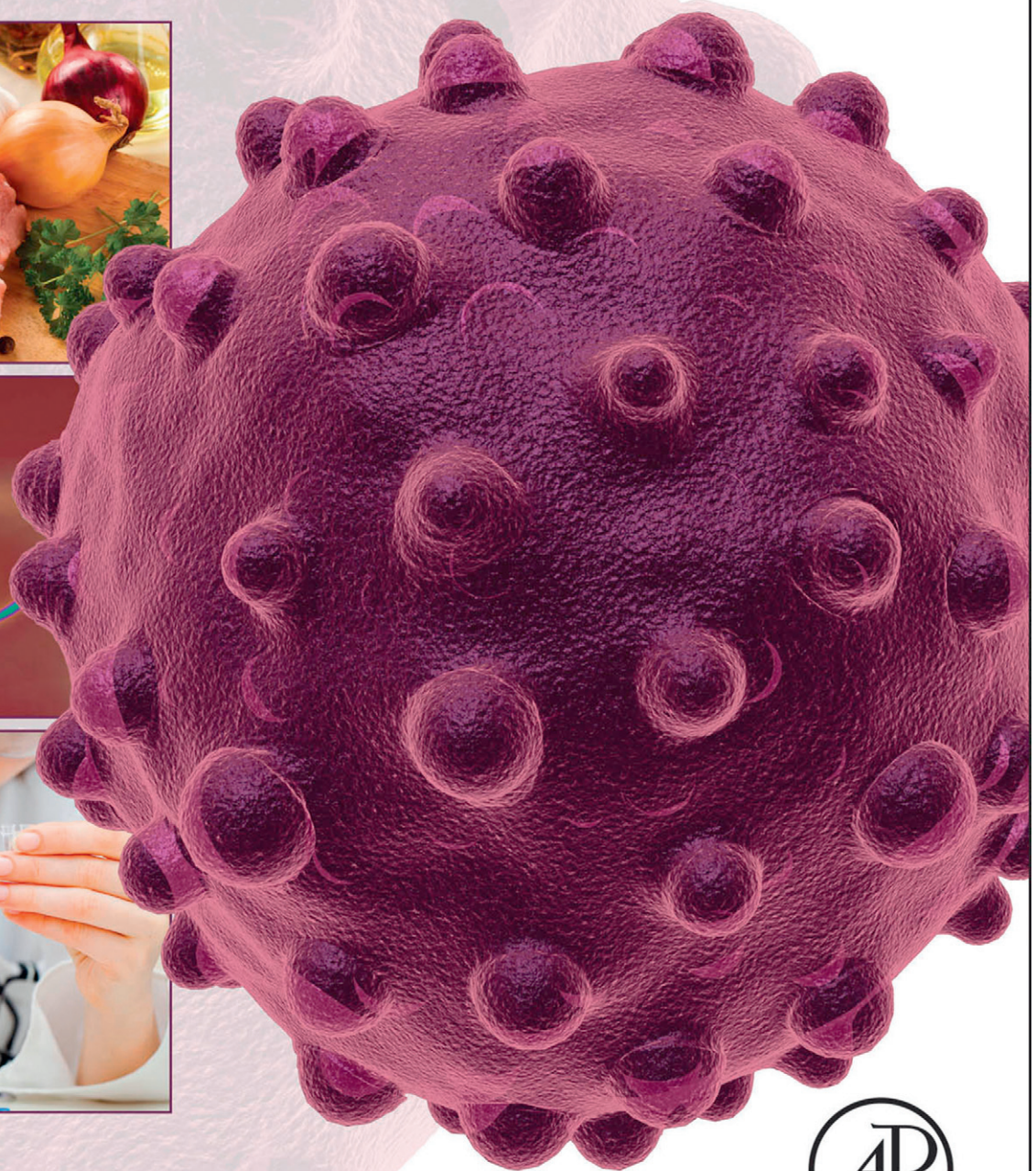
Encyclopedia of Food Safety

ENCYCLOPEDIA OF FOOD SAFETY

Edited by **Yasmine Motarjemi, Gerald Moy, Ewen Todd**



ENCYCLOPEDIA OF
FOOD SAFETY

VOLUME 3

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FOOD SAFETY

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VOLUME 3

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PREFACE

Why an Encyclopedia on Food Safety?

With the world's growing population, the provision of a safe, nutritious, and wholesome food supply has become a major challenge. To achieve this, effective risk management based on sound science and unbiased information is required by all stakeholders, including the food industry, governments, and consumers themselves. In addition, the globalization of the food supply requires the harmonization of policies and standards based on a common understanding of food safety among authorities in countries around the world.

Furthermore, reports of food safety incidents and foodborne disease outbreaks in one country are disseminated almost instantaneously through the 24/7 news cycle to consumers in other countries all over the world. Consequently, food safety managers in government and industry are sometimes called on to respond to queries from politicians, the media, and the general public even before they may be aware of the problem. Taking effective intervention measures and communicating the basis of their decisions and actions are essential for maintaining confidence in the safety of the food supply.

In all the above circumstances, sound scientific information is the key to effectively and efficiently assess, manage, and communicate on food safety risks. Yet, professionals and other specialists working in this multidisciplinary field are finding it increasingly difficult to keep up with developments outside their immediate areas of expertise. The time and staff needed to provide this information are beyond the resources of most individuals and organizations. Therefore, a single source of concise, reliable, and authoritative information on food safety has, more than ever, become a necessity.

This is the role that the Encyclopedia on Food Safety sought to fulfill by gathering all of the world's knowledge and expertise covering the entire spectrum of food safety topics into one comprehensive reference work. This was done with the objective of facilitating the work of those working in the field of food safety and related fields, such as nutrition, food science and technology, and environment. The Encyclopedia also provides a platform for experts to share their state-of-the art expertise and experience with the rest of the food safety community. Furthermore, the Encyclopedia's online feature is designed for rapid search and retrieval of relevant information.

Who Will Benefit from the Food Safety Encyclopedia?

The Encyclopedia will be useful for professionals and other specialists working in, but not limited to, the following institutions:

- Regulatory and enforcement agencies.
- Food industry.
- Trade and industry organizations.
- Audit and certification bodies.
- Academic institutions.
- Private and governmental scientific and research institutions.
- International and nongovernmental organizations with an interest in food.

What Does the Encyclopedia of Food Safety Contain?

With some 280 articles, the Encyclopedia provides comprehensive coverage a broad range of food safety topics, which may be grouped under the following general categories:

- History and basic sciences that support food safety.
- Foodborne diseases, including surveillance and investigation.
- Foodborne hazards, including microbiological and chemical agents.
- Substances added to food, both directly and indirectly.
- Food technologies, including the latest developments.
- Food commodities, including their potential hazards and controls.
- Food safety management systems, including their elements and the roles of stakeholders.

In developing the Encyclopedia, the editors and members of the Editorial Advisory Board have aimed to ensure that the Encyclopedia provides:

- Contributions by the foremost authorities in their fields.
- Unbiased and concise overviews on a multitude of food safety subjects.
- References for further information
- Specialized and general definitions for food safety terminology.

While the editors have made every effort to ensure that the Encyclopedia reflects the most complete and up-to-date information available, new scientific findings, and advances in food safety occur continuously. In undertaking a project of this scale and with the inevitably delays that occur during production, the editors acknowledge that some topics may have been omitted or insufficiently addressed. Therefore, the feedback of readers to point out any such errors or oversights will be greatly appreciated and will facilitate the development of future editions.

Acknowledgments

The lead editors would like to thank the Editorial Advisory Board members, section editors, and particularly, the authors who have generously contributed their time and talent to the development of this Encyclopedia. We are indebted to the Elsevier secretariat, which has assisted in the production of this work since its inception. Finally, a special note of thanks goes to our families whose patience and support are greatly appreciated.

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DEDICATION

This Encyclopedia is dedicated to our children, our grandchildren, and all the world's future generations who we hope will enjoy the benefits of a safe and nutritious food supply, produced with fair management of people working in industry and ethical treatment of animals.

FOREWORD I

Today's food system is one of the humanity's great achievements. It includes millions of commercial actors all over the world who produce, process, transport, store, market, and serve food that feeds billions of people daily. The complexity, diversity, and scope of the food system are almost beyond comprehension – ranging from small producers and processors serving local communities to vast global enterprises producing food for millions and managing extended international supply chains – all aimed at meeting high consumer expectations for safe, nutritious, and affordable food.

For all of its successes, the food system is full of challenges. Food insecurity and hunger remain major problems worldwide, and, for those with ready access to the foods of their choice, it is too easy to choose products high in salt, fat, and added sugar. Food safety – the task of avoiding chemical and microbiological contamination of food that can make people sick – is another persistent and dynamic challenge. In fact, new products in the marketplace, new patterns of production and supply, new consumer behaviors and new bacterial and chemical hazards – coupled with high consumer expectations – conspire to make food safety one of the central challenges of today's food system.

People working in the food system know this. Prominent illness outbreaks and contamination incidents take a toll on the public's health and cause a loss of confidence that can steer consumers away from healthy foods, like fresh fruits and vegetables, and impose big economic losses on food producers and processors. And the food system is responding with a heightened awareness of food safety at all levels of the food system and tremendous effort across the system to improve food safety. Much progress is being made.

One of the most important food safety developments of the last quarter century has been the emergence of a widely shared, science-based understanding of foodborne illness, its causes, and how it can be prevented. This begins with the understanding that the current burden of foodborne illness is unacceptable because it is largely preventable. It is preventable if we see food safety as a food system issue and recognize that microbiological and chemical hazards can enter the food supply at any point in the system along the pathway from the farm through processing, transport, storage, and retail sale. Likewise, opportunities to minimize hazards and help prevent food safety problems exist throughout the system, which means that everyone in the system shares responsibility for the safety of the food we eat.

Fulfilling this responsibility requires that we understand as much as we can about food safety hazards and their causes, devise the appropriate, science-based preventive controls for particular hazards and food production settings, monitor their effectiveness, and adjust the controls as needed based on experience. In short, progress on food safety depends fundamentally on a strong base of knowledge and continuous learning to systematically prevent food safety problems. And participants across the global food safety community are actively seeking and applying the knowledge needed to produce safe food and meet high consumer expectations.

This food safety encyclopedia provides a comprehensive overview of what we know about food safety hazards and control measures. We have more to learn, but the knowledge compiled in this encyclopedia demonstrates that we know a lot and that what we know can help empower participants in today's food system to fulfill their food safety responsibility. Although the food safety challenge is global and continuing, and may seem daunting, it can be met if all who share responsibility for food safety take advantage of the knowledge we have, participate in continuous learning, and place first priority every day on protecting the safety of food. That will be good for the food system – and for the consumers it serves.

Michael R Taylor
Deputy Commissioner for Foods and Veterinary Medicine, US Food and Drug Administration (FDA), Silver Spring, MD, USA

FOREWORD II

Food is one of the most basic requirements to sustain life. However, the safety of food and water cannot be taken for granted. Owing to both manmade and natural processes, an array of chemical and microbiological disease-causing agents find their way into food through multiple routes. When contaminated, such food can endanger or even destroy life. Therefore, from time immemorial, humankind has waged a constant battle against foodborne disease. Over many centuries of human development, people invented technologies that helped them fighting this battle, such as cooking, smoking, sun drying, canning, and freezing, to mention but a few. But like any scientific advance, some of these technologies presented their own food safety issues.

In a number of holy books, religious proscriptions for handling food contributed to food safety. In addition, many centuries ago, some governments already recognized that they had responsibilities in this domain and many laws were enacted to ensure the purity of certain foods. But it was only at the end of the nineteenth century, following scientific developments in the field microbiology and other areas of food science, that 'modern' food regulatory activities started.

In 1948, the availability, accessibility, and affordability of food were recognized as a basic human right by the United Nations in its Universal Declaration of Human Rights (Article 25, 1948). Implicit in this concept is the assumption that the food is first and foremost safe to consume, i.e., absence of health damaging properties. It is therefore not surprising that in the same year, the World Health Organization (WHO) was established as a specialized agency of the United Nations with a broad health mandate that included the specific responsibility to "develop, establish and promote international standards with respect to food…". Subsequently in 1963, WHO together with the Food and Agriculture Organization of the United Nations established an intergovernmental body to develop international standards for food – the Codex Alimentarius Commission. Today Codex stands as a major achievement in the promotion of food safety worldwide with an extensive collection of health and safety recommendations for food that are international recognized and referenced by the World Trade Organization and its member countries.

Thirty years ago, in 1983, WHO, again jointly with FAO, convened an Expert Committee on Food Safety to review the global food safety situation and provide guidance for governments, the food industry and consumers on how to cope with the inherent hazards and risks of our food supply. Based on available data and evidence at the time, the committee concluded that "illness due to contaminated food is perhaps the most widespread health problem in the contemporary world and an important cause of reduced economic productivity."

Unfortunately, this rather alarming statement appears to be still true today. Recent studies examining the morbidity and mortality of foodborne diseases have confirmed the significant public health burden posed by these diseases. In developed countries, one-quarter to one-third of the population are made ill each year because of foodborne diseases. In the developing world, the burden is much more severe. For example, diarrheal diseases are now estimated to cause 2.43 million deaths a year. According to WHO statistics, this is the second leading cause of mortality in low-income countries and kills more people than human immunodeficiency virus infection/acquired immunodeficiency syndrome (HIV/AIDS), malaria, or tuberculosis.

In addition, the large number of food safety crises, which occur with increasing frequency, is contributing to the growing public demand for better health protection from contaminated food. This has prompted governments to strengthen their food safety legislation, improve capacities and infrastructure, and tighten control measures. Examples of governmental measures include the creation of European Food Safety Agency by the European Union in 2002, the Food Safety Modernization Act in the USA of 2011 and, most recently, 2013, the commitment of the Premier of the People's Republic of China, Mr. Li Keqiang, to act with an 'iron fist' to improve food safety.

These positive developments are, unfortunately, contrasted by the fact that in many other countries, mostly developing countries, food safety does not receive the attention it deserves. In this regard, the medical profession and public health community appear to be slow in accepting the role that contaminated food plays in the epidemiology of diarrhea, particularly in infants and young children. The treatment of hospitalized cases and outpatients is rarely seen as an opportunity for educating patients and their families on why foodborne diseases occur and how they can be prevented. Two publications published in WHO's Bulletin in 1993 and 2003 urged the health sector to take steps to correct this oversight. Yet even today progress has been disappointing. For example, in the 2009, United Nations Children's Fund (UNICEF) and WHO published a document entitled 'Diarrhea: Why children are still dying and what can be done,' that again overlooked food safety as one of the most important interventions for these diseases. Consequently, in a recent publication in a prestigious *Medical Journal of Gastroenterology*, the issue had to be raised again and omission corrected. It can only be hoped that the public health and donor communities will eventually adopt a more holistic approach for the prevention of diarrheal diseases, which includes essential food safety interventions.

It is for this and many other reasons that I enthusiastically welcome the initiative of Elsevier to publish this Encyclopedia of Food Safety under the editorial leadership of Drs. Yasmine Motarjemi and Gerald Moy (my former WHO colleagues) as well as Dr Ewen Todd, a world renowned expert in food safety. The laudable collaboration and support of the Editorial Advisory Board, Section Coordinators, and the many authors who have freely devoted their time to advance the cause of food safety through the development of this Encyclopedia is also acknowledged.

With such a collection of information, whoever needs first-hand, reliable, and authoritative information on food safety does not need to consult various books, periodicals, or

websites. All of what is presently known in this domain can be found in this comprehensive work. In particular, the Encyclopedia will be useful for decision-makers, managers, officials, and scientists working in government, the food industry, academia, and nongovernmental organizations.

This Encyclopedia may be particularly important for colleagues in developing countries to not only improve food safety for their people but also convince politicians and other policy makers of the pivotal role of food safety in health and development. Without this awareness, the ultimate goal of safe food for all cannot be achieved.

F Käferstein
Former Director, Food Safety and Food Aid Programme (1980–98), World Health Organization, Wiesbaden, Germany

Further Reading

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EDITOR-IN-CHIEF

Yasmine Motarjemi holds a Masters degree in Food Science and Technology from the University of Languedoc, Montpellier, France (1978) and a Doctoral degree in Food Engineering from the University of Lund, Sweden (1988).

After her research and academic career at the University of Lund, in 1990, she joined the World Health Organization in Geneva as Senior Scientist. In WHO, she was responsible for the surveillance and prevention of foodborne illnesses (including education of professional food handlers and consumers), the development of the food safety assurance systems (e.g., Hazard Analysis and Critical Control Point system), and for assistance to the WHO Member States in strengthening their national food safety programme. She also contributed to the development of the risk analysis process. She has served in the Secretariat of various sessions of the Codex Alimentarius Commission and its Committees.

From 2000 to 2011, she held the position of Assistant Vice President in Nestlé where she worked as the Corporate Food Safety Manager. In this capacity, she has, among others, developed the Nestlé Food Safety Management system and managed various emerging food safety issues and crises.

She is the author, co-author, or editor of numerous peer-reviewed articles, books, training manuals, and other publications. Her latest books are Food Safety Management: A Practical Guide for the Food Industry (Elsevier 2014) and Invisible Things (original in French under the title: Les Invisibles), a book on food safety for children.

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Dr Gerald G Moy, since his retirement from the World Health Organization in 2008, is a scientific adviser with Food Safety Consultants International working with numerous governments and international organizations. His expertise includes assessment of national food safety programs, risk assessment of chemical hazards in food, total diet studies, food safety during mass gatherings, and food defense. Dr Moy is the editor-in-chief for recently published book Total Diet Studies (Springer) and is currently preparing a chapter on food and water protection for a book on key public health considerations during mass gatherings. He serves on the International Scientific Advisory Committee of the China National Center for Food Safety Risk Assessment, the Technical Advisory Group of the World Food Program Technical Advisory Group, and the WHO International Virtual Advisory Group on Mass Gatherings. He is the author of numerous book chapters, articles, and publications and serves on the editorial boards for several food safety journals.

He received his BS in chemistry from the University of Wisconsin and his PhD in physical organic chemistry from Oregon State University, followed by a post-doctoral fellowship in biophysics at the University of New Mexico. He is a Fellow in International Academy of Food Science and Technology and a recipient of the 2009 Great Wall Friendship Award for his contributions to food safety during the Beijing Olympics.

Ewen CD Todd is the President of Ewen Todd Consulting and the former Professor in the Department of Advertising, Public Relations and Retailing, and he is also the Adjunct Professor in the Departments of Food Science and Human Nutrition and Large Animal Clinical Sciences at Michigan State University (MSU). He was former directors of the Food Safety Policy Center and the National Food Safety and Toxicology Center at MSU. At both these centers, Dr. Todd coordinated research in microbiology, toxicology, epidemiology, risk assessment, social science, and policy in the area of food safety, distance education programs, and outreach in the community. Previously, he was in the Bureau of Microbial Hazards, Health Products and Food Branch, Health Canada, Ottawa where he was a research scientist for 33 years working on methods development for pathogens in foods, foodborne disease investigation and reporting, costs and surveillance of disease, illnesses caused by seafood toxins, and risk assessment of foodborne pathogens. He also helped develop risk management strategies for the Department including producing videos and pamphlets on food safety education. Some of his recent research has been working on *Listeria* and *E. coli* O157 transfer coefficient and modeling projects, hygiene in child care centers, schools, and elder care facilities. He has also collaborated with government agencies and academia in Spain, Kuwait, Saudi Arabia, Lebanon, Cambodia, Korea, Japan, and China on food safety issues, and is an expert witness in legal suits involving food safety. He has published extensively on many different aspects of food safety, including 11 recent papers on food workers and hand hygiene. He is active in the International Association for Food Protection (IAFP) and other organizations, and speaks and organizes symposia at national and international meetings. He is the associate editor for the Journal of Food Science and is a frequent reviewer of manuscripts submitted to several different scientific journals.

He has received the Government of Canada Distinctive Service Award for extraordinary teamwork and support to the Science and Technology Community; Recipient of the Excellence in Science Award for 1998 by Health Canada; Deputy Minister's Award of Team Excellence for the work done in promoting the Fight BAC! Campaign in Canada; the Professional Institute of the Public Service of Canada Gold Medal for Pure and Applied Science; and he is Fellow of the American Association for the Advancement of Science, the IAFP, and the MSU University Outreach and Engagement. He is also an honorary life member of the IAFP. He is a graduate of Glasgow University with a BSc in Bacteriology and a PhD in bacterial systematics.

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HOW TO USE THE ENCYCLOPEDIA

The material in this encyclopedia is organized into five broad sections, presented in four volumes. The sections consist of:

1. **History, science, and methods**: this section includes papers which help in the understanding of basic sciences underpinning food safety and foodborne diseases and their historical development.
2. **Hazards and diseases**: this section addresses the features of major foodborne hazards be they chemical, microbial, parasitical or physical and their health consequence.
3. **Food technologies**: this section explains the various food technologies and aspects related to their safety, or risks in their application.
4. **Foods, materials, and risks**: similarly, in this section, various groups of food products are described in terms of their risks and measures needed to ensure their safety.
5. **Food safety management**: finally, in this part, the building blocks of food safety management in the private and public sector are explained. The role of major international organizations is also reported.

To help realize the full potential of the material in the Encyclopedia the authors have provided five features to help you find the topic of your choice: a preface giving an overview of the encyclopedia and its objectives, a contents list by subject; an alphabetical contents list; cross-references to other articles; and a full subject index.

1 Contents List by Subject

Your first point of reference will probably be the contents list by subject. This list appears at the front of each volume, and groups the entries under subject headings describing the broad themes of quaternary science. This will enable the reader to make quick connections between entries and to locate the entry of interest. Under each main section heading, you will find several subject areas and under each subject area is a list of those entries that covers aspects of that subject, together with the volume and page numbers on which these entries may be found.

2 Alphabetical Contents List

The alphabetical contents list, which also appears at the front of each volume, lists the entries in the alphabetical order. This list provides both the volume number and the page number of each entry. On the opening page of an entry a contents list is provided so that the full details of any articles within the entry are immediately available.

3 Cross-references

All of the entries in the Encyclopedia have been extensively cross-references. The cross-references, which appear at the end of the entry, serve three different functions:

i. To indicate if a topic is discussed in greater detail elsewhere.
ii. To draw the reader's attention to parallel discussions in other entries.
iii. To indicate the material that broadens the discussion.

Example

The following list of cross-references appear at the end of the entry Characteristics of Foodborne Hazard and Diseases | Drug Resistant Pathogens.

> *See also*: Bacteria: *Listeria monocytogenes*; *Salmonella* Non-Typhi; *Salmonella* Typhi and *Salmonella* Paratyphi; Shiga Toxin-Producing *Escherichia coli* and Other Pathogenic *Escherichia coli*; *Shigella*; *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*. Characteristics of Foodborne Hazard and Diseases: Cost of Foodborne Diseases. Food Safety Assurance Systems: Cleaning and Disinfection. Public Health Measures: Food Safety in Hospitals and Other Healthcare Settings; Surveillance of Foodborne Diseases

Here you will find examples of all three functions of the cross-reference list: a topic discussed in greater detail elsewhere (e.g., *Listeria monocytogenes*; *Salmonella* Non-Typhi; *Salmonella* Typhi, and *Salmonella* Paratyphi; Shiga Toxin-Producing *Escherichia coli*), parallel discussion in other entries (e.g., Other Pathogenic *Escherichia coli*), and reference to entries that broaden the discussion (e.g., Food Safety Assurance Systems: Cleaning and Disinfection. Public Health Measures: Food Safety in Hospitals and Other Healthcare Settings).

4 Index

The index provides you with the page number where the material is located. The index entries differentiate between materials that is a whole entry, is part of an entry, or is data presented in a figure or a table. Detailed notes are provided on the opening page of the index.

5 Contributors

A full list of contributors is listed at the beginning of each volume.

GLOSSARY OF SELECTED TERMS

This Glossary of Selected Terms is a partial list of definitions for terms commonly used in the area of food safety. The terms selected are those that are important for communication among the various disciplines or are often subject to misunderstanding. Most of the definitions are taken from those recommended by international organizations or given by the authors contributing to this Encyclopedia. In cases where there are different definitions for a term, the Glossary presents the definition that is most consistent with usage by the majority of authors. Note that in some instances, slight differences between general definitions in this Glossary and those appearing in the individual articles may occur as the result of the specific context of the articles.

Acceptable daily intake The estimate of the amount of a substance in food or drinking water, expressed on a body weight basis, that can be ingested daily over a lifetime without appreciable health risk to the consumer.

Acute reference dose The estimate of the amount of a substance in food or drinking water, expressed on a body mass basis, that can be ingested in a period of 24 h or less without appreciable health risk to the consumer.

Adulteration (economic) A fraudulent action which is intended to omit a valuable constituent or substitute another substance, in whole or in part, for a valuable constituent; conceal damage or inferiority in any manner; or add any substance to increase its bulk or weight, reduce its quality or strength, or make it appear bigger or of greater value than it is (Note that in the US, adulterated food is generally defined as impure, unsafe, or unwholesome food.).

Antiseptic A substance that inhibits the growth and development of microorganisms. For practical purposes, antiseptics are routinely thought of as topical agents, for application to skin, mucous membranes, and inanimate objects, although a formal definition includes agents which are used internally, such as the urinary tract antiseptics.

As low as reasonably achievable A risk management approach that aims to keep exposure to a substance at the lowest level that is realistically achievable.

Asymptomatic shedder A person who does not exhibit the symptoms of an illness but excrete the pathogen (*see also* carrier).

Benchmark Reference point or standard against which performance or achievements can be assessed. A benchmark refers to the performance that has been achieved in the recent past by other comparable organizations, or what can be reasonably inferred to have been achieved in the circumstances.

Biomarkers Indicators of changes or events in human biological systems. Biomarkers of exposure refer to cellular, biochemical, or molecular measures that are obtained from biological media such as human tissues, cells or fluids, and are indicative of exposure to a substance. Biomarkers of effect refer to biological changes that represent an alteration in endogenous body constituents (e.g., depression of cholinesterase levels as an indicator of exposure to pesticides).

Carrier A person or animal that harbors a specific infectious agent without discernible clinical disease and serves as a potential source of infection. The carrier state may exist in an individual with an infection that is unapparent throughout its course (commonly known as healthy or asymptomatic carrier), or during the incubation period, convalescence and postconvalescence of an individual with a clinically recognizable disease (commonly known as an incubatory or convalescent carrier). Under either circumstance the carrier state may be of short or long duration (temporary or transient carrier, or chronic carrier) (*see also* asymptomatic shedder).

Case-fatality rate Usually expressed as the percentage of persons diagnosed as having a specified disease who die as a result of that illness within a given period. This term is most frequently applied to a specific outbreak of acute disease in which all patients have been followed for an adequate period of time to include all attributable deaths. The case-fatality rate must be clearly differentiated from the mortality rate (Compare with mortality rate).

Colony-forming unit A measure of viable bacterial or fungal cells in which a colony represents an aggregate of cells derived from a single progenitor cell.

Contaminant Any biological, chemical, or physical agent not intentionally added to food, which is present in food as a result of the production, manufacture, processing, preparation, transport, or holding of such food (Compare with hazard).

Control (noun) The state wherein correct procedures are being followed and critical criteria are being met.

Control (verb) To take all necessary actions to ensure and maintain compliance with criteria established in the Hazard analysis and critical control point system (HACCP) plan.

Control measure Any action and activity that can be used to prevent or eliminate a food safety hazard or reduce it to an acceptable level.

Corrective action Any action to be taken when the results of monitoring at the Critical Control Point (CCP) indicate a loss of control.

Crisis A predicted or unpredicted event which represents an immediate or future significant threat to an organization, its employees, consumers, and the public at large.

Critical control point A step at which control can be applied and is essential to prevent or eliminate a food safety hazard or reduce it to an acceptable level.

Critical limit (CL) A criterion which separates acceptability from unacceptability.

Detergent A chemical used to remove grease, dirt and food, such as washing-up liquid.

Disability adjusted life year (DALY) A metric used to express a health gap that extends the concept of potential years of life lost due to premature death to include equivalent years of 'healthy' life lost by virtue of being in states of poor health or disability. The DALY combines in one measure the time lived with disability and the time lost due to premature mortality. One DALY can be thought of as one lost year of 'healthy' life and the burden of disease as a measurement of

the gap between current health status and an ideal situation where everyone lives into old age free of disease and disability.

Disinfectant A chemical agent or a process that destroys, neutralizes, or inhibits the growth of pathogenic microorganisms (*see also* sanitizer).

Dose–response assessment The determination of the relationship between the magnitude of exposure (dose) to a chemical, biological, or physical agent and the severity and/or frequency of associated adverse health effects (response) in the exposed organism, system, or (sub) population in reaction to the agent.

Endotoxin A toxin present in intact bacterial cells and released when bacteria die or the cells are disrupted. A notable endotoxin is lipopolysaccharide, which is a major constituent of the outer cell membrane of Gram-negative bacteria and can cause toxic effect on lysis of bacteria. The term 'endotoxin' is to be differentiated from 'exotoxin', which is a toxin secreted in the surrounding medium and environment of the bacterial cell.

Enterotoxin A cytotoxin produced by bacteria that is specific for the mucous membrane of the intestine and causes diarrhea and/or vomiting associated with foodborne disease. Many infectious microorganisms produce enterotoxins in the gut, but some are produced external to the host (*see also* exotoxin and endotoxin).

Exotoxin A toxin that is secreted by bacteria. There are many different types of exotoxins. They can be released into the susceptible host (after infection and growth) or into the environment, including food (after contamination and growth). Those released into the intestines are typically heat labile (but some *E. coli* strains can produce both heat labile (HL) and heat stable (HS) toxins). *Clostridium perfringens* produces a HL enterotoxin after completion of sporulation in the host's intestines. *Staphyloccocus aureus* and *Bacillus cereus* enterotoxins produced in food are HS and cause vomiting and diarrhea, whereas toxins of *Clostridium botulinum* toxin, also produced in food, are HL and cause systemic neurological symptoms (*see also* exotoxin and endotoxin).

Epidemic The occurrence in a community or region of a group of illnesses which are similar in nature and clearly in excess of normal expectancy, and derived from a common or from a propagated source (Compare with pandemic).

Equivalence The situation where the application of two different food safety management measures lead to the same, or equivalent, public health outcomes.

Equivalence of sanitary measures (import–export of food) Equivalence is the state wherein sanitary measures applied in an exporting country, though different from the measures applied in an importing country, achieve, as demonstrated by the exporting country, the importing country's appropriate level of sanitary protection.

Exposure assessment The qualitative and/or quantitative evaluation of the likely ingestion of a biological, chemical, or physical agent in food as well as exposures from other sources if relevant.

Fecal–oral route A means of spreading pathogenic microorganisms from feces produced by an infected host to another host, usually via the mouth; for example, contact between contaminated hands or objects and the mouth.

Flow diagram A systematic representation of the sequence of steps or operations used in the production or manufacture of a particular food item.

Food Any substance, whether processed, semiprocessed, or raw, which is intended for human consumption, and includes drink, chewing gum, and any substance which has been used in the manufacture, preparation or treatment of 'food' but does not include cosmetics or tobacco or substances used only as drugs.

Food additive Any substance not normally consumed as a food by itself and not normally used as a typical ingredient of the food, whether or not it has nutritive value, the intentional addition of which to food for a technological (including organoleptic) purpose in the manufacture, processing, preparation, treatment, packing, packaging, transport, or holding of such food results, or may be reasonably expected to result, (directly or indirectly) in it or its byproducts becoming a component of or otherwise affecting the characteristics of such foods.

Food allergy A form of food intolerance in which there is evidence of an abnormal immunological reaction to the food (Compare with food intolerance).

Food establishment Any building or area in which food is handled and the surroundings under the control of the same management.

Food handler Any person who handles, prepares, or serves food, be they domestic food handlers, such as preparing family food, or professional food handlers, such as those working in food service establishments (cooks and waiters), retail stores, supermarkets, etc. (*see also* food worker).

Food hygiene All conditions and measures necessary to ensure the safety and suitability of food at all stages of the food chain.

Food industry The term includes primary manufacturing and processing industry as well as some other establishments involved in the food chain.

Food intolerance A reproducible, unpleasant reaction to a food or food ingredient, including reactions due to immunological effects, biochemical factors, such as enzyme deficiencies and anaphylactic reactions that often include histamine release (Compare with food allergy).

Food poisoning (or acute foodborne intoxication) A disease caused by a toxin or a chemical in food with symptoms usually appearing within 24 h after ingesting the agent. This term is commonly misused as a synonym for foodborne disease, which covers both infections and intoxications.

Food safety Assurance that food will not cause harm to the consumer, when it is prepared and/or eaten according to its intended use (Compare with food suitability and food hygiene).

Food safety hazard A biological, chemical, or physical agent in, or condition* of, food with the potential to cause an adverse health effect (Compare with contaminant). *Refers also to property of a food.

Food safety objective (FSO) The maximum frequency and/or concentration of a hazard in a food at the time of consumption that provides or contributes to the appropriate level of protection (ALOP) (Compare with Performance objective).

Food suitability Assurance that food is acceptable for human consumption according to its intended use (Compare with food safety and food hygiene).
Food worker Individuals who harvest, process, prepare and serve food, i.e., across the whole food chain to retail/foodservice; it is broader than that of a food handler, who typically works in foodservice establishments typically foodservice; however, the two terms are used interchangeably in the literature (*see also* food handler).
Foodborne disease Any disease of an infectious or toxic nature caused by or thought to be caused by consumption of contaminated food, including drink.
Foodborne pathogens Disease-causing microorganisms that are transmitted to humans via foods.
Generally recognized as safe Status of a substance that is generally recognized, among qualified experts, as having been adequately shown to be safe under the conditions of its intended use (used mainly in the USA).
Genomics The study of an organism via decoding the entire genetic sequence of the organism.
Genetic modification A process of altering the genetic makeup of an organism by techniques of modern biotechnology.
Genetically modified organism (GMO) AGMO or genetically engineered organism is an organism whose genetic material has been altered using genetic engineering techniques.
Good animal husbandry practice A system of management controls that need to be adopted at the level of primary producers to ensure production of food of animal origin that is safe for human consumption and acceptable for trade in the world market.
Good hygienic practice A system of management controls that need to be adopted at production, processing, storage, distribution, and preparation to ensure safety and suitability of products of consumption.
Good laboratory practice A system of management controls for laboratories and research organizations to ensure the quality, integrity, consistency, and reliability of results.
HACCP plan A document prepared in accordance with the principles of HACCP to ensure control of hazards which are significant for food safety in the segment of the food chain under consideration (see also HACCP).
Hazard A biological, chemical, or physical agent in, or condition*; of, food with the potential to cause an adverse health effect (Compare with contaminant). *Refers also to a property of a food.
Hazard analysis The process of collecting and evaluating information on hazards and conditions leading to their presence to decide which are significant for food safety and therefore should be addressed in the HACCP plan.
Hazard analysis and critical control point system A preventive system which identifies, evaluates, and controls hazards which are significant for food safety.
Hazard characterization The qualitative and/or quantitative evaluation of the nature of the adverse health effects associated with a biological, chemical, or physical agent which may be present in food. For a chemical agent, a dose–response assessment should be performed. For a biological or physical agent, a dose–response assessment should be performed if the data are obtainable.
Hazard identification The identification of the type and nature of adverse effects that a biological, chemical, or physical agent in food is capable of causing in an exposed population.
Incidence rate The number of new cases of a condition arising in a defined group within a given period or the number of new infections per unit of person–time at risk (Compare with prevalence).
In vitro In an artificial environment outside the living organism.
In vivo Within a living organism.
Lethal dose 50% The dose of a substance that would be expected to kill half of a population of exposed organisms.
Margin of exposure Ratio of the no-observed-adverse-effect level or benchmark dose lower confidence limit for the critical effect to the theoretical, predicted, or estimated exposure dose or concentration.
Maximum residue limit The maximum concentration of residues resulting from the use of a pesticide or veterinary drug that is acceptable in or on a food.
Minimum infective dose The lowest number of microorganisms required to cause an infection in the host.
Monitoring (CCP) The act of conducting a planned sequence of observations or measurements of control parameters to assess whether a CCP is under control.
Monitoring (general) Continuous or repeated observation, measurement and evaluation of health, and/or environmental or technical data for defined purposes, according to prearranged schedules in space and time, using comparable methods for sensing and data collection.
Morbidity rate An expression of the number of illnesses in a population at risk over a given period of time (usually one year).
Mortality rate An expression of the number of deaths in a population at risk over a given period of time (usually one year).
Nanomaterials Materials engineered at the nanoscale to have novel functionality or properties. Such properties will typically, but not exclusively, be demonstrated in the size range 1–100 nm, but this size range should be considered approximate.
Nanoparticles Particles with one or more external dimensions in the range 1–100 nm, but this size range should be considered approximate.
Nanotechnology The manipulation or materials at the nano level.
Notifiable disease A disease that must, by law or by ministerial decree, be reported to a government authority.
Outbreak A group of at least two cases of a single illness that are demonstrated by epidemiological investigation to have a single common exposure or source.
Pandemic Epidemic occurring over a very wide area, crossing international boundaries (often more than one continent) and usually affecting a large number of people.
Pasteurization A process involving heat treatment at a prescribed time–temperature combination to kill vegetative forms of pathogens that may be present, while causing minimal changes in the composition, flavor, and nutritive value of food. However, with advances and the development

of new food technologies, the term is sometimes used for nonthermal technologies leading to the same effect.

Pathogen An organism capable of causing disease.

Pathogenesis The course of a disease from its origin to its manifestation; more specifically it refers to the cellular events and reactions, and other pathologic mechanisms occurring in the development of the disease.

Pathogenicity Ability of a microorganism to cause disease in a host (Compare with virulence).

Performance criterion The effect in frequency and/or concentration of a hazard in a food that must be achieved by the application of one or more control measures to provide or contribute to a performance objective (PO) or an FSO.

Performance objective The maximum frequency and/or concentration of a hazard in a food at a specified step in the food chain before the time of consumption that provides or contributes to an FSO or ALOP, as applicable (Compare with Food Safety Objective).

Pesticide Any substance intended for preventing, destroying, attracting, repelling, or controlling any pest including unwanted species of plants or animals during the production; storage; transport; and distribution and processing of food, agricultural commodities, or animal feeds or which may be administered to animals for the control of ectoparasites. The term includes insecticides, herbicides, fungicides, rodenticides and algicides as well as plant growth regulators, defoliants, desiccants, and agents for thinning fruit or preventing the premature fall of fruit.

Prerequisite program Practices and conditions needed prior to and during the implementation of HACCP and which are essential to food safety.

Prevalence The number of persons in a population who have a disease at a specified point in time or over a specified period of time (Compare with incidence rate).

Primary production Those initial steps in the food chain up to and including, for example, harvesting, slaughter, milking, and fishing.

Processing aid Any substance or material, not including apparatus or utensils, and not consumed as a food ingredient by itself, intentionally used in the processing of raw materials, foods, or its ingredients, to fulfill a certain technological purpose during treatment or processing and which may result in the nonintentional but unavoidable presence of residues or derivatives in the final product.

Processing contaminant Undesirable contaminants that are formed during the treatment of food as a result of the interaction of their natural components or their ingredients.

Provisional maximum tolerable daily intake (PMTDI) The health-based reference value used for contaminants with no cumulative properties. Its value represents permissible human exposure as a result of the natural occurrence of the substance in food and in drinking water. In the case of trace elements that are both essential nutrients and unavoidable constituents of food, a range is expressed, the lower value representing the level of essentiality and the upper value the PMTDI.

Provisional tolerable monthly intake The health-based reference value used for a food contaminant with cumulative properties that has a very long half-life in the human body. Its value represents permissible human monthly exposure to a contaminant unavoidably associated with otherwise wholesome and nutritious foods.

Provisional tolerable weekly intake The health-based reference value used for food contaminants such as heavy metals with cumulative properties. Its value represents permissible human weekly exposure to those contaminants unavoidably associated with the consumption of otherwise wholesome and nutritious foods.

Quality management Quality management includes all the activities that organization use to direct, control, and coordinate quality. These activities include formulating a quality policy and setting quality objectives. They also include quality planning, quality control, quality assurance, and quality improvements.

Recommended dietary allowance The average daily dietary nutrient intake level that is sufficient to meet the nutrient requirements of nearly all (97–98%) healthy subjects in a particular life stage and gender group.

Reservoir An animal species that specifically harbors an infectious agent over long periods, often without harm to the host.

Risk A function of the probability of an adverse health effect and the severity of that effect, consequential to a hazard(s) in food.

Risk analysis A process of decision making (usually government) for managing food safety, consisting of three components: risk assessment, risk management, and risk communication.

Risk assessment A scientifically based process for evaluating risks associated with foodborne hazards, consisting of the following steps: (i) hazard identification, (ii) hazard characterization, (iii) exposure assessment, and (iv) risk characterization.

Risk assessment policy Documented guidelines on the choice of options and associated judgments for their application at appropriate decision points in the risk assessment such that the scientific integrity of the process is maintained.

Risk characterization The qualitative and/or quantitative estimation, including attendant uncertainties, of the probability of occurrence and severity of known or potential adverse health effects in a given population based on hazard identification, hazard characterization, and exposure assessment.

Risk communication The interactive exchange of information and opinions throughout the risk analysis process concerning risk, risk-related factors, and risk perceptions, among risk assessors, risk managers, consumers, industry, the academic community, and other interested parties, including the explanation of risk assessment findings and the basis of risk management decisions.

Risk estimate The quantitative estimation of risk resulting from risk characterization.

Risk management The process, distinct from risk assessment, of weighing policy alternatives, in consultation with all interested parties, considering risk assessment and other factors relevant for the health protection of consumers and for the promotion of fair trade practices, and, if needed, selecting appropriate prevention and control options.

Risk manager A person or an organization (usually government) with the authority to decide on the acceptability of risk and, if necessary, measures needed for their management.

Risk profile The description of the food safety problem and its context.

Safe (food) A level of risk that is deemed to be acceptable by some standard. The question of safety always involves the question of to whom the risk is acceptable, and by what criteria that party judges it so.

Sanitizer Type of antimicrobial (disinfectant) that kills or irreversibly inactivates microorganisms present on a surface, especially designed for use on food-processing equipment. The US Environmental Protection Agency further defines a sanitizer as providing at least 99.9% reductions of all microorganisms on a surface (*see also* disinfectant).

Shelf-life The predicted time at which a product will change from acceptable to unacceptable quality. It is influenced by factors such as raw ingredient quality, processing conditions, packaging practices, and storage conditions. Typically, shelf-life is determined by a combination of microbial, sensory, and chemical methods. 'Shelf-life' can be expressed on food labels by a variety of dates, including 'expiry', 'use by', 'sell by', 'best before', and 'consume by', depending on the applicable legislation.

Step (HACCP) A point, procedure, operation, or stage in the food chain including raw materials, from primary production to final consumption.

Strain An isolate of the same type of microorganism possessing different properties.

Surveillance The systematic, ongoing collection, collation, and analysis of data on specific diseases in a defined population, to guide public health decisions.

Surveillance (active) Public health surveillance that regularly reaches out to diagnostic laboratories or to clinicians to actively collect reports of specific diagnoses of infections.

Surveillance (passive) Public health surveillance that collects reports of specific diagnoses from clinicians or diagnostic laboratories, which they are required or requested to submit because of notifiable diseases regulations.

Time–temperature abuse A situation where food has not been cooked for long enough or at a sufficient high temperature to reduce contaminants to safe levels, or food has been stored for a time or at a temperature that permits bacteria to proliferate.

Traceability/product tracing The ability to follow, forward as well as backward, the movement of a food through specified stage(s) of production, processing, and distribution.

Uncertainty In risk assessment, imperfect knowledge concerning the present or future state of an organism, system, or (sub) population under consideration.

Validation (analytical methods) Practice undertaken to substantiate or confirm methods or procedures perform as expected and in a reliable manner and consistently meet expectations.

Validation (control measures) Obtaining evidence that a control measure or combination of control measures, if properly implemented, is capable of controlling the hazard to a specified outcome.

Validation (HACCP) Obtaining evidence that the elements of the HACCP plan are effective.

Variability Heterogeneity of values over time, space, or different members of a population. Variability implies real differences among members of that population.

Verification (general) The application of methods, procedures, tests, and other evaluations, in addition to monitoring and to determine whether a control measure is or has been operating as intended.

Verification (HACCP) The application of methods, procedures, tests, and other evaluations, in addition to monitoring and to determine compliance with the HACCP plan.

Veterinary drug Any substance applied or administered to any food-producing animal, such as meat or milk producing animals, poultry, fish, or bees, whether used for therapeutic, prophylactic, or diagnostic purposes, or for modification of physiological functions or behavior.

Virulence The degree of pathogenicity of a microorganism as indicated by case-fatality rates and/or its ability to invade the tissues of the host; the competence of any infectious agent to produce pathologic effects. The virulence of a microorganism is a measure of the severity of the disease it causes (Compare with pathogenicity).

Waterborne disease A disease resulting from the contamination of water either by pathogenic viruses, bacteria or protozoa, or by chemical substances. These agents are directly transmitted to people when the water is used for drinking, preparing food, recreation, or other domestic purposes.

Withdrawal period (veterinary drugs) The interval between the time of the last administration of a veterinary drug and the time of the collection of edible tissue or products from a treated animal that ensures the concentration of residues in food comply with the maximum residue limit for the drug.

Zoonosis A disease that can be passed directly or indirectly from animals, whether wild or domesticated, to humans. Also called zoonotic disease.

ABBREVIATIONS OF TECHNICAL TERMS

This is a nonexhaustive list of commonly used abbreviations in the area of food safety.

ADI	Acceptable daily intake.
ADME	Absorption, distribution, metabolism, and excretion.
AI	Adequate intake.
ALARA	As low as reasonably achievable.
ALOP	Appropriate level of protection.
ARfD	Acute reference dose.
BMD	Benchmark dose.
BMDL	Benchmark dose at lower confidence limit.
CCP	Critical control point.
CFR	Case fatality rate.
CFU	Colony forming unit.
CIP	Cleaning in place.
DALY	Disability adjusted life year.
DGGE	Denaturing gradient gel electrophoresis.
DNA	Deoxyribonucleic acid.
EAR	Estimated average requirement.
ED_{50}	Effective dose 50%.
ELISA	Enzyme linked immunosorbent assay.
EMRL	Extraneous maximum residue limit.
FSO	Food safety objective.
GAHP	Good animal husbandry practice.
GAP	Good agricultural practice.
GHP	Good hygienic practice.
GAqP	Good aquacultural practice.
GC	Gas chromatography.
GC–MS	Gas chromatography–mass spectrometry.
GHP	Good hygienic practice.
GLP	Good laboratory practice.
GM	Genetically modified.
GMO	Genetically modified organism.
GMP	Good manufacturing practice.
GPVD	Good practice in the use of veterinary drugs.
GRAS	Generally recognized as safe.
HAB	Harmful algal bloom.
HACCP	Hazard analysis and critical control point.
HPLC	High performance liquid chromatography.
HPLC–MS	High performance liquid chromatography–mass spectrometry.
HPP	High pressure processing.
HTST	High temperature short time.
HUS	Hemolytic uremic syndrome.
IEDI	International estimated daily intake.
IESTI	International estimated short term Intake.
LD_{50}	Lethal dose 50%.
LOAEL	Lowest observed adverse effect level.
LOD	Limit of detection.
LOQ	Limit of quantitation.
MFFB	Moisture on a fat free bases.
ML	Maximum level.
MLST	Multilocus sequence typing.
MLVA	Multiple locus variable number tandem repeat analysis.
MOE	Margin of exposure.
MRL	Maximum residue limit.
mRNA	Messenger ribonucleic acid.
MS	Mass spectrometry.
NEDI	National estimated daily intake.
NOAEL	No observed adverse effect level.
NOEL	No observed effect level.
OPRP	Operational prerequisite programme.
PC	Performance criterion.
PCR	Polymerase chain reaction.
PDCA	Plan do check act.
PEF	Pulsed electric fields.
PFGE	Pulsed field gel electrophoresis.
PMTDI	Provisional maximum tolerable daily intake.
PO	Performance objective.
PRP	Prerequisite program.
PrP	Protease resistant protein.
PTMI	Provisional tolerable monthly intake.
PTWI	Provisional tolerable weekly intake.
QPS	Qualified presumption of safety.
RDA	Recommended dietary allowance.
RNA	Ribonucleic acid.
SMEs	Small- and medium-sized enterprises.
SOP	Standard operating procedure.
SPS Agreement	Agreement on the Application of Sanitary and Phytosanitary Measures.
TBT Agreement	Agreement on Technical Barriers to Trade.
TDI	Tolerable daily intake.
TDS	Total diet study.
TEF	Toxic equivalency factor.
TEQ	Toxic equivalence.
TMDI	Theoretical maximum daily intake.
TSE	Transmissible spongiform encephalopathy.
UHT	Ultra high temperature.
UL	Upper limit.
UV	Ultra violet.

PESTICIDE RESIDUES

Contents

Conazoles

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Glossary

ADME An acronym in pharmacokinetics for absorption, distribution, metabolism, and excretion of xenobiotics within an organism.
Constitutive androstane receptor (CAR) A member of the nuclear receptor superfamily and is a key regulator of xenobiotic and endobiotic metabolism.
Cytochrome P450 aromatase The enzyme essential for the conversion of androgens to estrogens.
Cytochrome P450 superfamily (officially abbreviated as CYP) A large and diverse group of enzymes. The function of most CYP enzymes is to catalyze the oxidation of organic compounds.
Dystocia Impeded or difficult birth. Dystocia and delays in parturition are toxicities common to many conazole fungicides, they may be related to aromatase inhibition.
Lanosterol 14 α-demethylase (or CYP51A1, P45014DM) A cytochrome P450 (family 51, subfamily A, polypeptide 1) enzyme that converts lanosterol to cholesterol.

Chemical Group(s) and Mode of Pesticidal Action

The conazole fungicides represent a large group of compounds widely used in agriculture for protection and treatment of fungal diseases in crop plants and as pharmaceuticals in human and veterinary medicine. The information below refers to conazoles used as plant protection products.

According to their chemical structure, conazole fungicides are classified into triazoles and imidazoles, but their antifungal activity is due to the same molecular mechanism:

- Imidazoles: imazalil (enilconazole as pharmaceutical), oxpoconazole, pefurazoate, prochloraz, and triflumizole.
- Triazoles: azaconazole, bitertanol, bromuconazole, cyproconazole, difenoconazole, diniconazole, epoxiconazole, etaconazole, fenbuconazole, fluquinconazole, flusilazole, flutriafol, hexaconazole, imibenconazole, ipconazole, metconazole, myclobutanil, penconazole, propiconazole, prothioconazole, simeconazole, tebuconazole, tetraconazole, triadimefon, triadimenol, and triticonazole.

The common feature in the chemical structure of imidazole fungicides is the imidazole ring (Figure 1) and in the structure of triazole fungicides it is the 1,2,4-triazole ring (Figure 2).

The target site of conazole fungicides it is the inhibition of C14-demethylase in sterol biosynthesis (*erg11/cyp51*). The antifungal effect of conazoles is due to inhibition of sterol

Figure 1 Imidazole.

Figure 2 1,2,4-triazole.

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14-demethylase in fungi, thereby blocking the biosynthesis of ergosterol. The lack of ergosterol is detrimental because it is an essential sterol component in the membranes of fungi.

The CYP51 gene is functionally conserved and is the only member of the cytochrome P450 superfamily (CYP) having catalytically identical orthologues in plants, fungi, prokaryotes, and higher species. It encodes for lanosterol demethylase activity, critical for sterol biosynthesis in mammals. In humans, the sterol 14-demethylase, i.e. CYP51, is expressed in many different tissues. It is, therefore, plausible that the mechanism by which the triazoles perform their fungicidal activity is the same as that responsible for some of the toxic effects in mammals.

Uses

The major uses of conazoles are for protective and curative treatment of fungal diseases in cereals, oil seeds, fruit trees, grapes, vegetables, sugar beets, etc. They are used for seed dressings and by pre- and postharvest application to crops. The conazole fungicides are highly effective against many different fungal diseases, especially powdery mildews, rusts, and many leaf-spotting fungi.

The first representatives of the conazole fungicides were introduced in the 1970s. The most used imidazoles are imazalil and prochloraz, introduced in 1973 and 1977, respectively. Triazoles are the largest class of fungicides. The first launched triazole in 1973 was triadimefon followed soon by triadimenol and bitertanol. Numerous other triazoles have been discovered and placed on the market since then. The current ranking of global sales is: tebuconazole, epoxiconazole, propiconazole, difenoconazole, flusilazole, tetraconazole, fluquinconazole, and flutriafol.

There are no available data on the volume of conazole fungicides used in the agriculture world-wide. In the USA, there is also no precise information on the proportion of conazoles from all used fungicides.

For the period 1992–03 the conazole fungicides were in third place in the ranking of all used chemical classes of fungicides in the European Union. They are also reported to be in the top five chemical classes used in cereals.

Relevant Absorption, Distribution, Metabolism and Excretion Characteristics

Imidazoles such as prochloraz and imazalil show rapid and nearly complete oral absorption, extensive distribution, rapid and complete excretion.

Most triazoles are also rapidly absorbed and widely distributed, with the highest concentration usually in kidney and in liver.

Triazoles are extensively metabolized. Three common metabolites of triazoles have been identified: 1,2,4-triazole (free triazole), triazole alanine, and triazole acetic acid. Triazole alanine and triazole acetic acid are formed in plants, and 1,2,4-triazole in both plants and mammals (rat).

Formation of 1,2,4-triazole in the rat is less than 20% for the majority (approximately 80%) of the triazole fungicides for which data are available. Two compounds (tetraconazole and flusilazole) demonstrate relatively high conversion (68–77%) in rat metabolism studies. As a plant metabolite, and given the wide use of triazole fungicides, free triazole is found in a variety of food commodities, including animal byproducts. 1,2,4-triazole appears to be relatively stable in the environment, and may be found in rotational crops as well as in water. Based on the currently available toxicology data, the toxicological effects of 1,2,4-triazole are sufficiently different from those of the conjugates triazole alanine and triazole acetic acid. Triazole alanine and triazole acetic acid have generally not been found to be significant metabolites in rats, lactating goats, or laying hens.

Relevant Toxic Effects From Acute Exposure and Repeated Exposure

The acute oral toxicity of conazoles to mammals varies from moderate to low (LD50 values between 300 and more than 2000 mg kg^{-1} body weight). The toxicity after acute dermal exposure is low (LD50 higher than mg kg^{-1} body weight).

A number of adverse effects have been observed in laboratory animals after repeated administration of triazoles, such as developmental effects, effects on reproduction, hepatotoxicity, hepatocarcinogenicity in mice, and production of other types of tumors (thyroid, testis), via nongenotoxic mechanisms.

Developmental effects: To some extent, triazoles show a typical pattern of developmental toxicity in laboratory animals. They are usually embryotoxic, cause delayed development (decreased fetal weight and delayed ossification), and also induce the following malformations and variations in rats:

- Craniofacial or brain malformations (cleft palate, hypognathia, macroglossia, exophtalmus, and hydrocephalus) observed with bitertanol, cyproconazole, diniconazole, epoxiconazole, flusilazole, propiconazole, and triadimefon.
- Variations of the urinary tract (dilated ureter and renal pelvis, absent renal papillae, hydronephrosis, and distension of urinary bladder) observed with cyproconazole, flusilazole, hexaconazole, propiconazole, tetraconazole, triadimefon, and metconazole.
- Additional cervical ribs observed with bromuconazole, diniconazole, epoxiconazole, flusilazole, myclobutanil, hexaconazole, penconazole, prothioconazole, tetraconazole, triadimenol, and triticonazole.

Most of the developmental effects in rats occur at high dose levels which are maternally toxic. In the rat, it was noted that malformations such as cleft palate and variations such as absent renal papillae were seen at high doses. The finding of increased incidence of skeletal variations was considered to indicate some embryotoxicity. The other skeletal variations seen were of less toxicological concern.

Reproductive effects: Reproductive toxicity, generally observed at parentally toxic doses includes impaired fertility, prolonged gestation, dystocia, reduced survival and reduced pup/litter weight, and perinatal mortality. There is evidence for aromatase inhibition from *in vitro* and *in vivo* studies.

Effects on reproduction are reported with bitertanol, cyproconazole, epoxiconazole, fenbuconazole, flusilazole, flutriafol, metconazole, myclobutanil, penconazole, prothioconazole, tetraconazole, triadimenfon, triadimenol, and triticonazole.

Hepatotoxicity, hepatocarcinogenicity in mice and other types of tumors (thyroid, testis): Triazoles have been shown to have effects on the liver to various degrees (changes in the activities of a number of P450 enzymes, hypertrophy resulting in increased liver weight, chronic inflammation, and necrosis) and several of them induce mouse hepatocellular tumors and rat thyroid follicular cell tumors. There is evidence that tumor formation is via nongenotoxic mechanisms and that the tumors may be a consequence of effects on the liver.

Hepatocellular adenomas and adenomas/carcinomas have been observed in mice exposed to bromuconazole, cyproconazole, difenoconazole, diniconazole, epoxiconazole, fenbuconazole, fluquiconazole, flusilazole, metconazole, propiconazole, tebuconazole, tetraconazole, triadimefon, and triadimenol.

The mechanism of hepatic toxicity of conazoles in mammals is still not very well characterized because multiple effects have been identified. The conazoles had effects on nuclear receptors as evidenced by increased expression and enzymatic activities of a series of related cytochrome P450s.

As discussed above, the fungicidal activity of triazoles is a consequence of their direct inhibition of CYP51 (lanosterol-14-alpha-demethylase). In mammals, CYP51 is part of the pathway leading to the biosynthesis of cholesterol which is the primary sterol in the cell membrane of mammals and is required for the sex steroid hormone and vitamin D synthesis. Triazoles can also inhibit several other P450 enzymes, including members of the CYP1A, CYP2C, and CYP3A subfamilies, as well as CYPC19 and CYP26, though specificity varies with structure. Hence, the effects of triazoles on mixed function oxidase activity are a balance between induction and inhibition of a variety of CYP enzymes.

The imidazole fungicide prochloraz is also a potent inducer of hepatic microsomal monooxydase system in rats and mice. The spectrum of induction is similar to phenobarbital induction.

In rodents, one consequence of activation of nuclear receptors such as constitutive androstane receptor (CAR) is hepatic hyperplasia, and this might underlie some of the effects of the triazoles reported in the liver.

Conazoles are generally not genotoxic in standard *in vitro* and *in vivo* tests.

Effect in humans: There are no reported effects in humans similar to those described in experimental animals. A few adverse effects in persons involved in manufacturing and use of prochloraz and its formulations included ocular and dermal irritation after heavy exposure to plant protection products containing prochloraz.

Imazalil (enilconazole) was used at oral therapeutic doses of up to 1200 mg over 6 months for the treatment of chronic fungal infection due to *Alternaria alternata* in a single patient (female). The drug was tolerated without evident toxicity based on hematology and clinical observations with the exception of the induction of nausea at high-dose levels. Very limited studies of pharmacokinetics were carried out: at a dose of 400 mg day^{-1}, the maximum serum concentration was approximately 2 μg ml^{-1}, whereas at a dose of 1200 mg, the maximum serum concentration was approximately 4 μg ml^{-1}. The half-life was approximately 2 h, and administration of 1200 mg daily for 1 month did not result in accumulation. No changes in clinical chemical parameters were seen.

Occurrence in Food

Risks from long-term and short-term dietary intakes of residues of conazole fungicides have been assessed by the Joint FAO/WHO Meeting on Pesticide Residues.

For the assessment of the long-term intake of residues, the international estimated daily intake (IEDI) was calculated for different diets representative for relevant global regions. For the assessment of the short-term intake of residues, the international estimated short-term intake (IESTI) was calculated for relevant food commodities for which residue data and consumption data were available.

Table 1 Dietary risk assessment for selected conazoles (2000–10)

Compound	*Long-term risk assessment*		*Short-term risk assessment*	
	ADI (mg per kg bw)	*IEDI (% of the maximum ADI)*	*ARfD (mg per kg bw)*	*IESTI (% of the ARfD)*
Bitertanol	0–0.01	2–10	Not necessary	Not calculated
Cyproconazole	0–0.02	1–2	0.06	Ch: 0–4% GP: 0–5%
Difenoconazole	0–0.01	0–10	0.3	Ch: 0–10% GP: 0–7%
Fenbuconazole	0–0.03	0–20	Not necessary	Not calculated
Flusilazole	0–0.007	2–10	0.02	Ch: 0–100% GP: 0–40%
Prochloraz	0–0.01	7–10	0.1	Ch: 0–3% GP: 0–1%
Propiconazole	0–0.07	0–2	0.3	Ch: 0–70% GP: 0–30%
Tebuconazole	0–0.03	1–8	Not necessary	Not calculated

Abbreviations: ADI, acceptable daily intake; IEDI, international estimated daily intake; ARfD, acute reference dose; IESTI, international estimated short-term intake.

The long-term and short-term intakes of residues of selected conazoles are unlikely to present a public health concern (Table 1).

See also: Foodborne Diseases: Overview of Chemical, Physical, and Other Significant Hazards

Further Reading

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Relevant Websites

http://www.frac.info/frac/publication/anhang/FRAC_Code_List_2010.pdf
Fungicide Resistance Action Committee: Chemical groups (structures and pesticidal mode of action of conazole fungicides, FRAC Code List.

http://www.frac.info/frac/publication/anhang/FRAC_MoA_Poster%202010_final_version.pdf
Fungicide Resistance Action Committee: FRAC classification 2010, mode of action and chemical structures.

Use

http://www.croplifefoundation.org/Documents/PUD/NPUD%202002/Fung%20&%20Herb%202002%20Data%20Report.pdf
CropLife Foundation: Pesticide Use in US Crop Production (2002 with Comparison to 1992 & 1997).

Pesticide Industry Sales and Usage: 2000 and 2001 Market Estimates, United States.

http://epp.eurostat.ec.europa.eu/cache/ITY_OFFPUB/KS-76-06-669/EN/KS-76-06-669-EN.PDF
Office for Official Publications of the European Communities, 2007. Eurostat: The use of plant protection products in the European Union.

http://www.epa.gov/oppbead1/pestsales/
US Environmental Protection Agency: (2004) Biological and Economic Analysis Division.

Triazole Metabolites

http://www.epa.gov/opprd001/factsheets/tetraHHRA.pdf
US Environmental Protection Agency (2006) Human health aggregate risk assessment in support of reregistration and registration actions for triazole-derivative fungicide compounds.

Toxic Effects of Conazoles

http://dar.efsa.europa.eu/dar-web/provision
European Food Safety Authority (EFSA): Draft assessment reports on individual conazoles.

http://www.efsa.europa.eu/en/scdocs.htm
European Food Safety Authority (EFSA): EFSA conclusions on individual conazoles.

http://www.efsa.europa.eu/en/scdocs/doc/1167.pdf
European Food Safety Authority (EFSA): EFSA Panel on Plant Protection Products and their Residues (PPR Panel): Scientific opinion on risk assessment for a selected group of pesticides from the triazole group to test possible methodologies to assess cumulative effects from exposure throughout food from these pesticides on human health on request of EFSA.

http://www.fao.org/agriculture/crops/core-themes/theme/pests/pm/lpe/en/
Joint FAO/WHO Meeting on Pesticide Residues (JMPR): List of pesticides evaluated by JMPR.

Occurrence in Food

http://www.fao.org/agriculture/crops/core-themes/theme/pests/pm/jmpr/jmpr-rep/en/
Joint FAO/WHO Meeting on Pesticide Residues (JMPR): Reports and evaluations.

PESTICIDE RESIDUES

Dithiocarbamates

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Glossary

Food Any substance, whether processed, semi-processed, or raw, that is intended for human consumption, and includes drink, chewing gum, and any substance which has been used in the manufacture, preparation or treatment of 'food' but does not include cosmetics, tobacco, or substances used only as drugs. (In the context of this topic level contribution, drinking water is food).

Hazard A biological, chemical or physical agent in, or condition of, food with the potential to cause an adverse health effect.

Monitoring (general) The act of conducting a planned sequence of observations or measurements of control parameters to assess whether a control measure is under control.

Risk A function of the probability of an adverse health effect and the severity of that effect, consequential to hazard(s) in food.

Introduction

Dithiocarbamates are a class of organ sulfur compounds of which 21 known compounds are employed as pesticides. They are analogs of carbamates in which both oxygen atoms are replaced by sulfur atoms and are generated by reacting primary and secondary amines with carbon disulfide under alkaline conditions. Dithiocarbamates readily form octahedral complexes with metal ions such as Cu(II), Fe(II) and Fe(III), Co(II), Mn(II), and Ni(II). Some of the most used dithiocarbamates are summarized in Table 1. Based on the carbon skeleton structure, dithiocarbamates are categorized into three groups namely dimethyldithiocarbamates (DMDs), ethylene-bis-dithiocarbamates (EBDCs), and propylene-bis-dithiocarbamates.

Most of these compounds are rapidly degraded in the environment to yield thioureas such as ethylenethiourea (ETU), ethyleneurea (EU), and propylenethiourea. ETU is of toxicological concern due to its carcinogenicity, teratogenicity, and antithyroid properties. Maneb, mancozeb, and metiram can induce thyroid cancer in laboratory animals possibly via the formation of ETU. Thus, for risk assessment purposes these pesticides are grouped together.

The powerful fungicidal effect of EBDs is due to their metabolites or decomposition products, such as ethylene-bis-isothiocyanate, ethylenethiuram, and carbonyl sulfide. The mechanism of the pesticidal activity of dithiocarbamates is inhibition of catalytic and regulatory thiol groups of cytoplasm constituents by organic electrophiles (isocyanates, carbonyl disulfide, and isothiocyanates) generated by metabolism of the parent molecules or by the coordinated metal ions (Zn(II) and Mn(II)), which occurs at nearly neutral pH (higher than 5.5–6.0). The wide range of action and the poor biocidal specificity of these compounds toward fungi, bacteria, plants, and insects, as well as the undesired toxicity against mammals, are all due to this mechanism.

Sources and Uses of Dithiocarbamates

Dithiocarbamates are synthetic compounds most of them having been developed during and after World War II. There is no known natural source of these compounds. Dithiocarbamates are mainly used in agriculture as pesticides, although a close chemical analog (disulfiram, Antabuse) is used as a human pharmaceutical agent in the treatment of alcohol abuse. As pesticides, they are effective against a broad spectrum of plant diseases caused by fungi and are mostly used to protect fruits and vegetables. They are nonsystemic fungicides which stay on the surface of the crop after application and prevent fungal growth on them, without penetrating into the vegetable tissue. The residual products from their application include ETU, EU, imidazole derivatives, diisothiocyanates, diamines, disulfides, and the coordinating metals, so that washing or fruit peeling to remove these products is generally sufficient to reduce or avoid consumer exposure. Some dithiocarbamates are also used for vector insect control in public health and in the household.

Table 1 The most commonly used dithiocarbamates

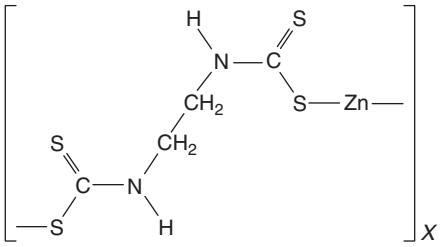
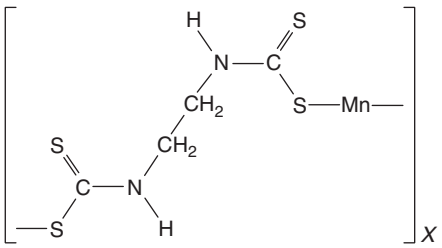
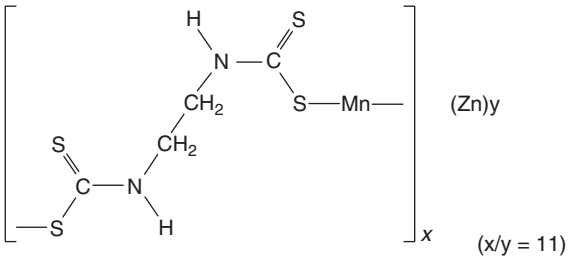
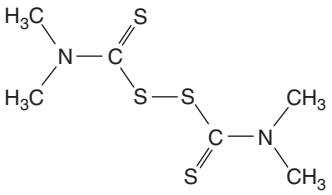
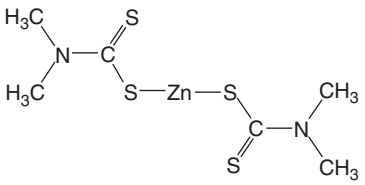
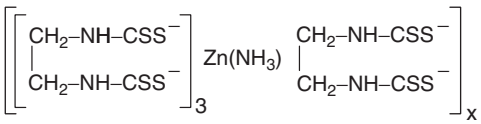
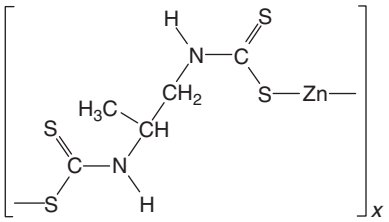
Description of Substance	*Chemical Structure*
Zineb IUPAC: Zinc ethylenebis(dithiocarbamate) CAS no.: 12122-67-7 Formula: $C_4H_6N_2S_4Zn$ MW 275.74	
Maneb IUPAC: Manganese ethylenebis(dithiocarbamate) CAS no.: 12427-38-2 Formula: $C_4H_6MnN_2S_4$ MW 265.3	
Mancozeb IUPAC: Manganese ethylenebis(dithiocarbamate) (polymeric) complex with zinc salt. CAS no.: 8018-01-7 MW 266.31	
Thiram IUPAC: Tetramethylthiuram disulfide CAS no.: 137-26-8 Formula: $C_6H_{12}N_2S_4$ MW 240.4	
Ziram IUPAC: Zinc bis(dimethyldithiocarbamate) CAS no.: 137-30-4 Formula: $C_6H_{12}N_2S_4Zn$ MW 305.83	
Metiram IUPAC: Zinc ammoniate ethylenebis(dithiocarbamate)-poly(ethylenethiuram disulfide) CAS no.: 9006-42-2 Formula: $C_4H_6N_2S_4Zn$ MW 1088.7	
Propineb IUPAC: Polymeric zinc propylenebis(dithiocarbamate) CAS no.: 12071-83-9 Formula: $(C_5H_8N_2S_4Zn)_x$ MW 289.8	

Table 2 Consumption of dithiocarbamate pesticides (in 100 kg) in different periods

Region	*Dithiocarbamates*			
	1974–76	*1981*	*1982*	*1983*
Africa				
Egypt	30			
Zimbabwe		795		
North/Central America				
Canada	10 977			
Mexico	4531	38 350	34 000	33 050
USA		60 000	50 000	
South America				
Argentina		4890	8370	
Uruguay	1454	822	1114	1668
Asia				
Brunei		3	2	2
Cyprus	701	2242	1538	
India	16.193	14.650	17.130	
Israel	4177	3110	3370	3580
Jordan		27.500	28.748	
Korea Republic	5027	18.380	18.233	
Kuwait	6			
Oman		115	62	120
Pakistan	24	370	881	
Turkey	5906	8901	9346	
Europe				
Austria	2751	2334	2322	2207
Czechoslovakia	6927	8678	6501	
Denmark	2187		11 485	13 747
Finland	504			
Greece	12 763			
Hungary	37 347	29 476	31 932	43 415
Italy	145 697	121 808	97 238	
Malta		350		
Norway	438	383	372	285
Poland	4007	11 386	14 102	12 517
Portugal	8114	8358	7592	
Sweden	3283	3800	4380	

Source: http://www.inchem.org/documents/ehc/ehc/ehc78.htm

Dithiocarbamates are the most often used pesticides in EU and other parts of the world (Table 2) and are most frequently detected in monitoring programs. Given their wide range of applications, they are produced in great quantities. The worldwide consumption is between 25 000 and 35 000 metric tons per year.

In the industry some products are also used as slimicides in watercooling systems, in sugar, pulp and paper manufacturing, and as vulcanization accelerators and antioxidants in rubber product manufacturing. Because of their chelating characteristics, they are used as scavengers of thiol-binding metals in wastewater treatment.

Absorption, Metabolism, and Excretion of Dithiocarbamates

As a general rule, dithiocarbamates can be absorbed via the skin, the mucous membranes and the respiratory and gastrointestinal tracts, whereas metal-complex alkylenebisdithiocarbamates are poorly absorbed both from the gastrointestinal tract and through the skin. Dithiocarbamates such as EBDCs are metabolized via hepatic microsomal enzymes to produce ETU. The elimination of ETU is largely through the kidney, and its elimination half-life is variable, ranging between 32 and 100 h, according to the species involved. Toxicokinetic data of these compounds in humans are not univocal: In some studies ETU can be detected in urine several days after exposure, whereas other studies suggest a short half-life of elimination.

Metabolism of dialkyldithiocarbamates results in the formation of dialkylthiocarbamic acid, free or as the *S*-glucuronide conjugate, of carbon disulfide and dialkyl amine as the decomposition products of the former, of formaldehyde, of sulfate as the final catabolic product of excess thiolic sulfur. The metabolic decomposition of EBDCs mammals results in formation, in addition to ETU, of carbon disulfide, ethylenediamine (EDA), ethylene bisthiuram disulfides, of hydrogen sulfide and ethylenebisthiocyanate. ETU is further broken down to carbon and nitrogen precursors which are incorporated into compounds such as oxalic acid, glycine, and urea. Dithiocarbamates and their metabolic products are found in the liver, kidneys, and the thyroid gland, but accumulation of these compounds does not take place because of their rapid metabolism.

Toxic Effects of Dithiocarbamates in Laboratory Animals

In rats, some dithiocarbamates tested at high dose-induced effects on reproduction (reduction of reproductive capacity) and on endocrine function. In teratogenicity studies dithiocarbamates induced an increase in resorption sites, and somatic and skeletal malformations in mice and rats. The doses needed to produce these effects were usually higher than 200 mg kg_{bw}^{-1} in rats and above 100 mg kg_{bw}^{-1} in mice, so being of questionable relevance for human toxicity in realistic exposure conditions. High levels of dithiocarbamates have also produced increased thyroid weight, reduction in colloid in thyroid follicles, thyroid hyperplasia, and nodular goiter. Prolonged administration of ETU to rats caused thyroid neoplasm. These neoplasms are observed as a consequence of inhibition of the production of thyroid hormones, with a consequent hyperincretion of the thyroid stimulating hormone (TSH), bringing about a goitrogenic effect and thyroid tumor. This carcinogenic activity of ETU was considered by International Agency for Research on Cancer (IARC) to be specifically linked with the different physiology of thyroid hormones in rodents, with no apparent risk to humans at doses below those able to cause a goitrogenic effect. For this reason, ETU has been allocated in IARC group 3. At dose above 50 mg kg_{bw}^{-1} (a level still unrealistic for even occupational human exposure) dithiocarbamates produce neurotoxic effects characterized by ataxia and paralysis of the hind legs, demyelination and degeneration of peripheral nerves in rats and rabbits.

The amine constituents of some dithiocarbamates may be converted *in vivo* to *N*-nitroso derivatives, which are considered to be both mutagenic and carcinogenic.

Health Effects of Dithiocarbamates in Human

In human beings the acute toxicity of dithiocarbamates is low, so that acute risk for consumers is consequently negligible. Toxic consequences of long-term exposure have been mainly studied in exposed agricultural workers, who may face skin sensitization, diffuse erythema, and eczematoid epidermatitis of the eyelids and inguinal regions when coming into contact with these compounds. Because of their metal-chelating properties, dithiocarbamates may cause a redistribution of toxic thiol-binding heavy metals such as lead and cadmium, in organs such as the brain. In very heavily exposed workers, similarly to animals, an inhibition in the production of thyroid hormones has been observed, with a compensative incretion of TSH. Regular contact with dithiocarbamates can cause functional changes in the nervous and hepatobiliary systems, and skin contact may induce contact dermatitis. An increase in T-cell proliferative responses to mitogens has been pointed out in mancozeb applicators in vineyards, thus suggesting a slight immunomodulatory effect of EBDCs.

Monitoring of Dithiocarbamates in Food

The hazard associated with the use of dithiocarbamates for the general population is the residue left on the foodstuff, which can be conveniently reduced or eliminated by washing or peeling fruit and vegetables before eating or cooking. As ETU is teratogenic, carcinogenic, mutagenic, and immunotoxic in test animals and accumulates in the thyroid of animals fed contaminated fodder, this exposure pathway may be a potential health hazard to humans.

Residues of dithiocarbamates in foodstuffs are determined by generation of carbon disulfide (CS_2) on hot acid digestion of the whole sample, followed by reaction of CS_2 with Cu(II) to yield a cupric complex which is quantified by spectrophotometry. Not only this method does not identify the specific dithiocarbamate (DTC) compound, but, worse, may also suffer from interference from CS_2 generated on hot acid digestion from natural compounds, such as phenyl isothiocyanate and other glucosinolates, originally present as micronutrients in the plant material, thus leading to false positives and overestimation of exposure. Also determination of the DTC-bound metal is used; however, the method suffers from most of the same inconveniences (no specificity for individual products and interference from other, even natural, sources of the metal in the foodstuff). As ETU cannot generate CS_2, this approach cannot be used to measure this major target compound, in monitoring the specific group of EBDs, the only able to generate ETU as metabolite. For this reason in some countries monitoring is performed through the

Table 3 Recommended ADIs (mg kg_{bw}^{-1}) and MRLs (mg kg^{-1}) for some commodities

Dithiocarbamate	*Commodity*	*ADI*	*MRL*
Ferbam	Cucumber	0–0.003	2
	Edible offal (mammalian)		0.1
	Eggs		0.05
Propineb	Grapes	0–0.007	5
	Meat (from mammals other than marine mammals)		0–0.05
Thiram	Melon except watermelon	0–0.01	0.5
	Milk		0.5
Ziram	Onion, bulb	0–0.003	0.5
	Pecan		0.1
	Peppers, sweet		7
	Pome fruits		5
	Potato		0.2
	Poultry meat		0.1
	Poultry edible offal		0.1
	Stone fruit		7

Abbreviations: ADI, acceptable daily intake; MRLs, maximum residue levels.
Source: Adapted from JMPR (2004) Joint FAO/WHO Meeting on Pesticide Residues (JMPR) Rome, 20–29 September 2004. Available at: http://www.who.int/ipcs/food/jmpr/en/2004_jmpr_summaryreport.pdf

Table 4 ADIs (mg kg_{bw}^{-1}), TADIs (mg kg_{bw}^{-1} day) and ARfD (μg kg_{bw}^{-1}/day) for some dithiocarbamates and their metabolites

Dithiocarbamate	*ADI*	*TADI*	*ARfD*	*Source*
Zineb		0–0.005		JMPR (1977)
		0–0.03		JMPR (1993)
	0.005			AUS (1992)
Maneb		0–0.005		JMPR (1977)
		0–0.03		JMPR (1993)
	0.05		0.2	Dir. 05/72
Mancozeb		0–0.005		JMPR (1977)
		0–0.03		JMPR (1993)
			0.006	AUS (1992)
	0.05		0.6	Dir. 05/72
Ziram	0–0.02			JMPR (1977)
			0.003	JMPR (1996)
	0.003			JMPR (2004)
	0.006		0.08	Dir. 03/81
Thiram		0–0.005		JMPR (1977)
	0–0.01			JMPR (1992)
	0.003			JMPR (1996)
	0.01		0.6	Dir. 03/81
			0.004	AUS (1995)
Ferbam	0–0.02			JMPR (1977)
	0.003			JMPR (1996)
	0.003			JMPR (2004)
Methiram		0–0.03		JMPR (1993)
	0.03			Dir. 05/72
Propineb	0.007			JMPR (1993)
	0.007		0.1	Dir. 03/39
Propylenethiourea	0.0003		0.003	JMPR (2010)
	0.0003		0.003	Dir. 03/39
	0–0.004			JMPR (1993)
Ethylenethiourea				

Abbreviations: ADI, Acceptable daily intake; ARfD, acute reference dose; TADI, temporary acceptable daily intake.
Source: Adapted from JMPR (2004) Joint FAO/WHO Meeting on Pesticide Residues (JMPR) Rome, 20–29 September 2004. Available at: http://www.who.int/ipcs/food/jmpr/en/2004_jmpr_summaryreport.pdf

Table 5 Dithiocarbamates in food samplesfrom different countries

Sample reference number	*Food*	*Country*	*Residue (mg kg^{-1})*	*MRL (mg kg^{-1})*
9658/2007	Herbs	UK	7.2	5
9689/2007	Herbs	UK	5.4	5
9800/2007	Herbs	UK	8.2	5
6654/2007	Passion fruit	South Africa	0.09	0.05 (LOD)
6797/2007	Passion fruit	Kenya	0.1	0.05 (LOD)
6654/2007	Passion fruit	Kenya	0.06	0.05 (LOD)
7384/2007	Passion fruit	South Africa	0.08	0.05 (LOD)
7409/2007	Passion fruit	Columbia	0.06	0.05 (LOD)
8507/2007	Passion fruit	South Africa	0.1	0.05 (LOD)
8366/2007	Passion fruit	Columbia	0.07	0.05 (LOD)
616/2007	Passion fruit	Columbia	0.4	0.05 (LOD)

Abbreviations: LOD, Limit of detection; MRL, maximum allowable residue.
Source: http://www.pesticides.gov.uk/uploadedfiles/Web_Assets/PRC/2007_PRC_Annual_Report.pdf

determination of ETU concentration in selected food commodities. Following suitable cleanup and gas chromatography (GC) conditions, individual EBDCs can be measured in various foodstuffs with a minimum detection limit of 10 ppb or 10 μg kg^{-1}. As derivatization is necessary for the GC analysis, additional ETU may be formed during reactions at elevated temperatures as a result of decomposition of EBDC residue present in the sample. As an alternative, liquid chromatography has also been applied using for detection either a nonselective UV absorbance (which necessitates a complicated sample cleanup) or a selective electrochemical detection, for which sample cleanup is much simpler.

Dietary Intake of Dithiocarbamates

Exposure of the general population to dithiocarbamates and to their breakdown products results from occasional residues in the diet. To evaluate the potential risk of dietary exposure to dithiocarbamates, the estimated intakes need to be compared with a toxicological reference value of one compound within the dithiocarbamate class or to a group of compounds with the same mechanism of toxicity, assumed to have been present in the food analyzed. Dithiocarbamates degrade rapidly after application to crops, yielding degradation products such as ETU, ethylene thiuram disulfide and [2, 1-C]-1, 2, 4-dithiazole-3-thione. Cooking of some foods treated before with EBDCs, have shown to result to a high level of ETU after cooking. Thus, boiling of foods containing the parent compounds can result in a high level of ETU. A group ADI for EBDCs is normally assigned as no distinction of specific parent material can be made during analysis.

Maximum allowable residue level, acceptable daily intake, and acute reference dose of dithiocarbamates have been set by Joint FAO/WHO Meeting on Pesticide Residues and other regulatory authorities to safeguard health of consumers of various foodstuffs (**Tables 3–5**).

See also: Foodborne Diseases: Overview of Chemical, Physical, and Other Significant Hazards. Risk Analysis: Risk Communication: Chemical Hazards; Risk Management: Application to Chemical Hazards

Further Reading

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PESTICIDE RESIDUES

Herbicides

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Glossary

Acceptable daily intake Estimated maximum amount of an agent, expressed on a body mass basis, to which individuals in a (sub)population may be exposed daily over their lifetimes without appreciable health risk.

Acute reference dose Estimated maximum amount of an agent, expressed on a body mass basis, to which individuals in a (sub)population may be exposed on a single day or at a single meal without appreciable health risk.

Epidemiology The study of factors associated with the frequency and distribution of disease or other health-related effects, and their causes, in a defined human population.

Herbicide active substance A chemical used to control or kill weeds.

Herbicide formulation A product consisting of one or more herbicide active substances and other chemicals (such as solvents or surfactants) that enhance the application or effectiveness of the active substance.

Mechanism of action A detailed description of how a chemical produces a biological effect, for example, by acting at a specific receptor or biomolecule.

Mode of action The general description of how a chemical produces a biological effect, for example, by acting on the thyroid.

Chemical Groups and Modes of Pesticidal Actions

Herbicides represent a diverse group of chemicals, which are used to control weeds. The Herbicide Resistance Action Committee (HRAC) lists over 250 active substances as having herbicidal activity, divided into 24 groups/subgroups based on the mode of herbicidal action (examples are in Table 1. The European Union (EU) pesticides database lists 324 herbicides. Some herbicidal modes of action are plant specific (e.g., synthetic plant hormones) but others have close correlates in mammals (e.g., Group D, electron cycling, and free radical generation) which are related to the observed toxicity.

In addition to having a range of chemistry, herbicides can be classified based on the method of application and mode of action, such as:

- Selective – affects certain plants only (e.g., broad-leaved weeds) without damaging others.
- Broad spectrum – act on all types of weeds.
- Systemic – transported through the weed.
- Contact – act only on the part of plant exposed to them, generally faster acting than systemic herbicides.
- Preemergence – acts to stop seed germination.
- Postemergence – acts on the seedlings or mature weed.
- Residual – persists in the soil to act on germination.

Many herbicide active substances are acids, which are formulated as either esters (e.g., aryloxyphenoxypropionate derivatives (FOPs)) or salts (e.g., glyphosate). Esterification can aid penetration of the plant cuticle followed by hydrolysis to liberate the acid.

It is not possible within the confines of this article to cover the modes of action of all herbicides, and the reader is referred to the HRAC website as an initial source of additional detail.

Table 1 Examples of HRAC herbicide groupings

HRAC group	*Mode of herbicidal action*	*Chemical classes*	*Herbicide (ISO name)*
A	Acetyl CoA carboxylase inhibition	Aryloxyphenoxypropionate (FOPs)	Haloxyfop
		Cyclohexanedione	Tralkoxydim
B	Acetolactate synthetase inhibition	Sulphonyl urea	Amidosulfuron
		Imidazolinone	Imiazethapyr
C	Inhibition of photosynthesis at photosystem II	Triazine	Atrazine
		Urea	Linuron
D	Photosystem-I- electron diversion	Bipyridylium	Paraquat
F	Bleaching	Triketone	Mesotrione
G	Inhibition of 5-enolpyruvyl shikimate-3-phosphate (EPSP) synthetase	Glycine	Glyphosate
O	Action-like indole acetic acid (synthetic auxin)	Phenoxycarboxylic acid	2,4-D

Uses

The range of uses of herbicides is broad as are application rates, varying by active substance and weed type. Herbicide formulations also vary greatly in composition from simple aqueous solutions, granules containing only active substance and binding agent, to those containing many coformulants to aid solubility, adherence to the weed and/or penetration through the plant cuticle. Application can be before planting or during crop growth (with selective herbicides), and in the case of some products a short-time before harvest for desiccation (e.g., glyphosate applied at before the harvesting of oil seed rape).

The total mass of herbicide applied in the UK has fallen by approximately 30% over the past two decades (Figure 1) despite a 30% increase in area treated This is presumably due to new products being effective at lower application rates.

In the USA, 178 000 t of herbicide was applied in 2002 a reduction from approximately 203 000 t in 1992. However, the use of glyphosate rose sixfold during the same period, as it replaced a number of older herbicides, to represent over 25% of the total mass of herbicides applied. Overall herbicide usage in the EU does not appear to have decreased significantly between 1992 and 2003. However, owing to different methods used to acquire the data, drawing any conclusions by comparing across the usage surveys should be performed with caution.

In addition to agricultural use, herbicides have uses in controlling weeds on lawns, around trees in urban areas, and for controlling weeds/algae on hard surfaces such as paths, roadways, and railways.

Relevant absorption, distribution, metabolism, and excretion (ADME) Characteristics

The ADME characteristics of herbicides vary between classes as well as individual chemicals. Details on the ADME of individual compounds can be found in publications such as World Health Organization (WHO)/International Programme on Chemical Safety (IPCS) Environmental Health Criteria documents, European Food Safety Authority (EFSA) conclusions, or United States Environmental Protection Agency (USEPA) documents. A few examples are given below.

Paraquat is poorly absorbed orally with approximately 10–20% of the dose systemically available in rats; similar results have been estimated from some human poisoning cases. In dogs there is evidence of saturation of absorption at moderate dose levels, with absorption of approximately 50% reported at 0.1 mg kg^{-1} body weight (bw) and approximately 30% at 2–5 mg kg^{-1} bw. Dermal absorption is $<1\%$. Paraquat concentrations in the lung and kidney were significantly higher than those in plasma following oral, intraperitoneal, or intravenous dosing. Paraquat is actively transported into the lung via a polyamine transporter. Paraquat metabolism is limited with the majority of the absorbed dose excreted in urine as paraquat. Kidney damage produced by paraquat contributes to enhanced retention in tissues. Patients excreting more than 1 mg paraquat per hour for 8 h or more, or have paraquat plasma concentrations of >0.3 mg l^{-1} 15 h post ingestion are likely to die.

Phenoxycarboxylic acids, such as 2,4-D, are generally well absorbed in (approximately 90%) following oral dosing, though dermal penetration is approximately 5%; however, the kinetics vary between different esters and salts. In humans, oral absorption of 2,4-D is moderately rapid with peak plasma levels seen at approximately 12 h. 2,4-D is widely distributed, can pass the placenta readily, and has been shown to bind reversibly to plasma proteins. In human poisoning cases, highest levels were found in the liver and kidneys. 2,4-D is excreted unmetabolized primarily in urine. With esters of phenoxy-acids the initial metabolism is hydrolysis to release the free acid and alcohol.

The oral absorption of glyphosate is incomplete, with approximately 30% absorbed from a bolus dose and approximately 10% when incorporated into rat diet. Dermal absorption of glyphosate is low (typically $<5\%$). Absorbed glyphosate is widely distributed, with some evidence of low-level accumulation of ^{14}C in the bone. Excretion of absorbed glyphosate is almost entirely in the urine without being metabolized.

Atrazine is well absorbed orally in rats, widely distributed, and initial excretion is rapid – predominantly in urine (approximately 80%). There is accumulation in erythrocytes

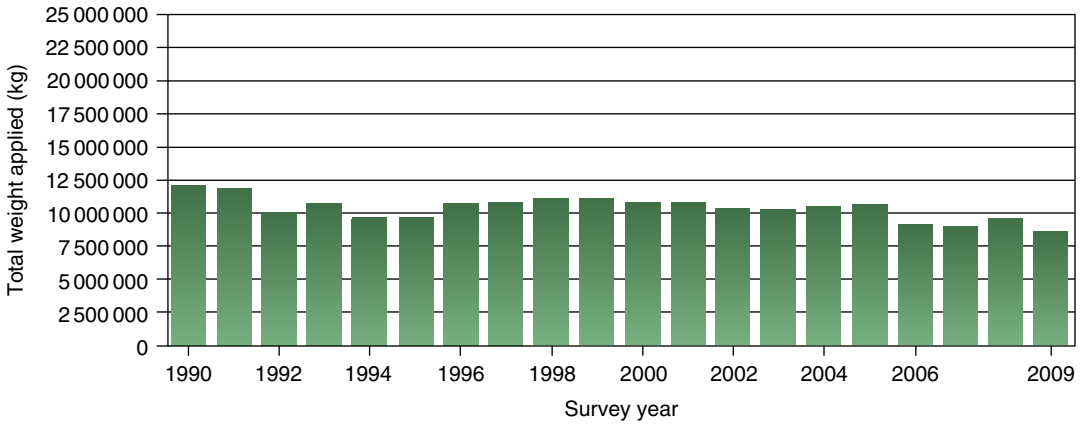

Figure 1 Mass of herbicides applied in the UK.

due to binding to the β-chain of rodent hemoglobin, which is considered to be of limited relevance to other species. Dermal absorption is $<5\%$ through human skin *in vitro* or *in vivo*. The metabolism of atrazine is extensive, with the main reactions being dealkylations.

Relevant Toxic Effects: From Acute Exposure and Repeated Exposure

The toxicity of herbicides varies between classes as well as individual chemicals. Details on the toxicity of individual compounds can be found in publications such as WHO/IPCS Environmental Health Criteria documents, EFSA conclusions, or USEPA documents. Very few herbicides have been evaluated by Joint FAO/WHO Meeting on Pesticide Residues.

Paraquat is acutely toxic by the oral (LD50s 20–500 mg kg^{-1} bw), dermal (LD50s $<100->2000$ mg kg^{-1} bw), and inhalation routes (LC50s 1–10 mg m^{-3}). Following repeated oral exposures the most sensitive organ is the lung where inflammation and progressive pulmonary fibrosis are seen at doses of approximately 1 mg per kg bw per day; higher doses produce kidney lesions. Initial lung changes are on type I pneumocytes. Paraquat is not genotoxic, carcinogenic, teratogenic, or toxic to reproduction. Investigative work using perilethal, parenteral doses has shown lesions of the *substantia nigra* consistent with those in patients with Parkinsonism.

2,4-D is of moderate oral toxicity (LD50s 300–1000 mg kg^{-1} bw) but of low dermal toxicity. In repeat dose studies the target organ is the kidney. 2,4-D is not genotoxic, carcinogenic, teratogenic, or toxic to reproduction.

Glyphosate is of low acute toxicity, but some formulations are moderately toxic. In repeat dose studies effects are seen only at high dose levels (e.g., 5000 ppm in the diet), predominantly reductions in bw gain, with salivary gland changes in some studies, and following chronic exposure liver hypertrophy and necrosis. Glyphosate is not genotoxic, carcinogenic, teratogenic, or toxic to reproduction.

Atrazine is of low acute toxicity. Positive results for skin sensitization have been reported in guinea-pigs and humans. Repeat dose studies did not present a consistent picture, reduced bw gain was seen together with slight effect on erythrocyte parameters, equivocal effects on the ovary and in dogs cardiotoxicity at a high dose level. The weight of evidence is that atrazine is not genotoxic, it is not teratogenic and does not affect reproduction without other toxicity. Atrazine is not carcinogenic in mice but produced mammary gland tumors in female Sprague Dawley (SD) rats. Investigative studies have shown the mechanism of tumor production, involves decrease in prolactin and leuteinizing hormone leading to suppressed estrus cycling and ovulation, which is unlikely to be relevant to humans due to the differences in the hypothalamic–pituitary–ovarian pathway between SD rats and humans.

Human Data

Paraquat has been involved in a significant number human poisoning cases, over 1000 of which have resulted in death. Some of these cases resulted from occupational exposures associated with poor hygiene practices and/or faulty equipment. Many cases were deliberate or accidental oral exposures. In cases of very high exposure death is due to kidney/multiorgan failure and/or pulmonary edema. With low to moderate ingestion, death due to pulmonary fibrosis can occur several days to weeks after exposure. The lethal oral dose of paraquat is approximately 2 g for an adult (approximately 30 mg kg^{-1} bw), equivalent to approximately 40 ml of a concentrated paraquat product. In survivors of paraquat poisoning there is evidence of complete recover of pulmonary function. There have been no reports of death attributed to inhalation of paraquat.

2,4-D has been involved in a number of human poisoning cases. In most instances the actual exposure is unknown but exposures to 100 ml of formulated product can cause death. The primary target organs are the kidney and heart. Birth defects and other adverse health effects ascribed to a 2,4-D containing formulation known as 'Agent Orange' have been shown to be the due to the presence of dioxins, which can be present, primarily as a byproduct of the synthesis of 2,4,5-T, the other herbicidal component of 'Agent Orange.'

Glyphosate containing products have been involved in a number of deaths predominantly from deliberate ingestion of several hundred milliliters of product. In many cases the initial signs (gastrointestinal erosion and oral ulceration) have been linked a surfactant rather than glyphosate *per se*. Death has been attributed to cardiac or respiratory arrest; at sublethal doses there is evidence of renal damage.

Epidemiological investigations have presented inconsistent results for reported associations between diseases and both generic herbicide use or use of specific compounds. Some positive associations have included phenoxy acids and non-Hodgkin's lymphoma; paraquat and Parkinsonism; and atrazine production and prostate cancer. However, other investigators have found no such correlations.

Occurrence in Food

Herbicide residues in crops are generally low, with many maximum residue levels set at the limit of quantification. This is because any significant residues present in a crop will normally damage the crop and reduce yield before they would engender any health risk. Genetically modified, herbicide-resistant crops permit the use of certain herbicides (e.g., glyphosate) while crops are growing. This can result in significant residues of the herbicide and/or metabolites.

Herbicides are not sought routinely in surveillance and monitoring schemes, but glyphosate was detected, although at low levels, in approximately 10% of the cereal samples analyzed for it in 2007 in the EU. Dietary exposures to herbicides are generally well below reference doses; however, if the herbicides are not degraded in the environment they might be transported to water sources resulting in human exposures via drinking water. In many countries, limits/guideline levels are applied to pesticide levels in drinking water (e.g., a total of 0.5 $\mu g\ l^{-1}$ in the EU; WHO).

See also: Foodborne Diseases: Overview of Chemical, Physical, and Other Significant Hazards

Further Reading

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http://www.hracglobal.com/Publications/ClassificationofHerbicideSiteofAction/tabid/222/Default.aspx
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general.http://pusstats.csl.gov.uk/
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USEPA: Pesticide registration status home page.

http://www.inchem.org/pages/ehc.html
World Health Organization – Environmental Health Criteria Series.

Inorganic and Other Metal-Containing Compounds

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Glossary

Absorption, distribution, metabolism, and excretion The absorption, distribution, metabolism, and excretion of a compound provides information on the extent and rate of absorption of a herbicide; tissues or organs receiving the highest exposures; the extent of metabolism and the types of metabolites formed; and the rate and extent of excretion (clearance) from the body.

Acceptable daily intake The estimated maximum amount of an agent, expressed on a body mass basis, to which individuals in a (sub)population may be exposed daily over their lifetimes without an appreciable health risk.

Acute reference dose The estimated maximum amount of an agent, expressed on a body mass basis, to which individuals in a (sub)population may be exposed during a meal or a day without appreciable health risk.

International estimated daily intake The long-term (lifetime) intake that is obtained from the World Health Organization (WHO) Global Environment Monitoring System (GEMS)/Food Regional Diets, which is based on per capita consumption.

International estimated short-term intake The short-term intake based on a large portion of a commodity that is consumed on a single day both by the general population and by children aged 6 years and under. The large portion values are obtained from actual food consumption survey data for individuals identified as 97.5 percentile consumers of the commodity among eaters only.

LD50 The LD50 (median lethal dose or 'lethal dose, 50%') is the dose of a chemical that would be expected to kill half of a population of exposed organisms.

National estimated daily intake The national estimated daily intake (NEDI) is a prediction of the daily intake of a pesticide residue, which is based on the most realistic estimate of residue levels in food and the best available data on food consumption for a specific population. The proportion of the commodity treated or imported may be used to correct residue estimates. When adequate information is available, monitoring and surveillance data or total diet studies may also be used.

Supervised trial median residues, Supervised trial median residues-processing Supervised trial median residues (STMR) is a median value of the residues of a pesticide measured after a supervised trial performed according to good agricultural practices. A supervised trial median residues-processing (STMR-P) is for a processed commodity calculated by applying a modification factor for the process (e.g., peeling or cooking) to the STMR calculated for the raw agricultural commodity.

Theoretical maximum daily intake The theoretical maximum daily intake (TMDI) is a prediction of the maximum daily intake of a pesticide residue, assuming that residues are present at the maximum residue levels (MRLs) and that average daily consumption of foods per person is represented by regional diets.

Introduction

Inorganic and organometallic compounds have been used as pesticides. At present, in most developed countries, only copper compounds are used in significant amount as fungicides. The others have been banned for either toxicological or environmental reasons. It is believed that some organotins are still used in some developing countries. Copper compounds, if used as directed, do not pose health risks. However, the estimated long-term intake in the European Union is not much below the acceptable daily intake, when other non-pesticidal sources of copper are considered. Organotins have a nonnegligible acute toxicity. Also, triphenyltin has immunotoxic and, possible, carcinogenic effects, and cyhexatin/azocyclotin have embryotoxic properties. However, estimated intakes are below reference values.

Chemical Groups and Modes of Pesticidal Actions

Copper

Copper and copper compounds share the similar pesticidal activities and toxicity, the latter is associated with copper content. They are used as fungicides in several crops, mainly grapes and tomatoes, and seed treatment.

Tin

Organic tin compounds, such as triphenyltin (fentin) acetate and triphenyltin (fentin) hydroxide, are used as fungicides, whereas others such as cyhexatin and the closely related azocyclotin are also used as acaricides in several crops. Their efficacy depends mostly on the organic part rather than on the metal itself.

Bismuth

Compounds of bismuth (in particular bismuth subcarbonate) have been used in agriculture combined with other pesticides, because they tend to increase the insects feeding on poisoned foliage.

Mercury

Mercuric chloride and organic mercury can be used as fungicides in agriculture and other settings, including seed treatment. Mercuric chloride has also insecticidal activity.

Uses

Copper

Copper compounds are also allowed to be used in the so called 'organic' farming. Scarce data are available on the amount of copper compounds used. In the UK there was an increase from an average yearly use of 27 t in the 1990s to a little more than 40 t in the last 10 years.

Tin

Cyhexatin, azocyclotin, triphenyltin (fentin) acetate, and triphenyltin (fentin) hydroxide are no longer registered in the EU and USA. However, their use still persist in other parts of the world. For this reason, a brief description isprovided below. Only data for fentin (hydroxide and acetate) are available from the UK showing that approximately 45 t $year^{-1}$ were used in early 1990s down to approximately 30 t in 2003 when it was banned.

Bismuth

Bismuth compounds have been used in the past in certain crops against foliar diseases, but its use is now discontinued.

Mercury

Mercuric chloride and organomercury compounds are no longer used as pesticides, at least in all developed countries. In view of this fact, and because mercury is discussed in other chapters of the encyclopedia, it will not be discussed in this article.

Relevant Absorption, Distribution, Metabolism, and Excretion Characteristics

Copper

As a micronutrient essential for life, copper follows a specific homeostatic regulation mechanism, which allows excess copper to be excreted mainly through the bile; once absorbed, copper is bound to proteins (up to 93% being ceruloplasmin), so that the free copper is normally not found neither in the blood nor in the cells. It also forms metalloproteins that function as enzymes. Copper levels in blood and tissues are generally stable; the body is able to maintain a balance of dietary copper intake and excretion that allows normal physiological processes to take place. The liver is the principal organ for regulation of copper, and main excretion is via the bile. Liver copper levels increase significantly following dosing with T_{max} at 12 h; elimination is rapid, with levels returning to control by 48 h after dosage. Oral absorption of copper varies according to the diet; for humans, a copper-adequate diet results in 36% absorption,whereas a low-copper diet and a high-copper diet results in 56% and 12% absorption. Metabolism does not occur. Copper does not accumulate, except in cases of genetic disease or chronic administration of high doses, where copper accumulates in the liver.

Tin

- Azocyclotin (tri(cyclohexyl)-1H-1,2,4-triazole-1-yltin) and cyhexatin (tricyclohexyltin hydroxide) are chemically related organotin compounds; azocyclotin breaks down to cyhexatin and 1,2,4-triazole. Azocyclotin has similar systemic toxicological properties to cyhexatin and may also have additional properties attributable to the 1,2,4-triazole that is formed. Oral doses of azocyclotin and cyhexatin were absorbed to a limited extent in rats: 12% and 2–10%, respectively. Azocyclotin completely breaks down to cyhexatin, and then the cyclohexyl rings are split to produce dicyclohexyltin and monocyclohexylstannoic acid. These are further hydroxylated and/or destannylated.
- Absorption of fentin is not complete and excretion is rapid with no indication of accumulation. Fentin acetate is rapidly hydrolized to fentin hydroxide, and then it is dephenylated and mainly excreted via feces. Some free tin may also be formed but it could not be quantified.

Relevant Toxic Effects: From Acute Exposure and Repeated Exposure

Copper

Acute oral LD50 in the rat is between 490 and 1280 mg kg^{-1} body weight (bw) for copper hydroxide, between 950 and 1860 mg kg^{-1} bw for copper oxychloride, between 300 and 500 mg kg^{-1} bw for tribasic copper sulphate and copper (I) oxide, and higher than 2000 mg kg^{-1} bw for the Bordeaux mixture. Copper hydroxide and the Bordeaux mixture may cause toxic effects by inhalation and present a risk of serious damage to eyes. Target organs of excess copper after oral administration are the liver and kidneys. Genotoxicity and carcinogenicity are not of concern on oral administration. Copper did not produce adverse effect on fertility, reproductive parameters or development, or neurotoxicity in animals. In humans, with genetic condition where the gene for copper transporter protein is inactive (as in Wilson's disease), copper progressively accumulates in the liver and brain, and in later stages of the disease (which is fatal due to liver failure if not treated) it shows signs of neurotoxicity. In genetically normal humans and laboratory animals, the homeostatic mechanisms that regulate copper prevent any accumulation in the brain and neural tissues so that copper does not show a neurotoxic potential. There was no evidence for adverse effects from oral exposure due to customary diets worldwide, or for any adverse effects of copper on pregnancy, parturition, lactation, or growth

and development in humans. There was evidence of toxicity particularly to neonates who were repeatedly exposed to milk heated in copper vessels or exposed to acid fruit stewed in copper vessels. A single case of long-term administration of excess copper as a dietary supplement resulting in liver failure is described in the literature. The upper limit of copper intake without adverse effect is not well defined, but is well recognised to be between 10 and 12 mg Cu per person per day, although some reports described the upper limit dose exposure for long period of time as being not adverse in a few individuals. Based on the WHO values established for human copper intake, the acceptable daily intake (ADI) – or more appropriately designated as 'upper limit for copper intake' for copper is 0.15 mg Cu kg^{-1} bw day^{-1}, and no acute reference dose (ARfD) was allocated based on the toxicological profile.

Tin

- Azocyclotin and cyhexatin have moderate acute toxicity by the oral route, the LD50 in rats are 209–265 mg kg^{-1} bw, respectively. The most sensitive effect seen in long-term studies of toxicity/carcinogenicity with azocyclotin and cyhexatin in mice and rats was decreased body weight, whereas at higher doses in short-term studies, the main toxicological effects seen in rats were local effects on the gastric mucosa, hematological changes, and hepatotoxicity. In long-term studies, cyhexatin also caused increased retinal atrophy. No neurotoxic, genotoxic, or carcinogenic effects were observed with either compound. They were not teratogenic, but cyhexatin caused embryotoxicity in rabbits. A group ADI of 0–0.003 mg kg^{-1} bw and a group ARfD of 0.02 mg kg^{-1} bw for both compounds was established by the 2005 JMPR.
- Fentin in rats after acute oral administration has an LD50 of approximately 160 mg kg^{-1} bw. In developmental toxicity studies, fentin cause resorptions in pregnant rabbits at dose levels only slightly higher than it caused maternal effects on body weight. Multigeneration reproductive toxicity study in the rats showed increased susceptibility in the offspring (based on offspring toxicity [decreased litter size, liver and spleen weight] was seen at a dose lower than parental toxicity [decreased body weight gain]). No malformations were observed in any study. EPA has classified fentin as a probable human carcinogen (B2) based on evidence of carcinogenicity in mice (liver tumors) and rats (pituitary and testicular tumors). However, fentin is unlikely to be genotoxic *in vivo*. In short-term and long-term toxicity studies show fentin immunosuppressive properties (lymphopenia and lymphocyte depletion of spleen and thymus, resulting in altered humoral and cellular immunity). The US EPA established an ADI of 0–0.00003 mg kg^{-1} bw and an ARfD of 0.001 mg kg^{-1}.

Occurrence in Food

Copper

Copper is present in almost all foods, and most human diets naturally include between 1 and 2 mg per person per day of copper, with some containing up to 4 mg per person per day. In plants, copper is absorbed from soil through the roots. On foliar application, the transportation and distribution of copper in plants are limited. For instance, residue levels in treated crops such as tomatoes ranged from 1.9 to 3.9 mg kg^{-1}, table grapes from 2.2 to 12 mg kg^{-1}, and wine grapes from 2.2 to 56 mg kg^{-1}. The levels of copper in untreated crops (background) ranged from 0.15 to 1.2 mg kg^{-1} in tomatoes and 0.54 to 4.8 mg kg^{-1} in grapes. In the EU, the following MRL have been set: 5 mg kg^{-1} for tomatoes, 20 mg kg^{-1} for table grapes, and 50 mg kg^{-1} for wine grapes. The Theoretical Maximum daily intake (TMDI) was 245%, 38% and 22% of the ADI of 0.15 mg kg^{-1} bw day^{-1} for French adults, toddlers, and infants, respectively. Wine was by far the highest contributor to the copper intake estimated for adults. The TMDI for the general population based on the WHO consumption data was estimated as 62% of the ADI. The refined national (Germany and UK) estimated intakes (NEDI) for adults, children, toddlers, and infants were highest for the UK toddler at 26% of the ADI.

As exposure to copper is not due to residues from the use as a plant protection product, an aggregate exposure assessment was performed in EU, including background levels of copper in food and drinking water, based on a French dietary survey. This aggregate estimate shows that, in particular for children, the overall dietary copper exposure is not negligible and approximates the established toxicological reference value.

Daily copper intakes of adult European consumers due to untreated food correspond to 13–40% of the proposed ADI, whereas for children between 3–14 years correspond to 51% of the ADI. When in addition, the median residue levels (STMR) in treated grapes and tomatoes are considered, and the average content in water is replaced by the highest copper content of 0.5 mg l^{-1} in drinking water in France, the calculated NEDI represent 40%, 103% and 92% of the ADI for the French adults, infants, and toddlers, respectively.

Tin

- Azocyclotin and cyhexatin residues are generally low. Both the long-term (IEDI) and short-term (IESTI) risk assessments were reassuring. In fact, the IEDIs were calculated for the five GEMS/Food regional diets from the STMR and supervised trial median residues-processing (STMR-P) values for fruits and processed products, and they ranged from 0 to 5% of the ADI, whereas the IESTIs ranged from 3 to 20% of the ARfD.
- Fentin acute dietary risk from food in the USA is up to 34% of the ARfD, whereas it is $<5\%$ for the ADI (chronic noncancer dietary risk). The chronic cancer dietary risk from food was estimated to be 1.1×10^{-6}–8.7×10^{-8} for the general US population, depending on the exposure scenario chosen.

See also: Foodborne Diseases: Overview of Chemical, Physical, and Other Significant Hazards. Institutions Involved in Food Safety: FAO/WHO Codex Alimentarius Commission (CAC); Food and Agriculture Organization of the United Nations (FAO); World Health

Organization (WHO). Public Health Measures: Monitoring of Contaminants. Toxic Metals: Trace Metals – Chromium, Nickel, Copper, and Aluminum

Further Reading

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EFSA Reports of the PRAPeR Meetings.

http://ec.europa.eu/sanco_pesticides/public/index.cfmevent=activesubstance.selection
European Union Pesticides Database.

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Joint FAO/WHO Meeting on Pesticide Residues (JMPR).

http://www.epa.gov/pesticides/reregistration/status.htm
USEPA Pesticide Registration Status Home Page.

http://www.inchem.org/pages/ehc.html
World Health Organization – Environmental Health Criteria Series.

Organophosphates and Carbamates

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Glossary

Absorption, distribution, metabolism, and excretion Absorption, distribution, metabolism, and excretion of a herbicide provides information on the extent and rate of absorption of a herbicide; tissues or organs receiving the highest exposures; the extent of metabolism and the types of metabolites formed; the rate and extent of excretion (clearance) from the body.

Acceptable daily intake Estimated maximum amount of an agent, expressed on a body mass basis, to which individuals in a subpopulation may be exposed daily over their lifetimes without appreciable health risk.

Acute reference dose Estimated maximum amount of an agent, expressed on a body mass basis, to which individuals in a subpopulation may be exposed during a meal or in one day without appreciable health risk.

International estimated daily intake Long-term (lifetime) intakes are obtained from the World Health Organization (WHO) Global Environment Monitoring System (GEMS)/Food Regional Diets that are based on per capita consumption.

International estimated short-term intake) Short-term intakes are based on a large portion of a commodity that is consumed on a single day both by the general population and by children aged 6 and under. The large-portion values are obtained from actual food consumption survey data for individuals identified as 97.5 percentile consumers of the commodity among eaters only.

LD50 Lethal dose 50%: the dose of a chemical that would be expected to kill half of a population of exposed organisms.

Pesticide formulation A product consisting of one or more active substances and other chemicals (such as solvents or surfactants) that enhance the application or effectiveness of the active substance.

Chemical Groups and Modes of Pesticidal Action

Organophosphates (OP) share a common chemical structure, but they differ significantly in the details of their structure, and hence in their properties and uses. Similar considerations can be applied to carbamates (CA). OP and CA insecticides represent two groups of chemicals that are usually dealt with together because they act on the same molecular target, neural acetylcolinesterase (AChE), in both mammals and insects. Their structural difference is reflected in some differences in the interaction with AChE, which in any case results in the inhibition of its enzymatic activity. There are only a few OPs (e.g., merphos) and a number of carbamates that are not significant AChE inhibitors and are not used as insecticides. For instance, the OP merphos was used as a cotton defoliant, the dithiocarbamates (e.g., mancozeb, maneb) are used as fungicides, the carbamates with a bulky substitution on the nitrogen (e.g., phenmedipham) or the thiocarbamates (e.g., molinate) are used as herbicides. These compounds will not be dealt within this article.

Uses

The range of uses of OP and CA insecticides is very broad, as are the application rates, varying by active substance, the crop to be used on, and the pest. It should also be noted that OPs and CAs are used in public health against vectors of diseases such as the malaria mosquitoes. In this case, some of these compounds are also allowed to be used indoors, based on certain toxicological and chemicophysical characteristics. In addition, other OPs, or even OPs used as insecticides, – were used for the treatment of human diseases such as parasitosis, myasthenia, or glaucoma.

OPs and CAs are the most used insecticides after the banning of most organochlorines such as DDT. Their use is now declining, especially for OPs, except in developing countries. In fact, in Europe (UK and Germany) the estimated sales of OPs declined from approximately 525 t to 258 t from 1995 to 2009, a reduction of a little more than 50%. Similarly in Japan, the decline was >40% from 2000 to 2009 (3350 t), in the USA >50% from 1995 to 2006 (17 000 t) and >70% from 1980 to 2006. On the contrary, in developing countries such as Bangladesh, there was a steady increase from 1995 to 2000 (+160%), to 2005 (+510%), to 2009 (+1000%) to approximately 8300 t. The trends for CAs were similar, except in Europe (UK and Germany) where there were minimal changes from 1995 to 2009, with sales being approximately 190 t. In 2009, sales were 494 t in Japan, and 23 000 t in Bangladesh.

Relevant ADME Characteristics

The absorption, distribution, metabolism, and excretion (ADME) characteristics of OPs and CAs vary. Most OPs and CAs are rapidly absorbed, metabolized, and excreted, but some OPs have a relatively long time of excretion (a few days as compared to a few hours). Hence, their effects after acute poisoning may last longer (e.g., fenitrothion). In urine, OPs are rarely excreted and only metabolites are generally found. The most known and usually determined ones are the alkylphosphates that are derived from most OPs; therefore finding them in urine gives little information on the individual compound to which the subject has been exposed. Some specific metabolites can be measured, including 3,5,6-trichloro-2-pirydinol from chlorpyrifos and p-nitrophenol from parathion. Details on the ADME of individual compounds can be found in publications such as WHO/International Programme on Chemical Safety (IPCS) Environmental Health Criteria, Food and Agriculture Organization (FAO)/WHO Joint Meeting on Pesticide Residues (JMPR) publications, European Food Safety Authority (EFSA) conclusions, or US Environmental Protection Agency documents.

Relevant Toxic Effects: From Acute Exposure and Repeated Exposure

The toxicity of OPs and CAs is due to the accumulation of acetylcholine at nerve terminals because of acetylcholinesterase inhibition. The compounds only differ in potency and duration of the effects. In particular, inhibition of AChE caused by CAs is rapidly (minutes–hours) reversible whereas that caused by OPs is essentially irreversible and takes many hours or even days to recover. For these reasons, repeated exposures to OPs over consecutive days may cause a cumulation of effects, whereas in the case of CAs, only acute, reversible effects can be observed that recover before the exposure during the following day. However, acute toxicities, as defined by the LD50, vary significantly and those of CAs overlap with those of OPs. For instance, the OP LD50 may vary from approximately 3 mg kg^{-1} bw (mevinphos) to $>10\,000$ mg kg^{-1} bw (malathion) similar to those of CA that range from approximately 1 mg kg^{-1} bw (aldicarb) to hundreds of mg kg^{-1} bw.

The signs of the cholinergic syndrome are typical and should be easily recognized by the physician. These include excessive salivation, lacrimation, sweating, urinary urgency, miosis, blurred vision, abdominal cramps, muscle fasciculation, and heart arrhythmias. More severe poisoning include bronchoconstriction, blurred vision, dizziness, confusion, convulsions, and coma. Besides unspecific supportive treatment, there are antidotes available against OP poisoning (atropine and oximes) that are not suggested for the self-limiting, rapidly reversible poisoning by CAs. Supportive treatment includes anticonvulsants such as benzodiazepines, and mechanical ventilation. If appropriate therapy is provided on time, and especially convulsions and hypoxia are prevented, full recovery occurs and no long-term sequelae are expected.

Children have been reported to be more sensitive to toxic effects than adults. However, this higher sensitivity applies only to acute high exposures and is due to the lower capacity of the detoxifying machinery that is overcome in children at somewhat lower doses that in adults. On the contrary, at low doses, such as those derived from residues in food, there appears to be no significant difference in sensitivity between the young and adults. Several OPs and CAs have been banned in industrialized countries because of high, acute toxicity (e.g., mevinphos, aldicarb) that caused both occupational and, rarely, foodborne poisoning. However, it should be noted that those episodes were subsequent to gross misuse of the compounds.

Certain OPs have been reported to cause a peripheral polyneuropathy, known as organophosphate-induced delayed polyneuropathy (OPIDP). However, only few of OPIDP-causing OPs are still on the market (e.g., methamidophos, dichlorvos) and they cause it only at doses causing severe, life-threatening acute poisoning, which can never occur as a consequence of their presence as residues in food.

The possibility of long-term effects following prolonged exposure to OPs (much less so to CAs) has been raised by several studies. However, except for individuals suffering from a previous acute poisoning, there is no compelling epidemiological or experimental evidence that this might be the case. In any case, such a possibility has only been raised as a consequence of nonfood exposure (e.g., occupational or environmental).

Details on the toxicity of individual compounds can be found in publications such as WHO/IPCS Environmental Health Criteria, FAO/WHO JMPR, EFSA conclusions, or US Environmental Protection Agency documents.

Occurrence in Food

OP and CA residues in food crops can be found in concentrations that depend on the compound, use rate, and crop.

The evaluation of active substances by the Joint FAO/WHO Meeting on Pesticide Residues includes a dietary risk assessment, based on the evaluation of the toxicological properties and the residue aspects of the compounds. For the assessment of the long-term intake of residues, the international estimated daily intake (IEDI) was calculated for different diets representative for relevant global regions. For the assessment of the short-term intake of residues, the international estimated short-term intake (IESTI) was calculated for relevant food commodities for which residue data and consumption data were available.

In summary, the long-term intake of residues of selected OPs and CAs (Table 1) is unlikely to present a public health concern, except that in a few cases, when based on a conservative approach as that used by the FAO/WHO. However, the short-term intake of residues of most of the OPs and CAs requires a closer scrutiny to identify which crops pose the higher risk (i.e., % acute reference dose (ARfD) >100) and may present a public health concern. For instance, a refinement of the assessment can be carried out by actually measuring (monitoring) the residue levels in marketed crops. This can also be applied to the few instances where the acceptable daily intake is exceeded. For instance, OPs and CAs are sought routinely in surveillance and monitoring schemes, and are found in less than 10% of the samples in the EU and at levels that are generally below (even well below) the maximum residue limits . Therefore, dietary exposures to OPs and

Table 1 Dietary risk assessment for selected OPs and CAs

Compound	*Long-term risk assessment*		*Short-term risk assessment*	
	ADI (mg kg^{-1} bw)	*IEDI (% of the maximum ADI)*	*ARfD (mg kg^{-1} bw)*	*IESTI (% of the ARfD)*
Acephate (OP)	0–0.003	2–7	0.04	Ch: 0–390 GP: 0–170
Aldicarb (CA)	0–0.003	6–20	0.003	Ch: 330–560 GP: 140–230
Carbofuran (CA)	0–0.001	24–60	0.001	Ch: 0–830 GP:120–510
Carbaryl (CA)	0–0.008	6–20	0.02	Ch: 330–560 GP: 140–230
Carbosulfan (CA)	0–0.01	24–60	0.02	Ch: 0–830 GP:120–510
Chlorpyrifos (OP)	0–0.01	3–26	0.1	Ch: 0–40 GP: 0–10
Chlorpyrifos-methyl (OP)	0–0.01	8–26	0.1	Ch: 0–40 GP: 0–10
Diazinon (OP)	0–0.005	7–36	0.03	Ch: 6–90 GP: 3–30
Dimethoate (OP)	0–0.002	110–120	0.02	Ch: 20–760 GP: 10–320
Fenitrothion (OP)	0–0.006	280–780	0.04	Ch: 2–240 GP: 1–150
Fenthion (OP)	0–0.007	1–10	0.01	Ch: 0–2 GP: 0–1
Malathion (OP)	0–0.3	0	2.00	Ch: 0–7 GP: 0–2
Methamidophos (OP)	0–0.004	4–36	0.01	Ch: 0–410 GP: 0–150
Methiocarb (CA)	0–0.02	2–5	0.02	Ch: 0–50 GP: 0–70
Parathion (OP)	0–0.004	7–20	0.01	Ch:1–140 GP: 0–400
Parathion-methyl (OP)	0–0.003	3–30	0.03	Ch: 0–80 GP: 0–20
Phosalone (OP)	0–0.02	0–40	0.03	Ch: 0–40 GP: 0–10
Phosmet (OP)	0–0.01	0–90	0.02	Ch: 1–50 GP: 0–10
Pirimicarb (CA)	0–0.02	3–24	0.1	Ch: 0–70 GP: 0–10
Pirimiphos-methyl (OP)	0–0.3	10–50	0.2	Ch: 0–70 GP: 0–40
Profenofos (OP)	0–0.03	2–42	1	Ch: 0–10 GP: 0–6
Terbufos (OP)	0–0.0006	0–17	0.002	Ch: 3–30 GP: 0–30
Triazophos (OP)	0–0.001	0–30	0.001	Ch: 0–560 GP: 0–790

Abbreviations: Ch: Children 6 years and below; GP: General population.

CAs as calculated from monitoring data (i.e., levels closer to what the population is in fact exposed) are generally well below the chronic and acute reference doses.

See also: Disciplines Associated with Food Safety: Food Safety Toxicology. Institutions Involved in Food Safety: FAO/WHO Codex Alimentarius Commission (CAC); Food and Agriculture Organization of the United Nations (FAO); World Health Organization (WHO). Risk Analysis: Risk Assessment: Chemical Hazards; Risk Assessment: Principles, Methods, and Applications

Further Reading

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http://www.fao.org/agriculture/crops/core-themes/theme/pests/pm/jmpr/jmpr-rep/en/
Food and Agriculture Organization/World Health Organization.

http://www.epa.gov/pesticides/reregistration/status.htm
US Environmental Protection Agency.

PESTICIDE RESIDUES

Organochlorines

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Glossary

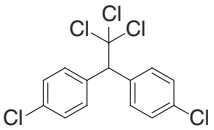
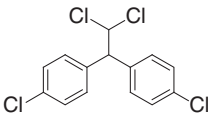
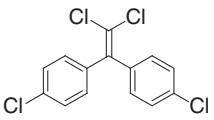
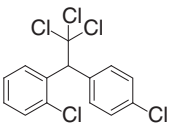
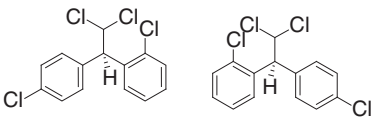
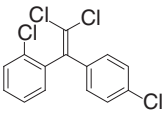
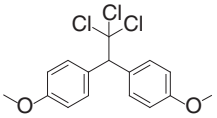
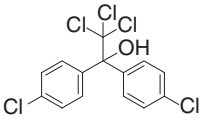
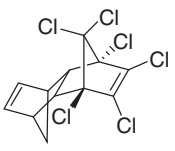
Acceptable daily intake The dose of a specific contaminant that may be absorbed daily by an individual for the whole life without an appreciable increase of the health risk.
Acute reference dose An estimate of a chemical substance in food or drinking water expressed in a body weight basis that can be ingested over a short period of time usually during one meal or in one day without appreciable health risk to the consumer.
Bioaccumulation The gradual build up over time of a chemical within a living organism, which occurs either because the chemical is taken up faster than it can be metabolized and excreted.
Biomagnification The increasing concentration of a substance in the tissues of organisms at successively higher levels in a food chain. As a result, organisms at the top of the food chain have higher levels (body burden) of a persistent toxin or pollutant than those at lower levels.
Contaminant Any biological or chemical agent, foreign matter, or other substances not intentionally added to food which may compromise food safety or suitability.
Food safety The assurance that food will not cause harm to the consumer when it is prepared and/or eaten according to its intended use.
Lipophilic It means having a strong attraction for fats/able to dissolve, be dissolved in, or absorb lipids (fats).
Monitoring The act of conducting a planned sequence of observations or measurements of control parameters to assess whether a control measure is under control.
Provisional tolerable daily intakes Maximum doses that are set for substances that are anticipated to be without health risk to humans when taken daily over the course of time. They are expressed in one day time unit.
Risk management The process, distinct from risk assessment, of weighing policy alternatives, in consultation with all interested parties, considering risk assessment and other factors relevant for the health protection of consumers and for the promotion of fair trade practices, and, if needed, selecting appropriate prevention and control options.

Introduction

Organochlorinated pesticides (OCPs) are a structurally heterogeneous class of organic compounds composed primarily of carbon, hydrogen, and chlorine atoms. The chlorine atoms on the organic moieties contribute to the high stability, slow biodegradation, and high lipid solubility of most OCPs, properties that, in turn, lead to their accumulation in animal tissues, including humans. OCPs of a prominent environmental and public health concern include dichlorodiphenytrichlorolethanes (DDT complex), hexachlorocyclohexanes (HCH), cyclodienes, hexachlorobenzene (HCB), and other insecticides such as chlordecone and mirex. Some typical compounds are illustrated in Table 1.

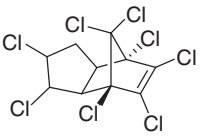
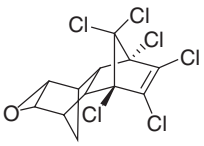
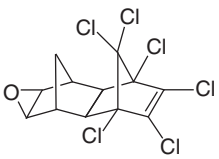
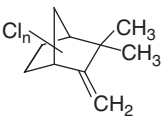
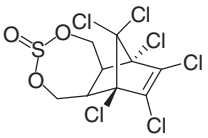
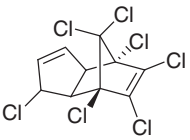
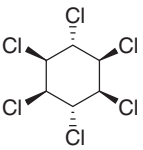
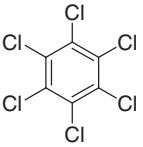
OCPs have broad spectrum insecticidal activity and primarily act as excitatory neurotoxins, by altering the movement of ions across the nerve cell membranes, thus changing the ability of the nerve to fire. For example, DDT act through neurotoxicity by causing repetitive firing of nerve cells and makes them unable to fire in response to a signal. Lindane kills insects by stimulating the central nervous system (CNS) causing trembling, hyperexcitation, loss of coordination, paralysis, and eventually death. Cyclodienes act by blocking the chloride channel of gamma aminobutyric acid (GABA) receptors and inhibiting GABA-ergic neurotransmission. Dicofol inhibits electron transport during energy-producing mitochondrial electron transport and oxidative phosphorylation. By interrupting this process the insects fail to store energy for the later use. Pentachlorophenol acts mainly through an inhibition of phosphorilative oxidation, with a consequent block in energy storage and the fungicide HCB acts through an inhibition of porphyrins metabolism.

Table 1 Structures of some organochlorine pesticides of major environmental and toxicological concern

Description of substance	*Chemical structure*
1. Dichlorodiphenyl-ethanes *p,p′*- DDT IUPAC: 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane CAS No.: 50-29-3 Molecular formula: $C_{14}H_9Cl_5$; Formula weight: 354.49	
p,p′-DDD IUPAC: 1,1-dichloro-2, 2-bis (4-chlorophenyl)ethane CAS. No.: 72-54-8 Molecular formula: $C_{14}H_{10}Cl_4$; Formula weight: 318.03	
p,p′-DDE IUPAC: (1,1-dichloro-2, 2bis (4-chlorophenyl) ethylene) CAS No.: 72-55-9 Molecular formula: $C_{14}H_8Cl_4$; Formula weight: 320.05	
o,p′-DDT IUPAC: 1,1,1-trichloro-2-(*o*-chlorophenyl)-2-(*p* chlorophenyl)-ethane CAS No.: 789-02-6 Molecular formula: $C_{14}H_9Cl_5$; Formula weight: 354.49	
o,p′-DDD (Mitotane) IUPAC: 1,1-dichloro-2-(*o*-chlorophenyl)-2-(*p* chlorophenyl)ethane CAS No.: 53-19-0 Molecular formula: $C_{14}H_{10}Cl_4$; Formula weight: 320.05	
o,p′-DDE IUPAC: 1,1-dichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethylene CAS No.: 3424-82-6 Molecular formula: $C_{14}H_8Cl_4$; Formula weight: 318.03	
Methoxychlor IUPAC: 1,1,1-Trichloro-2,2-bis(4-methoxyphenyl)ethane CAS No.: 72-43-5 Molecular formula: $C_{16}H_{15}Cl_3O_2$; Formula weight: 345.65	
Dicofol (Kelthane) IUPAC: 2,2,2-trichloro-1,1-bis(4-chlorophenyl)ethanol CAS No.: 115-32-2 Molecular formula: $C_{14}H_9Cl_5O$; Formula weight: 370.49	
2. Cyclodienes Aldrin IUPAC: 1,2,3,4,10,10-Hexachloro-1,4,4a,5,8,8a-hexahydro-1,4:5,8-dimethanonaphthalene CAS No.:309-00-02 Formula: $C_{12}H_8Cl_6$; Formula weight: 364.92	

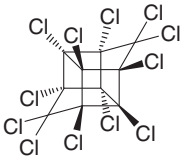
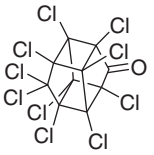
(*Continued*)

Table 1 Continued

Description of substance	*Chemical structure*
Chlordane IUPAC: 1,2,4,5,6,7,8,8-octachloro-2,3,3°,4,7,7°-hexahydro-4,7-methano-1H-indene CAS No.: 12789-03-6 Molecular formula: $C_{10}H_6Cl_8$; Formula weight: 409.78	
Dieldrin IUPAC: 3,4,5,6,9,9-Hexachloro-1°,2,2°,3,6,6°,7,7°-octahydro-2,7:3,6-dimetanonapth[2,3-*b*]oxirene CAS No.: 60-57-1 Molecular formula: $C_{12}H_8Cl_6O$; Formula weight: 380.91	
Endrin IUPAC: 3,4,5,6,9,9,-Hexachloro-1a,2,2a,3,6,6a,7,7a-octahydro-2,7:3,6-dimethanonaphth[2,3-*b*]oxirene CAS No.: 72-20-8 Molecular formula: $C_{12}H_8Cl_6O$; Formula weight: 380.92	
Toxaphene IUPAC: Toxaphene CAS No.: 8001-35-2 Molecular formula: $C_{10}H_{10}Cl_8$; Formula weight: 413.82	
Endosulfan IUPAC: 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepine-3-oxide CAS No.: 115-29-7 Molecular formula: $C_9H_6Cl_6O_3S$; Formula weight: 406.95	
Heptachlor IUPAC: 1,4,5,6,7,8,8-Heptachloro-3a,4,7,7a-tetrahydro-4,7-methanol-1*H*-indene CAS No.: 76-44-8 Molecular formula: $C_{10}H_5Cl_7$; Formula weight: 373.32	
3. Hexachlorocyclohexane isomers Lindane (γ-HCH also known as γ-BCH) IUPAC: (1*r*,2*R*,3*S*,4*r*,5*R*,6*S*)-1,2,3,4,5,6-hexachlorocyclohexane CAS No.: 58-89-9 Molecular formula: $C_6H_6Cl_6$; Formula weight: 290.83	
4. Hexachlorobenzene Hexachlorobenzene IUPAC: Hexachlorobenzene CAS No.: 118-74-1 Molecular formula: C_6Cl_6; Formula weight: 284.78	

(*Continued*)

Table 1 Continued

Description of substance	*Chemical structure*
5. Others Mirex IUPAC: 1,1a,2,2,3,3a,4,5,5a,5b,6-dodecachloroacta-hydro-1,3,4-metheno-1H-cyclobuta[cd]pentalene CAS No.: 2385-85-5 Molecular formula: $C_{10}Cl_{12}$; Formula weight: 545.5	
Chlordecone (Kepone) IUPAC: 1,1a,3,3a,4,5,5,5a,5b,6-Decachlorooctahydro-2*H*-1,3,4-(methanetriyl)cyclobuta[*cd*]pentalen-2-one CAS No.: 143-50-0 Molecular formula: $C_{10}Cl_{10}O$; Formula weight: 490.64	

Uses of OCPs

OCPs have been used for several applications and were widely dispersed into the environment over the past 50 years. Now, many of these compounds are ubiquitously found as trace contaminants in soil, sediment, air, biota, and food, even in places where they were never used, including Antarctica. DDT has been employed as an insecticide in agriculture and public health. Banned for use in agriculture, its only permitted use is for indoor spraying of walls to control the malaria vector mosquito. It is estimated that 600 000 t of DDT was used domestically in the USA 30 years before its ban. Over 80% of the pesticide used during 1970–72 was applied to cotton crops and the remainder was used on peanut and soybean crops. Chlordane, heptachlor, and pentachlorophenol are used to treat wood to prevent pest damage. Under the Stockholm Convention on Persistent Organic Pollutants (POPs), the production and use of most of the older OCPs are restricted or banned. However, several OCPs are still being used.

For example, the annual production of endosulfan is estimated to range between 18 000 and 20 000 t worldwide. In the USA, 400 t is estimated to be used annually for domestic purpose. The main uses include control of pests in vegetables, fruits, cereal grains and cotton, ornamental shrubs, trees, vines, and ornamental plants. In Africa it is commonly used in cotton production at a dose rate between 0.1 and 0.2 t km^2, whereas in India it is used to control pests on cashew plantations at a dose rate of 0.12 t km^2. The estimated annual production in India is 9500 t, where 4500–5000 t is consumed domestically. Codex Alimentarius recommends maximum residue limits for endosulfan on several food commodities.

Another OCP in use is dicofol with an estimated global production of 5500 t per annum. In 2000, the use of dicofol in Europe was estimated to be 290 t, the same estimated annual use in North America. The average application rate is 0.044 t km^2. Dicofol is used for foliar spray on agricultural crops, such as beans, grapes, citrus, cucurbits, tomatoes, apple and cotton, and ornamentals. It is also used for mite control. DDT can be found as an impurity of dicofol because it is produced as an intermediate during its manufacture.

Lindane has been used to protect crop seeds from insects, for pest control in forests, on livestock and household pets for control of ticks and other pests, and in homes to control ants and other household pests. It is also used to control head lice and scabies in humans. The estimation of global production between 1950 and 2000 was 600 000 t, of which the majority was used in agriculture (Table 2).

OCPs entering the environment persist for long periods, in particular where ambient temperatures and UV irradiation are low. Owing to their appreciable volatility and chemical resistance to degradation, they are transported in the atmosphere, contaminating places very far from the sites of application or use, in general from tropical countries to the coldest areas of the world. Owing to health and environmental concerns associated with their biomagnification and accumulation, most OCPs were banned from use in most countries since 1970s. More recently, the production and use of 10 OCPs along with polychlorinated biphenyls and dioxins were banned under Stockholm Convention on POPs, but some exceptions were allowed (Table 3).

Relevant Absorption, Distribution, Metabolism and Excretion Characteristics

When OCP-contaminated food is consumed, the OCPs are rapidly absorbed from the small intestine and distributed throughout the body. Because OCPs are poorly biodegradable and strongly soluble in body lipids, they accumulate in body tissues with high lipid content, such as the adipose tissue, the brain, the liver, the kidney, and the myocardium. Thereafter a continuous exchange between blood and tissues takes place. In pregnant women these chemicals cross the placenta. In lactating women OCPs are secreted into breast milk, thus transferring the mother's burden to the young through breastfeeding.

Most of the limited OCPs biodegradation and elimination from the body takes place slowly through the kidney.

Table 2 An extended list of persistent organic pollutants banned by the Stockholm Convention

Chemical	*Pesticides*	*Industrial chemicals*	*Byproduct*	*Annex*
Aldrin	+			A
Chlordane	+			A
Chlordecone	+			A
DDT	+			B
Dieldrin	+			A
Endrin	+			A
Heptachlor	+			A
Lindane	+			A
Mirex	+			A
Technical endosulfan and its related isomers	+			A
Toxaphene	+			A
Hexabromobiphenyl		+		A
Hexabromodiphenyl ether and heptabromodiphenyl ether (commercial octabromodiphenyl ether)		+		A
Perfluorooctane sulfonic acid, its salts and perfluorooctane sulfonyl fluoride		+		B
Tetrabromodiphenyl ether and pentabromodiphenyl ether (commercial pentabromodiphenyl ether)		+		A
α-HCH	+	+		A
β-HCH	+	+		A
Pentachlorobenzene	+	+		A & C
HCB	+	+	+	A
PCBs		+	+	A
Chlorinated dioxins			+	C
Chlorinated furans			+	C

Abbreviations: DDT, dichlorodiphenyltrichloroethanes; HCB, hexachlorobenzene; HCH, hexachlorocyclohexanes; PCBs, polychlorinated biphenyls.
Annex A – Intentionally produced chemicals that need to be eliminated.
Annex B – Intentionally produced chemicals with restrictions.
Annex C – Unintentionally produced chemicals.

Table 3 Estimated dietary intake (ng per day) of some OCPs through seafood consumption by the general population of different countries

Country	*DDTs*	*CHLs*	*HCHs*	*HCB*
Indonesia	1100	10	18	10
China	882	14.4	28.2	11.4
Sweden	256	87	35	36
Italy	197	–	13	4.6
Spain	–	–	–	12.4
South Korea	270	13	2.1	11

Note: DDTs = Σ *p,p′*-DDE, *p,p′*-DDD, *p,p′*-DDT, *o,p′*-DDD; CHLs = Σoxy-chlordane, *trans*-chlordane, *cis*-chlordane, *trans*-nonachlor, *cis*-nonachlor; HCHs = $\Sigma\alpha$-, β-, γ-HCH.
Abbreviations: CHLs, chlordanes; DDT, dichlorodiphenyltrichloroethanes; HCB, hexachlorobenzene; HCH, hexachlorocyclohexanes.
Source: Adapted from Moon H, Kim H, Choi M, Yu J, and Choi H (2009) Human health risk of polychlorinated biphenyls and organochlorine pesticides resulting from seafood consumption in South Korea, 2005–2007. *Food and Chemical Toxicology* 47: 1819–1825.

Although they are excreted to some extent in the bile, most are reabsorbed. Most OCPs have long elimination half-lives and can be detected in fat years after the last exposure. DDT has a half-life of approximately 3.4 years in adipose tissue. Dichlorodiphenyldichloroethylene (DDE), the principal DDT metabolite, is more stable than DDT and is rarely excreted from the body, but both are excreted in breast milk. Dieldrin has a half-life of approximately 267 days in blood, which is suspected to be shorter than those of heptachlor epoxide and α-, β-, and γ- isomers of HCH. Note that α- and β-HCH have no insecticidal activity and are contaminants of technical grade lindane. Comparing the three HCH isomers, β-HCH is eliminated very slowly from the body and thus has a significant contribution to the total HCH body burden. Its half-life in human blood is estimated to be approximately 7.2 years.

Prolonged half-lives in the body have some exceptions: for example, estimated half-life of endrin is 24 h, whereas that of lindane and chlordane is between 10 and 20 days. Aldrin is readily metabolized to dieldrin so that residues are rarely found in food and animals. 1,3-dichloropropene is excreted in urine as a mercapturic acid derivative and thioethers within few hours after absorption. Thus the degree of accumulation depends on metabolic rate, where slowly metabolized OCPs exhibit the highest accumulation rate and persist in the tissues for long periods of time.

Occurrence in Food

As a consequence of widespread environmental contamination and biomagnification along the food chain, OCPs are

Table 4 Total DDT intake compared with the PTDI

Country	*Year*	*Daily intake (ng per kg bodyweight)*	*% of PTDI*
Australia	1980	0.390	3.9
	1987	0.026	0.26
Egypt	1988	13.700	137.0
Finland	1984	0.041	0.42
	1986	0.026	0.26
Guatemala	1982	0.260	2.6
	1984	0.200	2.0
	1985	0.065	0.66
	1988	0.031	0.32
India	1981	3.900	39.0
	1983	3.600	36.0
Japan	1980	0.056	0.56
	1982	0.070	0.74
	1984	0.030	0.3
	1986	0.020	0.2
	1988	0.020	0.2
The Netherlands	1984	0.004	
	1985	0.004	0.04
New Zealand	1982	0.003	0.03
Switzerland	1983	0.03	0.3
Thailand	1980	1.600	16.0
	1987	0.0008	0.008
United Kingdom	1980	0.050	0.5
	1981	0.035	0.36
	1985	0.05	0.5
United States	1980	0.360	3.6
	1982	0.033	0.34
	1985	0.036	0.36
	1986	0.019	0.2

Abbreviations: ADI, acceptable daily intake (0–0.02 mg per kg bodyweight); DDT, dichlorodiphenyltrichloroethanes; PTDI, provisional tolerable daily intake (0–0.01 mg per kg bodyweight).
Source: WHO (2003) Health risks of persistent organic pollutants from long range transboundary air pollution. *Joint WHO/Convention Task Force on the Health Aspects of Air Pollution*. Geneva: WHO.

present in foods at levels which show significant variability among countries and different areas within individual countries, in particular, in areas previously or currently used for intense agricultural activities with high levels of OCP application. The dietary intake thus varies among countries and population groups within countries, as exemplified by the data reported in **Tables 3–5**. World Health Organization has reported that the highest dietary intake of DDT and the older OCPs was in developing countries where some OCPs are still used or where their use was only recently banned.

Human exposure mainly occurs through trace environmental contamination of food, which accounts for more than 90% of total OCPs burden. Most food contamination occurs in meat and meat products, fish, milk, and other dairy products. Deposition of airborne OCPs on pasture is considered the primary pathway from which these pollutants enter the food chain,

Table 5 Acceptable/tolerable intakes of selected organochlorinated pesticides (mg per kg bodyweight) as evaluated by JMPR

Compound	*ADI*	*PTDI*	*Year of evaluation*
Aldrin		0–0.0001	1994
Chlordane	0–0.0005		1994
Chlorobenzilate	0–0.02		1980
DDT		0–0.01	2000
Dicofol	0–0.002		1992
Dieldrin		0–0.0001	1994
Endosulfan	0–0.006		1998
Endrin		0–0.0002	1994
Heptachlor		0–0.0001	1994
Hexachlorobenzene	ADI withdrawn		1978
Lindane	0–0.001		1997

Abbreviations: ADI, acceptable daily intake; JMPR, Joint FAO/WHO meeting on pesticide residues; PTDI, provisional tolerable daily intake.

although in regions with highly contaminated soil, green vegetables may significantly contribute to human exposure. Food-producing animals bioaccumulate OCPs in their adipose tissue through ingestion of contaminated pasture or the feed derived from contaminated fatty materials. OCPs are transferred to animal milk and enter into human diet through milk and dairy products. Although fish and seafood is a small fraction of human diet, it is a major route of exposure for Arctic populations who live on marine mammals, which are at the top of the marine trophic network, which serves to biomagnify the OCPs.

In the past, phenoxyacetates, widely used herbicides were found to be contaminated by dioxin. In particular formulations containing 2,4,5-trichlorophenoxy acid (2,4,5-T) were found to be contaminated by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin as well as other dioxin isomers. This caused episodes of significant environmental contamination where the highest levels were observed in 1970s, when phenoxy herbicides found high levels of use in rice crops.

Monitoring of OCPs in Food

Multiresidue analysis is usually employed to determine the levels of OCPs in foods for health risk assessment. Validated methods, including sampling, sample storage and transport, and sample processing and analysis, have been issued by several international agencies and national regulatory bodies. Following appropriate sample extraction and purification, analyses are usually performed by gas chromatography with electron-capture detection. For some OCPs, the use of high-resolution gas chromatography–mass spectrometry with stable-isotope labeled analogs as internal standards is preferred. Key analytical issues to obtain reliable data are not only sensitivity in the parts-per-trillion (nanogram per kilogram) range but also resolution of specific isomers, because of their toxicities, may be orders of magnitude different.

Health Effects of OCPs

The toxicity of OCPs in humans differs significantly and the CNS is the main target organ, where they cause either

depression or stimulation. Organochlorines disturb the neuronal membrane causing hyperexcitability of the nervous system. Cyclodienes, HCH, and toxaphene inhibit GABA-mediated chloride influx in the CNS, whereas DDT affects potassium and voltage-dependent sodium channels. Owing to its uncoupling effect on phosphorylative oxidation, pentachlorophenol acute toxicity is characterized by sudden liberation of energy by the body, with hyperpyrexia, tremors, profuse sweating, and convulsions.

DDT is capable of inducing alterations on reproduction and development in animals. This property is attributed to a hormone-altering action of DDT isomers and metabolites. *o,p′*-DDT isomer has the strongest estrogen-like properties, whereas *p,p′*-DDE has antiandrogenic properties and has been shown to alter the development of reproductive organs when administered perinatally to rats. Scarce and inconsistent evidence is available in humans, and the significance for human health of the estrogenic properties identified in laboratory animals remains unclear. However, certain studies suggest the need for further investigation. For example, one study reported a significant reduction of anal position index among babies born from mothers who suffered a significant increase of maternal *p,p′*-DDE serum levels during the first trimester of pregnancy. Other studies found that DDT exposure and altered menstrual cycles and human perinatal exposure to DDT at levels $>0.20\,\mu g\,l^{1}$ was associated with adverse effects on neurodevelopment up to the age of 4 years. Prepubertal exposure to DDT was associated with breast cancer in some studies, but not confirmed by others.

HCB has been shown to cause hepatic, neurological, developmental, endocrine, and immunological toxicity in human. In 1970s, consumption of bread produced with HCB contaminated seeds in Turkey caused an epidemic of chemical porphyria. The latter is caused by the ability of HCB to inhibit hepatic delta-aminolevulinate synthetase, delta-aminolevulinate dehydratase, and uroporphyrinogen decarboxylase enzymes, which are responsible for porphyrins metabolism. All isomers of HCH have been shown to produce liver and kidney effects with α- and β-isomers being much more toxic. Endosulfan has significant teratogenic effects in highly exposed laboratory animals, but there is no evidence of a similar effect in humans. *In vitro* studies have shown that this OCP can promote proliferation of human breast cancer cells, but there are no epidemiological studies linking exposure to endosulfan and increase of cancer risk in humans.

Epigenetic effects, attributable to an altered expression of specific genes due to impairment of DNA methylation balance, have been hypothesized for some persistent organic pesticides. Low doses of DDT have been shown to affect DNA methylation in experimental animals, whereas in studies carried out on Greenlandic Inuit populations suggest a significant inverse linear relationship between DNA methylation and plasma concentrations of DDT, DDE, β-benzenehexachloride, oxychlordane, α-chlordane, and mirex.

Health-based guidance values have been established by WHO and other international organizations (**Tables 4** and **5**). These are used as guidance during evaluation of safety through comparison with exposure estimated from measured levels of the chemical in specific foods and estimated dietary intake of those foods.

Risk Management of OCPs

Compounds with a potential for accumulation, such as DDT/DDE, dieldrin/aldrin, endrin, HCB, and β-HCH, can be found in animal products for weeks or months after exposure of the animal. Therefore, the risk for residues in animal products is considered to be high. Lindane (γ-HCH) and chlordane pose intermediate risks for residues in animal products. However, endosulfan, deltamethrin, and methoxychlor are rapidly metabolized and they are seldom found in animal products. The restricted use or banning of most OCP compounds in Western countries have limited possible threats they might pose to health.

Cessation of direct application as pesticides on animals and reducing their presence in their feed, housing, or pasture is the best way of controlling residues in animal products. In particular, control of OCP levels in feedstuffs is an important risk management intervention. If acceptable limits for residues are exceeded, removal of the contaminated items from the human food chain is necessary. For animals that are contaminated, extending the time before these animals or their products can be used for food can allow levels in their tissues to deplete. For growing animals, reduction in contamination levels due to dilution may also help to achieve a product in compliance with legal residue limits.

Conclusion

OCPs are a class of pesticides that were widely used in agriculture and public health because of their effectiveness over prolonged periods of time. However, health and environmental concerns have resulted in a ban on the production and use of most OCPs. Despite the ban, the persistence of residues of OCPs in the environment still results in the contamination of food, particularly foods of animal origin. Residues can also be found in the human body and in human milk around the world. Thus continued research is needed on these contaminants, their body burden and potential health effects, and ways to reduce their bioavailability in food. Moreover, effective policies are needed to control their manufacture and release into the global environment.

See also: Foodborne Diseases: Overview of Chemical, Physical, and Other Significant Hazards

Further Reading

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www.chemicalbodyburden.org/cs_organochl.htm
Pesticides Pesticide Action Network North America (PANNA), Case study: Organochlorine.

Pyrethroids

R Pfeil, Federal Institute for Risk Assessment, Berlin, Germany

Glossary

CS-syndrome An acute poisoning syndrome characterized by choreoathetosis and salivation, typically produced by high doses of type II pyrethroids.

Paresthesia An abnormal sensation, such as prickling, itching, or, when more severe, burning of the skin.

Pyrethrins Several esters extracted from the flowers of three species of *Chrysanthemum*, used chiefly as an insecticide.

Pyrethroids Any of a class of synthetic, pyrethrin-like insecticides with a mechanism of action similar to that of naturally occurring pyrethrins.

T-syndrome An acute poisoning syndrome characterized by whole-body tremors, typically produced by high doses of type I pyrethroids.

Type I pyrethroids Pyrethroids lacking an α-cyano group in the molecule.

Type II pyrethroids Pyrethroids containing an α-cyano group in the molecule.

Chemical Groups and Modes of Pesticidal Action

Pyrethroids are synthetic insecticides derived from the naturally occurring pyrethrins, the six insecticidal compounds of pyrethrum isolated from the *Chrysanthemum* genus of plants.

Like pyrethrins, pyrethroids are composed of two basic structural moieties, an acid and an alcohol. In most pyrethroids, the acid portion consists of chrysanthemic acid, a cyclopropane ring bonded to a carboxylic acid moiety, and a variety of halogenated and nonhalogenated substituents, which were introduced to increase their photostability. The alcohol portion is either a primary or a secondary alcohol, which is bound to a variety of heterocyclic structures. In addition, several pyrethroids have a cyano group bound to the α-methylene of the alcohol, which results in enhanced insecticidal potency of the compound. Compounds lacking the α-cyano substituent are termed type I pyrethroids (e.g., allethrin, bifenthrin, etofenprox, permethrin, phenothrin, resmethrin, and tefluthrin), whereas compounds with an α-cyano group are termed type II pyrethroids (e.g., cyfluthrin, cyhalothrin, cypermethrin, deltamethrin, esfenvalerate, fenpropathrin, and τ-fluvalinate).

With a few exceptions, pyrethroids have 1-3 asymmetric carbon atoms. Compounds containing an asymmetric center may exist in two forms that are mirror images (enantiomers) and which are described as *R*- or *S*-configuration. For cyclopropanecarboxylic acid derivatives, only isomers with the *R*-configuration at C-1 position of the cyclopropane ring have substantial insecticidal activity, whereas the 1*S* isomers are not insecticidal. Furthermore, compounds containing two asymmetric centers at C-1 and C-3 position of the cyclopropane ring consist of two pairs of isomers, which are designated as *cis* and *trans*, based on the orientation of substituents of C-1 and C-3 in relation to the cyclopropane ring or the similarly restricting structure that may have been introduced to replace this ring.

The insecticidal properties of pyrethroids are due to their ability to disrupt the electrical signaling in the nervous system, an effect that is also relevant to mammals. Pyrethroids modify the gating characteristics of voltage-sensitive sodium channels in neuronal membranes to delay their closure. In general, type I pyrethroids prolong sodium channel opening only long enough to cause repetitive firing of action potentials (repetitive discharge) following a single stimulus. In contrast, type II pyrethroids prolong sodium channel opening for such long periods that the membrane potential becomes depolarized to the point that the generation of an action potential is impossible (depolarization-dependent block). Type I pyrethroids have a greater insecticidal activity when the ambient temperature decreases, whereas type II pyrethroids become more effective with increase in temperature.

Pyrethroids also decrease chloride channel currents, thereby increasing excitability, and it is likely that this action contributes to most characteristics of poisoning with type II pyrethroids. At relatively high concentrations, pyrethroids can also act on γ-aminobutyric acid (GABA)-gated chloride channels, which may be responsible for the seizures seen with severe type II pyrethroid poisoning.

Uses

Natural pyrethrins and the early pyrethroids (e.g., allethrin, resmethrin, and tetramethrin) are sensitive to photodegradation and have thus rarely been used in agriculture, with the exception of uses in glasshouses or in organic farming.

In commercial formulations, pyrethrins are often mixed with piperonyl butoxide, a synergist that increases insecticidal efficacy (but also toxicity) by the inhibition of pyrethrin metabolism. Additionally, the early pyrethroids are used as effective domestic insecticides that possess very low mammalian toxicity.

The introduction of light-stable pyrethroids in the early 1970s permitted to control numerous pests of agricultural significance. Pyrethroids are nonsystemic insecticides (i.e., no uptake through leaves and roots) with contact and stomach action and a rapid knockdown effect. They are effective against a wide range of chewing, sucking, and boring insects, particularly Coleoptera, Diptera, Heteroptera, Homoptera, Lepidoptera, Orthoptera, and Thysanoptera species in many crops (e.g., cereals, citrus, corn, cotton, fruits, hops, grapes, oilseed rape, potatoes, soya beans, sugar beet, vegetables, forestry, and ornamentals).

Owing to their high insecticidal activity, pyrethroids can be applied at dose rates as low as 2.5 g ha^{-1}, whereas the usual application rates vary widely between 5 and 200 g ha^{-1}, depending on the different intrinsic activities and/or different content of active isomers of the compounds.

In USA, approximately 900 tons active ingredient (a.i.) of permethrin and approximately 450 tons a.i. of cypermethrin have been applied annually to agricultural, residential, and public health usages. The great majority of use (>70% for permethrin and >85% for cypermethrin) occurred in nonagricultural settings. The annual usage of nonphotostable pyrethroids was <14 tons a.i. for allethrin, <23 tons a.i. for resmethrin, and 7–14 tons a.i. for tetramethrin, with the majority used for nonagricultural purposes.

In Germany, approximately 100 tons a.i. of pyrethroids have been marketed in plant protection products in 2009, with highest domestic sales reported for β-cyfluthrin, λ-cyhalothrin, etofenprox, α- and ζ-cypermethrin, bifenthrin, deltamethrin, esfenvalerate, and tefluthrin.

Apart from their use in agriculture, pyrethroids are of great importance for control of insect pests in public health and animal health. For example, in vector control programs, more than 520 tons a.i. of pyrethroids have annually been used worldwide.

Relevant Absorption, Distribution, Metabolism, and Excretion Characteristics

Because pyrethroids exhibit a wide structural diversity, there are some inherent differences in their absorption, distribution, metabolism, and excretion characteristics. Also, oral absorption data from toxicokinetic studies may be influenced significantly by the dosing vehicle and the dosing volume.

Absorption of pyrethroids from the gastrointestinal tract is rapid and usually incomplete, with estimates of approximately 30–70% of an orally administered dose for most compounds. First-pass metabolism may contribute significantly to a decrease in bioavailability. Once absorbed, pyrethroids distribute rapidly throughout the body and particularly into tissues with high-lipid content, including the central nervous system (CNS). For permethrin, deltamethrin, and λ-cyhalothrin, maximum plasma concentrations were achieved within 2–4 h. Plasma and brain half-life estimates were between 10 and 39 h and 14 and 35 h, respectively, whereas the half-life time in fat was up to 30 days. Owing to the slow elimination, pyrethroids may accumulate in fatty tissues when administered repeatedly.

The clearance rate is notably dependent on the structure, with *trans*-isomers hydrolysis being catalyzed by hepatic carboxylesterases, whereas hydrolysis of *cis*-isomers by oxidases is a relatively slow mechanism. Thus, the half-lives of *trans*-isomers are generally shorter by 2- to 10-fold than of their *cis*-counterparts. The slower breakdown of *cis*-isomers may contribute, in addition to their higher affinity to the sodium channel complex, to their greater mammalian toxicity. The rate of metabolism is also influenced by the presence of an α-cyano group, which slows both hydrolysis and oxidation.

Pyrethroids are metabolized rapidly by ester hydrolysis to their acid and alcohol components. This reaction is followed by hydroxylation and conjugation to glucuronides and sulfates, which are excreted in the urine. Generally, the metabolic reactions involved are exclusively detoxification processes, and data available suggest that the major esterase metabolites of pyrethroids lack neurotoxic activity.

Relevant Toxic Effects: From Acute Exposure and Repeated Exposure

The acute toxicity of pyrethroids to mammals varies widely, depending on the structure of the compound and the route of administration. Following acute oral exposure, the toxicity is strongly influenced by the dosing vehicle. Administration in aqueous suspensions gives normally lower acute oral toxicities than administration in vegetable oils, presumably because the low aqueous solubility of pyrethroids limits their bioavailability when administered in aqueous suspensions. With a few exceptions, most pyrethroids that are currently used in agriculture have oral lethal dose, 50% (LD50) values between 50 and 500 mg per kg body weight when administered in vegetable oils to rats and are therefore classified as 'moderately hazardous.' Pyrethroids with oral LD50 values of more than 500 mg per kg body weight, which are, therefore, classified as 'slightly hazardous' or 'unlikely to present acute hazard in normal use,' are mostly type I pyrethroids.

In general, pyrethroids have low acute toxicities following dermal exposure due to their limited absorption through the skin, whereas administration by routes that favor delivery to the CNS (e.g., intravenous injection) enhances the expression of acute toxicity.

Pyrethroids have been classified according to the acute intoxication syndromes they produce in rodents after administration of near-lethal to lethal doses. Signs of intoxication generally fell into one of two categories, which were designated the T-syndrome (tremor) and the CS-syndrome (choreoathetosis and salivation). In general, type II pyrethroids containing an α-cyano group produce the CS-syndrome, whereas type I pyrethroids lacking the α-cyano group tend to produce the T-syndrome. However, exceptions to this classification scheme have been noted because a few compounds produce elements of both syndromes (tremor and salivation).

The clinical effects produced by type I pyrethroids are characterized by aggressive sparring, increased sensitivity to external stimuli, fine tremors progressing to the whole body, spontaneous muscular contractions, hyperthermia, and death. The effects are generated largely by action on the CNS, but there is also evidence for repetitive firing in sensory nerves, which may be the mechanism causing paresthesia and the hyperexcitable state.

Because type II pyrethroids prolong the mean open time of sodium channels compared with type I compounds, they produce a more complex poisoning syndrome that involves salivation, increased extensor tone in the hind limbs, incoordination, tremors progressing to generalized choreoathetosis (writhing spasms), loss of consciousness, tonic seizures, and death. Unlike the type I pyrethroids, type II compounds generally decrease rather than increase the startle response to sound. Also, type II pyrethroids do not typically produce the repetitive activity in sensory nerves seen with type I compounds.

In repeated-exposure toxicity studies, the main target organ of pyrethroids was the nervous system, with clinical signs and neurobehavioral effects similar to those observed in acute toxicity studies. Microscopic lesions in peripheral nerves occurred only at lethal doses. Other findings at the highest dose levels were reduced body weight gain and liver enlargement, but this did not occur with all pyrethroids.

There is no evidence from epidemiological studies that pyrethroids induce cancer in humans. Also, long-term studies in experimental animals have not revealed a distinct carcinogenic hazard relevant to humans, although increased incidences of certain tumors in rodents have been observed occasionally.

Pyrethroids were generally not genotoxic in standard tests in bacteria, yeasts, mammalian cells *in vitro*, and rodents *in vivo*.

Pyrethroids did not impair the mating capacity and fertility in experimental animals. No evidence of teratogenicity was observed in experimental animals, even at doses producing clinical signs of maternal toxicity.

Neonatal rats are approximately 6–17 times more sensitive than adults to acute high-dose toxicity of type I and II pyrethroids, probably due to their incompletely developed detoxification enzyme system of the liver rather than greater sensitivity of the neonatal CNS.

Developmental neurotoxicity studies in mice exposed to pyrethroids during postnatal days 10–16 identified changes in motor activity and muscarinic acetylcholine receptor density in the brain of adult mice. However, a review of 22 developmental neurotoxicity studies in rodents did not reveal a consistent pattern of pyrethroid-induced effects on the CNS of a developing rodent, and further well-designed studies are needed to identify the potential neurotoxicity of pyrethroids after exposure during early development periods.

Human cases of systemic poisoning are rare and usually result from accidental exposure or intentional ingestion of pyrethroids, particularly the more potent type II compounds. Massive ingestion gives rise to sore throat, nausea, vomiting, and abdominal pain within minutes. Further systemic effects occurring 4–48 h after exposure include dizziness; headache; fatigue; increased secretions; and in rare, severe cases convulsions and coma.

Dermal exposure can result in transient paresthesia with many pyrethroids, presumably due to hyperactivity of cutaneous sensory nerve fibers, whereas inhalation exposure can result in upper airway irritation.

Occurrence in Food

The evaluation of active substances by the Joint Food and Agriculture Organization/World Health Organization Meeting on Pesticide Residues includes a dietary risk assessment, based on the evaluation of the toxicological properties and the residue aspects of the compounds. For the assessment of the long-term intake of residues, the International Estimated Daily Intake was calculated for different diets representative for relevant global regions. For the assessment of the short-term

Table 1 Dietary risk assessment for selected pyrethroids

Compound	*Long-term risk assessment*		*Short-term risk assessment*	
	ADI (mg per kg body weight)	*IEDI (% of the maximum ADI)*	*ARfD (mg per kg body weight)*	*IESTI (% of the ARfD)*
Bifenthrin	0–0.01	8–20	0.01	Ch: 0–430 GP: 0–230
Cyfluthrin, β-cyfluthrin	0–0.04	0–2	0.04	Ch: 0–240 GP: 0–120
Cyhalothrin, λ-cyhalothrin	0–0.02	3–10	0.02	Ch: 0–60 GP: 0–40
Cypermethrins (including α- and ζ-cypermthrin)	0–0.02	7–30	0.04	Ch: 0–40 GP: 0–20
Deltamethrin	0–0.01	20–30	0.05	Ch: 0–130 GP: 0–58
Esfenvalerate	0–0.02	50–70	0.02	Ch: 0–10 GP: 0–3
Fenpropathrin	0–0.03	3–80	Not established	Not calculated

Abbreviations: ADI, acceptable daily intake; IEDI, international estimated daily intake; ARfD, acute reference dose; Ch, children 6 years and below; GP, general population.

intake of residues, the International Estimated Short-Term Intake (IESTI) was calculated for relevant food commodities, for which residue and consumption data were available.

In summary, the long-term intake of residues of selected pyrethroids (Table 1) is unlikely to present a public health concern. Also, the short-term intake of residues of most of the selected pyrethroids is unlikely to present a public health concern, with the exception of ifenthrin (IESTI $>100\%$ for strawberries), cyfluthrin (IESTI $>100\%$ for head cabbage and/or broccoli), and deltamethrin (IESTI $>100\%$ for spinach and Chinese cabbage for children). However, higher than 100% should not necessarily be interpreted as giving rise to a health concern because the assessments are based on conservative assumptions and, therefore, refinements of those estimates are possible.

See also: Analytical Methods: Overview of Methods of Analysis for Chemical Hazards. Disciplines Associated with Food Safety: Food Safety Toxicology. Foodborne Diseases: Overview of Chemical, Physical, and Other Significant Hazards. Institutions Involved in Food Safety: FAO/WHO Codex Alimentarius Commission (CAC); Food and Agriculture Organization of the United Nations (FAO); World Health Organization (WHO). Pesticide Residues: Conazoles; Dithiocarbamates; Herbicides; Organochlorines; Organophosphates and Carbamates. Risk Analysis: Risk Analysis of Hazards in Food: An Overview; Risk Assessment: Chemical Hazards; Risk Assessment: Principles, Methods, and Applications; Risk Management: Application to Chemical Hazards

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WHO: Joint FAO/WHO Meeting on Pesticide Residues (JMPR) Publications.

VETERINARY DRUGS RESIDUES

Contents

Veterinary Drugs – General

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Glossary

Acceptable daily intake The amount of total residue that can safely be consumed per day over a human's lifetime. The ADI is calculated by dividing the no observed adverse effect level (from the most appropriate toxicological study) by a safety factor.

Marker residue The residue selected for assay by the regulatory method. In general, the marker residue is a subset of the total residue; for example, the marker residue could be the parent drug, a metabolite, or a combination of residues.

Maximum residue limit The maximum concentration of a marker residue or other residue indicated for monitoring that can legally remain in a specific edible tissue of a treated animal.

No observed adverse effect level The highest dose level of a drug tested that produces no observable effects. It is expressed usually in mg kg^{-1} bw day^{-1}.

Regulatory method The aggregate of all analytical procedures for quantifying and confirming the presence of the marker residue in the target tissue of the target animal.

Target animal The species and animal production class for which the veterinary drug has been authorized.

Target tissue The edible tissue selected to monitor for residues in the target animals.

Total residues The aggregate of all compounds that result from the use of an animal drug, including the drug, its metabolites, and any other substances formed in or on food because of such drug use.

Veterinary drug residue Any compound present in edible tissues of animals that results from the use of a drug and includes the drug, its metabolites, and any other substance formed in or on food because of the drug's use.

Withdrawal period The duration of time following administration of a veterinary drug required to assure that drug residues in animal-derived foods are below a determined maximum residue limit.

Veterinary Drugs and History of Use

Throughout recorded history, humans have endeavored to treat diseases with medicinal substances. The development of drugs for treating animals occurred simultaneously with the development of human drugs, and little distinction was made between human and animal medicine until recent times. As early as 2000 BC, the Egyptians recorded in the Kahun papyrus prescriptions for treating animal diseases and uterine diseases of women. However, it was not until the Twentieth Century that governments began to regulate the manufacturing, labeling, conditions of use, and sale of human and veterinary drugs. The Food and Drug Administration in the United States was the first government agency in the world to attempt broad scientific review of foods and drugs, and in the present day, most developed countries have adopted similar regulatory schemes.

In 1996, the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) was established under the auspices of the World Organisation for Animal Health to harmonize the studies and data requirements for veterinary drug marketing authorization across countries and regions. With regard to food safety, the role of VICH is to harmonize technical requirements but not to assess data or establish any safety standards. That

responsibility rests with Codex Alimentarius Commission, which establishes maximum residue limits (MRLs) for residues from veterinary drugs in foods of animal origin on international level primarily based on risk assessments performed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA).

The basic tenets of veterinary drug regulation dictate that the drug must be safe for the animal, effective for its intended use, and manufactured to a high standard of quality. The demonstration of drug safety, efficacy, and quality is a rigorous and demanding process that relies on data generated through high quality, well-controlled, scientific studies. For veterinary drugs intended for use in food-producing animals, the safety assessment also requires that food from animals treated with the drug will not harm consumers. This article will focus on the food safety evaluation of veterinary drugs used in food-producing animals (risk assessment) and the procedures used to mitigate any potential harm to consumers caused by residues of veterinary drugs in animal-derived foods (risk management).

Drug Use in Food-Producing Animals

The Codex Alimentarius Commission defines a veterinary drug as any substance applied or administered to any food-producing animal such as meat or milk of animals, poultry, fish, or bees, whether used for therapeutic, prophylactic or diagnostic purposes, or for modification of physiological functions or behavior. This broad definition includes drugs for:

- treatment of diseases or other pathological conditions.
- prevention of diseases or other pathological conditions.
- diagnosis of diseases.
- zootechnical purposes such as estrus synchronization, induced ovulation, chemical castration, etc.
- improved production such as increase in growth rate, feed efficiency, milk production, etc.
- behavior modification, such as tranquilizers.

In the European Union, some substances such as anticoccidial compounds administered in animal feed are regulated as feed additives; however, these substances are considered to be veterinary drugs within the Codex Alimentarius Commission.

Veterinary Drug Residues and Food Safety

To protect consumers from adverse health effects caused by foodborne residues of veterinary drugs, developed and many developing countries maintain strict controls on authorization, labeling, and use of veterinary drugs in food-producing animals. National authorities further conduct surveillance programs to detect drug residues in animal tissue at levels greater than the maximum legal limit, i.e., MRLs, and take enforcement actions against those responsible for the violation. The Codex Alimentarius Commission has examined the most commonly used veterinary drugs and established appropriate MRLs for meat, poultry, fish, milk, eggs, and honey. These international standards not only assist government authorities in protecting their domestic food supply but also greatly facilitate international trade in animal-derived foods.

Dietary exposure to veterinary drug residues can cause acute or chronic adverse health consequences depending on the drug and dose. Acute responses can result from anaphylactic or pharmacologic reactions; however, these appear to be rare events. Anaphylactic reactions have been reported in previously sensitized individuals following consumption of milk or meat containing residues of penicillin. Outbreaks of food poisoning in several countries have been caused by consumption of liver from cattle and pigs treated illegally with the beta agonist drug clenbuterol to increase growth rate. Symptoms included palpitations, nausea, vomiting, dizziness, chest tightness, uneasiness, trembling, and instability.

Of greater concern are the adverse health consequences caused by chronic dietary exposure to drug residues at subacute doses. In authorizing the marketing and use of veterinary drugs, regulatory bodies establish the conditions of use, often called good veterinary practice, to ensure that any residues in animal-derived foods will not cause harmful effects when consumed over a lifetime. Hazard identification and characterization for veterinary drug residues are essentially the same as for food additives, and pesticides use the same methods and evaluation criteria. However, the safety assessment is made more complicated by the pharmacokinetics of the drug in the target animal to which the drug was administered. Drugs administered orally may be completely, partially, or not absorbed at all. Following absorption, drugs are distributed to the various tissues and organs. Lipophilic drugs will concentrate in tissues and fluids with high fat content, such as muscle and milk. Hydrophilic drugs tend to accumulate in highly perfused tissues such as the kidneys. Many drugs bind to plasma proteins thus restricting their distribution to the intravascular space. Drugs are metabolized to various degrees depending on their chemical structure and the capacity of the target animal species to transform the drug. Finally, drugs and their metabolites are eliminated by the animal through various routes. The most important routes are renal and hepatic, but lipophilic drugs tend to be excreted in milk as fat deposits are mobilized during lactation. All these factors affect the concentration of residues in animal-derived foods and must be taken into account in calculating the MRL and withdrawal period.

Toxicological Testing

Toxicological testing for veterinary drugs is based on the same battery of tests used in evaluating food additives and pesticides. Testing should include an assessment of systemic toxicity, reproduction toxicity, developmental toxicity, genotoxicity, carcinogenicity, and effects on the human intestinal flora. In general, oral administration is the route of choice for *in vivo* tests.

Systemic Toxicity Testing

Repeat-dose toxicity testing should be performed to define:

1. Toxic effects based on repeated and/or cumulative exposures to the compound and/or its metabolites,

2. Incidence and severity of the effect in relation to dose and/or duration of exposure,
3. Doses associated with toxic and biological responses, and
4. A dose with no observed toxic or biological response.

Reproduction Toxicity Testing

Multigeneration reproduction studies are designed to detect any effect on mammalian reproduction. These include effects on male and female fertility, mating, conception, implantation, ability to maintain pregnancy to term, parturition, lactation, survival, growth and development of the offspring from birth through to weaning, sexual maturity, and the subsequent reproductive function of the offspring as adults.

Developmental Toxicity Testing

Developmental toxicity testing is used to detect any adverse effects on the pregnant female and development of the embryo and fetus consequent to exposure of the female from implantation through the entire period of gestation to the day before cesarean section. Such adverse effects include enhanced toxicity relative to that observed in nonpregnant females, embryo-fetal death, altered fetal growth, and structural changes to the fetus.

Genotoxicity Testing

A battery of genotoxicity tests are used to identify substances that have the capacity to damage the genetic information within cells. Substances that are considered to be genotoxic should be regarded as potential carcinogens. Those that cause genetic damage in germ cells also have the potential to cause reproductive/developmental effects.

Carcinogenicity Testing

Compounds that are suspected to have carcinogenic potential are tested by the oral route for carcinogenicity. The decision to conduct carcinogenicity testing is based on all available data including results of genotoxicity testing, structure activity relationship information, and results of repeat-dose and mechanistic studies. Carcinogenicity testing is performed using a carcinogenicity bioassay. However, information derived from a combined assay for carcinogenicity and chronic toxicity may also be acceptable.

Testing for Effects on the Human Intestinal Flora

For compounds with antibacterial properties, testing is conducted to determine the effects of residues of the drug on the human intestinal flora.

ADI Determination

The toxicological tests are designed to determine the dose at which the compound produces an adverse effect and a dose which produces no observed adverse effect. If the drug is not a carcinogen, the no observed adverse effect level of the most sensitive effect in the most sensitive species is divided by a safety factor (usually 100) to calculate the acceptable daily intake (ADI) for drug residues.

MRL Determination

To assess exposure to veterinary drug residues from the diet, it is necessary to determine the daily consumption of animal-derived foods by the general population. Table 1 lists the consumption values for a 60 kg adult that the JECFA has established to calculate MRLs. This model diet is intended to provide a conservative estimate of dietary exposure.

The safe concentration is the maximum concentration of total residues (parent drug and metabolites) in each edible tissue that when consumed in the amounts, listed in Table 1, will not exceed the ADI.

$$\text{Safe concentration} = \frac{\text{ADI } \mu\text{g kg}^{-1} \text{ daily} \times 60 \text{ kg}^{-1}}{\text{grams consumed day}^{-1}} \quad [1]$$

Because it is not practical for regulatory purposes to monitor total residues in edible tissues, a single analyte, designated as the marker residue, is selected whose concentration is in a known relationship to the concentration of the total residue in the last tissue (target tissue) to deplete to its safe concentration. The target tissue and marker residue are selected so that the absence of marker residue above a designated concentration (MRL) will confirm that each edible tissue has a concentration of total residue at or below its safe concentration.

$$\text{MRL} = \text{Safe concentration } \mu\text{g g}^{-1} \frac{\text{Marker residue } \mu\text{g g}^{-1}}{\text{Total residue } \mu\text{g g}^{-1}} \quad [2]$$

In the United States, MRLs are considered to be food safety standards because they are derived from the ADI; however, the Codex Alimentarius Commission, EU, and other countries perform a second calculation based on good veterinary practice. If the levels of residues estimated from supervised trials, when the drug is administered according to good veterinary practice, are below the MRL as calculated in eqn [2], then the levels determined by good veterinary practice will dictate the

Table 1 Daily consumption values

Edible product	*Grams consumed*
Muscle	300
Liver	100
Kidney	50
Fat	50
Eggs	100
Milk	1500
Honey	50

MRL, provided that practical analytical methods are available for routine residue analysis. Finding residues above MRLs based on good veterinary practice indicates that the veterinary drug has been misused but does not necessarily imply a food safety risk.

Establishing Withdrawal Periods

The withdrawal period is a risk management tool designed to ensure that veterinary drug residues will deplete to levels at or below the MRL when the drug is used in accordance with good veterinary practice.

Establishing a withdrawal period requires data collected from residue depletion studies in the target animal population. The studies are designed to simulate the conditions of use paying particular attention to the proposed dosing regimen, normal husbandry conditions, animal gender, and animal maturity. Animal treatment should be consistent with the intended product label including, for injectable products, the location and injection method. The highest intended treatment dose is administered for the maximum intended duration. Tissues for residue analysis are collected from animals as a function of time after the final treatment.

For residue studies in tissues, marker residue data are obtained from the target tissue of at least 16 animals with four animals being slaughtered at each of four evenly distributed time points. For residue studies in milk, sufficient data are provided by at least 20 animals with milk collected from all animals at evenly spaced time points.

Residue concentrations are transformed logarithmically, and their depletion is plotted over time followed by linear regression analysis. The withdrawal period is the point on the regression line in which the marker residue is at or below the MRL in 99% of the target animal population with a confidence level of 95%. This practically assures that food from an animal treated with a drug in accordance with good veterinary practice will not contain residues above the MRL. The combination of MRLs, withdrawal periods, and good veterinary practice is essential to maintaining a safe food supply.

Risk Management at the International Level

Codex established the Codex Committee on Residues of Veterinary Drugs in Food in 1986. Since that time, Codex has adopted 590 MRLs for some 59 veterinary drugs. Most countries use Codex MRLs as a basis for establishing their national regulations for veterinary drug use. Although, harmonization has been achieved for a wide variety of veterinary drugs, differences in food safety policies among countries have resulted in difference in MRLs, permitted uses, and marketing authorizations, some of which have disrupted trade, such as the dispute between the USA and EU on the use of hormones in beef cattle. Another contentious trade issues is the use of ractopamine in finishing swine.

See also: Institutions Involved in Food Safety: FAO/WHO Codex Alimentarius Commission (CAC); Food and Agriculture Organization of the United Nations (FAO); World Health Organization (WHO). Public Health Measures: International Standards and Harmonization of Food Safety Legislation. Risk Analysis: Risk Assessment: Chemical Hazards. Veterinary Drugs Residues: Anabolics; Anthelmintics; Antibacterials; Coccidiostats; Control of Helminths; Ectoparasiticides

Further Reading

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VETERINARY DRUGS RESIDUES

Antibacterials

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Glossary

Actinomycete A group of Gram-positive bacteria with high guanine and cytosine content in their DNA.
Efflux pumps Proteinaceous transporters localized in the cytoplasmic membrane of all bacterial cell types that can contribute to antimicrobial resistance. Transmembrane spanning proteins that vary in structural complexity.
Metaphylaxis The administration of drugs to herds of animals as therapy for sick animals combined with prophylaxis in healthy animals.
Mobile genetic element (MGE) MGEs include plasmids and conjugative transposons and these DNA structures can contain one or more antimicrobial resistance-encoding gene(s).
Prophylaxis The administration of drugs to herds of animals to prevent the emergence of disease.
Transposons DNA sequences that move from one location on the genome to another and are frequently called 'jumping genes.'
Zoonosis Any infectious disease that can be transmitted between species from animals to humans or from humans to animals (the latter is sometimes called reverse zoonosis or anthroponosis).

Use of Antibacterial Compounds in Food-Producing Animals

Food-Producing Animals

Traditionally, the important food-producing animal species include cattle, pigs, and poultry. Other minor species are sheep, ducks, goats, llamas/alpacas/camels, deer, rabbits, and some wild game. To assure the long-term care, health, and welfare of food-producing animal species, antibacterial compounds are used for treatment. These same drugs can also be given prophylactically to prevent infectious diseases in both domestic and food-producing animals, including fish. On occasion, certain antibacterial classes can be used as growth promoters to improve feed conversion efficiency when poultry, pigs, and feedlot cattle are being intensively reared. In such cases, antibacterial drugs are administered to all animals in the group, flock, or herd, via the animals' feed or drinking water.

Antibacterial Drugs Licensed for Use in Food-Producing Animals

In general, antibacterial compounds are used for three purposes in animals: therapeutic use to treat sick animals; prophylactic use to prevent infection in flocks/herds of food-producing animals; and as growth promoters to improve feed utilization and reduce production time. In 2011, Bush and coworkers indicated that more than 50% of the antibiotics produced are included as components in animal feeds to promote growth. This practice has been ongoing since the 1950s, despite efforts to prevent such use by several agencies including the World Health Organization (WHO) and the European Union (EU).

Europe has adopted a proactive stance on the use of antimicrobials. On 1 July 1999, the EU banned the use of most antimicrobial agents for growth promotion in an effort to preserve the efficacy of important human drug classes. The EU also established the European Antimicrobial Resistance Surveillance System to monitor levels of resistance in important human pathogens. On 1 January 2006, the EU banned the feeding of all antimicrobials and related drugs to food-producing animals for growth-promoting purposes. Currently, in Europe, no antimicrobial agents can be used in farm animals for growth promotion purposes. In 2004, the US Food and Drug Administration (USFDA) banned enrofloxacin (a fluoroquinolone (FQ) prescribed for therapeutic purposes and not growth promotion) in chickens and turkeys on the grounds that its use contributed to the increase in FQ resistance, being detected in bacterial pathogens isolated from humans. Subsequently, this move was followed in April 2012 by an order that prohibits certain uses of the cephalosporin class of antimicrobial drugs in cattle, pigs, chickens, and turkeys, in an effort to preserve the efficacy of cephalosporins for the treatment of infections in humans. Table 1 provides a list of the 16 antimicrobial classes along with specific drugs in each class that are available as commercial preparations for use in food-producing animals. The agents listed in Table 1 are approved by the USFDA. Agents approved for use in other countries vary greatly depending on national regulations.

Table 1 Commercially available antimicrobial classes and compounds approved by the US Food and Drug Administration, for use in food-producing animals

Aminocoumarins
Novobiocin
Aminoglycosides
Apramycin
Gentamicin
Neomycin
Spectinomycin
Amphenicols
Florfenicol
Diaminopyrimidines
Ormetoprim
Fluoroquinolones
Danofloxacin
Enrofloxacin
Glycolipids
Bambermycins
Ionophores
Laidlomycin
Lasalocid
Monensin
Narasin
Salinomycin
Semduramicin
Lincosamides
Lincomycin
Pirlimycin
Tetracyclines
Chlortetracycline
Oxytetracycline
Tetracycline
Macrolides
Carbomycin
Erythromycin
Oleandomycin
Tilmicosin
Tulathromycin
Tylosin
Penicillins
Amoxicillin
Ampicillin
Cloxacillin
Penicillin
Tiamulin
Pleuromutilins
Bacitracin
Polymixin B
Polypeptides
Carbadox
Quinoxalines
Virginiamycin
Streptogramins
Sulfachloropyridazine
Sulfadiazine
Sulfonamides
Sulfadimethoxine
Sulfamerazine
Sulfamethazine
Sulfaquinoxaline

Discovery of Antibacterial Drug Classes, Their Mode of Action, and Use in Food-Animal Production

Discovery of Antibacterial Drugs

The origin of antimicrobial agent discovery can be traced to 1929 when Alexander Fleming recognized the antibacterial activity of a substance secreted by *Penicillium notatum* on a contaminated culture plate. Subsequently, the first synthetic chemotherapeutic agents, the sulfonamides, were developed by Gerhard Domagk in 1935. Following these events, Selman Waksman announced the discovery of a new antibiotic, streptomycin, which was produced by the actinomycete, *Streptomyces griseus*. Over the following decade, microorganisms producing chloramphenicol, neomycin, and tetracyclines were isolated and studied. By the 1970s, it was widely accepted that antibacterial therapy signaled the end of bacterial infections as a significant cause of mortality in human and animal populations. However, this misguided optimism was dispelled by the emergence of antimicrobial resistance, resulting from the widespread use of these therapeutic agents. Veterinary public health professionals are now confronted by resistant bacterial pathogens and the transfer of these resistant microbes, or resistance elements contained therein, from animals to humans. The latter could be considered as a modern zoonosis.

Modes of Action of Antibacterial Compounds

Antibacterial compounds used in the treatment of bacterial infections fall into relatively few groups or families according to their principal mechanism of action. There are five recognized modes of action that can be outlined briefly, as follows:

Inhibition of Cell Wall Synthesis

Because peptidoglycan is a unique component of bacterial cell walls, antibacterial agents that prevent the cross-linking of peptidoglycan chains inhibit cell wall synthesis and are considered to be selectively toxic for bacteria. β-Lactams are a class of antibacterial agents that can target bacterial cell wall synthesis, and these compounds specifically act on the transpeptidation step during the synthesis of peptidoglycan. These β-lactam antibiotics bind cell receptors known as penicillin binding proteins, thereby inhibiting their activity. In addition to interfering with transpeptidation, many of these drugs promote autolysin activity causing cell lysis. The penicillins and cephalosporins comprise the largest and most important class of antibacterial drugs which inhibit the cell wall synthesis. Bacitracin and glycopeptides also interfere with cell wall synthesis by preventing the cross-linking steps required during this process.

Disruption of Cell Membrane Structure

Comparatively few antibacterial drugs act on the bacterial cell membrane. As antibacterial agents of this type are more toxic for animal cells when compared to other classes, their use is generally limited to topical application(s). Polypeptides represent one group that interfere with the normal functioning of the bacterial cell membrane.

Inhibition of Nucleic Acid Synthesis

Quinolone and FQ antimicrobial agents act by binding to bacterial topoisomerases and interfering with DNA replication and supercoiling of the chromosomal structure. Rifamycins target a subunit of the RNA polymerase enzyme and inhibit bacterial DNA-dependent RNA synthesis.

Inhibition of Protein Synthesis

Protein synthesis is a process targeted by a number of different classes of antibacterial agents. The selective toxicity of some relates to the key differences in structure between prokaryotic and eukaryotic ribosomes. This mode of action is characteristic of aminoglycosides, aminocyclitols, amphenicols, macrolides, lincosamides, streptogramins, and tetracyclines.

Inhibition of a Unique Bacterial Metabolic Pathway

Sulfonamides and trimethoprim target the folic acid biochemical pathway of bacteria. These antibacterial compounds are termed folic acid pathway inhibitors. Sulfonamides interfere with the formation of folic acid, an essential precursor for nucleic acid synthesis. The synthetic pyrimidine derivative, trimethoprim, inhibits the activity of dihydrofolate reductase, a later step in this anabolic pathway. When used in combination, the action of each agent is potentiated resulting in enhanced activity against bacteria. Potentiated sulfonamides are selectively toxic for bacteria because animals can absorb preformed folic acid from their feed. Figure 1 provides a brief schematic summary of the different classes of antibacterial drugs and their corresponding target sites within the typical bacterial cell.

Use of Antibacterial Drugs in Food-Animal Production

Antibacterial drugs are used in food-producing animals primarily to control infectious diseases caused by various pathogenic bacteria. Therapeutic use generally involves the treatment of sick animals on an individual basis, but on occasion it can require the use of medicated feed or water to treat a group, herd, or flock of animals.

It is not uncommon for veterinarians to administer antibacterial drugs to animals which are not showing any clinical signs of infection, but which are at high risk of becoming infected. Herds or flocks of food-producing animals may be given antibacterial drugs if there is a risk of an outbreak of infectious disease due to exposure to a particular disease agent and/or when unfavorable host or environmental conditions arise. Application of antibacterials to prevent infection is referred to as prophylaxis, and in food-animal production, this is a common practice during stressful periods of an animal's life such as following transport of (beef) animals to feedlots or at the weaning stage of production for pigs. Prophylactic or metaphylactic use of antibacterials can aid in the control and prevention of animal diseases in both food and companion animals. However, use of antibacterials should never be intended to replace the requirement for good management practices, given that their use may eventually lead to resistance.

Importantly, veterinary public health professional bodies are now developing and adopting guidelines and codes of practice for the prudent use of antibacterial compounds. These guidelines highlight the need for proper laboratory confirmed diagnosis, specific therapy, and judicious use of these chemotherapeutic agents for a defined period.

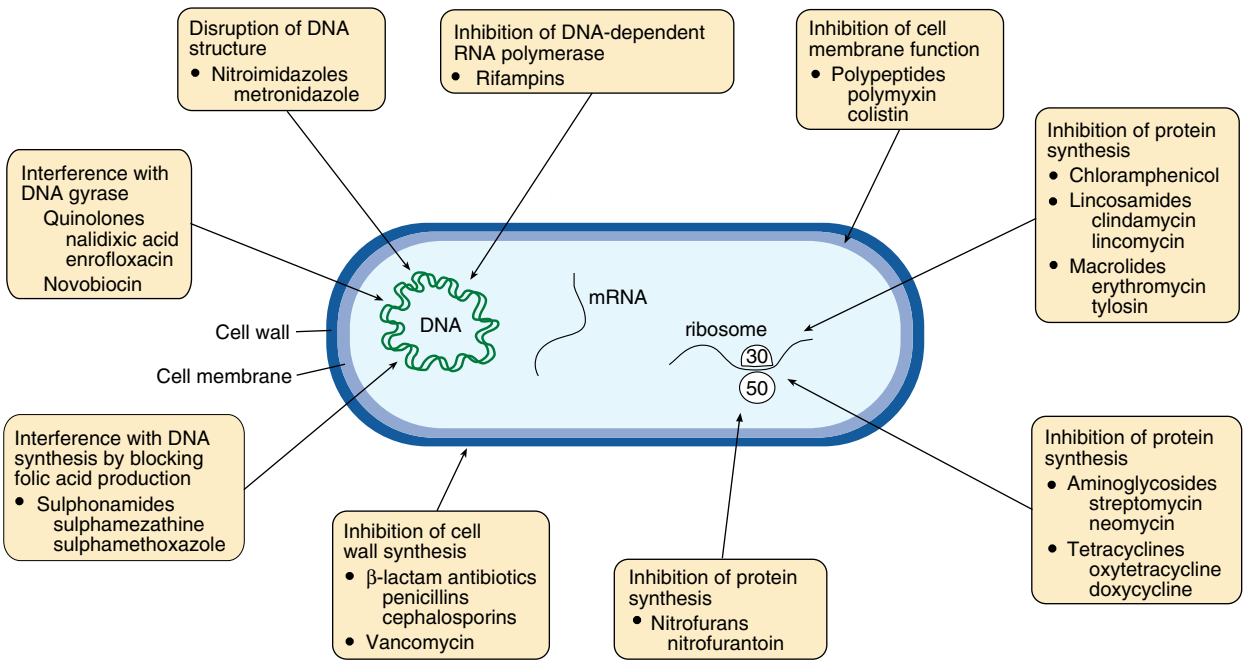

Figure 1 Antibacterial agents and their corresponding targets within the bacterial cell. Reproduced with permission from Quinn PJ, Markey BK, Leonard FC, *et al.* (2011) *Veterinary Microbiology and Microbial Diseases*. Oxford: Wiley-Blackwell Publishers.

Antibacterial Resistance and Public Health

Linking the Use of Antibacterials in Food-Animal Production and Public Health

Several different classes of antibacterials are now recognized (Table 1) and many of these are used in both animal and human medicine. Bacteria of food-animal origin elaborating a resistance phenotype to a particular drug class, could compromise chemotherapeutic treatment in a human when either the same drug or a different member of this drug class is used. Table 2 identifies the major classes of antimicrobial agents used in both animal and human medicine.

Furthermore, bacteria of animal origin can act as a reservoir of resistance genes, which are frequently carried on mobile genetic elements (MGEs) and these MGEs can be transmitted to other animal and human pathogens alike. In a recent study on bovine *E. coli* isolates recovered from Irish farms, resistance to ampicillin, neomycin, streptomycin, sulfonamide, and tetracycline was associated with integrons and MGEs, such as transposons and conjugative plasmids, which highlight the importance of the commensal microflora of food-producing animals as a reservoir of transferable multidrug resistance. It has been suggested that those antibacterials that are important in human medicine should not be used therapeutically in food-producing animals, particularly for mass medication.

Antibacterial Resistance Mechanisms

Four mechanisms account for bacterial resistance to antibacterials. These include the following:

1. Enzymatic degradation of the drug (often referred to as drug inactivation).
2. Structural modification of the drug target (drug-target modification).
3. Reduced permeability of the drug.
4. Active export of the drug (reduced intracellular accumulation associated with reduced membrane permeability or increased drug efflux).

Bacterial resistance can be defined in broad terms, as either innate (intrinsic) or acquired (extrinsic). Innate resistance relates to the general physiology of a bacterium arising from its phenotype and can include the complexity of the bacterial cell wall structure, activity of a bacterium's efflux pumps, or enzymatic inactivation of an antibacterial agent. In *Salmonella* one of the main mechanisms of antimicrobial resistance is the overexpression of efflux pumps. Chemically modulating the activity of efflux pumps may present a new therapeutic approach to target resistant bacteria, through the use of efflux pump inhibitors in combination with the antibacterials to which the microorganism is already resistant. In contrast, acquired resistance can arise from a mutation in a resident gene or the acquisition of exogenous genetic material encoding resistance genes via conjugation; transformation, and/or transduction. These mechanisms involve different MGEs including conjugative plasmids and transposons; the uptake of naked DNA and bacteriophages carrying resistance genes. The same class of antimicrobial-resistant genes or closely related genes can often be identified in different bacterial species, pointing to the fact that horizontal gene transfer occurs between bacteria of the same genus and between unrelated genera. Thus, resistance genes have the potential to spread widely among both pathogenic and commensal bacteria, alike. Figure 2 provides a schematic summary of the general mechanisms involved in the development of antimicrobial resistance. Each of these resistance mechanisms has one or more genetic components and these genes may be chromosomally located or carried on an MGE.

Cross-Resistance

This occurs when a single resistance mechanism confers resistance to more than one member of an antimicrobial class. In some cases where compounds have overlapping targets in the organism, resistance to chemically unrelated agents can occur. For example, if resistance to any of the three antimicrobial classes, macrolides, the lincosamides, or streptogramin B is present in an organism, the use of one of these antimicrobials will result in the selection and maintenance of resistance to all three antimicrobial classes (cross-selection). This is because all three of these classes have target sites on the bacterial ribosome and a change in the structure of ribosomal RNA results in cross-resistance to all three classes of the drug.

Table 2 Major classes of antimicrobials shared by food-producing animals and humans

Class of antimicrobial	*Selected examples of agents within each class*
β–lactams	Penicillin, amoxicillin, and ceftiofur
Macrolides and lincosamides	Tylosin[a] and tilmicosin[a], tulathromycin[a], and lincomycin
Aminoglycosides	Gentamicin and neomycin
Colistin	Colistin sulfate and colistimethate sodium
Fluoroquinolones	Enrofloxacin[a], danofloxacin[a], ciprofloxacin
Tetracyclines	Tetracycline; oxytetracycline, and chlortetracycline
Sulfonamides	Sulfamethoxazole and sulfadiazine
Streptogramins	Quinupristin/dalfopristin[b] and virginiamycin[a]
Polypeptides	Bacitracin
Phenicols	Florfenicol[a]
Pleuromutilin	Tiamulin[a] and retapamulin[b]

[a]Used in animals only.
[b]Used in humans only.

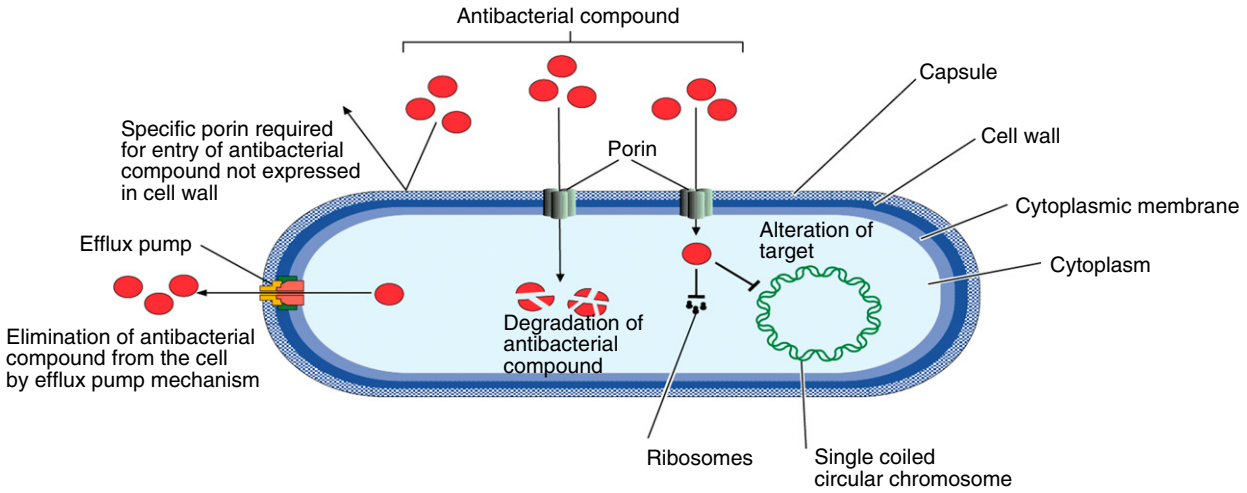

Figure 2 The general mechanisms involved in the development of antimicrobial resistance. Reproduced with permission from Quinn PJ, Markey BK, Leonard FC, *et al.* (2011) *Veterinary Microbiology and Microbial Diseases*. Oxford: Wiley-Blackwell Publishers.

Coresistance

This occurs when two or more resistance genes are physically linked in the same bacterium and each confers resistance to members of separate antimicrobial classes. An example is the resistance elements in *Salmonella* Typhimurium DT104 that confer resistance to ampicillin, chloramphenicol, streptomycin, sulfonamide, and tetracycline (ACSSuT phenotype). Use of any of these drugs will select for the retention of resistance to the other antimicrobials also. The above concepts help to explain some of the resistance problems which arise in human medicine through the use of antimicrobial agents in animals, even when these agents are not used in clinical therapy.

Resistance Linked to the Use of Antibacterials in Agriculture and Overlaps with Public Health

Resistance to antimicrobials associated with their use in animals is an issue of concern to all health care professionals. Much of this relates to the potential for transfer of antimicrobial-resistant pathogens via the food chain along with the risk of transfer of antimicrobial resistance-encoding genes from animal enteric flora to human pathogens. Consequently, it is not surprising that reports are now describing a reduction in the efficacy of antibacterial therapy in animals colonized with resistant bacteria. For several decades, many scientific studies have commented on the fact that use of antibacterials in food-producing animals can be linked to the emergence of resistant bacterial infections in humans. In 2000, a WHO report on infectious disease stated 'Since the discovery of the growth-promoting and disease-fighting capabilities of antibiotics, farmers, fish-farmers, and livestock producers have used antibiotics in everything from apples to aquaculture. This ongoing and often low-level dosing for growth and prophylaxis inevitably results in the development of resistance in bacteria in or near livestock, and also heightens fear of new resistant strains jumping between species.'

There is heightened public health concern about the increase in antimicrobial resistance, not least because studies have begun to report new multidrug-resistant strains of bacteria that have not in the past been associated with food or food-producing animals. An example of this is the emergence of livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA). This bacterium represents a new health threat for persons in the pig/livestock industries. Although the risk of foodborne infection with LA-MRSA appears to be extremely low, this superbug can be transmitted to humans in direct contact with animals and consequently, MRSA can no longer be considered just a hospital problem. Similarly, public health concerns have also arisen due to an increase in the reporting of foodborne zoonotic bacteria associated with resistance to extended spectrum β-lactam antibiotics including organisms expressing AmpC enzymes. In particular these resistance markers have been associated with plasmids that can be transferred between strains of *E. coli* and *Salmonella* species.

Residues of Antibacterial Drugs in Food Matrices

When administered to food-producing animals and others, veterinary drugs can be metabolized. Breakdown products or residues of these drugs can include the unaltered parent compound itself and/or its metabolites and these can be detected in edible portions of an animal food product. Residues may also include associated excipients contained in a veterinary drug preparation. Concerns about antibacterial residues in foods of animal origin center around human health and economic effects. Food-containing drug residues, when consumed, may elicit a severe allergic reaction in sensitive individuals. In addition, the presence of residues may lead to changes in the human intestinal microflora and act to select for resistance.

Residues of antimicrobial compounds in foods of animal origin could also negatively affect some food-processing procedures. As an example, these chemical contaminants could inhibit the activity of starter cultures used in the production of

certain fermented meats, yogurt, and cheeses, leading to economic losses for food producers. Furthermore, if consumers lose confidence in the safety of the food supply, arising from the threat of resistant bacteria or drug residues, it can be expected that they will reduce their consumption, a feature which would result in economic losses to the broader food industry.

Control of Residues

As a means of controlling residues in food of animal origin, an essential step would be to educate veterinary professionals and others through suitable programs. Steps should be taken to encourage the veterinarian and livestock producer to eliminate the nontherapeutic use of antimicrobial compounds in food production as has been undertaken in Denmark.

It would be beneficial to establish surveillance systems to monitor antimicrobial resistance in human and food-animal populations, antimicrobial drug consumption in food animals in addition to suitable simple and robust field tests for the identification of drug residues in edible animal products. Maximum residue limit (MRL) is defined as the highest concentration of a contaminating metabolite resulting from the use of a veterinary medicinal product which may be legally permitted or recognized as acceptable in or on a food. In Europe, tests are regularly performed to detect the presence of antibacterial residues in food. Before the animal or animal products can be sold for slaughter/consumption, a withholding period must be observed which is of sufficient duration to allow for any residues to be eliminated, such that antibacterial residues are no longer detected at concentrations above the MRL. These time periods are mandated in order to reduce the exposure for humans to residues of veterinary medicinal compounds in foods of animal origin. In addition, many professional veterinary bodies worldwide are examining ways to reduce the overuse and misuse of antibacterials in food-producing animals and develop novel nonpharmacological-based preventive strategies, including good hygiene measures.

Conclusion

Antimicrobial-resistant pathogens are a major challenge for animal and human health alike. Transmission of antimicrobial-resistant bacteria or their corresponding genes from food-producing animals to humans must be controlled. Efforts to promote the judicious use of these important chemotherapeutic agents in animals should be encouraged. A collaborative engagement, involving all stakeholders along the farm-to-fork continuum would serve as a first step toward reducing the use of antimicrobial compounds in agriculture along with seeking to educate the public and health care professionals on the prudent use of antibacterial drugs. The latter step will become even more important in the future, if we are to attempt to preserve the efficacy of existing compounds, and ensure the welfare of animals and the safety of animal food products for the benefit of public health.

See also: Characteristics of Foodborne Hazard and Diseases: Drug Resistant Pathogens. Disciplines Associated with Food Safety: Food Microbiology. Foodborne Diseases: Overview of Biological Hazards and Foodborne Diseases; Overview of Chemical, Physical, and Other Significant Hazards. Risk Analysis: Food Safety Training and Health Education: Principles and Methods. Safety of Food and Beverages: Meat and Meat Products. Veterinary Drugs Residues: Veterinary Drugs – General

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VETERINARY DRUGS RESIDUES

Anthelmintics

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Glossary

Anthelmintic Any drug that destroys or causes the expulsion of parasitic intestinal worms.
Chromatography Physical method of separation in which the components to be separated are distributed between two phases, one of which is stationary (stationary phase) whereas the other (the mobile phase) moves in a definite direction.
Helminth Parasitic worm found in the intestines of vertebrates, especially roundworms, tapeworms, and flukes.
Liquid chromatography A separation technique in which the mobile phase is liquid.
Mass spectrometry The art of measuring atoms and molecules to determine their molecular weight.
Maximum residue limit Maximum concentration of residue established by a standard-setting body in a food product obtained from an animal that has received a veterinary medicine or has been exposed to a biocidal product for use in animal husbandry.
Metabolite Any intermediate or product resulting by metabolism or by a metabolic process.
Nematode Unsegmented worms of the phylum Nematoda with elongated rounded body, pointed at both ends.
Pharmacokinetics A branch of pharmacology dedicated to the study of the time course of drug absorption, distribution, metabolism, and excretion.
Transformation products Chemical species resulting from environmental or metabolic processes.

Introduction

There are several types of veterinary drugs that can be applied to livestock, such as aminoglycosides, macrolides, tetracyclines, β-agonists, and anthelmintics. Among these, anthelmintics (also called parasiticides, endectocides, and nematocides), are usually used to treat parasitic worms infections, including flatworms (tapeworms and flukes) and roundworms (nematodes), which usually infect human, livestock, and crops, affecting food production. For instance, gastrointestinal nematodes are parasites that cause important economic losses to livestock worldwide due to reduced appetite, lower body weights, reduced egg production (poultry), and death.

To combat helminthiasis, several approaches have been proposed including regulation of parasitic vectors in populations, zootechnical strategies, and breeding of resistant animals. However, treatment with anthelmintics is the option most widely used as vaccines have proven ineffective till date. Before 1940s, only natural compounds including arecoline, lead arsenate, nicotine, and carbon tetrachloride were applied, but they are also toxic to the host. In 1960s and 1970s, organophosphate anthelmintics were introduced, although some of them have been removed from the market due to their toxicity.

In general, broad-spectrum anthelmintics are effective, although most of them are usually used for specific infections. In fact, the term anthelmintic refers to the spectrum of pharmaceutical activity and not to a common chemical substructure of these drugs. Furthermore, the efficacy of these products depends on the pharmacokinetics in the host, including the complex interaction between formulation and route of administration, physicochemical properties of the compound and the metabolites generated.

New compounds have been developed to overcome drug resistance to conventional anthelmintics caused by direct exposure of helminths to the drug at therapeutic doses.

Moreover, the presence of anthelmintic residues in livestock may have serious consequences on consumers, and international organizations and governments have established maximum residue limits (MRLs) of these compounds in several matrices, in order to assure food safety. The unauthorized or incorrect use of anthelmintics can result in the introduction of harmful residues into the food chain. As a consequence, the development of sensitive analytical methods that fulfill the requirements established by governments and international standard-setting bodies is needed. For that purpose, advanced analytical methods mainly based on liquid chromatography (LC) coupled to mass spectrometry (MS) have been widely used for the determination of anthelmintic residues in edible tissues, in order to assure food safety as well as to increase the knowledge of the pharmacokinetics of these compounds.

Types of Anthelmintics

Anthelmintics are usually classified into several types on the basis of similar chemical structure and mode of action. Basically, three main families can be distinguished: benzimidazoles, nicotinic receptor agonists, and macrocyclic lactones.

Most members within each group have similar effects, although they are small chemical differences.

Benzimidazoles

They are considered as the first chemical class of modern anthelmintics. This group includes benzimidazole carbamates, thiabendazole analogs, triclabendazole and prodrug netobimin, and a phenylguanidine derivative which is rapidly converted into albendazole *in vivo*. Thiabendazole was first discovered in 1961, and subsequently other benzimidazoles were introduced as broad-spectrum anthelmintics such as albendazole, fenbendazole, flubendazole, mebendazole, oxfendazole, and triclabendazole.

It can be observed in Figure 1, that they have a bicyclic ring system in their structures in which benzene is fused to the 4 and 5 positions of the heterocycle (imidazole).

They are the largest chemical family to treat endoparasitic diseases in domestic animals and they are widely used for prevention and treatment of parasitic infections (i.e., nematodes) in aquaculture and livestock with an excellent nematocidal activity. Furthermore, some of them have been used as pre- or postharvest fungicides. In general they interfere with the worm's energy metabolism at the cellular level. Thus, they bind to a specific building block, β-tubulin, preventing its incorporation into certain cellular structures.

Nicotinic Receptor Agonists

This class of anthelmintics is formed by several classes of compounds with different chemical structures as shown in Figure 1. Thus, they include imidazothiazoles, such as levamisole, and tetrahydropyrimidines, such as pyrantel and morantel. They cause spastic muscle paralysis of the worm due to prolonged activation of the excitatory nicotinic acetylcholine receptors on body wall muscle. This type of compound only affects adult and larval population worms, whereas benzimidazoles are also able to kill worm eggs.

Macrocyclic Lactones (Avermectins and Milbemycins)

Macrocyclic lactones are considered as the last 'traditional' class of anthelmintics introduced consisting of two closely related chemical groups: avermectins, which include abamectin, doramectin, ivermectin, emamectin, eprinomectin, and selamectin; and milbemycins (also called nemodectins), with moxidectin being the most representative compound. The structure of these type of compounds are shown in Figure 2, and it can be observed that avermectins are complex macrocyclics containing a 16-membered ring, linked to a disaccharide, showing different polarity, whereas, milbemycins do not have saccharide substituents.

Ivermectin was the first macrocyclic lactone introduced as a anthelmintic by Merck in the 1980s. It is a semisynthetic derivative of avermectin, which is a large macrocyclic lactone fermentation product of *Streptomyces avermitilis*. It was the first drug to kill migrating larval stages of worms as well as adults, and therefore, other companies developed analogs such as doramectin, selamectin, etc.

In general, each drug has two homologs, with the major component comprising more than 80%, which is usually used

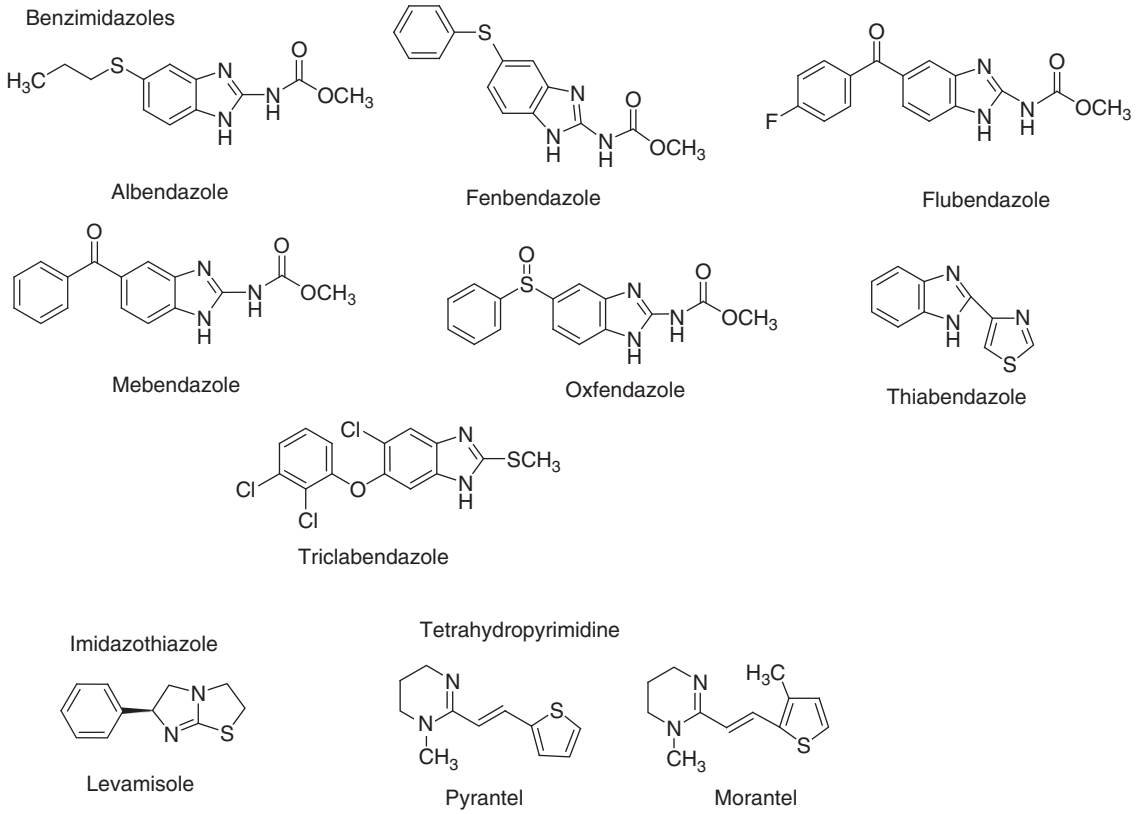

Figure 1 Chemical structure of benzimidazoles and nicotinic receptor agonists.

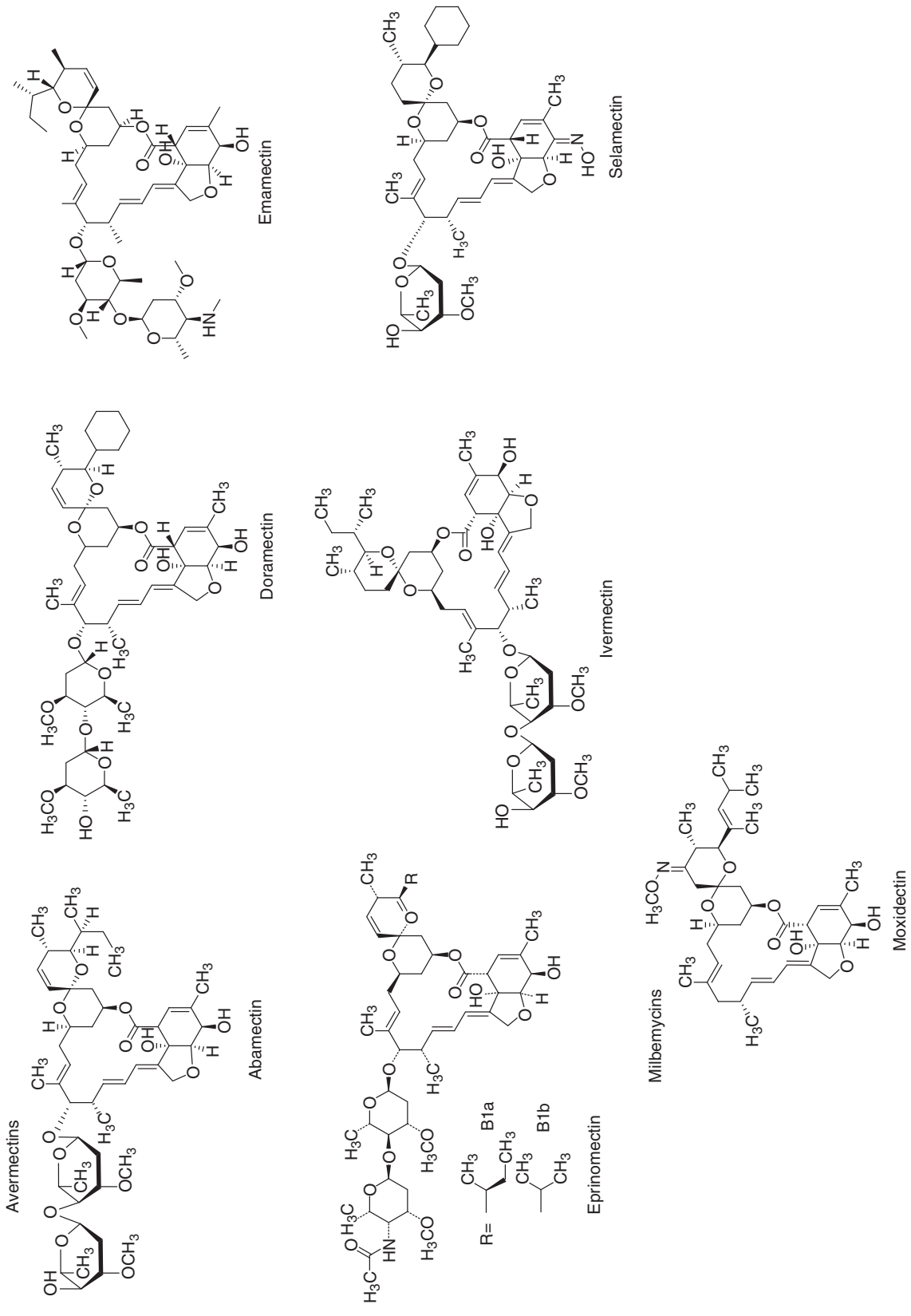

Figure 2 Chemical structure of macrocyclic lactones.

as the marker compound to calculate total residues in edible tissues. Thus, abamectin is a mixture of avermectins containing more than 80% of avermectin B1a and less than 20% of avermectin B1b, being the a and b series sec-butyl and isopropyl homologs, respectively. By contrast, ivermectin can be obtained by the reduction of abamectin (*cis*-hydrogenated product at the 22,23 position) and has higher activity and broader spectrum. Abamectin, ivermectin, and doramectin only contain C, H, and O, and further modifications of the avermectin structure have been obtained by introducing other atoms, such as N, to achieve different insecticidal properties with reduced withdrawal times.

Meanwhile, moxidectin was introduced in 1997. Isolated from *Streptomyces cyanogrise* and *Streptomyces hygroscopicus*, it is able to kill certain worms resistant to ivermectin.

Finally it must be highlighted that macrocyclic lactones have been used against a wide range of nematode and arthropod parasites in livestock. Basically they interfere with gamma-aminobutyric acid-mediated neurotransmission, causing paralysis and death of the parasite. In fact, they are the most potent killer of worms and are more persistent in their effect. Although they have a wide margin of safety for livestock, and are effective against all stages of worms, they are ineffective against cestodes (tapeworms) and trematodes (liver flukes).

Other Anthelmintic Agents

In addition to these major anthelmintic classes, there are some specific compounds that are more effective for treating certain infections. Thus, emodepside, nitazoxanide, piperazine, paraherquamide, and praziquantel can also be used as anthelmintics. For instance, emodepside is effective against parasites that are resistant to benzimidazoles, levamisole, and ivermectin.

Furthermore, some parasitic worms are resistant to conventional classes of anthelmintics, and therefore, new classes of anthelmintics, with new modes of action, are being proposed. Thus, a new class of anthelmintic, named aminoacetonitrile derivative (AAD) has been developed. For instance, in 2009 Novartis introduced (Zolvix®), which is the AAD monepantel. It is effective against some nematodes resistant to other drugs, because its mode of action, which is based on a nematode-specific clade of acetylcholine receptor subunits, is different. This new class is well tolerated and has low toxicity to mammals. Another example is tribendimidine, with high activity against *Ascaris lumbricoides* and *Necator americanus*. No mutagenic and clastogenic effects were observed compared with other anthelmintic agents, and therefore it is considered safe with a broad range of activities.

Anthelmintic Toxicity and Legislation

Although anthelmintics are effective against worms, they may also affect the host itself based on the same biochemical mechanism that operates against the parasite or by specific mechanism to the host. The widespread use of anthelmintics implies the possibility of the presence of residues in edible tissues, and toxic effects could be associated with chronic exposure to these compounds, such as teratogenicity, congenital malformations, diarrhea, anemia, pulmonary edema, or necrotic lymphadenopathy.

Among the different classes of anthelmintics discussed in the Introduction, it has been observed that avermectins and milbemycins are usually safe, considering they usually modify certain types of communication between two nerve cells. This type of communication is characteristic in nematodes and arthropods but in vertebrates it only exists in the central nervous system and these compounds do not penetrate the brain and spinal cord. Thus, ivermectin has been well tolerated in humans and no indication of associated central nervous system toxicity has been observed at doses up to 10 times the highest US Food and Drug Administration-approved dose of 200 $\mu g\ kg^{-1}$.

In relation to benzimidazoles, several studies have been carried out to evaluate the toxicity of these compounds, considering that they have a wide range of toxicity including teratogenic effects, congenital malformations, and anemia effects. Furthermore, some of the transformation products of this class of compounds show higher toxicity than the parent compound. For instance, hydroxymebendazole has been found to be more embryotoxic than mebendazole. Furthermore, albendazole sulfoxide has teratogenic effects in several species including humans, but at different concentrations. However, several toxicological effects between the two isomers of this compound were noted. Thus, the (+) enantiomer was the predominant enantiomer in the bovine, ovine, and caprine and in which teratogenicity and clinical development abnormalities have not been reported after conventional usage.

Meanwhile, European Medicines Agency (EMA) has indicated that albendazole should be regarded as a potential mutagen and no level had been identified at which there would be 'no mutagenic risk' to consumers of meat containing albendazole residues. However, it has been observed that there is a threshold value below which aneuploidy genesis will not occur, ensuring that conventional therapeutic use of albendazole as well as other similar compounds will not pose a risk in humans. In fact, it is recognized that there is no indication of harmful effects of this compound in humans and animals despite its worldwide use. For instance, there are no reports of a teratogenic action in humans, not even in women dosed during pregnancy.

In relation to other benzimidazoles it has been noted that mebendazole has low systemic toxic potential, but at high doses it can cause anemia and liver damage. Considering the toxic effect that this type of compound can provoke, considerable effort has been focused on the development of a new generation of benzimidazoles, which are more soluble and produce minimal acute damage to the animal – even at very high doses.

By contrast, praziquantel is unlikely to be of toxicological concern to humans and the European Union (EU) has granted marketing authorization of this compound for use on several animals without the need to set an MRL.

Finally, it must be emphasized that levamisole, pyrantel, and morantel are more toxic, considering that they affect transmission of neural impulses of the host, but at higher doses. In fact, levamisole has the narrowest margin of safety, although toxicity is usually due to excess dosage. Thus, EMA

suggest an MRL of 10 μg kg^{-1} of this compound in muscle, fat, and kidney from bovine, ovine, porcine, and poultry.

Although these compounds have toxic effects at high doses in laboratory animals, levels detected in food are generally below toxicity thresholds and pose no risk to consumers, though special attention should be paid to the presence of this type of residues in milk.

Legislation

Although anthelmintics are more toxic to parasites than mammals, food produced from treated animals should not contain residues of such drugs which would pose a food safety risk. Therefore, national and international organizations establish MRLs of these drugs in foodstuffs. In this sense, only a limited number of products are licensed for treatment of animals during the lactating period and have a MRL set under European Commission Regulation 37/2010, in order to protect consumers from risks related to these compounds. An overview of the established MRLs are listed in Table 1. It can be observed that different MRLs have been established for the anthelmintic, depending on the matrix and different marker residue. Thus, the marker residue of most benzimidazoles in foodstuffs is defined as the sum of the parent and its persistent metabolites. For instance, the marker residue definition of albendazole includes the sum of albendazole sulfoxide, albendazole sulfone, and albendazole 2-amino-sulfone, whereas for oxfendazole, the marker residue is defined as the sum of extractable residues, which maybe oxidized to oxfendazole sulfone.

Furthermore, it must be emphasized that flubendazole is the only benzimidazole registered for poultry in different countries, although other benzimidazoles such as albendazole are used to treat helminth infections in poultry because of cost reasons. In fact, MRLs have been established for this molecule in several target tissues of poultry, including eggs (400 μg kg^{-1}).

Like the EU, other countries have established legislation and regulations regarding human health, food safety, and environmental protection. In the USA, MRLs or tolerances for veterinary drugs in foodstuffs can be found in the Code of Federal Regulations, namely Title 21 (Food and Drugs, 500–600). For instance, ivermectin has been approved in the USA for use in several animal species, including nonlactating cattle. Doramectin and moxidectin are approved for use in beef cattle and nonlactating dairy cows, whereas eprinomectin was recently approved for use in beef and dairy cattle. Furthermore, in 2006 the USA approved febendazole for the control of nematodes in turkeys, establishing a MRL of 6000 μg kg^{-1} in liver and 2000 μg kg^{-1} in muscle, fenbendazole sulfone being the marker residue.

In Canada, the Department of Health is in charge of administering a variety of laws, and develops and enforces regulations. It has also established MRLs for monitoring of residues of veterinary drugs in food. For instance, in this country, abamectin, doramectin, eprinomectin, ivermectin, and moxidectin are used to treat food animals such as bison, cattle, deer, sheep, swine, and reindeer against nematodes and arthropods; emamectin is used to control sea lice in fish farms and selamectin is used for the treatment of pets against heart- and roundworms. Only eprinomectin and moxidectin are permitted for use in dairy cattle, with no milk withholding time, considering that eprinomectin was designed to exhibit a low milk/plasma ratio, and moxidectin is less toxic with a larger acceptable daily intake.

China has also established legislation regarding the presence of anthelmintics in edible tissues. For instance, the MRLs for triclabendazole in edible ruminant tissues were set at 200, 300, and 300 μg kg^{-1} in muscle, liver, and kidney tissues of bovines respectively, whereas in goats, the MRL was set at 100 μg kg^{-1} in the same tissues.

It must be highlighted that, depending on the country or government organization, the MRLs set for the same combination compound/matrix maybe different. For instance, eprinomectin has an MRL in milk of 20 μg kg^{-1} in Canada and EU, whereas the MRL is lower (12 μg kg^{-1}) in the USA. Meanwhile, in China, MRLs are set at 100 μg kg^{-1} for avermectin, ivermectin, and doramectin in bovine liver and 20 and 100 μg kg^{-1} for avermectin and doramectin, respectively in bovine muscle, whereas in the EU, MRLs in bovine liver are 20, 100, 100, and 600 μg kg^{-1} for avermectin, ivermectin, doramectin, and eprinomectin, respectively.

In relation to benzimidazoles, for instance, China and EU have established MRLs for these compounds, which range from 50 to 400 μg kg^{-1} and 10 to 5000 μg kg^{-1} respectively, depending on the matrix and compound. Thus, albendazole and related products (albendazole sulfoxide, sulfone, and amino sulfone) have an MRL of 100 μg kg^{-1} in milk, muscle, and fat, whereas it is set at 5000 μg kg^{-1} for kidney and liver. However, for flubendazole, an MRL of 400 μg kg^{-1} was set in eggs.

Finally, it must be emphasized that although most residue surveillance programs test for the presence of anthelmintic residues in edible tissues, few positive results were found. Thus, the presence of anthelmintics in muscle in several countries of Europe were tested and of 1061 beef samples, only 2.45% contained detectable amounts of anthelmintic residues at concentrations ranging from 0.2 to 171 μg kg^{-1}. However, none of the positive results were above EU MRL or action level, indicating that the risk of exposure of the European consumer to this type of drugs is negligible.

Furthermore, albendazole and metabolites have been analyzed in porcine muscle and livers, but only two metabolites, albendazole sulfone and albendazole 2-amino-sulfone were detected in some porcine livers but at concentrations lower than the MRLs established by the EU.

Pharmacokinetics of Anthelmintics

After administration, anthelmintics are usually absorbed into the bloodstream and transported to different parts of the body, including the liver, where they maybe metabolized through oxidation and cleavage reactions, and excreted in the feces and urine. The characterization of the kinetic behavior, metabolic fate, excretion, and residue profile of anthelmintics in the target animal will contribute to its optimized use as well as the determination of the length of the withdrawal time.

However, the administration of anthelmintics does not always result in the expected therapeutic success, because

Table 1 MRLs for some anthelmintic drug residues in several matrices under European Commission Regulation 37/2010

Compound	*Marker residue*	*Animal species*	*MRL* ($\mu g\ kg^{-1}$)	*Target tissues*
Albendazole	Sum of albendazole sulfoxide, albendazole sulfone, and albendazole 2-amino sulfone, expressed as albendazole	All ruminants	100	Muscle
			100	Fat
			1000	Liver
			500	Kidney
			100	Milk
Fenbendazole	Sum of extractable residues which maybe oxidized to oxfendazole sulfone	All ruminants, porcine, and Equidae	50	Muscle
			50	Fat
			500	Liver
			50	Kidney
		All ruminants	10	Milk
Flubendazole	Sum of flubendazole and (2-amino 1H-benzimidazol-5-yl)(4fluorophenyl)methanone	Poultry and porcine	50	Muscle
			50	Skin and fat
			400	Liver
			300	Kidney
	Flubendazole	Poultry	400	Eggs
Mebendazole[a]	Sum of mebendazole methyl (5-(1-hydroxy, 1-phenyl)methyl-1H-benzimidazol-2-yl)carbamate and (2-amino-1H-benzimidazol-5-yl)phenylmethanone, expressed as mebendazole equivalents	Ovine, caprine, and Equidae	60	Muscle
			60	Fat
			400	Liver
			60	Kidney
Oxfendazole	Sum of extractable residues which maybe oxidized to oxfendazole sulfone	All ruminants, porcine, and Equidae	50	Muscle
			50	Fat
			500	Liver
			50	Kidney
		All ruminants	10	Milk
Thiabendazole	Sum of thiabendazole and 5-hydroxy thiabendazole	Bovine and caprine	100	Muscle
			100	Fat
			100	Liver
			100	Kidney
			100	Milk
Triclabendazole[a]	Sum of extractable residues which maybe oxidized to keto-triclabendazole	All ruminants	225	Muscle
			100	Fat
			250	Liver
			150	Kidney
Abamectin[a]	Avermectin B1a	Bovine	10	Fat
			20	Liver
		Ovine	20	Muscle
			50	Fat
			25	Liver
			20	Kidney
Doramectin	Doramectin	All mammalian food producing species	40	Muscle
			150	Fat
			100	Liver
			60	Kidney
Emamectin	Emamectin B1a	Fin fish	100	Muscle and skin
Eprinomectin	Eprinomectin B1a	Bovine	50	Muscle
			250	Fat
			1500	Liver
			300	Kidney
			20	Milk
Ivermectin[a]	22,23-dihydro-avermectin B1a	All mammalian food producing species	100	Fat
			100	Liver
			30	Kidney

(*Continued*)

Table 1 Continued

Compound	*Marker residue*	*Animal species*	*MRL* ($\mu g\ kg^{-1}$)	*Target tissues*
Moxidectin	Moxidectin	Bovine, ovine, and Equidae	50	Muscle
			500	Fat
			100	Liver
			50	Kidney
		Bovine and ovine	40	Milk
Levamisole[b]	Levamisole	Bovine, ovine, porcine, and poultry	10	Muscle
			10	Fat
			100	Liver
			10	Kidney
Morantel	Sum of residues which may be hydrolyzed to N-methyl-1,3-propanediamine and expressed as morantel equivalents	All ruminants	100	Muscle
			100	Fat
			800	Liver
			200	Kidney
			50	Milk

[a]Not for use in animals from which milk is produced for human consumption.
[b]Not for use in animals from which milk or eggs is produced for human consumption.
Abbreviation: MRLs, maximum residue limits.

host-related factors can modify pharmacokinetic behavior and efficacy of the selected compound. Thus, the factors affecting drug pharmacokinetics can be distinguished between two groups: interindividual (species, sex, and genetics) and intraindividual (age, gestation, stress, medication, food, and environment) factors. Interindividual factors remain constant during the life of an organism, whereas intraindividual ones change during the life. Basically they are related to physiological and pathological state of an organism. For instance, it has been observed that the metabolic interconversion between fenbendazole sulfide and sulfoxide differs in horses compared to ruminants, and the bioavailability and residence time were lower and shorter in horses than in ruminants.

Extensive work has been carried out to study the pharmacokinetic and metabolism of certain benzimidazoles. In general, benzimidazoles are limited in absorption from the gastrointestinal tract due to the poor solubility of these drugs and the absorption is generally fast (from 2–7 h with flubendazole to 6–30 h for other compounds). Mebendazole is absorbed from the gastrointestinal tract and is intensively metabolized in sheep through ketoreduction and decarbamylation followed by conjugation.

Furthermore, it is known that the absorption and the efficacy are influenced by their administration with food. Thus, the bioavailability of benzimidazole sulfides is markedly reduced in ruminants which have had unrestricted access to food compared with those given restricted access prior to treatment. Sometimes in order to minimize the metabolic oxidation of benzimidazole sulfides and sulfoxides to sulfones, metabolic inhibitors are added. For instance, piperonyl butoxide has been added to modify the pharmacokinetic profile of febendazole.

The pharmacokinetics of albendazole has been studied in several animal species, although few studies have been carried out in poultry. In general, it is rapidly metabolized in all species, and the plasma levels of oxidized metabolites (sulfoxide and sulfone) are much higher than the parent drug. In fact, the sulfoxide transformation product is considered the active metabolite responsible for the therapeutic activity of albendazole, whereas albendazole sulfone is considered an inactive compound. Furthermore, it can be noted that the coadministration of other drugs such as cimetidine and praziquantel affects the kinetics of albendazole probably by the induction of the second sulfonation step. In poultry it has been observed that the maximum concentration was obtained at 1 h and it was only detected for 6 h after treatment. However, albendazole sulfoxide was detected for 25 h after treatment. Furthermore, these results were compared with those obtained in other species such as calf, dogs, goats, pigs, and sheep, and the maximum concentration (C_{max}) and time to reach it (T_{max}) are lower in chickens. Therefore, it was concluded that albendazole was absorbed to a greater extent and more quickly in poultry than in mammalian species. Finally, only albendazole metabolites can be measured in eggs if single oral dose was given. However, if the treatment is applied during 7 days, albendazole and its metabolites can be simultaneously detected in yolk, whereas albendazole cannot be detected in egg albumin. The absence of albendazole in albumin can be explained considering the relatively higher lipid solubility of albendazole compared with its metabolites. In the same way, it has been observed that the concentration of flubendazole in egg yolk was about five times higher than in the albumin.

Other compounds that have been studied are mebendazole and triclabendazole. Thus, some metabolites such as hydroxymebendazole (reduced metabolite) and aminomebendazole (hydrolyzed metabolite) can be found in food from animals treated with mebendazole, with the highest concentrations in liver of the reduced metabolite. In fact, this metabolite can be detected in liver for 14 days after administration. In relation to triclabendazole, it is rapidly removed from blood by the liver, and it is oxidized to the sulfoxide and sulfone, which are the main metabolites detected in plasma.

For nicotinic receptor agonists, it has been shown that the absorption and excretion of levamisole is fast and it is not affected by the route of administration because of its high solubility. It has been observed that the levels of this drug in blood cattle are maximum in less than 1 h, and 90% of the total dose is excreted in urine in 24 h. However, pyrantel is poorly soluble in water and its metabolism is fast, with the metabolites excreted rapidly in the urine. Morantel is absorbed rapidly from the upper small intestine of sheep and metabolized rapidly in liver (17% of the initial dose is excreted in the urine as metabolites within 96 h after dosing).

In general, macrocyclic lactones are hydrophobic and they are distributed throughout the body and some of them can be concentrated in adipose tissue. Depending on the species, they have an elimination half-life of 32–178 h. In this sense, a pharmacokinetic study of emamectin benzoate has been carried out in Atlantic salmon and it was shown that emamectin B1a was the major residue component (>80% in edible tissues). Therefore, this compound has been used as marker residue in edible tissues of Atlantic salmon for monitoring emamectin benzoate.

Further metabolic studies have been carried out with other anthelmintic drugs. Thus, the metabolism of praziquantel has been studied in several tissues of rainbow trout maintained at different temperatures (12 and 18 °C), observing that absorption was faster at higher temperatures, whereas the elimination of the drug was less dependent on the temperature. Thus, after 32 h, 67–96% of the drug has been eliminated from the tissues.

In relation to the elimination of anthelmintics, biliary secretion is an important pathway for elimination of macrocyclic lactones. Thus, they are primarily excreted in the feces and the remainder (<10%) in the urine. However, more lipophilic compounds are also excreted in milk. Biliary route is the most important pathway for secretion and recycling of benzimidazoles to the gastrointestinal tract, as well as praziquantel, which is partly excreted by bile fluid and partly as water soluble metabolites through the kidneys. Furthermore, the intestinal clearance of albendazole sulfoxide exhibits a stereoselective elimination of the (−) form.

The excretion of anthelmintics in the feces of livestock has given rise to concern as avermectins have adverse effects in dipteran flies and coleopteran beetles. However, benzimidazoles are unlikely to affect dung-dwelling arthropods and the environmental impact is not limited to specific effect on scavenger insects. For instance, the fecal concentration of febendazole and its metabolites was determined in horses, noting that no drug could be detected at 12 h post dosing. The maximal concentration was monitored at 24 h, and no target compounds were detected after 72 h. In feces the highest concentration was observed for the parent compound.

Analytical Methodology

Anthelmintic residues are mainly determined applying LC techniques coupled to several detectors, although some benzimidazoles have been determined by gas chromatography (GC). However, GC is not suitable for quantification because of thermal decomposition in the chromatograph or nonreproducible ionization in the source of the MS.

Benzimidazoles could be detected using ultraviolet (UV) or fluorescence detection, considering that most of them (albendazole, flubendazole, and thiabendazole) have natural fluorescence. Furthermore in relation to UV, the use of diode array is very useful, considering that individual analytes have characteristic spectra. Levamisole can also be detected by UV.

Meanwhile, avermectins can be detected using fluorescence detection including a derivatization reaction using trifluoroacetic acid, and this methodology has been used for the determination of emamectin in Atlantic salmon. Although the limits of detection provided by fluorescence detectors are below the MRLs established by governments, there are some problems related to the derivatization step such as instability of fluorescent derivatives, slow and/or incomplete formation of derivatives, and the low reproducibility of the results.

Furthermore, and according to current legislation in Europe, MS should be used for confirmation purposes in the determination of residues in foodstuffs. Therefore, conventional detection has been replaced by MS applying several detectors, such as single quadrupole, triple quadrupole, time of flight (TOF), and Orbitrap. In this sense, several ionization techniques, such as electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), and atmospheric pressure photoionization, have been used. Moreover, the application of MS analyzers allows the identification and quantification of metabolites of anthelmintics in the samples.

However, in the case of avermectins, the use of MS is not a straightforward strategy. These compounds are heavy (molecular weight ranging from 600 to 900 Da), but most of them only contain one nitrogen atom in their structure. In fact, ivermectin, doramectin, and abamectin only contain C, H, and O, which have negative effects on the ionization. This implies that several assays have been carried out to study the best ionization of these compounds. For instance, it has been indicated that for abamectin, the determination of ammonium adduct by ESI is the best option, although other studies have indicated that the use of APCI with negative ionization provided $[M-H]^-$ as the predominant ion for ivermectins. In general, it has been reported that avermectins, which are devoid of nitrogen atoms, produce significantly higher abundances for $[M+Na]^+$ than $[M+H]^+$. Furthermore, another difficulty is that sodium adducts are difficult to break and therefore several authors have evaluated alternative ionization processes, observing that negative ESI or APCI could overcome many of these problems.

In relation to the LC separation, C18 or C8 columns are mainly used. Moreover, the selection of the mobile phase is critical for the correct ionization of the target compounds. Acetonitrile and methanol are usually used for the determination of anthelmintics, although for avermectins, the use of methanol increases the sensitivity of the compounds in relation to acetonitrile. The pH of the mobile phase maybe critical in order to get suitable chromatographic separation, and ammonium formate or acetate could provide suitable results. Furthermore, in the past decade, ultra high-performance LC has been widely used in order to reduce analysis time, obtaining narrower peaks, and increasing sample

throughput. Furthermore, the combination of LC with tandem MS (MS/MS) allows the selective determination of these compounds.

Finally, it must be emphasized that sample preparation is the critical step for the simultaneous determination of several analytes in complex matrices. In relation to the extraction of these compounds from several matrices, different methodologies have been applied. Thus, liquid–liquid partition using acetonitrile has been used, allowing the deproteination of proteins. Solid phase extraction (SPE) has also been evaluated, utilizing C18, polymer sorbents or strong cation cartridges. However, they are laborious and time-consuming, and new approaches could be applied. Thus, immunoaffinity chromatography (IAC) could be used as a valuable tool for clean-up, allowing a fast and selective extraction of avermectins from extracts of bovine and swine liver. Furthermore, the combination of IAC with LC–MS provides sensitive and selective methods. However, matrix solid phase dispersion has been applied for the determination of 37 anthelmintic drugs and metabolites in muscle. In addition, pressurized liquid extraction has been applied for the determination of benzimidazoles in tissues of animals such as swine, cattle, sheep, and chickens. Finally, it must be noted that low temperature clean-up has been used for the determination of ivermectins and moxidectin in bovine muscle.

Furthermore, benzimidazoles are usually extracted by relatively polar aqueous extraction solutions. However, some metabolites are covalently bound to matrix components (liver or kidney) and chemical or enzymatic hydrolysis should be included in the extraction procedure, although simplified methods using anhydrous acetonitrile could be used.

Nowadays, generic extraction conditions can be applied for the simultaneous extraction of several types of compounds in a single analytical method. However, the inclusion of some anthelmintics such as avermectins is not easy considering the low sensitivity obtained by the use of LC–MS/MS, and special attention should be paid to these compounds, when they are included in multiresidue methods. In this sense, QuEChERS approach (acronym for Quick, Easy, Cheap, Effective, Rugged and Safe), which is based on acetonitrile extraction followed by a phase separation induced by the addition of salts and a clean-up step based on dispersive SPE, has been successfully applied for the extraction of this type of compounds from edible tissues.

Conclusions and Future Trends

As described in this article, the use of anthelmintics to treat helminth parasites and the resulting residues in edible tissues is a problem of major concern. Furthermore, the development of resistance to the currently used anthelmintics is an issue of increasing importance. In this sense, the development of new compounds, such as AAD, are needed in order to improve the characteristics of conventional classes of anthelmintics such as lower toxicity, favorable pharmacokinetic properties, and broad-spectrum capacities.

Furthermore, additional studies should be carried out to more thoroughly examine the pharmacokinetics and metabolism of these compounds, especially during pregnancy. The possibility of elimination of some nonpolar compounds by milk should be also evaluated in order to minimize the risk of the presence of anthelmintic residues in this matrix. To assure a sustainable inventory of effective anthelmintics, monitoring of drug resistance and research with combinations of current or future drugs will be necessary.

Moreover and despite the evaluation of the metabolism of anthelmintics in vertebrates, much attention should be paid to the evaluation of metabolism of these products in parasitic helminths.

In relation to analytical methodologies, current methods should include known metabolites in routine analyses in order to fulfill current legislation. However, anthelmintic metabolism is complex, and it is necessary to develop and validate new analytical methods that allow the accurate determination of new metabolites in foodstuffs. For that purpose, high resolution MS analyzers such as TOF or Orbitrap are very helpful, considering that they acquire full-scan spectra, and target, nontarget and unknown analysis that could be carried out in one single analysis.

Finally and considering the different MRLs established by several countries or organizations for the same combination anthelmintic/matrix, international legislation should be harmonized, in order to avoid some problems during export.

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See also: Analytical Methods: Overview of Methods of Analysis for Chemical Hazards. Disciplines Associated with Food Safety: Food Safety Toxicology. Food Safety Assurance Systems: Food Safety and Quality Management Systems. Institutions Involved in Food Safety: FAO/WHO Codex Alimentarius Commission (CAC); Food and Agriculture Organization of the United Nations (FAO); World Health Organization (WHO). Public Health Measures: Food Control and Public Health Laboratories; Fundamentals of Food Legislation. Risk Analysis: Risk Assessment: Chemical Hazards. Veterinary Drugs Residues: Antibacterials; Control of Helminths; Veterinary Drugs – General

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VETERINARY DRUGS RESIDUES

Anabolics

M Smith, US Food and Drug Administration (FDA), Rockville, MD, USA

Glossary

Anabolic steroids A class of steroid substances that increase protein synthesis within cells, resulting in the buildup of cellular tissue (anabolism), particularly in muscle.

Beta-agonist A class of substances that act on adrenergic receptors, thereby causing smooth muscle relaxation, dilation of bronchial passages, vasodilation in muscle and liver, and release of insulin, and that have anabolic effects in animals.

Codex Alimentarius Commission The governing body of the joint UN World Health Organization (WHO)/Food and Agriculture Organization (FAO) food standards program that develops standards, codes of practice, guidelines, and other recommendations to ensure the health of consumers and fair practices in the international food trade.

Joint FAO/WHO Expert Committee on Food Additives (JECFA) The independent expert scientific review body that provides risk assessments and scientific support for Codex food additive, contaminant, and veterinary drug residue standards.

Somatotropins Protein hormones consisting of large complex peptides. Natural somatotropins are produced in the pituitary gland and function in regulating metabolic processes and increasing anabolism.

World Trade Organization (WTO) The international organization that seeks to supervise and liberalize international trade.

Introduction

Very few issues involving veterinary medicines have resulted in more controversies over the years than those surrounding the use in animals of drugs that promote anabolism and the safety of food produced from those treated animals. Although the USA/Canada–European Union (EU) beef hormones trade dispute has faded from the headlines, there are still significant differences of opinion concerning the outcome of that World Trade Organization (WTO) action. Disagreements within the Codex international standard-setting deliberations have also continued over the use of hormones and other anabolics such as somatotropins and the beta-agonists, ractopamine and zilpaterol, in food-producing animals, including the development of maximum residue limits (MRLs) in foods.

Somatotropins

There was little interest in using somatotropins commercially in animal production until in the 1980s, when they could be produced in large quantities through recombinant deoxyribonucleic acid (DNA) technology. After a comprehensive review of its safety and efficacy, including with regard to human food safety, recombinant bovine somatotropin (bST) received approval by the US Food and Drug Administration (FDA) in November 1993 as an animal drug intended to increase milk production in dairy cows. Worldwide at this time, Mexico, Brazil, and approximately 20 other countries have also approved bST for commercial sale, whereas a number of other countries do not permit its use (e.g., Canada, Australia, Japan, and all EU countries). As part of the conditions for approval in the USA, the drug's sponsor initiated a post-approval monitoring program to determine if the incidence of mastitis and antibiotic use was manageable under actual use conditions and if label directions were adequate. This program was significant because it represented the most extensive postapproval study ever conducted for any animal product that FDA has approved. When FDA evaluated the data collected in this program, it concluded that 'the effects of the use of bST were in close agreement with the effects observed in the sponsor's preclearance studies' and that bST was safe and exhibited no adverse effects on the milk supply.

But questions have continued to be raised over the years by others including the European Commission and Health Canada. One issue for which Canada expressed concern in April 1998 related to a 90-day oral toxicity study in rats. Canada maintained that this study was 'misreported' by the FDA and alleged that there was significant absorption of oral bST based on serum antibody levels in and toxicity to the rats. This assertion, as well as the circumstances under which it was made public, became highly controversial, and, following publication of the Canadian report, several groups and individuals in the USA raised questions about the safety of milk from bST-treated cows. In response to these concerns, FDA prepared the 'Report on the FDA's Review of the Safety of Recombinant bST.' The report affirmed the original review of the 90-day rat oral toxicity study, concluding again that there

were 'no biologically significant observed effects in either the thyroid or the prostate.' In addition, FDA conducted a review of the report cited by Health Canada of the antibody response to oral bST. Although it concurred that oral exposure to high doses of bST results in antibody production, FDA concluded that 'there is no evidence for biologically significant absorption of intact bST from the gastrointestinal tract.'

FDA also reviewed the March 1999 European Commission Directorate General XXIV 'Report on Public Health Aspects of the Use of bST' and took issue with the conclusions of the report with respect to the safety of insulin-like growth factor 1 (IGF-1). IGF-1 is a hormone similar in structure to insulin. It plays an important role in bST stimulating milk production in cows. Concern centers on the fact that some studies of IGF-1 have shown a correlation between abnormally high levels of circulating IGF-1 and the development and growth of human cancers. The FDA asserted that the conclusions 'do not appear to be consistent with the current state of scientific knowledge.' Specifically, the report stated that establishing an *in vivo* quantitative dose–effect relationship for IGF-1 is virtually impossible because of the diverse biological effects attributable to the intrinsic activity of IGF-1. But the FDA found that there are standard procedures for assessing the hazard associated with all types of compounds that exert a broad variety of metabolic effects. FDA concluded the data provide ample evidence that 'the amount of IGF-1 and truncated forms excreted in milk following the administration of bST to dairy cows is safe for all consumers, including infants and that additional exposure data are not necessary.' The EU also cited the increased risk of mastitis from use of bST and therefore the possibility of increased use of antibiotics and possible concomitant increased antimicrobial resistance, and continued a moratorium on the drug's sale that had been in place since 1990. A permanent EU ban started in January 2000. Referring to the animal protection goals of EU Council Directive 98/58/EC, the bST ban was based solely on a regard for the welfare requirements of animals, and no concerns about the safety of milk produced from treated animals were expressed when the prohibition was put into effect.

FDA's determination that food products from cows treated with bST are safe for consumers has been supported by numerous scientific and regulatory bodies including the Joint FAO/WHO Expert Committee on Food Additives (JECFA). In 1992, the JECFA concluded that 'the lack of oral activity of bST and IGF-1 and the low level and nontoxic nature of the residues of these compounds, even at exaggerated doses, results in an extremely large margin of safety for humans consuming dairy products from bST-treated cows.' In 1998, the JECFA reaffirmed the safety of milk and meat from bST-treated cows. The Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF), acting on the advice of the JECFA, recommended that the Codex Alimentarius Commission should adopt an MRL for bST in food. But the European Commission opposed the advancement of an MRL for bST within Codex, and proposed to retain the draft MRL at Step 7 until another review by the JECFA. Nevertheless, the CCRVDF decided in June 1999 to take the proposal forward for discussion in the Codex Commission, but the matter ended up being withdrawn from the agenda. Final approval of bST through Codex has been delayed ever since that time. During its session in July 2012, the Codex Commission continued to hold bST at Step 8 but asked for an updated review of the scientific data to be undertaken by the JECFA, thereby establishing a process for possible progression of MRLs for bST at a future time.

Porcine somatotropin (pST) is a growth hormone naturally produced in pigs and is also produced through recombinant DNA technology. pST injections or implants containing this hormone cause pigs to gain more muscle and less fat in comparison with untreated animals. This hormone has been approved for use in a number of countries including Australia, Mexico, Peru, and Vietnam but has not yet been approved in the USA. In 1999 after reviewing pST, the JECFA noted the following: the lack of increased concentrations of pST residues in the edible tissues of treated animals, the lack of a biologically significant increase in the intake of IGF-1 by humans who consume the edible tissues of treated animals, and the lack of toxicological concern with regard to the levels of residues of pST and exogenous IGF-1 likely to occur in treated pigs. The JECFA stated that pST can be used in pigs without appreciable risk to the health of consumers and recommended that it was not necessary to set any MRLs for edible tissues in pigs. The CCRVDF and the Codex Commission finalized these conclusions in 2003. pST is not approvable in the EU because of the blanket prohibition contained in Council Directive 96/22/EC for substances used in stock farming having a hormonal or thyrostatic action and for beta-agonists.

Steroid Hormones Used in Food-Producing Animals

The use of hormonally active steroids as growth promoters in farm animals can increase the production of veal and beef significantly (up to 15%). The USA and a number of other countries worldwide have approved some of these substances for use at low concentrations to increase the rate of weight gain and/or improve feed efficiency in beef cattle. In the USA, these drugs are available for over-the-counter purchase and are usually administered by livestock producers. The naturally occurring (endogenous) steroid hormones approved by the FDA (i.e., estradiol, progesterone, and testosterone) are formulated as implantable pellets and are designed to deliver the hormones at a slow, constant rate when injected subcutaneously under the skin of the animal's ear. Numerous scientific studies have demonstrated that, when these drugs are utilized in accordance with their approved conditions of use, concentrations of the hormones in edible food tissues remain within the normal physiological range that has been established for untreated animals of the same age and sex. Because of the slow release of very small amounts of the hormone and a short average half-life (approximately 10 min), it has been determined by FDA and other agencies that no preslaughter withdrawal time is necessary to protect the public health and that consumers are not at risk from eating food from animals treated with these compounds. For the synthetic steroid hormones that have been approved by the FDA and other agencies (i.e., trenbolone acetate, zeranol, and melengestrol acetate (MGA)), extensive toxicological testing in animals was required to determine safe levels in edible tissues because these substances are not metabolized as quickly as the naturally occurring steroid hormones. For all of the synthetic hormones,

the FDA required the manufacturers to provide evidence demonstrating that the amount of residue left in each edible tissue after treatment is below the level determined to be safe.

Hormones in Beef Trade Controversy

Starting in 1981, the EU began restricting the use in livestock production of natural hormones to therapeutic purposes only. In 1985, it also prohibited the use of synthetic hormones and imports of meat from animals that had been administered these hormones. The EU justified the ban as needed to protect the health and safety of consumers from the illegal and unregulated use of hormones in livestock production in several EU Member States. On 1 January 1989, the EU banned the import of US beef produced with the six FDA-approved hormones. This action dramatically reduced beef exports to EU Member States. For the USA the value of the lost exports was approximately $100 million, and it retaliated by imposing 100% duties on $100 million in EU products exported to the USA. This retaliation continued with some adjustments during the period 1989–96. Then in 1999 the USA imposed another 100% *ad valorem* duty on selected food products from EU countries.

In April 1996, the USA requested that the WTO establish a dispute panel to consider the US claim that the EU's prohibitions against the six US-approved hormones "adversely affect imports [into the EU] of meat and meat products and appeared to be inconsistent with the obligations of the European Communities under the WTO Agreement on the Application of Sanitary and Phytosanitary (SPS) Measures (SPS Agreement)." The WTO's dispute resolution procedures permit a WTO member government to request that a dispute resolution panel be established to determine whether measures maintained by another WTO member government violate its obligations under the WTO agreements. Such a panel, normally consisting of three individuals selected in consultation with the parties to the dispute, considers written submissions and oral arguments by the parties. According to the applicable WTO procedures, a panel may seek advice from experts selected in consultation with the parties to the dispute, particularly in a dispute involving scientific or technical issues. In May 1996, a panel was formed "to examine … the matter … and to make such findings as will assist … in making the recommendations or in giving the rulings provided for in the SPS (and other WTO) agreements." After consulting with scientific and technical experts, the panel issued its final report in June 1997, finding that the EU measures were not in accord with the SPS Agreement. Following an EU appeal of the initial findings of the panel, in February 1998 the WTO upheld the panel's findings and found specifically that the EU was inconsistent with Article 5.1 of the SPS Agreement. (Article 5.1 states: "Members shall ensure that their sanitary or phytosanitary measures are based on an assessment, as appropriate to the circumstances, of the risks to humans, animal or plant life or health, taking into account risk assessment techniques developed by the relevant international organizations.") The panel said the EU 'maintained measures which were not based on a risk assessment' as required by Article 5.1. The panel also stated that the EU actions were not in accord with Article 3.3 of the SPS Agreement. (Article 3.3 states: "Members may introduce or maintain sanitary or phytosanitary measures which result in a higher level of sanitary or phytosanitary protection than would be achieved by measures based on the relevant international standards, guidelines, or recommendations, if there is a scientific justification, or as a consequence of the level of sanitary or phytosanitary protection a Member determines to be appropriate in accordance with the relevant provisions of paragraphs 1 through 8 of Article 5.") The panel found that "by maintaining sanitary measures which are not based on existing international standards without justification under Article 3.3 of the SPS Agreement, it had acted inconsistently with the requirements … of that agreement." Finally, after arbitration proceedings and other delays, in July 1999 the WTO authorized the USA to begin collecting tariffs, by suspending its concessions on $116.8 million worth of imports from the EU, the amount that it lost each year due to the EU hormone ban.

Following these WTO findings, the EU announced it would conduct additional studies and risk assessments on hormones that would satisfy its Article 5.1 obligations. In November 2004, the EU filed a request for consultations with the USA asserting that the USA should have removed its retaliatory measures because the EU believed it had now removed the measures found to be SPS inconsistent by the WTO dispute panel. Specifically, the EU believed that it was now in conformity with the dispute panel because on 14 October 2003, it had amended Council Directive 96/22/EC (the prohibition on the use in stock farming of certain substances having a hormonal, thyrostatic, or beta-agonist action) to include all uses of these drugs. The EU further asserted that the amendment of this EU directive was based on comprehensive risk assessments, in particular on the opinions of its Scientific Committee on Veterinary Measures relating to Public Health. Underpinning these risk assessments were a number of EU-funded and -initiated studies and projects. The EU concluded that 'the avoidance of intake of oestradiol 17β is of absolute importance to human health and that, consequently, the placing on the market of meat containing this substance should be prohibited.' And with regard to the other hormones in dispute, the EU provisionally prohibited the placing on the market of meat containing these substances because it asserted that relevant scientific evidence was insufficient. The EU stated that assessments of the safety of hormone residues in meat are based on 'uncertain assumptions and inadequate scientific data'; i.e., data on residue levels in meat were based on studies using methods that were inadequate to measure precisely the low levels found in animal tissues and there was only limited information on the levels of the various metabolites of the steroids. The EU also questioned the reliability of data on daily production rates of human steroid hormones in healthy prepubertal children. The USA disagreed and denied both that the new Council Directive 96/22/EC was based on science and that it implemented the WTO panel's recommendations and rulings. The USA believed that, contrary to the EU's claim, there were no studies that demonstrated there was increased health risk from the consumption of meat from animals treated with growth-promoting hormones.

In January 2005, the EU requested the WTO to convene another dispute panel, and the first meeting of the new

panel and the disputing parties took place in September 2005. To highlight the broad interest that this trade case continued to have, it is interesting to note that it was the first time a WTO dispute panel meeting had been conducted and broadcast in full transparency. The EU reaffirmed its position that there were possible risks to human health associated with hormone-treated meat, given the available scientific data. The EU claimed it had complied with its WTO obligations and challenged the USA for maintaining its prohibitive import tariffs on EU products. The USA disputed whether the EU had conducted an adequate risk assessment to support its position and maintained that there was a clear worldwide scientific consensus supporting the safety to consumers of eating hormone-treated meat. In October 2008, the WTO issued a mixed ruling allowing the USA to continue its trade sanctions, but allowing the EU to maintain its ban.

Then in January 2009, the USA announced its intent to make changes to the list of EU products subject to increased tariffs under the dispute, including changes to the EU countries and products affected, and higher tariffs on some products. The EU claimed that these actions constituted an 'escalation' of the dispute. On 25 September 2009, following a series of negotiations, the USA and the EU signed a memorandum of understanding, which phased in certain changes over several years, including raising the amount of US beef certified as having no added hormones that would be permitted to be imported into the EU. In May 2011, the USA announced it was terminating higher duties for imported products listed under the dispute. In April 2012, agreement was reached to end this prolonged trade dispute when the EU Member States agreed to increase the quota of 'no hormone added' beef permitted to be imported into the EU and the USA agreed to drop its *ad valorem* punitive duties on EU products exported to the USA. Although this agreement is seen as a 'pragmatic' step forward, no decision was made, nor is likely to be made, by the WTO on the substantive public health issues surrounding this long-standing dispute.

International Risk Assessment and Management

In pressing its case in the hormone trade dispute with the EU, the USA relied significantly on the safety decisions made by the Codex Commission, the CCRVDF, and the JECFA.

For the six hormones at issue in this dispute, the JECFA had assessed five of the substances (all except MGA) and made MRL recommendations on four of them (excluding trenbolone) during its meeting in 1987. For trenbolone, further data were sought and a JECFA MRL recommendation was made in 1989. After failing to reach a consensus for several years, in June 1995 the Codex Commission adopted on the basis of a vote the standards for the five hormones. These standards apply exclusively with respect to cattle and meat and meat products of bovine origin, when these hormones are used for growth promotion purposes. The standard for MGA was not adopted at that time.

For the three natural hormones, the Codex considered it 'unnecessary' to establish MRLs because these hormones are produced endogenously at variable levels in human beings. The Codex found that residues resulting from the use of these substances as growth promoters in accordance with good animal husbandry practice are by comparison minimal and unlikely to pose a hazard to human health.

The JECFA had concluded in 1988 that 'residues arising from the use of testosterone and estradiol-17 as growth promoters in accordance with good animal husbandry practice are unlikely to pose a hazard to human health and that the amount of exogenous progesterone ingested in meat from treated animals would not be capable of exerting a hormonal effect, and therefore, any toxic effect, in human beings.' The term 'good animal husbandry practices' is recognized by the JECFA to mean 'the official recommended or authorized usage including withdrawal periods, approved by national authorities, of veterinary drugs under practical conditions.' This is an important condition given that some of the EU concerns raised as part of the WTO trade dispute made reference to the improper use of hormones.

With respect to two of the synthetic hormones (zeranol and trenbolone), the JECFA recommendations concluded that any toxic effects of these hormones are associated with their hormonal properties and that an acceptable daily intake (ADI) could thus be established on the basis of a no-hormonal-effect level. The JECFA adopted what it considered to be a conservative approach by studying animals highly sensitive to these hormonal substances and using a safety factor of 100. The JECFA recommendations included the following: an ADI of 0–0.5 $\mu g\,kg^{-1}$ body weight for zeranol and 0–0.0225 $\mu g\,kg^{-1}$ body weight for trenbolone, and for both hormones an MRL of 2.25 $\mu g\,kg^{-1}$ in bovine muscle and 10.25 $\mu g\,kg^{-1}$ in bovine liver. According to the JECFA, the MRLs thus obtained would not exceed the ADI or safe level at any time after implantation of the drug, regardless of the withdrawal period used.

The Codex Commission by a majority vote of 33 to 29 (seven countries abstaining) adopted the JECFA recommendations for these hormones. The Commission usually adopts its standards by consensus, but in this case after several years it became clear that consensus was not possible. The approval of these five hormones by the Codex provided important support for the USA in its subsequent case in the WTO against the EU. At its 2007 meeting, the CCRVDF could not reach consensus on the advancement of the proposed MRLs for MGA. But at that meeting the draft MRLs for MGA in cattle tissue were retained at Step 7 of the 8 step adoption process with the understanding that the EU would provide new data for a re-evaluation of MGA by the JECFA. Furthermore, if no new information was forthcoming, or if the JECFA reaffirmed its decision, the CCRVDF agreed that it would advance the MRLs for MGA to Step 8 at its next session. The JECFA reviewed additional data submitted by the EU and concluded that the new data did not provide any basis to reconsider the ADI. But the EU still maintained its concern regarding the advancement of the draft MRLs for MGA to Step 8 and stated that it continued to have safety concerns especially for particular susceptible population groups. This view was also supported by other delegations. But still other countries supported the findings of the JECFA's re-evaluation and the CCRVDF agreed to advance the draft MRLs for MGA in cattle tissues to the Codex Commission for adoption at Step 8.

The EU, China, Norway, and Switzerland expressed their opposition to this decision. After reviewing all positions again, the Commission at its July 2009 meeting adopted the draft MRLs. The delegations of the EU, Bosnia and Herzegovina, China, Croatia, Norway, and Switzerland expressed their strong opposition to the adoption of these MRLs.

Use of Ractopamine in Swine and Beef

Ractopamine hydrochloride is a drug that stimulates beta-adrenergic receptors. This class of substances has effects similar to epinephrine and also has anabolic effects tending to the conservation of lean muscle and reduction of fat. Ractopamine was approved by the FDA for use in swine in 1999 with the claims of increased rate of weight gain, improved feed efficiency, and increased carcass leanness in finishing swine fed a complete ration containing at least 16% crude protein from 150 to 240 lb body weight. An ADI of 1.25 μg kg^{-1} body weight in the human diet was established, with a tolerance for parent ractopamine in swine liver of 0.15 ppm (150 ppb, or 150 μg kg^{-1} of liver) and a tolerance of 0.05 ppm for parent ractopamine in swine muscle (50 ppb, or 50 μg kg^{-1} of muscle). No withdrawal time is required on this FDA approval. Ractopamine was subsequently approved by the FDA for use in cattle in 2003 and turkeys in 2008. Ractopamine has been approved for use as a veterinary drug in swine in 21 countries (e.g., Australia, Brazil, Canada, Hong Kong, Indonesia, Mexico, New Zealand, South Africa, South Korea, and the USA). In addition, Canada, Mexico, Indonesia, and the USA have also approved its use in beef. Japan has established an ADI and MRLs for pork and beef, but has not yet approved the product for use; however, Japan recognizes its use in other countries for import purposes. In August 2007, Taiwan determined that ractopamine could be safely used in cattle and swine and notified the WTO that it planned to adopt the recommended Codex MRL of 10 ppb. Taiwan then reconsidered this approach and in 2010 began enforcing a zero tolerance for ractopamine residues and testing levels in beef imports from the USA and other countries. In 2011 Taiwan approved beef imports if they contained no more than the Codex MRL (10 ppb). Taiwan is yet to adopt MRLs for ractopamine in pork.

The EU and China have not approved ractopamine for domestic use and do not allow residues of ractopamine in imported food. As described above in the Section Somatotropins, the EU prohibits the use of all beta-agonists intended solely for growth promotion without any therapeutic purposes. The EU also has stated that it is of the opinion that there are still unanswered safety questions and scientific concerns linked to the use of ractopamine. China developed residue data in swine, particularly in lung, which it believed raised concerns for the safety of food derived from animals treated with ractopamine.

Ractopamine was first evaluated by the JECFA in 1992, when it was determined that there were insufficient data on which to establish an ADI or recommend MRLs. Ractopamine was again evaluated by the JECFA in 2004 and an ADI of 0–1 μg per kg of body weight per day was established and it was determined that MRLs could be recommended to CCRVDF. Specifically, the JECFA established MRLs in muscle, fat, liver, and kidney of 10, 10, 40, and 90 ppb, respectively. At the JECFA meeting held in 2006, ractopamine was reevaluated and the previously recommended MRLs were reaffirmed. And as a result of a request in 2009 by the Codex Commission, a special electronic session of the JECFA was held in 2010 during which residue data in swine provided by China were evaluated.

In 2010, over the objection of several countries, the CCRVDF forwarded draft MRLs for residues of ractopamine to the Codex Commission to be considered at Step 8. At its 2010 meeting, the Commission failed for the third time to adopt recommended MRLs for ractopamine in pork and beef. The opposition to adoption was led by the EU, which objected because the EU prohibits the use of beta-agonists intended solely for growth promotion without any therapeutic purposes. The EU also stated that it was of the opinion that there were still unanswered safety questions and scientific concerns linked to the use of ractopamine. Although many delegations at the Commission meeting supported adoption of the recommended MRLs, consensus was not achieved and no vote was taken. The EU was joined by Russia, Switzerland, and Norway to oppose the MRLs. China also opposed adoption because of its continuing concerns about concentrations of the residue in lung, which is heavily consumed in some regions in China.

At the 2011 Commission meeting, some delegations maintained that there were still unanswered safety questions which required further studies, particularly with respect to the residues in lung tissue and scientific concerns linked to the use of ractopamine. They also opposed finalizing the MRLs because the decision did not have a broad consensus of agreement and therefore would undermine the credibility of Codex. China referred to their experimental findings on residues in pig lungs and voiced their concern with the safety of ractopamine, especially their concern with the risks related to residues in lung tissue and other offal tissues. China expressed the view that, before ractopamine MRLs are adopted, a risk assessment should be completed, one that fully considers the safety questions raised by Chinese consumption data. The WHO JECFA secretary reminded the Commission that the JECFA had evaluated extensive residue data, including the additional residue studies in pigs submitted by China, and that the JECFA again reaffirmed the MRLs for muscle, fat, liver, and kidney. With regard to the Chinese data, the JECFA was of the opinion that, when consumed, lung and other nonstandard tissues generally replaced the standard tissues (e.g., muscle meat) and were not added to the daily consumption of products of animal origin. The JECFA concluded that even if residue levels in lung were higher than in the other tissues, based on the estimated dietary exposure, they did not indicate a health concern. At the end of this and other discussions, the Commission attendees voted not to proceed on a vote to adopt the ractopamine MRLs and they were retained at Step 8.

The delay by holding the ractopamine MRLs at Step 8 raised some fundamental questions within Codex regarding the balance between science-based decision making and the role of other factors in setting Codex standards. The USA and a number of other countries believed that the scientific issues concerning the safety of ractopamine had been resolved

through multiple JECFA evaluations. They did agree that the JECFA should proceed to recommend an MRL for lung tissue but that work would not affect the pending MRLs in muscle, fat, liver, and kidney. Some countries expressed their belief that the opposition to adoption of MRLs for ractopamine by the EU and some other countries is primarily based on nonscientific issues.

At the Codex Commission meeting in July 2012, there remained no substantive changes in positions and no consensus on adoption of the MRLs. After the Delegation of Ghana requested a vote on adoption of the proposed MRLs, the Commission voted 69 to 67 to adopt the MRLs for ractopamine. The EU and eight other countries expressed their reservations for the adoption. The Commission also agreed to place the veterinary drug zilpaterol, another beta-agonist that is similar to ractopamine, on the priority list for future evaluation by the JECFA.

Illegal Use of Anabolics in Food-Producing Animals

During the 1980s, there were widespread press reports in Europe of black market sales of 'hormone cocktails' by a 'hormone mafia' as well as several reports of serious health effects from consuming meat from treated animals. More recent studies have concluded that in some EU Member States, an extended black market in veterinary anabolic hormones still exists. Illegal use of hormones has also been a problem in the USA. In 2004 the FDA responded to several cases of unapproved uses of growth-promoting hormone implants in nonruminating veal calves that went to slaughter. There are no FDA-approved growth-promoting hormone implants for veal calves, and the extra-label use for nontherapeutic purposes in veal of these growth-promoting hormonal implants is illegal. As a result of these investigations, the FDA took very aggressive monitoring and enforcement actions to ensure that this activity did not continue.

Clenbuterol is a beta-agonist that has the ability to increase muscle mass, but residues in tissues of treated animals can cause symptoms of acute poisoning in people who consume them. Although this drug was reviewed by the JECFA and Codex has adopted MRLs for some food-producing animals (cattle and horses), Codex recommends that it be used only after approval by national authorities and only in association with therapeutic uses and under well-controlled withdrawal times. Beta-agonists represent a class of drugs having similar modes of action, but not all have the same safety or toxicity profile. Clenbuterol is metabolized by oxidative and conjugative pathways and has a long plasma half-life. Ractopamine is a beta-agonist that is metabolized solely by conjugation, has a relatively short plasma half-life, and has low oral potencies in humans. Clenbuterol and some other beta-agonists having relatively slow rates of elimination demonstrate high oral potencies in humans. As a result of illegal use, clenbuterol in edible tissues of livestock represents a serious risk to consumers. At the concentrations of clenbuterol measured in some contaminated liver and meat samples, pharmacological effects may be expected in humans after consuming 100–200 g of product. As mentioned above in the Section Somatotropins, EU legislation forbids the use of beta-agonists as growth-promoting substances in cattle raised for human consumption. However, it is recognized that this EU ban has not eliminated illegal uses of beta-agonists in Europe. There are permitted uses of beta-agonists, including clenbuterol, in the EU and some other countries as a therapeutic treatment for inhibition of uterine contractions (tocolysis) for female cattle during calving and of respiratory diseases and tocolysis for horses not raised for human consumption. In 1990 symptoms, but no deaths, from clenbuterol residue-induced food poisoning were reported from investigations of separate events in Spain and France. After a number of food poisoning incidents involving pork and some pig organs in Shanghai (2006), in Guangdong Province (2009), and in Henan Province (2011), serious penalties were imposed in China against individuals promoting the use of clenbuterol in animal feed.

In 1991, following some serious violations, the FDA advised the public of the possibility of illegal clenbuterol use in domestic animals and of its concern about adverse effects on public health if residue was present in food. The FDA also alerted consumers and others about illegal importation of this drug or of meat containing residues. Over the years the FDA has found several individuals guilty of distributing clenbuterol for use in food-producing animals. Results of monitoring programs in EU Member States during 1992 and 1993 for the occurrence of residues of beta-agonists in food-producing animals vary substantially with respect to the percentages of positive samples, ranging from 0% to 7%. Investigators have reported that as late as 2010, 'dozens of illegal veterinary drugs are used in the EU and the number of active compounds is still expanding.' These substances include estrogenic, androgenic, and progestagenic compounds, as well as thyreostatic, corticosteroidal, and beta-adrenergic compounds. In the EU one estimate of the number of different illegal 'hormones' that have been identified ranged from 35 to 55. Because of the illegal black markets that exist for these substances, there are likely many counterfeit products that are being offered as well. In addition, Internet sales and more open borders, especially in Europe, have made these problems more acute in recent years. The illegal use of clenbuterol and other growth promotants in food-producing animals remains a serious problem worldwide.

Conclusion

Since 1995, with the enactment of the SPS Agreement, Codex MRLs have essentially become the de facto world standards for drug residues in food. This is because if other countries establish more stringent MRLs, those countries can be required to independently demonstrate that their MRLs are based on competent and valid risk analyses or take the chance of becoming subject to trade sanctions. Because of this increased importance of Codex standards in determining the outcome of trade disputes, the establishment of MRLs for animal drugs has become extremely contentious and difficult both in the JECFA deliberations and within the Codex Step 8 process. This has been especially true for drugs that affect muscle mass or otherwise increase food production. Although Codex standards are voluntary and countries are free to conduct their own

scientific reviews and risk assessments, many countries do rely on Codex standards to ensure the protection of consumers and promote fair trading practices. Adoption of Codex standards is especially important for countries that do not have the resources to carry out their own risk assessments. In addition, other countries that have not established veterinary drug MRLs, but want to import animal-derived food from countries that use and enforce Codex MRLs, can permit the entry of those products and have confidence in their safety.

Many controversies and serious differences of opinion continue regarding the safe use of anabolic substances in veterinary medicine, particularly when they are used for growth promotion and increased food production purposes. It is clear that differing decisions by national authorities and disagreements that continue to arise out of Codex and JECFA conclusions can lend support or nonsupport to opposing views about the safety of these drugs. The WTO trade dispute between the USA/Canada and EU over the safety of hormones has persisted without substantive resolution for almost 20 years. Some critics may have differences of opinion about how to interpret the scientific evidence supporting Codex or JECFA decisions. Some critics believe that Codex committees like CCRVDF should consider 'other relevant factors' such as animal welfare or environmental concerns. Still other critics may find fault in the selection procedures for the scientific experts who participate in the JECFA, including the overall composition of the JECFA, the scientific competence of the experts, and the potential conflicts of interest of the experts. Also, some critics see JECFA's reliance on unpublished industry data and lack of procedural transparency to be problems. Although Codex, JECFA and many of the countries that participate in Codex have dealt effectively with many of these criticisms, some of these issues are not easily resolved. But it is important that they continue to be addressed. Those individuals and countries participating in Codex and JECFA must work as effectively as possible to deal directly with these criticisms and other subjects that may undermine the credibility of Codex and JECFA.

Some countries are concerned that any lack of confidence or even misinformation about the scientific and public health protection basis for Codex standards will create more confusion and further undermine the work of Codex in the future. Many also believe that the widely publicized disagreements concerning the safety of anabolic drug residues in food have made worse, and even encouraged, certain illegal activities that seriously threaten public and animal health. If the scientific bases for health and safety standards are perceived by a vocal few to be invalid, whether they are viewed as overly protective or not protective enough, there may emerge a distrust by the public of the regulatory actions that result from those standards. Failing credibility may also result in increased problems related to a growing lack of compliance by product manufacturers and users. For example, increasing reports of illegal products being available on black markets in some countries and through Internet sales may indicate, in addition to inadequate enforcement capabilities, that some government regulatory requirements are viewed as arbitrary or scientifically unjustifiable. The availability and use of such illegal products clearly can greatly undermine public health efforts, exacerbate regulatory control problems, and significantly compromise the important goals that Codex and other food safety organizations and national regulatory agencies are pursuing.

Countries participating in and/or relying on Codex and JECFA programs must work in the most effective ways to assure the integrity and transparency of these programs and work hard to build more consensus regarding the decisions that are made by these organizations. This will help ensure that Codex decisions truly further the stated goals of Codex: protecting the public health and assuring fair food trade practices. A lesser result is not acceptable.

Disclaimer

Some antimicrobials are commonly used in livestock feed at low doses for growth promotion or feed conversion efficiency (e.g., bambermycins, zinc bacitracin, salinomycin, virginiamycin, monensin sodium, carbadox, and lasalocid sodium). Some of these drugs affect the movement of ions across biologic membranes in the gut or modify the rumen and the physiology of the colon microflora by, for example, increasing nitrogen utilization. Others may act to selectively modify the microbial populations within the animal alimentary tract to improve production efficiency and to maintain health by, for example, combating low-level infections. These substances are not generally classed as anabolic agents and are not included in the subject matter of this article.

The opinions and information in this review article are those of the author and do not represent the views and/or policies of the FDA.

See also: Foodborne Diseases: Overview of Chemical, Physical, and Other Significant Hazards. Organisms of Concern but not Foodborne or Confirmed Foodborne: Classical Swine Fever Virus. Safety of Food and Beverages: Meat and Meat Products. Veterinary Drugs Residues: Veterinary Drugs – General

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Food and Agriculture Organization of the United Nations.

VETERINARY DRUGS RESIDUES

Coccidiostats

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Glossary

Acceptable daily intake (ADI) The estimate of the residue, expressed in terms of μg or mg per kg of bodyweight, that can be ingested daily over a lifetime without any appreciable health risk.

Acute reference dose (ARfD) The estimate of the amount of a substance in food or drinking water, expressed on a bodyweight basis, that can be ingested in a period of 24 h or less without appreciable health risk to the consumer. It is derived on the basis of all the known facts at the time of evaluation. The ARfD is expressed in mg of the chemical per kg of bodyweight.

Adverse effect Change in morphology, physiology, growth, development, or lifespan of an organism which results in the impairment of functional capacity or impairment of the capacity to compensate for additional stress or increase in susceptibility to the harmful effects of other environmental influences.

Food-producing animals Defined as animals bred, raised, kept, slaughtered, or harvested for the purposes of producing food.

Marker residue A marker residue is that residue, the concentration of which decreases in a known relationship to the concentration of total residues in tissues, eggs, milk, or other animal tissues.

Maximum residue limits (MRLs) The maximum concentration of residue resulting from the use of a veterinary medicinal product (expressed in mg per kg or μg per kg on a fresh weight basis) which may be accepted by the community to be legally permitted or recognized as acceptable in or on food.

No observed adverse effect level (NOAEL) The greatest concentration or amount of an agent, found by study or observation, that causes no detectable adverse alteration of morphology, functional capacity, growth, development, or lifespan of the target.

Residues All pharmacologically active substances, expressed in mg per kg or μg per kg on a fresh weight basis, whether active substances, excipients, or degradation products, and their metabolites which remain in food obtained from animals.

Target tissue The edible tissue selected to monitor for the marker residue in the target animal.

Withdrawal period The interval between the time of the last administration of a veterinary drug and the time when the animal can be safely slaughtered for food or when milk or eggs can be safely consumed.

Coccidiosis

Coccidiosis is a parasitic disease, which can occur wherever animals are housed in small areas that are contaminated with coccidial oocysts. Coccidiosis has affected historically all species of wild and domestic birds. The nature of the parasitic infestation is present in all poultry farms, even in the presence of high sanitary standards and good management, with a high potential impact on animal welfare. Coccidia are without question the most important parasites of poultry in terms of distribution, frequency, and economic losses. Coccidiosis is an important enteric disease often caused by highly host-specific intestinal protozoan intracellular parasites which belong to the genus *Eimeria* (phylum Apicomplexa), and is characterized by high mortality. Some species tend to be more pathogenic in terms of the inoculation dose required to produce measurable effects, but all species of avian *Eimeria* produce weight loss, increased feed conversion ratios, loss of skin pigment, and decreased egg production. Avian coccidia of the genus *Eimeria* are extremely host specific. Chickens, turkeys, pheasants, and Japanese and bobwhite quails all have their own species. Each poultry species may be infected with several different *Eimeria* species (*Eimeria necatrix, Eimeria tenella, Eimeria acervulina, Eimeria brunetti, Eimeria maxima, and Eimeria mivati* in broiler chickens or chickens for fattening; *Eimeria meleagrimitis, Eimeria gallopavonis, Eimeria adenoides, and Eimeria dispersa* in turkeys; *Eimeria duodenalis, Eimeria colchici, and Eimeria phasiani* in pheasants). Although there are no exact prevalence and incidence data on clinical and subclinical coccidiosis in commercial poultry, it is widely acknowledged that the parasites are present in all commercial flocks.

The life cycle of the coccidium is complex (Figure 1). It begins when a susceptible bird ingests sporulated oocysts from the environment in contaminated litter, feed, and water. After ingestion, the sporozoites are released from the oocysts, and actively penetrate the host cells (intestinal mucosa or epithelial cells lining the gut wall), and develop intracellularly into multinucleate schizonts (also called meronts). The

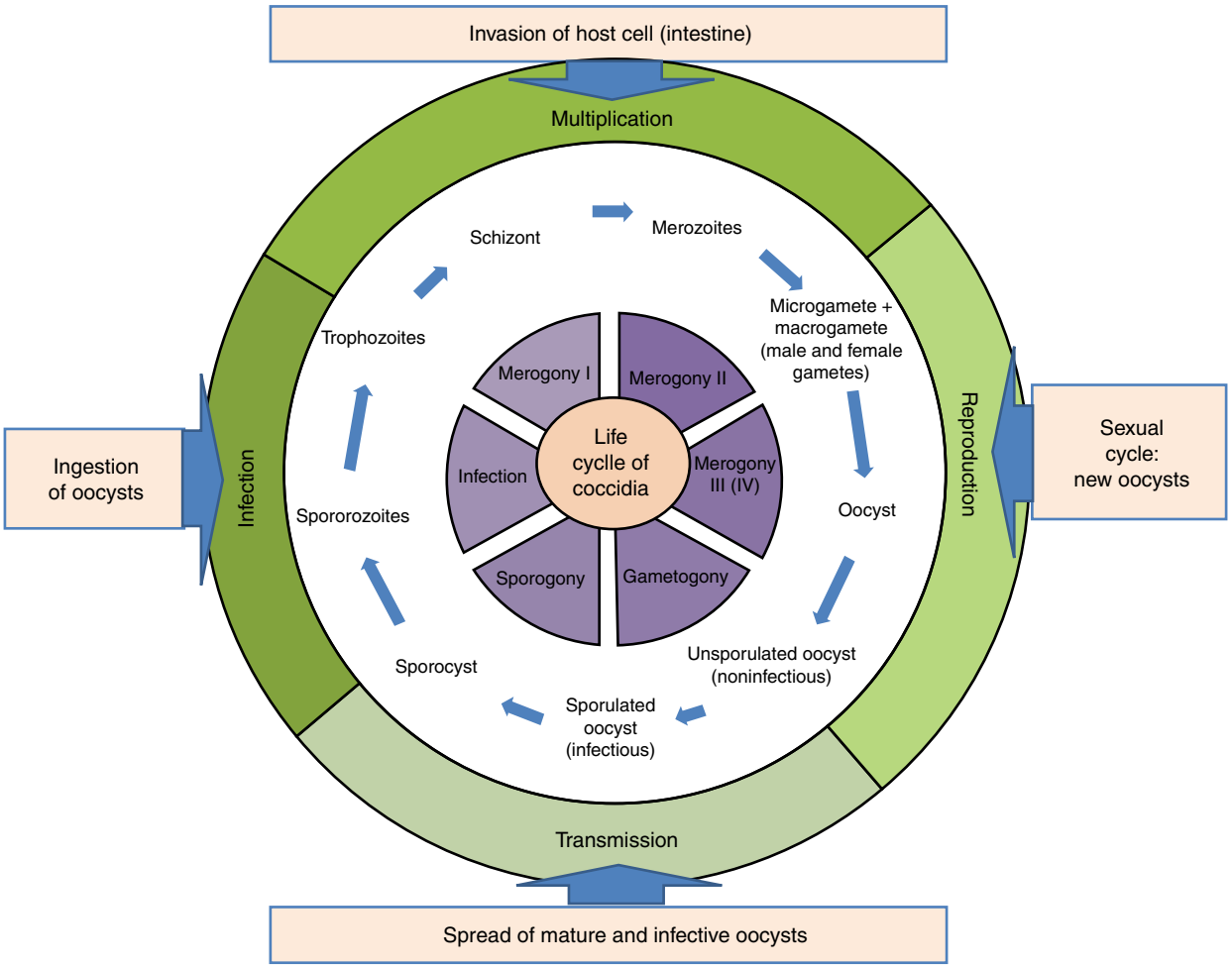

Figure 1 Life cycle and reproduction of Coccidia.

sporozoite transforms into trophozoite, which undergoes multiple karyokinesis and eventually produces numerous merozoites. This sequence of developmental events occurs a predetermined number of times (reinfective cycle), and each cycle is called a generation. Eventually the merozoites produced by the terminal generation of merozoites transform into sexual gametes. Some merozoites become differentiated into male and female gametes and the sexual part of the cycle is initiated. Immature sexual forms are known as gametocytes. The male gametocytes divide into a large number of mobile microgametocytes, whereas the development of macrogametocytes (female) leads to the production of a single microgametocyte. The male and female gametocytes unite resulting in the production of a zygote, which secretes a cyst wall and becomes an oocyst. The oocyst is excreted in the feces, usually in the nonsporulated condition. Sporulation occurs in the environment. Each oocyst ingested by the host may give rise to hundreds of thousands of infective oocysts in the feces within 7–12 days. Transmission from one farm to another is facilitated by the movement of personnel and equipment, and even new farms will have the parasite present within a few weeks after poultry are introduced.

Coccidiostats or Anticoccidials Drugs Used in Poultry

In intensive poultry production, the main method of controlling coccidiosis is through the addition of drugs used as anticoccidial drugs or coccidiostats in the feed at the authorized levels and observing the prescribed hygiene requirements. All coccidiostats inhibit reproduction and do not fully eliminate the parasite from the intestine of the animal avoiding losses due to acute and often lethal coccidiosis. Eggs are however, a special issue. Maximum age levels and restrictions on usage in layer feed may not always be enough to ensure that eggs are free of coccidiostats. The strategy of medications for layers is different because most anticoccidial therapy must cease at the time when the bird comes to lay eggs, to avoid the potential deposition of drug residues in eggs. The objective is to prevent clinical coccidiosis while still encouraging the development of active immunity during the first 14–20 weeks of life. Prophylactic medication with coccidiostats in the feed remains the major way of preventing coccidiosis. The biggest problem associated with this control is the development of resistance by the coccidium to all medications available for use.

Coccidiosis is generally well controlled using currently available polyether ionophore antibiotics, but the susceptibility of *Eimeria* species varies considerably with *E. maxima*, *E. acervulina*, and *E. tenella* considered the most difficult species to control. The introduction of the first polyether ionophore coccidiostat (monensin) in the 1970s represented a major achievement in the control of coccidiosis. Before this, coccidiosis outbreaks were common and more difficult to treat or prevent, as only nonpolyether ionophore coccidiostats were available and these were much less effective because of the rapid development of immunity by the parasite. The intensive use of most coccidiostats is accompanied by the development of tolerance or reduction in the susceptibility of the target population. Repeated exposure to the same coccidiostats can result in the selection of drug-resistant strains of *Eimeria* which might be expected to result in the reduced efficacy of coccidiostats. The efficacy of modern coccidiostats is affected by the severity of exposure to coccidia. Some drugs, such as the polyether ionophores, will still work if exposure to the coccidian is mild. If conditions change, the exposure level rises, these drugs are often not able to control resistant populations of the coccidian.

Outbreaks of clinical coccidiosis commonly occur when the coccidiostats are excluded from the feed during antibacterial therapy, typically at approximately 3–4 weeks of age. Combinations of antibacterials with coccidiostats help to prevent this type of outbreak. In addition, potentiated sulfonamides (e.g., sulfadimethoxine and ormetoprim in a 5:3 ratio), which have both coccidiostatic and antibacterial efficacy, can be useful. The main method of controlling coccidiosis is through the addition of coccidiostats to the feed. They need to be administered throughout the life of the bird as is in the case of chickens for fattening or broilers in order to protect against reinfection from the ever-present oocyst stage of the malady.

Coccidiostats or anticoccidial drugs can act at specific times during the life cycle or exert their effects at several phases. Coccidiostats can act on extracellular stages (sporozoites and merozoites) to prevent penetration of cells or on the intracellular stages to stop or inhibit development, and a few anticoccidials affect the sporulation of oocysts after they are excreted. There are two classes of coccidiostats: (1) Coccidiostatics, which arrest or inhibit growth of intracellular coccidian and give rise to latent infection after drug withdrawal and (2) Coccidiocides, which kill most of the coccidial stages. Some coccidiostatic drugs may be initially coccidiostatic but eventually coccidiocidal depending on factors as length of time on medication, dosage, and species of coccidia. Most coccidiostats currently used in the poultry production are coccidiocides.

Legislative Framework for Coccidiostats

The legislative background for coccidiostats or anticoccidial drugs in the European Union (EU) are regarded as feed additives or veterinary medicines products.

The requirements and procedures for the marketing authorization for veterinary medicinal products in the EU are primarily laid down in Directive 2001/82/EC and in Regulation (European Commission (EC)) No. 726/2004. These texts additionally lay down harmonized provisions in related areas such as manufacturing, wholesaling, or advertising of veterinary medicinal products. Currently, the EU is assessing the safety of all pharmacologically active substances that are administered to food-producing animals, and is attempting to set a single legally binding maximum residue limit (MRL) for each substance. These are laid down in Regulation (EC) No. 470/2009, on MRLs, Regulation (EC) No. 470/2009, repealing Council Regulation (European Economic Community (EEC)) No. 2377/90 and amending Directive 2001/82/EC, and of the Council and Regulation (EC) No. 726/2004. Under the Regulation (EEC) No. 2377/90 only those compounds listed in Annexes I, II, and III can be used in food-producing animals. Annex IV is the designation of drugs considered unsafe on consumer health grounds. Drugs in this last Annex are effectively prohibited for use in food-producing animals within the EU.

The Commission Regulation (EU) No. 37/2010 on pharmacologically active substances and their classification regarding MRL in foodstuffs of animal origin, in its annex included two tables. **Table 1** (allowed substances) lists the pharmacologically active substance, marker residue, animal species, MRL value, target tissues, other provisions (according to Article 14(7) of Regulation (EC) No. 470/2009), and therapeutic classification, and **Table 2** lists prohibited substances where an MRL cannot be established. This classification substitutes the 4 annexes of the Council Regulation (EEC) No. 2377/90.

In the EU, feed additives are regulated by different legislation than veterinary medicinal products. The legal basis for additives for use in animal nutrition is laid down in Regulation (EC) No. 1831/2003 that repeals Directive 70/524/EEC and for which authorization and prerequisites of coccidiostats for their use are defined for individual products (brand names) following review by the European Food Safety Authority (EFSA) of data provided by the applicant (**Tables 1** and **2**). Only additives that have been through an authorization procedure may be placed on the market. Authorizations are granted for specific animal species, specific conditions of use, and for 10 year periods. According to Article 6 of this regulation a feed additive shall be allocated to one or more of the following categories, depending on its functions and properties: (1) technological additives; (2) sensory additives; (3) nutritional additives; (4) zootechnical additives; and (5) coccidiostats and histomonostats. More recently, there have been included several coccidiostats of antibiotic origin to be used in chicken and turkey species under certain conditions. The EFSA is responsible for conducting the evaluation of the data submitted requesting authorizations. MRLs and postmarket monitoring plans may be established for a feed additive if deemed necessary.

Management of Resistance

A number of strategies have been developed to extend the useful life of coccidiostats, while still controlling coccidiosis. The programs used for coccidiostats are the following: (1) continuous, (2) shuttle, and (3) rotation. In some cases, birds

Table 1 Coccidiostat polyether ionophores authorized in the EU

Name of additive	*Trademark*	*Target species*	*Minimum/ Maximum (mg per kg per feed)*	*Withdrawal period (WP)*	*MRL (μg kg^{-1})*
Polyether ionophores					
Monensin sodium	125 mg kg^{-1} (Coxidin and Elancoban)	Chickens for fattening	100–125	1 day	25 (skin/fat) 8 (liver, kidney, and muscle)
	120 mg kg^{-1} (Elancoban)	Chickens reared for laying (maximum 16 weeks)	100–120		
	100 mg kg^{-1} (Coxidin and Elancoban)	Turkeys for fattening (maximum 16 weeks)	90–100		
Lasalocid sodium	125 mg kg^{-1} (Avatec)	Chickens for fattening	75–125	5 days	60 (muscle) 300 (skin/fat and liver)
		Chickens reared for laying (maximum 16 weeks)	75–125		
		Turkeys (maximum 12 weeks)	90–125		150 (kidney) 150 (eggs)
Maduramicin ammonium	5 mg kg^{-1} (Cygro)	Chickens for fattening	5	5 days	No MRL has been established
		Turkeys (maximum 16 weeks)	5		
Narasin	70 mg kg^{-1} (Monteban)	Chickens for fattening	60–70	1 day	50 (liver, kidney, muscle, and skin/fat)
Narasin + nicarbazin	40–50 mg narasin per kg + 40–50 mg nicarbazin per kg (Maxiban G160)	Chickens for fattening	80–100	0 days	50 μg narasin per kg liver, kidney, muscle, and skin/fat
Salinomycin sodium	70 mg kg^{-1} (Salinomax and Sacox)	Chickens for fattening	50–70	1 day	5 (liver, kidney, and muscle)
	(Kokcisan)		60–70		15 (skin/fat)
	50 mg kg^{-1} (Sacox)	Chickens reared for laying (maximum 12 weeks)	50	Not required	
Semduramicin sodium	25 mg kg^{-1} (Aviax)	Chickens for fattening	20–25	5 days	No MRL has been established

Abbreviation: MRL, maximum residue limit.

are given one coccidiostat continuously through succeeding flocks, but two or more coccidiostats (e.g., shuttle programs) may be given during the life of a flock being convenient to provide a particular coccidiostat for a period during which one type of feed is given. Shuttle and rotational programs can be used to block the development of drug resistance. A 'shuttle program' usually involves a change coccidiostats within a single grow-out period (between the starter and grower rations) (i.e., a chemical in the starter feed, a polyether ionophore in the grower feed, and no medications in the finisher feed) whereas a 'rotational program' has an objective to rotate the agents (i.e., rotation between medications at different times of the year) between grow-out periods (i.e., one class might be used in starter feed, another in growers, returning to the first for the finished diet followed by a withdrawal diet or nicarbazin during the fall and winter and a different medication in the spring and summers), all help to delay, or in some cases even avoid, the emergence of resistance. It is known that any resistant forms from a previous flock may survive in the litter for a short period, so drugs are usually included in the starter feed. These programs take advantage of the different coccidiostat properties with a different mode of action (e.g., between polyether ionophores that share a similar mode of action and nonpolyether ionophores), matching spectrum of activity, potency, and drug cost against risk of infection, while slowing the rate of development of resistance. It is generally accepted the rotational programs provide increased drug efficacy at the time of change by letting coccidia regain sensitivity during the off-time of each drug.

Classes of Coccidiostats or Anticoccidial Drugs

The coccidiostats or anticoccidial drugs can be grouped into two major classes. In the first class includes polyether ionophore antibiotics which are produced by fermentation with several strains of *Streptomyces* spp. and *Actinomadura* spp. comprising the following substances: monensin sodium, lasalocid sodium, maduramicin ammonium, narasin, salinomycin sodium, and semduramicin sodium. This class of polyether ionophores has limited antibacterial activity, especially against *Clostridium* and can interact with other antibiotics when they are concurrently administered. The second

Table 2 Coccidiostats nonpolyether ionophores authorized in the EU

Name of additive	*Trademark*	*Target species*	*Minimum/ Maximum (mg per kg per feed)*	*Withdrawal period (WP)*	*MRL ($\mu g\ kg^{-1}$)*
Nonpolyether ionophores					
Decoquinate	40 mg kg^{-1} (Deccox)	Chickens for fattening	20–40	3 days	No MRL has been established
Robenidine hydrochloride	36 mg kg^{-1} (Cycostat)	Chickens for fattening and Turkeys	30–36	5 days	No MRL has been established
Halofuginone hydrobromide	3 mg kg^{-1} (Stenorol)	Chickens for fattening Chickens reared for laying (maximum 12 weeks) Turkeys (maximum 16 weeks)	2–3	5 days	10 (muscle) 25 (fat) 30 (liver and kidney)
Diclazuril hydrochloride	1 mg kg^{-1} (Clinacox)	Chickens for fattening (maximum 12 weeks) Chickens reared for laying (maximum 16 weeks) Turkeys for fattening (maximum 12 weeks)	1	5 days Not required 5 days	No MRL has been established
Nicarbazin	50 mg kg^{-1} (Maxiban)	Chickens for fattening	40–50	5 days	200 (muscle, liver, kidney, and skin/fat)

Abbreviation: MRL, maximum residue limit.

class includes the nonpolyether ionophores (often referred as synthetic compounds or chemicals).

Polyether Ionophore Antibiotics

Polyether ionophores are produced by saprophytic fungi, predominantly *Streptomyces* spp. or *Actinomadura* spp. (i.e., maduramicin and semduramicin) containing mycelial biomass (maduramicin can be extracted into granular carrier); continuing commercial success is due to their broad-spectrum activity against pathogenic *Eimeria* spp. in poultry and also to a prolonged absence of serious problems with drug resistance.

In vitro studies with monensin, salinomycin, or lasalocid reveal that sporozoites of *E. tenella* are damaged after 24 h incubation, but are not if incubation is shortened (20 min); under laboratory conditions, polyethers allow a relatively high oocyst output due obviously to their lack of activity against sporozoites *in vivo*. It has been shown that these polyether ionophores do not only cause irreversible damage to free merozoites and mature schizonts of first- and second-generation coccidia but also to erythrocytic stages of various chloroquine-resistant malaria parasites.

Polyether ionophores can have both anticoccidial and antibacterial activity, and the group is used extensively in poultry. Other uses are as growth-promoting agents (nowadays forbidden in the EU) and as an active compound against clostridiosis.

Polyether ionophores (ion carriers) facilitate movement of some monovalent cations, such as sodium and potassium, and divalent cations such as calcium and magnesium across cell membranes. Polyether ionophores as a class are divided into two general groups based on the mode of ion transfer across membranes. These include channel formers and ion carriers. Channel-forming ionophores arrange themselves inside the membrane structure creating a hydrophilic channel for the ions. By this means, ions from outside the cell pass through the provided hydrophilic channel into the cell. There are two subclasses of ion carrier ionophores: (1) neutral ionophores and (2) carboxylic ionophores. Both neutral and carboxylic ionophores are compounds that form lipid-soluble dynamically reversible complexes with cations and by this means facilitate specific ionic transport across biological membranes. Neutral ionophores are highly toxic because they form charged complexes that are capable of perturbing biologic membranes and action potentials. The carboxylic polyether ionophores are open-chained oxygenated heterocyclic rings with a single terminal carboxyl group of moderate molecular weight (200–2000) which form zwitterionic complexes with cations and promote electrically neutral cation exchange diffusion that is tolerated better in intact organisms. They form lipid-soluble complexes with polar cations (K^+, Na^+, Ca^{2+}, and Mg^{2+}) enhancing their transport across biomolecular lipid membranes. The transport of specific ions (Na^+, K^+, and Ca^{2+}) across membranes is responsible for multiple pharmacologic effects at the cellular level. The carboxylic polyether ionophores mediate concentration-dependent, pH-sensitive transport of ions independent of membrane potentials. They act as 'exchange diffusion' carriers, transporting a cation during both stages of the exchange cycle.

Members of the polyether ionophores group are classified as either monovalent or divalent on the basis of their affinity for particular cations. The monovalent ionophores (salinomycin, monensin, narasin, maduramicin, and semduramicin) tend to combine more readily with Na^+ and K^+, whereas

lasalocid, a divalent ionophore combines with Na^+ and K^+, as well as with Ca^{2+} and Mg^{2+}. It is known that monensin, classified as an Na^+-selective ionophore, binds to Na^+ outside the cell, and carries it into the cell, then produces high intracellular Na^+ concentrations. Na^+ influx is compensated by the much slower K^+ efflux where an initial H^+ (proton) efflux may result in intracellular alkalosis. With salinomycin and narasin and others that show K^+ selectivity, the K^+ egress is countered by an initial H^+ ingress, which could result in intracellular acidosis. Overall, monensin forms monovalent complexes with Na^+, whereas salinomycin and narasin preferentially complex K^+ and lasalocid Ca^{2+}.

The carboxylic polyether ionophores direct their effects at both the asexual and sexual cycles of coccidian causing the normal transport of Na^+ and K^+ ions across surface membranes to fail. The polyether ionophores are coccidiocidal because of their ability to preferentially move ions, usually Na^+, into various stages of the parasite, altering ionic balance in the coccidian. Because coccidia intracellular parasites cannot make adenosine triphosphate (ATP) to drive their Na–K ATPase pumps, the coccidial organism loses the ability to osmoregulate and increasing intracellular sodium is transferred to other organelles. Polyether ionophores are not used in human beings for prophylaxis or therapy.

In general, polyether ionophores, when administered orally to chickens, are rapidly absorbed from the gut and widely distributed to the tissues. Its residues in food products are of special concern because of the high degree of toxicity of these drugs to many species.

Monovalent cationic

Monensin Monensin was the first carboxylic polyether ionophore introduced in the USA for the control of coccidiosis in 1971. Monensin belongs to the polyether monocarboxylic acid chemical family, and it is produced by the fermentation of a *Streptomyces cinnamonensis* strain. Monensin is used in the prevention of coccidiosis in poultry caused by *E. necatrix*, *E. tenella*, *E. acervulina*, *E. brunette*, *E. maxima*, and *E. mivati*. Monensin sodium is a coccidiostat agent that is authorized in the EU as a feed additive in poultry. Monensin sodium is active mainly against Gram-positive bacteria, is absorbed and metabolized extensively by chickens and turkeys, and its metabolic fate is similar in both species. Unchanged monensin represents a limited fraction of the excreted monensin-related compounds. The monensin residues were readily detected in liver (giving the highest residue concentrations), fat, and muscle between 0 and 2 days postdrug withdrawal. Monensin residues are sometimes not detectable at all in the eggs of treated hens. For practical reasons, the skin/fat has been retained as the target tissue in poultry. A great number of metabolites have been isolated from the excreta and tissues. The main excretion route for monensin is fecal and metabolites are produced by either single or combined demethylation, decarboxylation, and hydroxylation reactions.

Salinomycin Salinomycin was approved for chickens in 1983. It is a sodium salt of a polyether monocarboxylic acid produced by *Streptomyces albus*. Salinomycin sodium is authorized in the EU as a coccidiostat feed additive. It is used in the prevention of coccidiosis in poultry caused by *E. necatrix*, *E. tenella*, *E. acervulina*, *E. brunette*, *E. maxima*, and *E. mivati*. Salinomycin is active against certain Gram-positive bacteria, whereas Enterobacteriaceae are resistant. In addition, it is known that salinomycin has an inhibitory effect on *Clostridium perfringens*, therefore the use of salinomycin leads to a decrease in the incidence of necrotic enteritis in broiler chickens. Salinomycin is absorbed to a certain extent in the chicken and extensively metabolized although the concentration present in the edible tissues of the animals are very low and scarcely detectable. Salinomycin is considered as the marker residue and skin/fat as the target tissue. Salinomycin produces relatively low residue levels in the eggs compared with other polyether ionophores.

Narasin Narasin was approved for chickens in 1986. Narasin is a polyether antibiotic produced by the fermentation of a strain of *Streptomyces aureofaciens*. Narasin is authorized as a coccidiostat feed additive for the control of *Eimeria* infection in chickens for fattening. Narasin, at the levels used for the treatment of coccidiosis, is also effective in the prevention of necrotic enteritis in chickens. The antimicrobial spectrum of narasin is mainly limited to Gram-positive bacteria, including enterococci, staphylococci, and *C. perfringens*. Narasin is absorbed to an unknown extent and excreted rapidly via feces by the chicken. The main metabolic pathway in the chicken and rat involves oxidative processes leading to the formation of very similar metabolites in both species in terms of chemical structure and biological activity. For food control purposes narasin can be selected as a marker residue and skin/fat as the target tissue. Narasin is used also in combination with nicarbazin for chickens for fattening. The passage of narasin into egg yolk has been reported.

Maduramicin Maduramicin was approved for chickens in 1989. Maduramicin ammonium is a polyether carboxylic ionophore agent that is authorized as a coccidiostat feed additive in the chicken and turkey for the control of *E. adenoides, E. meleagrimitis, E. gallopavonis, and E. dispersa*. Maduramicin can inhibit the growth of Gram-positive microorganisms. Maduramicin and/or its metabolites are rapidly eliminated in chickens. Steady state is observed after 3 days in the excreta, but after 6 days in plasma. Maduramicin is the main compound excreted (26%). *O*-Demethylation represents the main metabolic pathway of maduramicin in chickens. Maduramicin is the marker residue in all tissues tested. Tissue residue kinetics of total residues and marker residue indicate a rapid decline of residues in all tissues.

Semduramicin Semduramicin was approved for chickens in 1994. It is a monocarboxylic acid polyether ionophore produced by the fermentation *of Actinomadura roseorufa* and is authorized in the EU as a coccidiostat feed additive against poultry *Eimeria*. Gram-negative aerobic and anaerobic bacteria were not affected by semduramicin. *Clostridium perfringens, Clostridium difficile, Eubacterium limosum*, and Groups C and E *Streptococci* were more susceptible. Following a 7-day oral administration of the ^{14}C-uniformally labeled compound at the dose corresponding to the intended use, i.e., 25 mg kg^{-1} in feedstuffs, the total radioactivity measured in

plasma at slaughter following a 6-h withdrawal period was very low (0.025 μg ml^{-1} equivalent semduramicin). No MRL has been established for semduramicin in animal tissues and eggs.

Divalent cationic

Lasalocid sodium Lasalocid has been marketed since 1977 for chickens. Lasalocid sodium belongs to the divalent polyether ionophore family and is produced by the fermentation of *Streptomyces lasaliensis* subsp. *lasaliensis*. It is a polyether carboxylic ionophore agent that is authorized as a coccidiostat in the EU. Lasalocid can selectively destroy intracellular sporozoites while remaining relatively non-injurious to the host cell. Lasalocid sodium is effective in controlling the coccidiosis in chickens and turkeys and has a selective antimicrobial activity against Gram-positive bacteria whereas many Gram-negative strains are naturally resistant. Lasalocid sodium is absorbed and metabolized into a number of metabolites; none of which have been identified in the excreta or in tissues. The liver is the target tissue. Lasalocid residues may persist in edible tissues for longer periods than either salinomycin or monensin and may require a substantially longer withdrawal period than any of the other polyether ionophore compounds. The marker residue has not been identified. Lasalocid produced the highest residue concentrations in eggs compared with other ionophores.

Nonpolyether Ionophores (Synthetic Compounds or Chemicals)

Decoquinate

Decoquinate was first described in 1968. It belongs to the quinolone derivative group, and has been used as a coccidiostat in various animal species. Other substances belonging to the quinolone derivative group are buquinolate and methylbenzoquate. Decoquinate is a 4-hydroxyquinolone (ethyl-6-decyloxy-7-ethoxy-4-hydroxyquinoline-3-carboxylate) used for the prevention of coccidiosis in broiler chickens caused by *E. tenella, E. necatrix, E. acervulina, E. mivati, E. maxima,* or *E. brunetti*. It acts on the sporozoite stage of the life cycle by disrupting electron transport in the mitochondrial cytochrome system of coccidia. Its main use is in shuttle programs and withdrawal feeds. Rapid emergence of resistance to this compound and others as buquinolate and nequinate has been their major weakness. For that reason, clinical use remained limited, as there was evidence of the emergence of resistance of coccidia against decoquinate when used for a longer period on the same farm. Decoquinate can have an anticoccidial effect on first-generation schizonts of *E. tenella*, adversely affect sporulation, and permit the development of immunity if fed at levels lower than its coccidiostatic levels. Decoquinate is only absorbed to a small extent from the gastrointestinal tract of chicken, and once absorbed, is rapidly cleared from blood and tissues via bile and to a much lesser extent in urine. Decoquinate is distributed widely in tissues. Skin/fat is the target tissue.

Robenidine

Robenidine hydrochloride (*N*1,*N*3-bis(*p*-chlorobenzylidene) amino]guanidine hydrochloride) belongs to a chemical group of guanidines and is used in poultry and rabbits. Initially it acts as a coccidiostat and arrests the development of the first-generation schizonts of *E. tenella* by preventing the formation of merozoites. An additional effect on gametogeny has been identified. Robenidine hydrochloride is authorized as a coccidiostat feed additive in the EU and it is primarily active against Gram-positive bacteria. It is absorbed to a limited extent and excreted rapidly. Its metabolic fate has been established involving the splitting of the molecule at the semicarbazide bonds, oxidation of the *p*-chlorobenzaldehyde formed to *p*-chlorobenzoic acid, and conjugation of the latter with amino acids. Unchanged robenidine is the major compound excreted by chicken and turkey. Robenidine is the marker residue in chicken and turkey. In chickens, it is not completely absorbed from the GI tract; the absorbed portion is well distributed to tissues and extensively metabolized. Excretion occurs over several days following oral administration. Residues are detected in the eggs, primarily the yolk, of treated hens for weeks after medication is withdrawn.

Amprolium

Amprolium is structurally similar to vitamin B_1. When it is administered orally to chickens, it has a low bioavailability, but the absorbed fraction is widely distributed into the tissues and rapidly eliminated in the urine and feces. Amprolium administered to laying hens is deposited primarily in the egg yolk, and residues can be detected in eggs for 10 days or more after cessation of treatment, depending on the dose and assay sensitivity.

No MRL has been established for this compound.

Halofuginone

Halofuginone (DL-*trans*-7-bromo-6-chloro-3-[3-(3-hydroxy-2-piperidyl)-acetonyl]-4(3H)-quinazolinone hydrobromide) is a potent anticoccidial that belongs to the chemical group of quinazolinones. It is effective against most asexual stages of *Eimeria* spp. in chickens and turkeys. It has both coccidiostatic and coccidiocidal effects. When halofuginone is given to laying hens at low doses in the feed, residues of this substance are not detectable in eggs, but at high doses, residues can be detected from days to weeks, depending on the concentration level added to the feed. Residues appeared at similar levels in eggs (yolk and albumen), but residues are more persistent in yolk.

Diclazuril

Diclazuril is a synthetic compound of the triazines group that is used as a feed additive and veterinary drug for the control of coccidiosis. Other substances of this group are clazuril (authorized as anticoccidial drug for use orally in pigeons) and toltrazuril. Chemically, the structure of diclazuril is 2,6-dichloro-α-(4-chlorophenyl)-4-(4,5-dihydro-3,5-dioxo-1,2,4-triazin-2(3H)-yl)benzeneacetonitrile. In chickens and turkeys infected by a single *Eimeria* strain or mixed *Eimeria* strains, diclazuril showed anticoccidial activity at all the doses tested (diclazuril acts on the asexual or sexual stages of coccidia blocking the excretion of oocysts). In birds, the highest plasma concentration of ingested radioactivity was observed 6 h after a single administration. Plasma radioactivity was due almost exclusively to unchanged diclazuril until 72 h

after dose. Diclazuril cannot be used in animals from which eggs are produced for human consumption.

Toltrazuril

Toltrazuril is a triazinetrione derivative used as an anticoccidial agent. It is widely used in chickens, turkeys, pigs, and cattle for the prevention and treatment of coccidiosis, by administration in drinking water. Following repeated oral administration to chickens of ^{14}C-toltrazuril during 2 days, 50% and 90% of the total radioactivity administrated was eliminated by 4.5 and 15.5 days after dosing, respectively. In turkeys, following repeated administration of unlabeled toltrazuril for 2 days, toltrazuril concentrations in plasma were low and the unchanged compound was not detected 24 h after the last dose. Toltrazuril sulfone was the major metabolite, and 8 days after the last administration in chickens, represented 100% of the total radioactivity in muscle and fat and 80% in the liver. Toltrazuril sulfone is the most appropriate marker residue for chicken and turkey. MRL has been established for this compound in poultry: 200 $\mu g\,kg^{-1}$ (skin/fat), 600 $\mu g\,kg^{-1}$ (liver) and 400 $\mu g\,kg^{-1}$ (kidney). Toltrazuril cannot be used in animals from which eggs are produced for human consumption.

Nicarbazin

Nicarbazin is an anticoccidial belonging to carbanilide group. Nicarbazin is marketed as a coccidiostat for chickens for fattening up to an age of 28 days. Nicarbazin is a synthetic complex composed of an equimolar amount of 4,4′-dinitrocarbanilide (DNC) and 2-hydroxy-4,6-dimethylpyrimidine (HDP). DNC is also known as *N*,*N*′-bis(4-nitrophenyl) urea. The mode of action seems to be via inhibition of succinate and the energy-dependent transhydrogenases, and the accumulation of calcium in the presence of ATP. Nicarbazin causes the destruction of second-generation schizonts suggesting a coccidiocidal mode of action. However, there were also findings indicating a coccidiostatic action or both. Nicarbazin is active on several *Eimeria* strains. Nicarbazin once ingested is rapidly separated into its two components, HDP and DNC. DNC from nicarbazin was considerably more available to the animal than DNC given alone or administered simultaneously with HDP in similar proportions as in nicarbazin. DNC appeared as the marker residue, and the liver was the target tissue. DNC residues declined rapidly from tissues following nicarbazin withdrawal. HDP-related residues are much lower than those derived from DNC. Nicarbazin should not be used in layers because of decreases in egg production.

Sulfonamides

Sulfonamides are simple synthetic compounds related to *para*-aminobenzoic acid (PABA). Trimethoprim and pyrimethamine do not contain sulfur. Many bacteria absorb *p*-aminobenzoate, convert it into dihydrofolate and tetrahydrofolate, and then ultimately use it to synthesize purines for nucleic acid synthesis. By virtue of their chemical resemblance to *p*-aminobenzoate, sulfonamides inhibit the synthesis of folates by blocking the conversion of PABA to dihydrofolic acid. Trimethoprim and pyrimethamine are diaminopyrimidine derivatives that inhibit the conversion of dihydrofolate to tetrahydrofolate by bacterial forms of dihydrofolate reductase.

Sulfonamides (bacteriostatic) and trimethoprim (bactericidal) are commonly used in combination where they are bactericidal, because (1) they inhibit two different reactions on the same metabolic pathway and thus exhibit synergistic activity and (2) the combination reduces the likelihood that resistance will develop. Sulfonamides have been used in poultry in the prevention and treatment of coccidiosis. The potentiated sulfonamides have a fairly broad spectrum of activity.

Many organisms once susceptible are now resistant to sulfonamides, often due to single-step mutations in the target enzyme. However, useful activity still includes many Gram-positive and Gram-negative organisms as well as *Chlamydia*. Most sulfonamides in this class are absorbed from the gastrointestinal tract and distribute into most tissues including the central nervous system. Mainly they are eliminated unchanged in urine, although some undergo modification in the liver before elimination in urine. The rate at which sulfonamides are eliminated in the urine varies according to the extent to which they are protein bound. Sulfamerazine and sulfadimethoxine are frequently combined with trimethoprim for oral administration in sporadic coccidiosis outbreaks.

Resistance to sulfonamides and trimethoprim is widespread and operates by various mechanisms (chromosomal mutation in the target enzyme, plasmid-mediated acquisition of alternative forms of the target enzymes, reduced permeability of the cell membrane, and altered means of PABA utilization).

Drug Residues from Coccidiostat Use

Residues are low levels of the drug and its metabolites which remain in the animal carcass or other food products of animal origin following drug administration.

For food-producing animals, studies are required to observe how rapidly residues of veterinary drugs are eliminated from the animal. The data obtained in these studies are available to predict a preslaughter withdrawal time appropriate to allow depletion of the residue to acceptable levels (i.e., MRLs) in the target tissues. To define the depletion profile for the major edible tissues and products (e.g., muscle, liver, skin/fat, and kidney of slaughtered animals as well as eggs) a minimum number of animals per slaughter time and eggs per time point are needed.

The MRL is the highest concentration of a residue for a particular chemical that is legally permitted in food. An appropriate withdrawal period (for products for food-producing animals) is then established by regulatory authorities to ensure that the residues are depleted to below the MRL.

Residues are a problem if they still persist at unacceptable levels at slaughter, as they then expose the consumer to potentially unsafe residues.

MRL

The MRLs (termed tolerances in the USA) are established to set a maximum level of the coccidiostatic substances that may remain in the animal without posing a risk to consumers of

food derived from the animal. MRLs are the points of reference for the establishment of withdrawal periods required for marketing authorizations of veterinary medicinal products used in food-producing animals. Withdrawal periods are essential for the control of residues in the food of animal origin.

To establish MRLs for a given drug requires provision of the following data: knowledge of dosage schedule (dose, frequency, and duration of treatment) and administration route; metabolic and pharmacokinetic data in laboratory animals and each of the target food-producing animals; distribution and residue depletion data for the major edible tissue (i.e., muscle, fat, liver, and kidney) in each target species using radiolabeled drug; validated analytical methods for the detection and quantitation of residues, including the marker residue; and data defining the effect of residues on food processing.

The toxicological data are used not only to characterize the biological properties of the molecule but also to identify a no observed adverse effect level (NOAEL), which in turn is used to calculate an acceptable daily intake (ADI), which represents the quantity of drug residue that can be safely consumed daily for a lifetime without an adverse effect on consumer health. For substances with an ADI derived from toxicological endpoints, all metabolites are considered to have the same toxicological significance as the parent drug unless that metabolite has greater toxicity than the parent drug. The MRL is then elaborated from this ADI along with knowledge of the depletion kinetics of the drug in the animal or its milk or eggs, and with reference to standard consumption values for particular types of food. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) uses a model diet (daily food basket) approach which overestimates daily consumption, which for poultry is: 300 g muscle, 100 g liver, 10 g kidney, 90 g fat, and 100 g eggs. The toxicity study (or studies) used to identify the NOAEL for the critical effect may be referred to as the pivotal study (or studies). To reduce the uncertainty associated with the imprecision of the NOAEL and to make full use of the dose–response curve, proposals have been made for a more precise calculation of a dose that produces a small increase in the level of adverse responses. This dose is referred to as a 'benchmark dose' (BMD) and could be used as an alternative to the NOAEL in order to derive an ADI. By agreement, a default safety (uncertainty) factor of 100 (a10-fold factor for interspecies differences (extrapolation from laboratory animals to humans) and a 10-fold factor for human interindividual variation) is normally used. When an NOAEL has not been identified for the most appropriate endpoint, the lowest observed adverse effect level (LOAEL) can be used. Drugs may also produce adverse effects after short-term high-level exposure and in such cases, JECFA will establish an acute reference dose (ARfD) in addition to the ADI. The identification of NOAELs, the calculation of ADI values, and the establishment of MRLs is a complex scientific process involving toxicology, pharmacology, microbiology, residue depletion kinetics, and analytical method, but the MRL values ensure that the ADI will not be exceeded by the consumer when eating foods of animal origin.

The MRL has been calculated to ensure that the dietary exposure is lower than the relevant ADI. The MRL is the point on the residue depletion curve at which the concentration of the marker residue in the target tissue depletes to describing the upper one-sided 95% confidence limit over the 95th percentile. Actually JECFA uses the median of the residue distribution to substitute for the MRL in the dietary exposure estimate (called the estimate daily intake) and changes the model for exposure assessment.

The EU MRL values for drugs used as anticoccidials in food-producing animals are listed in Table 3.

Drug Withdrawal/Withholding Periods

A critical factor in the medication of all food-producing animals is the mandatory withdrawal period, defined as the time during which the coccidiostat must not be administered prior the slaughter of the animal for consumption or the taking of food products produced, for example, eggs. Withdrawal periods are set to ensure that any remaining residues are below the MRL. This is an integral part of the regulatory authorities' approval process and is designed to ensure that no significant drug residue is present in the bird at slaughter based on the MRL established. Adherence to the withdrawal period provides assurance that food derived from treated animals will not exceed the MRL for the drug substance. The withdrawal period is intended to ensure that no harmful residues remain in edible tissues and products after slaughter. Failure to observe the preslaughter withdrawal period while using an animal drug is the major cause of violative drug residues in poultry and egg production in the EU. Even if the withdrawal period is only a few days or few hours, the resulting residues can violate the national regulations against sale of adulterated foodstuffs which can cause distortions in competition between Member States of the EU.

Carryover or Cross Contamination of the Feed of Coccidiostats

Different studies have shown that an entire contamination-free production of premixes and compound feeds in existing multiproduct plants is not possible in practice. Practical experience indicates that in feed mills, residual quantities of medicated feedingstuffs may be retained at various points along the production line and end up in the beginning of the production of another feed product, contaminating subsequent batches of feed as they are processed. This unavoidable carryover may occur at all stages of production and processing of feed but also during storage and transport. EFSA defined cross contamination as contamination of feeds that are produced after the production of a mixed feed, containing additives with residual amounts of the previous feed batch. For example, some feed additives and premixes with properties, such as strong adhesion to walls, particle size and density, and electrostatic properties, may influence cross-contamination behavior and affect how cross-contamination occurs. The electrostatic properties of some drugs, particularly those in powder form, aggravate the problem, making it more difficult to clean the equipment between batches. The technological equipment in the feed mill, such as the design of dosage and grinding and mixing equipment, can influence the

Table 3 EU MRLs for drugs used as anticoccidials (Regulation (EU) No. 37/2010 of 22 December 2009)

Pharmacologically active substance(s)	*Marker residue*	*Animal species*	*MRLs*	*Target tissues*	*Other provisions*	*Therapeutic classification*
Amprolium	Not applicable	Poultry	No MRL required	Not applicable	For oral use only	No entry
Clazuril	Not applicable	Pigeon	No MRL required	Not applicable	No entry	No entry
Decoquinate	Not applicable	Bovine and ovine	No MRL required	Not applicable	For oral use only Not for use in animals from which milk is produced for human consumption	No entry
Diclazuril	Not applicable	All ruminants and porcine	No MRL required	Not applicable	For oral use only	No entry
		Poultry	500 $\mu g\ kg^{-1}$ 500 $\mu g\ kg^{-1}$ 1500 $\mu g\ kg^{-1}$ 1000 $\mu g\ kg^{-1}$	Muscle Skin/fat Liver Kidney	Not for use in animals from which eggs are produced for human consumption	Antiparasitic agents/agents acting against protozoa
Halofuginone	Halofuginone	Bovine	10 $\mu g\ kg^{-1}$ 25 $\mu g\ kg^{-1}$ 30 $\mu g\ kg^{-1}$ 30 $\mu g\ kg^{-1}$	Muscle Fat Liver Kidney	Not for use in animals from which milk is produced for human consumption	Antiparasitic agents/agents acting against protozoa
Lasalocid	Lasalocid A	Poultry	60 $\mu g\ kg^{-1}$ 300 $\mu g\ kg^{-1}$ 150 $\mu g\ kg^{-1}$ 150 $\mu g\ kg^{-1}$	Muscle Skin + fat and liver Kidney Eggs	No entry	Anti-infectious agents/antibiotics
Monensin	Monensin A	Bovine	2 $\mu g\ kg^{-1}$ 10 $\mu g\ kg^{-1}$ 30 $\mu g\ kg^{-1}$ 2 $\mu g\ kg^{-1}$ 2 $\mu g\ kg^{-1}$	Muscle Fat Liver Kidney Milk	No entry	Anti-infectious agents/antibiotics

Toltrazuril	Toltrazuril sulfone	All mammalian food-producing species	100 μg kg^{-1} 150 μg kg^{-1} 500 μg kg^{-1} 250 μg kg^{-1}	Muscle Fat Liver Kidney	Not for use in animals from which milk is produced for human consumption	Antiparasitic agents/agents acting against protozoa
		Poultry	100 μg kg^{-1} 200 μg kg^{-1} 600 μg kg^{-1} 400 μg kg^{-1}	Muscle Skin + fat Liver Kidney	Not for use in animals from which eggs are produced for human consumption	
Sulfonamides (all substances belonging to the sulfonamide group)	Parent drug	All food-producing species	100 μg kg^{-1} 100 μg kg^{-1} 100 μg kg^{-1} 100 μg kg^{-1}	Muscle Fat Liver Kidney	The combined total residues of all substances within the sulfonamide group should not exceed 100 μg kg^{-1} For fin fish the muscle MRL relates to 'muscle and skin in natural proportions' MRLs for fat, liver, and kidney do not apply to fin fish Not for use in animals from which eggs are produced for human consumption	Anti-infectious agents/ chemotherapeutics
		Bovine, ovine, and caprine	100 μg kg^{-1}	Milk		
Trimethoprim	Trimethoprim	All food-producing species	50 μg kg^{-1} 50 μg kg^{-1} 50 μg kg^{-1} 50 μg kg^{-1} 50 μg kg^{-1}	Muscle Fat Liver Kidney Milk	For porcine and poultry species the fat MRL relates to 'skin and fat in natural proportions' Not for use in animals from which eggs are produced for human consumption	Anti-infectious agents/ chemotherapeutics

Abbreviation: MRLs, maximum residue limits.

Table 4 Maximum levels in foodstuffs (Regulation (EC) No. 124/2009, and Regulation (EU) No. 610/2012)

Substance	*Foodstuffs*	*Maximum content in $\mu g\ kg^{-1}$ (ppb) wet weight*
Lasalocid sodium	Food of animal origin from animal species other than poultry and bovine	
	– Milk	1
	– Liver	50
	– Kidney	20
	– Other food	5
Narasin	Food of animal origin from animal species other than chickens for fattening	
	– Eggs	2
	– Milk	1
	– Liver	50
	– Other food	5
Salinomycin sodium	Food of animal origin from animal species other than chickens for fattening and rabbits for fattening	
	– Eggs	3
	– Liver	5
	– Other food	2
Monensin sodium	Food of animal origin from animal species other than chickens for fattening, turkeys, and bovine (including dairy cattle)	
	– Liver	8
	– Other food	2
Semduramicin	Food of animal origin from animal species other than chickens for fattening	2
Maduramicin	Food of animal origin from animal species other than chickens for fattening and turkeys	
	– Eggs	12
	– Other food	2
Robenidine	Food of animal origin from animal species other than chickens for fattening, turkey and rabbits for fattening and breeding	
	– Eggs	25
	– Liver, kidney, skin and fat	50
	– Other food	5
Decoquinate	Food of animal origin from animal species other than chickens for fattening, bovine, and ovine except dairy animals	20
Halofuginone	Food of animal origin from animal species other than chickens for fattening, turkeys, and bovine except dairy cattle	
	– Eggs	6
	– Liver and kidney	30
	– Milk	1
	– Other food	3
Nicarbazin (residue: 4,4′-dinitrocarbanilide)	Food of animal origin from animal species other than chickens for fattening	
	– Eggs	300
	– Milk	5
	– Liver	300
	– Kidney	100
	– Other food	50
Diclazuril	Food of animal origin from animal species other than chickens for fattening, guinea fowl, rabbits for fattening and breeding, ruminants, and porcine	
	– Eggs	2
	– Liver and kidney	40
	– Other food	5

level of cross contamination. Cross contamination of feed batches can result in the exposure of nontarget animals and induce adverse health effects in these animals due to a specific sensitivity of mammalian species as compared to poultry. Residue formation in edible tissues of nontarget species may result in unexpected human exposure through the consumption of animal products.

The legal basis of this technological process in the EU is Regulation (EC) No. 183/2005 which provides that feed business operators shall ensure that establishments under

their control are approved by the competent authority in case these establishments are manufacturing and/or placing on the market coccidiostats and histomonostats or premixes containing coccidiostats and histomonostats.

The maximum tolerances for the presence of active substances contained in coccidiostats have been established in the food of animal origin and in feedingstuffs (Table 4).

Carryover or cross contamination has been evaluated. Regular investigations have been performed with some coccidiostats, including lasalocid and nicarbazin and showed the persistence of these compounds in various feed batches produced after the intentional incorporation of a polyether ionophore coccidiostat into feed. The health risk to nontarget species resulting from the consumption of cross-contaminated feed with coccidiostats at levels of 2%, 5%, or 10% was evaluated by the EFSA and a revision of the risk assessments has been done.

The MRLs for the coccidiostats lasalocid sodium, narasin, salinomycin sodium, monensin sodium, semduramicin, maduramicin, robenidine, decoquinate, halofuginone, nicarbazin, and diclazuril are updated in Table 3.

See also: Foodborne Diseases: Overview of Chemical, Physical, and Other Significant Hazards. Veterinary Drugs Residues: Veterinary Drugs – General

Further Reading

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VETERINARY DRUGS RESIDUES

Ectoparasiticides

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Glossary

Acaricide Chemical used for the control of ticks.
Ectoparasiticide Chemical used for the control of ectoparasites.
Endectocide A drug effective against both endo- and ectoparasites.
Insecticides Chemicals used for insect control.
Metaphylaxis The administration of drugs to herds of animals as therapy for sick animals combined with prophylaxis in healthy animals.
Prophylaxis The administration of drugs to herds of animals to prevent disease.
Synergist Chemical used to enhance the parasiticidal activity of certain active ingredients or help to overcome resistance.

Introduction

Food-producing animals are infected by a number of ectoparasites, including lice, ticks, mange mites, myiasis larvae, and nuisance flies. Infection with these parasites causes irritation leading to fleece and hide damage, due to the direct damage caused by the parasites and/or self-inflicted trauma. When infestation reaches critical levels it can also result in reduced feed intake, weight loss, poor body condition, reduced milk yield and wool production. In addition, many ectoparasites also act as vectors for several important diseases, such as babesiosis, theileriosis, and trypanosomosis. Control of the parasites is largely based on the use of chemicals that are usually applied topically to the skin and have a direct effect on the parasite. Some ectoparasiticides can also be given parenterally which will then act systemically when the parasite takes up the compound while feeding on the host.

Summary of Each of the Individual Drug Classes

Most ectoparasiticides are neurotoxins, exerting their effect on the nervous system of the target parasite. Those used in large animals can be grouped according to structure and modes of action into the organochlorines, organophosphates and carbamates, pyrethrins and pyrethroids, formamidines, macrocyclic lactones (MLs) (avermectins and milbemycins), insect growth regulators, and a number of miscellaneous compounds, including synergists such as piperonyl butoxide (Table 1).

Owing to widespread resistance and concerns regarding the environmental persistence of organochlorines, some products have been withdrawn from the market in many parts of the world. Organophosphates are extremely toxic in animals and humans leading to chronic toxicity, and several compounds possess teratogenic potential and are seldom used today. Carbamate insecticides are closely related to organophosphates but they cause a spontaneously reversible block on acetylcholinesterase. The main carbamate compounds used today are carbaryl and propoxur. Carbaryl has low mammalian toxicity but may be carcinogenic.

Natural pyrethrins are derived from the chrysanthemum plant and synthetic pyrethorids (SPs) are synthesized chemicals modeled on the natural pyrethrin molecule. The SPs are more stable with longer residual activity and higher potency, providing a rapid knock-down effect on insects. Some preparations contain piperonyl butoxide, which acts as a synergist by helping to prevent the pyrethrin or pyrethroid metabolism by microsomal mixed-function oxidase systems in insects. The mode of action appears to be an interference with Na^+ channels of both peripheral and central neurons, resulting in delayed repolarization and eventual paralysis.

The amidines (formamidines) are acaricides inhibiting monoamine oxidase, which is responsible for the metabolism of neurotransmitter amines in ticks and mites. Amitraz causes rapid detachment of blood feeding acarids. They are also octopamine receptor agonists in insects.

The avermectins and milbemycins belong to a family of compounds called the macrocyclic lactones and are natural fermentation products of soil dwelling streptomycete microorganisms. MLs are large complex ringed structures; avermectins have a 16-membered macrocyclic ring, containing a spiroketal group, a benzofuran ring, and disaccharide or monosaccharide functionalities as can be seen in Figures 1 and 2. Milbemycins are structurally similar to avermectins but lack the disaccharide group. Ivermectin was the first antiparasitic agent that showed broad-spectrum activity against both nematodes and arthropods. This unique broad-spectrum activity against both endo- and ectoparasites has resulted in the MLs being classified as endectocides. The activity against

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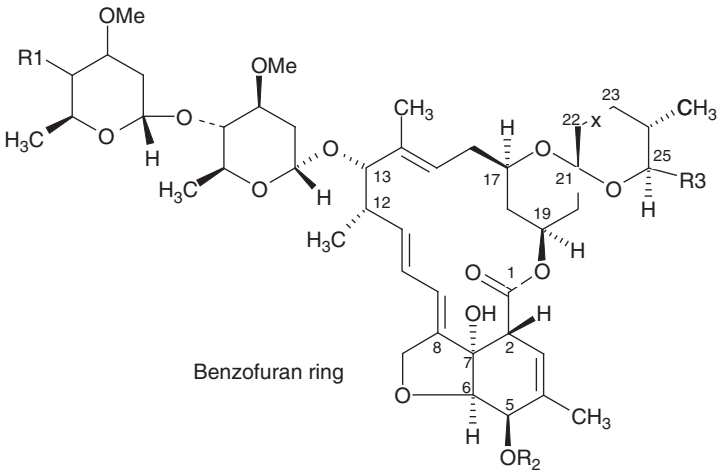
Table 1 Summary of major chemical classes of ectoparasiticides used in food-producing animals

Drug class	*First introduction*	*Examples*	*Application*	*Used*	*Safety*
Organochlorines	1940	Dichlorodiphenyltrichloroethane, dieldrin, and benzene hexachloride	Control of most ectoparasites. Long persistence in coat/fleece	Widely used to control flystrike (fly larvae) and sheep scab in sheep	Toxicity = central nervous system stimulation. No longer in use in many parts of the world due to human and environmental safety issues
Organophosphates	1950	Chlorfenvinphos, diazinon, malathion, and phosmet	Control of most ectoparasites. Long persistence in coat/fleece	Used against fly larvae, flies, lice ticks, and mites	Extremely toxic in animals and humans, some compounds also teratogenic
Pyrethrins and synthetic pyrethroids	1970	Cypermethrin, deltamethrin, flumethrin, and permethrin	Spray, dip, pour-on, or spot-on formulations	Biting and nuisance flies, lice, and ticks. Some compounds also effective against mange mites	Among the safest ectoparasiticides in mammals and birds but are highly toxic to fish and aquatic invertebrates
Formamidines	1960	Amitraz	Spray, dip, pour-on, or spot-on formulations	Control of ticks, lice, and mites in cattle, sheep, and pigs	Relatively wide safety margin in mammals; the most frequently associated adverse effect is sedation, which may be associated with an agonist activity of amitraz on α2-receptors in mammalian species
Macrocyclic lactones	1980	Avermectin – abamectin, doramectin, and eprinomectin. Milbemycin – moxidectin, and milbemycin oxime	Orally, parenterally, or topically as pour-on or spot-on formulations	Parenteral administration more effective against sucking lice and certain mites, whereas topically applied products more suitable for controlling biting lice and flies. Usually little effects against ticks. Used in cattle, sheep, and pigs	Generally wide safety margin. Persistent in body for extended periods
Insect growth regulators	1970	Methoprene, lufenuron, dicyclanil and cyromazine	Oral or topical spray formulations	Mainly used for blowfly control in sheep and fly control in poultry	They constitute a group of chemical compounds that do not kill the target parasite directly, but interfere with growth and development



Avermectin	R1	R2	R3	C_{22}-x-C_{23}
A_{1a}	OH	CH_3	$CHCH_3CH_2CH_3$	-CH=CH-
A_{1b}	OH	CH_3	$CHCH_3CH_3$	-CH=CH-
Avermectin B_{1a}	OH	H	$CHCH_3CH_2CH_3$	-CH=CH-
Avermectin B_{1b}	OH	H	$CHCH_3CH_3$	-CH=CH-
A_{2a}	OH	CH_3	$CHCH_3CH_2CH_3$	-CH_2-CHOH-
A_{2b}	OH	CH_3	$CHCH_3CH_3$	-CH_2-CHOH-
B_{2a}	OH	H	$CHCH_3CH_2CH_3$	-CH_2-CHOH-
B_{2b}	OH	H	$CHCH_3CH_3$	-CH_2-CHOH-
	Benzofuran ring			
Doramectin	OH	H	C_6H_{11}	-CH=CH-
Emamectin Benzoate B_{1a}	$C_6H_5COOHCH_3NH$	H	$CHCH_3CH_2CH_3$	-CH=CH-
Eprinomectin B_{1a}	$NHCOCH_3$	H	$CHCH_3CH_2CH_3$	-CH=CH-
Ivermectin B_{1a}	OH	H	$CHCH_3CH_2CH_3$	-CH_2-CH-

Figure 1 Chemical structures of avermectins.

ectoparasites is dependent on the product formulation of the active molecule and the method of application. The mode of action of avermectins and milbemycins is not completely understood.

Ectoparasiticide Resistance to Drugs and Impact on Public Health

Resistance of arthropods to the lethal effects of parasiticides has been known for more than a century. To date more than 500 species of resistant insects, mites, and ticks have been documented of which 40% are parasites of humans or animals.

The mechanism of resistance can be classified as either physiological or behavioral. Physiological mechanisms include increased metabolism of the insecticide (detoxification), decreased sensitivity of the target site, or decreased penetration into the target organism. The resistance mechanism generally involves one gene, although a combination of genes have also been implicated in certain cases.

Tolerance or resistance to all of the organophosphates currently in the market has been reported in specific ectoparasites (e.g., *Rhipicephalus (Boophilus) microplus* and adult blowflies) although largely restricted to certain parts of the world. The development of resistance to the organochlorines as well as environmental concerns has led to their withdrawal from the market.

Pyrethroid resistant strains of the cattle tick (*R. (B.) microplus)*, horn flies (*Haematobia irritans*), houseflies, and lice have been reported. Resistance against amitraz has also been documented in several strains of *R. (B.) microplus* in Australia,

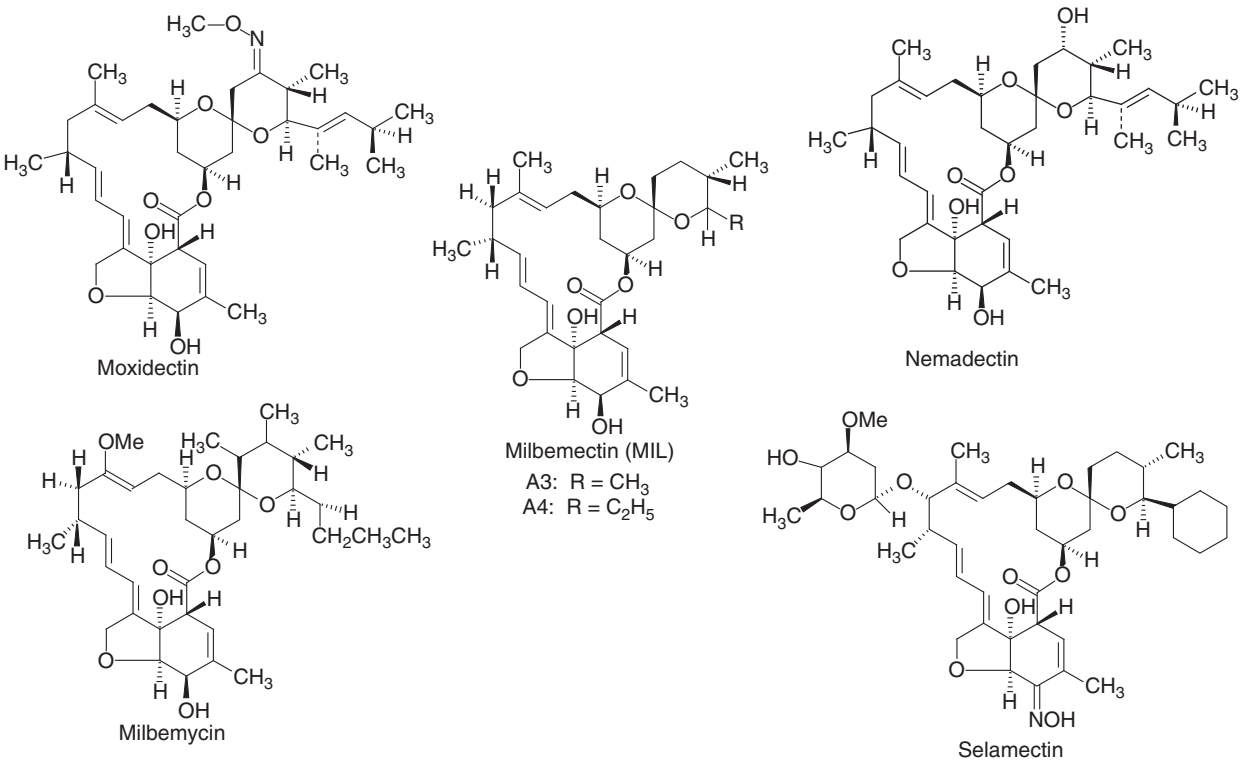


Figure 2 Structures of milbemycins and selamectin.

but no resistance in multihost ticks has thus far been reported. To date no data on the development of ectoparasite resistance is available for the MLs. In animal health resistance of ectoparasites against insect growth regulators are still rare.

Control of Drug Residues in Food of Animal Origin

Ectoparasiticides are commonly applied topically and are often lipophilic resulting in slow dermal absorption with long tissue and plasma half-lives. In addition, the natural grooming behavior of farm animals can result in significant oral absorption with increased systemic exposure in both treated and untreated animals. The chronic effects of ectoparasiticides from food intake on human health are not well defined, but there is increasing evidence of carcinogenicity, disruption in hormonal function, and genotoxicity. Restrictions are therefore applied to many of the ectoparasiticides indicated for use in food-producing animals to ensure that unacceptable residues are not present in products intended for human consumption. Consequently many international organizations and countries have issued their own pesticide maximum residual limits (MRLs) for the purpose of international trade. The Codex Alimentarius Commission (CAC), established by Food and Agriculture Organization and World Health Organization in 1963, develops harmonized international food standards, guidelines and codes of practice to protect the health of the consumers, and ensure fair trade practices in the food trade. Currently CAC has 185 member countries. Using MRLs as either criteria or guidelines many countries have mounted effective residue monitoring programs.

Analysis of ML Residues

Following administration, MLs are stored in the liver and fat tissue and slowly released, metabolized, and excreted. With the exception of eprinomectin, lactating cows or cows during the last 2 months of pregnancy cannot be treated with this drug class. MLs can be analyzed in biological matrices using a range of techniques including immunoassay, high-performance liquid chromatography (HPLC) with ultraviolet, or fluorescence detection (FLD) or liquid chromatography (LC) coupled to tandem mass spectrometry (LC–MS/MS). HPLC fluorescence has been the technique of choice for the analysis of ML residues for many years because of its excellent sensitivity and selectivity. However, MLs do not possess a native fluorophore and precolumn derivatization with non-fluorescent reagents is required before chromatographic analysis. A number of different derivatization approaches have been employed to reduce reaction time, enhance the stability of derivatives, and extend analysis to additional analytes. Eprinomectin is the most difficult ML to analyze and requires elevated reaction temperatures to produce a stable fluorescent derivative. In recent years, a number of groups have developed LC–MS/MS methods for detection of ML residues. In general, it has been shown that mobile phase additives have to be carefully controlled, with ammonium acetate or formate being desirable for production $[M+H]^+$ or $[M+NH4]^+$ ions, which are more suitable for low-energy collision-induced dissociation experiments. It has been shown by a number of groups that if formic or acetic acid are used in the mobile phase that $[M+Na]^+$ ions are predominate, which are difficult to fragment. ML residues can be analyzed down

to <1 ppb in meat or milk using HPLC–FLD or LC–MS/MS-based detection following simple sample preparation procedures. LC–MS/MS is becoming more popular because a wider range of drugs can be included in the analysis.

ML residues are occasionally detected in food, with ivermectin being the most frequently detected endectocide in bovine tissues, followed by milk and fish. Much attention has been placed on the analysis of ML residues down to low parts per billion levels in milk samples because of the perceived risk to developing neonates. This has been further fueled over reports that as much as 5% of the dose of some ML drugs such as ivermectin are excreted in the milk. In response, safer ML products such as eprinomectin and emamectin have been developed for applications in dairy cows and fish, respectively. Surprisingly in 2010–11, there were widespread reports of ivermectin residues in beef products exported from Brazil. At the time, no limit was set for ivermectin residues in muscle tissue in the European Union or the United States. In response, MRLs of 30 and 10 $\mu g\ kg^{-1}$ are now in place in these respective regions.

See also: Foodborne Diseases: Overview of Chemical, Physical, and Other Significant Hazards. Veterinary Drugs Residues: Anthelmintics

Further Reading

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VETERINARY DRUGS RESIDUES

Control of Helminths

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Glossary

Anthelmintics Drugs that acts against helminthic infections.
Anthelmintic resistance A heritable change in the susceptibility to the attack of an anthelmintic in a population of parasitic nematodes.
Cestodes Parasitic flatworms of the class Cestoda having a long flat body equipped with a specialized organ of attachment at one end. Also called tapeworms.
Endectocide A drug effective against both endo- and ectoparasites.
Nematodes Parasitic worms of the phylum Nematoda, having unsegmented, cylindrical bodies, often narrowing at each end, and including parasitic forms, such as the hookworm and pinworm. Also called roundworm.
Trematodes Parasitic flatworms of the class Trematoda that have a thick outer cuticle and one or more suckers or hooks for attaching to host tissue. Also called fluke.

Introduction

Domestic animals are affected by a large variety of helminth parasites which includes nematodes (roundworms), trematodes (flukes), and cestodes (tapeworms). Since the first discovery of the potent broad-spectrum anthelmintics, it has been widely used and has led to major changes in clinical parasitism, and millions of dollars are being spent annually in efforts to reduce the effects of parasitism in food-producing animals. Both cattle and sheep producers list internal parasites as a common cause of significant economic impact on animal production. Producers are inflicted by losses at the global level that run to many billions of dollars (liver fluke infestation alone causes losses of an estimated $3 billion worldwide).

Antiparasitic compounds are the dominant segment of the veterinary pharmaceuticals market with global sales of approximately $3.5 billion annually. Resistance to all major antiparasitic (anthelmintic) drug classes is now widespread in intestinal nematodes of sheep and goats. Modern anthelmintics generally have a wide margin of safety, considerable activity against immature (larval) and mature stages of helminths, and mostly a broad spectrum of activity (Table 1).

Summary of the Individual Drug Classes

Anthelmintics are separated into classes on the basis of similar chemical structure and mode of action. The major drug classes, their introduction onto the market and mechanism of actions are summarized in Table 2. After the introduction of the first benzimidazole molecule on the market, approximately 20 new benzimidazole compounds have been synthesized with potent anthelmintic activity, and are in use today to control helminths.

Levamisole is an anthelminthic and immunomodulator belonging to a class of synthetic imidazothiazole derivatives.

Macrocyclic lactones (ML) (including avermectin/milbemycin) are endectocides with broad activity against both external and internal parasites. Since the first introduction of ivermectin on the market (a semisynthetic derivative of avermectin), many

Table 1 Anthelmintic drug classes commonly used to treat helminth infections in food-producing animals

Chemical group	*Activity spectrum (Helminth group)*	*Food-producing animals*
Benzimidazole	Nematodes and some trematodes	Cattle, sheep, goats, pigs, and poultry
Imidazothiazole	Nematodes	Cattle, sheep, goats, pigs, and poultry
Tetrahydropyrimidine	Nematodes	Cattle
Avermectin/milbemycin	Endectocide	Cattle, sheep, goats, and pigs
Amino-acetonitrile derivatives	Nematodes	Sheep
Praziquantel	Cestodes and trematodes	Sheep and goats
Nitrophenolic compounds	Some nematodes and trematodes	Cattle and sheep
Salicylanilides	Some nematodes and trematodes	Cattle and sheep
Benzenesulfonamides	Trematodes	Cattle and sheep

Table 2 Spectrum of activity, mode of action, and toxicity of the different anthelmintic chemical classes, with comments on the appearance of resistance

Chemical group	*Discovery and first release onto market*	*Mode of action*	*Anthelmintic spectrum*	*Pharmacokinetics*	*Toxicity*	*Route of administration*	*Resistance situation*
Benzimidazole (BZ)	Thiabendazole was discovered in 1961 and subsequently a number of second generation BZ products had been commercially developed for use in domestic animals	Binds with parasite β-tubulin for subsequent disruption of tubulin-microtuble dynamic equilibrium	Broad-spectrum against lung and GI nematodes. Some activity against cestodes and trematodes. One compound (triclabendazole) only effects against *Fasciola*	Poor GI absorption	Low toxicity	Oral administartion	First reported in 1964. High resistance worldwide in sheep and goat trichostrongylid nematodes. Emerging resistance in cattle trichostrongylid nematodes reported
Imidazothiazole	The first veterinary product was tetramisole in 1967. Levamisole, the *l*-isomer of tetramisole is currently the only compound in this group available	Nicotinic acetylcholine receptor agonist	Broad-spectrum against lung and GI nematodes, mainly adult stages	Rapid absorption following parental administration. Limited absorption following oral or topical application. Rapid elimination	Narrow safety margin	Oral administration, pour-on or by injection	First reported in 1979. High resistance worldwide in sheep and goat trichostrongylid nematodes. Emerging resistance in cattle trichostrongylid nematodes reported.
Tetrahydro-pyrimidine	Pyrantel, introduced in 1966	Nicotinic acetylcholine receptor agonist	GI nematodes, narrow spectrum, mainly adult stages	Poor GI absorption in ruminants, good GI absorption in pigs	Good safety margin	Oral administration	No reports in food producing animals
Avermectin/ milbemycin	A series of macrocyclic lactone derivatives was introduced as an anthelmintic in 1981	Activity is mediated through γ-aminobutyric acid and/or glutamate gated chloride channels	Very broad-spectrum against ecto- and endoparasites (endectocide) – both GI and lung nematodes	Highly lipophilic and rapidly absorbed, but reduced absorption from GI (in ruminants)	Good safety margin in majority of target species	Oral administration, pour-on or by injection	First ivermectin resistance reported in 1988 and moxidectin in 1991. Resistance reported in sheep, goat, and cattle trichostrongylid nematodes. Ivermectin still effective in Europe and Canada. Moxidectin resistance so far only reported in goat trichostrongylid nematodes

Amino-acetonitrile derivatives	2010	Nematode-specific acetylcholine receptors	Broad-spectrum, recently introduced for use in sheep			Oral administration	–
Spiroindoles	2012	Nicotinic acetylcholine receptors	Narrow spectrum GI nematodes			Oral administration	–
Praziquantel	1978	Modulates cell membrane permeability and causes damage to parasite tegument	Narrow spectrum – adults cestodes in ruminants (at high dose rates) and schistosomes	Good GI absorption	Wide safety margin	Oral administration	–
Nitrophenolic compounds	Developed in the late 1960s as injectable fasciolicide for sheep and cattle	Intrinsic mode of action not established. Disrupts tegument of *Fasciola*	Narrow-spectrum, Highly effective against adult *Fasciola* and some abomasal nematodes	Rapid absorption following parental administration	Well tolerated in ruminants at recommended dose rates	Oral administration, but more effective when administered parentally	–
Salicylanilides	Rafoxanide was developed in 1969 and introduced for sheep and cattle in 1970	Complex. Uncouples oxidative phosphorylation in flukes but including other biochemical and physiological processes within the parasite	Narrow-spectrum. Effective against mature *Fasciola* with some compounds showing activity against immature flukes. Also activity against some adult nematodes and some compounds against cestodes and *Paramphistomum*	Well absorbed after both external and parenteral administration	Well tolerated in ruminants at recommended dose rates	Oral administration	–
Benzenesulf-onamides		Effect on gut and integument of flukes	Narrow-spectrum. Effective against adult *Fasciola*	Slow absorption following oral administration	Safe drug with high therapeutic index	Oral administration and injection	–

Abbreviations: BZ, benzimidazole; GI, gastrointestinal.

ivermectin analogs were introduced for the control of parasites, including: moxidectin, milbemycin oxime, doramectin, selamectin, abamectin, and eprinomectin. The MLs exhibit no activity against cestodes, trematodes, or protozoa. Fecal excretion is the main route of elimination of most of the ML; however, in lactating animals, up to 5% of the dose may be excreted in the milk.

Anthelmintic Resistance to Drugs and Impact on Public Health

The standards of anthelmintic efficacy usually demand that $\geq$95% of parasitic nematodes be removed with a single drug treatment, and below this level, the efficacy indicates the evidence of anthelmintic resistance. Drug resistance in parasites results from the selection of a subpopulation, which can tolerate the lethal effects of the drug that are normally effective against them. This resistance has a genetic basis due to target gene mutation/alteration of the drug receptor, but is often more complex involving other nonreceptor based mechanisms (Table 2). In livestock, anthelmintic resistance to the broad-spectrum drug, thiabendazole, was first reported by 1964. Today, significant levels of anthelmintic resistance have been recorded throughout the world to all three of the major anthelmintic classes used (benzimidazoles, imidothiazoles-tetrahydropyrimidines, and ML; see Table 2). Among sheep in particular, multidrug resistant nematode populations have become an important limitation for successful sheep production in many parts of the world. Anthelmintic resistance has also been reported in cattle and pig nematodes though not as widespread. Limited data is available on the resistance in cestodes against the drugs, but in the case of liver fluke, *Fasciola hepatica*, an important trematode infection of livestock in many temperate areas of the world, resistance (or decreased efficacy) to triclabendazole (a benzimidasole) has been reported.

The mode of action of the benzimidazole drugs can be directly linked to the interruption of microtubular function in the target parasite. Benzimidazole resistance is thus mainly caused by transversion mutations leading to specific amino acid substitutions in the β-tubulin protein at codon 200 (TTC to TAC; phenylalanine to tyrosine), codon 167 (TTC to TAC), or codon 198 (GAA to GCA; glutamate to alanine). To date, there is no convincing evidence of a role for tubulin mutations in *F. hepatica* resistance to triclabendazole; however, there is evidence to indicate that altered uptake and metabolism of the drug maybe involved.

A nicotinic acetylcholine receptor (ACh – the primary excitatory transmitter in nematodes) on the muscle cells of nematodes is associated with the sensitivity to levamisole. Levamisole, monepantel, and derquantel are cholinergic agonists that selectively produce depolarization and spastic contraction, followed by the paralysis of body muscle cells of nematodes. Monepantel, however, acts on a different class of nicotinic receptor than levamisole and derquantel. Resistance to levamisole is associated with mutation of the nicotinic acetylcholine receptor subunits, involved in forming the levamisole receptor.

Both classes of ML mediate their nematocidal effect by interacting with a range of ligand-gated ion channels of the muscles of the pharynx and somatic musculature. As a result, nematodes become paralyzed and starve to death. Resistance to ML is more complex and the mechanism of resistance to these compounds in parasitic nematodes seems to involve changes in the activity of multidrug ATP binding cassette (ABC) transporters such as P-glycoprotein.

Treatment regimens against nematodes in humans often do not achieve the same high level of efficacy and therefore it may be much more difficult to notice an anthelmintic failure. Current mass treatment campaigns against onchocercosis, lymphatic filariosis, schistosomosis, and soil-transmitted helminths in humans are based on the use of drugs being employed for decades in the veterinary field. Data suggestive of praziquantel resistance in *Schistosoma mansoni*, and ML resistance in both *Onchocerca volvulus* and soil transmitted helminths has been reported. The cost of developing a new class of broad-spectrum anthelmintic for use in food-producing animals is becoming prohibitive and very few new classes of anthelmintic have been developed in the past 40 years. New anthelmintic drug classes, such as monepantel, derquantel, and emodepside that have been developed do not seem to have the breadth of spectrum in terms of target hosts and parasite species as the MLs and benzimidazoles, and have not so far been developed for use in humans.

Control of Anthelmintic Drug Residues in Food of Animal Origin

Anthelmintic drug residues can potentially occur in food following the administration of veterinary medicinal products to farm animals or from cross contamination at feed mills. Maximum residue limits (MRL) have been set for the anthelmintic drug marker residues in edible tissues (namely, liver, muscle, kidney, and fat), eggs, and milk as markers of food safety. MRLs also play an important role in the harmonization of trade between regions worldwide including the EU, North America, Asia, Australia, and New Zealand. In addition, withdrawal periods have been set for veterinary medicinal products to ensure that residues have depleted below the MRL before tissues entering the food chain. A range of regulatory control measures to ensure food safety include the licensing of veterinary medicinal products, on-going national surveillance programs, networks of reference laboratories, and performance criteria for analytical methods.

To monitor food safety, analytical methods have been developed for analysis of anthelmintic drug residues in food. Most of the early methods for the analysis of anthelmintic residues were based on high performance liquid chromatography coupled to ultraviolet, fluorescence, or electrochemical detection. In recent years, there has been a move to liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) technology, which allows the analysis of a wider range of anthelmintic drug residues at parts-per-billion. LC-MS/MS is advantageous because simpler sample preparation approaches can be employed and the sample throughput can be improved vastly. Several analytical methods have been developed that can analyze as many as 40 anthelmintic residues in both milk and animal tissues. Kinsella et al. (2011) have recently reported the application of this technology to the analysis of the new anthelmintic drug,

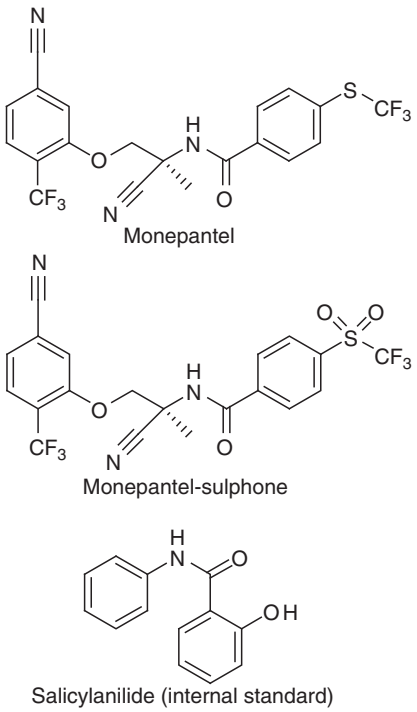

Figure 1 Structure of monepantel, monepantel sulphone, and the internal standard salicylanilide.

monepantel and its sulphone metabolite in milk and animal tissue. Figure 1 shows the chemical structures of monepantel, monepantel sulphone, and salicylanilide, which was used an internal standard in the method.

Residue Findings and Implications

Anthelmintic residues are occasionally detected in food samples and are typically found in $<1\%$ of tissue samples. The results of early residue surveillance indicate oxfendazole to be one of the most frequently detected anthelmintic residues, found mainly in ovine tissues. Many countries have commenced testing for triclabendazole residues in animal tissue, which has led to some reports of noncompliant residues in ovine tissue samples. Recently, anthelmintic analysis was extended to include the flukicide residues, namely, closantel, clorsulon, niclosamide, nitroxynil, oxyclozanide, and rafoxanide. From the inception of this testing, there have been some reports of MRL violations for closantel and rafoxanide residues in ovine tissue samples. However, in some countries, the most significant development has been the reported detection of low levels of closantel, nitroxynil, rafoxanide, and triclabendazole residues in milk samples. As a result of these positive milk samples, new MRLs have been established for four flukicide residues in milk.

See also: Foodborne Diseases: Overview of Chemical, Physical, and Other Significant Hazards. Veterinary Drugs Residues: Veterinary Drugs – General

Further Reading

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NUTRIENTIAL HAZARDS

Contents

Micronutrients: Vitamins and Minerals

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Glossary

Dietary supplement A product that is taken orally and that contains one or more ingredients (such as vitamins, minerals, amino acids) that are intended to supplement the diet by increasing the daily dietary intake of such nutrients and cannot be considered as a food or a meal. Dietary supplements must be clearly labeled as such, indicating that they are only intended for oral administration.

Food biofortification Technologies for enhancing the micronutrient content of staple crops by means of biological processes such as conventional selective breeding and genetic engineering techniques.

Food fortification The addition of one or more essential nutrients to food, whether or not it is normally contained in the food, in order to prevent or correct a demonstrated deficiency of one or more nutrients in the population or specific population groups.

Nutrient bioavailability The amount of nutrient ingested dose that is absorbed and used for normal body functions. Nutrients must reach the site of action in their active form in order to exert their metabolic function; bioavailability of nutrients is governed by both external and internal factors.

Recommended dietary allowances The average daily dietary nutrient intake level that is sufficient to meet the nutrient requirements of nearly all (97–98%) healthy subjects in a particular life stage and gender group.

Upper intake level (UL) The maximum intake from food that is unlikely to pose risk of adverse health effects from excess in almost all (97.5%) apparently healthy subjects in an age and sex-specific population group. ULs should be based on long-term exposure from food, including fortified food products.

Introduction

Food safety is an umbrella term for those conditions that ensure the availability of safe and healthy food when prepared and/or consumed according to their intended use. In Europe, and in general in the most developed societies, the availability of enough amount of food is not the main problem, so attention is addressed toward food safety.

To achieve an appropriate dietary regime in these societies, however, is not an easy task as most of the people believe that they have an adequate diet and an optimal nutrient contribution can be warranted by slightly varying food habits. Moreover, sedentary lifestyles and the current esthetic standards constrain to low calories ingestion, which is associated with an inferior nutrient intake. In addition, stress, smoking, pollution, drugs and alcohol consumption, etc., increase the nutrient requirements, which in many cases are not achieved with the usual diet. Furthermore, recommended intakes of some nutrients for pregnant and lactating women are virtually impossible to be fulfilled by standard diets. In fact, diets of more than two-thirds of the world's population lack one or more essential mineral elements. This problem can be overcome by dietary diversification, mineral supplementation, food fortification, or by increasing the concentration and/or bioavailability of mineral elements in place (biofortification).

In the case of fortified foods, it is essential to characterize and assess the risk of overconsumption, which must be regulated by food safety authorities. In other words, it is important to define which foods can be enriched, the fortifying substances and sources that can be used, maximum enrichment levels, and maximum daily allowance.

Nowadays, thanks to the scientific advances related to the analysis of food composition and the knowledge about the metabolic processes in human body, it is possible to define the concepts of nutritional normality, deficiency, and toxicity. Therefore, the reference values of nutrient intake are focused not only on the prevention of risks associated with nutrient deficiencies but also on the prevention of chronic and degenerative diseases, with the ultimate aim of health promotion. Various international organizations have established Recommended dietary allowances to be used as a basis for establishing appropriate dietary guidelines (Table 1).

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Table 1 Recommended intake for vitamins and minerals (per day) (FAO/WHO, 2002)

	Vitamins													*Minerals*					
	B_1 (mg)	B_2 (mg)	B_3 (mg)	B_6 (mg)	B_9 (μg)	B_{12} (μg)	C (mg)	A (μg)	D (μg)	E[k] (mg)	K (μg)	B_5 (mg)	B_8 (μg)	Ca (mg)	Mg (mg)	Fe (mg)	Zn (mg)	I (μg)	Se (μg)
Infants and children																			
0–6 months	0.2	0.3	2	0.1	80	0.4	25	375	5	2.7	5	1.7	5	300[a]–400[b]	26–36[b]	–[d]	1.1–6.6[c]	15[f] (30[fg])	6
7–11 months	0.3	0.4	4	0.3	80	0.5	30	400	5	2.7	10	1.8	6	400	53	6–19[ce]	0.8–8.3[c]	135	10
1–3 years	0.5	0.5	6	0.5	160	0.9	30	400	5	5	15	2	8	500	60	4–12[c]	2.4–8.4[c]	75	17
4–6 years	0.6	0.6	8	0.6	200	1.2	30	450	5	5	20	3	12	600	73	4–13[c]	3.1–10.3[c]	110	21
7–9 years	0.9	0.9	12	1	300	1.8	35	500	5	7	25	4	20	700	100	6–18[c]	3.3–11.3[c]	100	21
Males (years)																			
10–18	1.2	1.3	16	1.3	400	2.4	40	600	5	10	35–65	5	25	1300	250	10–38[ch]	5.7–19.2[c]	135–110[h]	34
19–50	1.2	1.3	16	1.3	400	2.4	45	600	5	10	65	5	30	1000	260	9–27[c]	4.2–14[c]	130	34
51–65	1.2	1.3	16	1.7	400	2.4	45	600	10	10	65	5	30	1000	260	9–27[c]	4.2–14[c]	130	34
>65	1.2	1.3	16	1.7	400	2.4	45	600	15	10	65	5	–	1300	230	9–27[c]	4.2–14[c]	130	34
Females (years)																			
10–18	1.1	1.1	16	1.2	400	2.4	40	600	5	7.5	35–65	5	25	1300	230	9–65[ch]	4.6–15.5[c]	140–100[h]	26
19–50	1.1	1.1	14	1.3	400	2.4	45	500	5	7.5	55	5	30	1000	220	20–59[c]	3.0–9.8[c]	110	26
51–65	1.1	1.1	14	1.5	400	2.4	45	500	10	7.5	55	5	30	1300	220	8–23[c]	3.0–9.8[c]	110	26
>65	1.1	1.1	14	1.5	400	2.4	45	600	15	7.5	55	5	–	1300	190	8–23[c]	3.0–9.8[c]	110	26
Pregnancy (trimester)																			
1st	1.4	1.4	18	1.9	600	2.6	55	800	5	–[j]	55	6	30	–[j]	220	–[i]	3.4–11[c]	200	–[j]
2nd	1.4	1.4	18	1.9	600	2.6	55	800	5	–[j]	55	6	30	–[j]	220	–[i]	4.2–14[c]	200	28
3rd	1.4	1.4	18	1.9	600	2.6	55	800	5	–[j]	55	6	30	1200	220	–[i]	6–20[c]	200	28
Lactation (months)																			
0–3	1.5	1.6	17	2.0	500	2.8	70	850	5	–[j]	55	7	35	1000	270	10–30[c]	5.8–19[c]	200	35
3–6	1.5	1.6	17	2.0	500	2.8	70	850	5	–[j]	55	7	35	1000	270	10–30[c]	5.3–17.5[c]	200	35
6–12	1.5	1.6	17	2.0	500	2.8	70	850	5	–[j]	55	7	35	1000	270	10–30[c]	4.3–14.4[c]	200	42

[a]Breastfeeding.
[b]Infant formula feeding.
[c]As a function of % bioavailability.
[d]Neonatal iron reservoirs are sufficient to meet the iron requirement for the first 6 months in full term infants.
[e]Bioavailability of dietary iron during this period varies greatly.
[f]Owing to the high variability in bodyweight at these ages, the recommended intake is expressed as μg per kg bodyweight per day.
[g]For preterm infants.
[h]Depending when the growth spurt starts.
[i]It is recommended that iron supplements in tablet form are given to all pregnant women because of the difficulties to correctly evaluate iron status during pregnancy. In the nonanemic pregnant woman, daily supplements of 100 mg of iron (e.g., as ferrous sulfate) provided during the second half of pregnancy are adequate. In anemic women, higher doses are usually required.
[j]It is maintained that the recommendation corresponds to the age group of the mother.
[k]Available data are insufficient to establish recommended intake for vitamin E. Thus, only 'acceptable intakes' are shown in the table.
Source: http://www.fao.org/docrep/004/Y2809E/y2809e00.HTM

The possible risks associated with micronutrients (minerals and vitamins) deficit and toxicity are described as follows.

Minerals

Minerals are chemical elements essential for normal metabolic functioning of the human body, so they are considered essential. They must be provided by the diet (food and drink) on regular and daily basis, ranging from grams per day for major minerals (macro) to milligrams or micrograms per day for trace elements and other microminerals, some of which can be probably considered as essential elements or contaminants.

All minerals have the ability to produce toxicity symptoms if they are consumed over its maximum tolerable intake level (tolerable upper intake level (UL)). On the contrary, the absence of one of them produces specific deficiency diseases, which are often adjusted with suitable dietary supplementation. The deficiency prevalence of only four minerals is actually known: those of iron and iodine are widespread among the population, whereas the lack of zinc and selenium occurs just in some population groups under very specific conditions. For all other minerals, deficiency takes place in exceptional situations.

Macrominerals

Calcium (Ca)

It is the most abundant mineral in the human body and 99% of body Ca is found in the bone system; the rest is present in the intracellular and extracellular fluids. It participates in important functional processes such as the formation and maintenance of bones and teeth, and it is also involved in the nerve impulse transmission, neuronal excitability, neurotransmitter formation, and blood coagulation.

Milk and dairy products are the major dietary sources of calcium. They are also important because they contain substances that promote calcium's absorption, such as vitamin D, lactose, and proteins. Other Ca dietary sources are fish, whole grains, nuts, and legumes.

Ca deficiency can be caused by inadequate dietary intake of this mineral, vitamin D deficiency, a very low dietary Ca/P ratio, and high Ca losses. The main consequence of this deficit is inadequate mineralization of the bone matrix or osteoporosis, which can cause rickets in children and youths. It is also associated with osteomalacia, growth arrest, hypertension, increased cancer risk (especially in colon), and seizures.

In normal situations, dietary toxicity of Ca is rare, but it can occur by supplement consumption. Doses more than 2 g day^{-1} can cause hypercalcemia, with more or less severe effects depending on its intensity. Besides interfering with the absorption of other divalent metals (such as iron, magnesium, manganese, and zinc), hypercalcemia can lead to constipation, nausea, polyuria, and kidney stones and, in extreme situations, loss of muscle tone, coma, and death.

Phosphorus (P)

Approximately 80% of the total body content of P is contained, together with Ca, in the bone structure and teeth; the rest of this mineral is mostly present in soft tissues, and a very low amount (1%) is dissolved in the extracellular fluid. Its main function is structural, as it is part of hydroxyapatite in calcified tissues, phospholipids in biological membranes, nucleotides, and nucleic acids. Other functions include acid–base balance in blood as phosphate buffer, temporary reserve and transfer of energy from metabolic substrates, and participation in phosphorylation processes.

Meat, fish, dairy products, nuts, legumes, and cereals are good dietary sources of phosphorus. Processed foods also contain considerable amounts of this mineral as it is present in various additives that are added therein.

Inadequate intake of P results in the development of hypophosphatemia, whose characteristic symptoms are muscle weakness, bone loss, decreased growth, poor dental development, rickets, and osteomalacia.

On the contrary, P excess or hyperphosphatemia is not common in healthy subjects, but it can be found in patients with renal diseases, which may lead to the production of calcium phosphate calculi in the body tissues and to muscular symptoms (such as tetany). Recently, a higher P intake has been detected, which has been associated with an increased intake of cola drinks and food with P-containing additives.

Magnesium (Mg)

Approximately 65–70% of this mineral is bound to Ca and P as bone constituents, whereas the rest is distributed in various organs and tissues, in fact, it is the second most abundant intracellular cation. This mineral participates mainly in the metabolism and maintenance of bone structure, in various enzyme, nervous, and cardiac functions, and in the regulation of blood pressure.

Foods with a high Mg content include whole grains, legumes, green leafy vegetables, tofu, meat, fruit, and dairy products.

Mg deficiency is observed only in two situations: as a secondary complication of a primary disease (like cardiovascular or neuromuscular diseases) and as a result of rare genetic disorders of homeostasis. This deficiency is usually associated to tachycardia, which can be accompanied by weakness, muscle spasms, disorientation, nausea, vomiting, and seizures.

Toxicity resulting from an excessive Mg intake ($>$350 mg day^{-1}) is rare in healthy people, but it can occur in patients with renal failure, which can cause diarrhea, nausea, and abdominal pain.

Sodium (Na)

It is the principal cation of the extracellular fluid, 30–40% being fixed in the skeleton. It is an essential regulator of extracellular fluid (cardiac output or blood pressure), and it also plays an important role in regulating the osmolality, the acid–base balance, cell membrane potential, and active transport across cell membranes.

Most ingested Na comes from sodium chloride (common table salt, of which 40% is sodium), which is added during food cooking or industrial processing. Food products prepared by salting and curing (such as hams, sausages, and salted fish) are characterized by a high Na content.

Na deficiency is rare, even with low-sodium diets. Under extreme conditions of intense and persistent sweating, trauma, chronic diarrhea, or kidney disease, it may be a necessary supplementation treatment with salt.

Na excessive consumption is associated with the development of hypertension and edema. However, acute toxicity related to dietary Na is not a concern as kidneys excrete Na excess.

Potassium (K)

It is the main cation of the intracellular liquid, 98% being present inside the cell. It is, therefore, essential for the acid–base balance of the body, water maintenance; transmission of nerve impulses, and contraction of smooth, skeletal, and cardiac muscles.

It is widely distributed in legumes, dried fruits, and nuts. Potatoes, spinach, bananas, and avocados are also good dietary sources.

There are several situations that can cause hypopotassemia, such as insulin excess, chronic kidney disease, diarrhea, vomiting, and excess of laxatives. This deficiency can affect nerve and muscle activities and can lead to renal, metabolic, and heart alterations.

Excessive consumption of potassium can cause gastrointestinal disturbs; this is not considered a real toxic effect unless kidneys do not function properly. In this case, it could lead to a cardiac arrest in an extreme situation. Thus, K supplements can play an important role in the chronic heart failure.

Chlorine (Cl)

It is the main anion present in the extracellular fluid. Along with sodium, sulfate, phosphate, and bicarbonate salts, it maintains the acid–base balance of body fluids, performing a passive role in this regulatory system. In addition, it is involved in the formation of digestive gastric juices.

Most of the ingested chlorine comes from sodium chloride or table salt (60% chloride), which is added to food in its various preparation stages. Food with a high Cl content includes seaweed, rye, tomatoes, lettuce, celery, and olives.

Within a balanced diet, it is difficult for chloride deficiency situations to appear. However, vomiting, chronic kidney disease, renal failure, and respiratory acidosis can lead to Cl deficiency. On the contrary, the only known dietary cause of hyperchloremia is dehydration due to water deficiency. Consumption of large amounts of chlorine (in salt form) has been associated with arterial hypertension in predisposed subjects.

Microminerals/Trace Elements

Iron (Fe)

Fe acts as a catalyst for a broad spectrum of metabolic functions. It is a component of hemoglobin and it is required for oxygen transportation, essential for cellular respiration. In myoglobin, it represents the muscle oxygen reservoir. It is also a component of enzymes, such as cytochrome (essential for energy production) and others required for immune system functioning.

There are two types of dietary Fe: (1) nonheme Fe (the most abundant one), present in both plants and animal tissues and (2) heme Fe (more bioavailable), from hemoglobin and myoglobin in food from animal origin. Nonheme Fe is found mainly in vegetables, whole grains, nuts, and oil seeds, whereas heme Fe is present in viscera, red meat, fish, and seafood.

The increase of iron and prebiotics in the plant edible parts is expected to improve human health in both industrialized and developing countries exposed to endemic infections. In collaboration with the HarvestPlus Challenge Program, the high-iron rice group is using biotechnology to generate new rice varieties to increase Fe concentrations in the grain.

Fe deficiency remains the most common nutritional deficiency in industrialized countries despite dietary improvements. This occurs when the ingested amount does not meet the body requirements, due to insufficient dietary intake, poor absorption, or excessive physiological losses. It is manifested by iron deficiency anemia, which usually causes pallor signs, splenomegaly, and a lying weight curve in infants.

Fe toxicity in healthy people is unusual as there is a quite effective homeostasis mechanism. However, toxicity problems can arise if the subject has a genetic disease (hemochromatosis) and/or if repeated blood infusions are carried out. Abuse of Fe supplements (>45 mg day^{-1}) may also pose a health risk due to free radical reactions resulting from this excess. Consequently, there may be an increased risk of bacterial infection, neoplasia, arthropathy, cardiomyopathy, and endocrine disruption.

Zinc (Zn)

It is the most abundant intracellular trace element, and it is involved in three main function groups in the human body: catalytic, structural, and regulatory. It is, therefore, fundamental for the regulation of gene expression, for the organism growth and development, and for the immune system function.

It is widely distributed in foods and the main Zn sources are viscera, meat, seafood, whole grains, nuts, and oil seeds. The most bioavailable Zn is provided by foods from animal origin.

The clinical manifestations are observed in communities or people who eat small amounts of animal protein. It can cause growth retardation, sexual and skeletal immaturity, neuropsychiatric disorders, dermatitis, alopecia, diarrhea, increased infection susceptibility, and loss of appetite.

Zinc deficiency caused by inadequate dietary intake is a global nutritional problem in human populations, especially in developing countries. Biofortification of wheat and other staple foods with Zn is, therefore, an important challenge and a high-priority research task. The results of many studies suggest that foliar Zn application in wheat represents an effective approach to provide more dietary Zn from wheat-derived products.

Regarding its toxicity, it has been reported that at doses higher than 40 mg day^{-1}, Zn can interfere with copper, thus leading to a decrease of some enzymatic activities. Doses more than 100 mg day^{-1} can cause diarrhea, cramps, nausea, vomiting, and depression of the immune system. These levels can be reached with the intake of certain vitamin and mineral preparations that already contain Zn.

Iodine (I)

It is an essential constituent of the thyroid hormones (thyroxine (T_4) and triiodothyronine (T_3)), which play an essential role in gene transcription so as to regulate the basal metabolic rate as well as body growth and development. The thyroid gland actively absorbs iodide from the blood stream to synthesize these hormones and release them back into the blood.

Seafood (fish, shellfish, and seaweed) concentrate iodine from seawater, and they are generally the richest source of such mineral. In some populations, milk has become the main iodine source due to the use of iodized salt and I-fortified feed

for cattle breading. Processed foods can also provide some extra iodine, as some additives already contain it.

Iodine deficiency is one of the most serious public health problems according to the World Health Organization, as it can cause mental and physical retardation of children (cretinism) if it occurs during fetal development. Other disorders related to hypothyroidism include goiter (hypertrophied thyroid gland), mental slowing, depression, weight gain, and low basal body temperatures.

The toxicity of this mineral may be associated with impaired immunity leading to excessive production of thyroid hormones. A high consumption of algae or dietary supplements used for weight loss can also lead to an excessive I intake. Symptoms of I toxicity include: increased basal metabolic rate, voracious appetite, thirst, weight loss, general weakness, heat intolerance, nervousness, and heart problems.

However, dietary iodine intake is generally lower than recommended daily levels because the amount and consumption frequency of iodine-rich foods are not enough to satisfy the requirements. Therefore, the introduction of iodized salt has been the basis of international iodine deficiency eradication efforts because it is an inexpensive source of stable iodine content, and salt is consumed in relatively similar quantities worldwide. Furthermore, agronomic biofortification has proven to be effective on iodine enrichment of fodder, rice, potatoes, carrots, tomatoes, and leaf vegetables.

Copper (Cu)

It is a component of several enzymes, cofactors, and proteins in human body; approximately 90% of Cu is located in muscles, bones, and liver. It contributes to the formation of red blood cells and the maintenance of blood vessels, nerves, bones, and immune system; it is also present in antioxidant enzymes such as superoxide dismutase.

Rich food sources of this mineral include viscera (especially liver), seafood, legumes, nuts, seeds, and drinking water.

Deficiency is rare, but children with poor Ca diets are more predisposed, especially if they also have diarrhea or malnutrition. There are also diseases that decrease Cu absorption, such as celiac disease, cystic fibrosis, or tracking of restrictive diets. It manifests with anemia, neutropenia, and severe bone demineralization.

Acute toxicity in humans is very rare and it usually occurs by contamination of drinking water and food due to Cu pipes and utensils. Symptoms include vomiting, diarrhea, hemolytic anemia, liver and kidney damages, and even death. It can also appear as a rare hereditary disease (Wilson's disease) with signs of liver damage.

Selenium (Se)

Selenium is an essential micronutrient as it is component of the amino acids selenocysteine and selenomethionine and it functions as cofactor for reduction of antioxidant enzymes such as glutathione peroxidase. It is also involved in the thyroid gland functioning and stimulates the immune system.

Fish, shellfish, and viscera are good sources of this mineral, followed by meat and eggs. Despite the low Se content of cereals and their derivatives, they are good sources of this mineral because of their high consumption. Cereal biofortification by means of Se-enriched fertilizer is an efficient way to increase their relatively low Se level, as it has been successfully used for potatoes and carrots.

Selenium deficiency is very rare, but it can occur in patients with severe intestinal dysfunctions or subjected to total parenteral nutrition; populations dependent on food grown in selenium-deficient soils are also at risk. Symptoms include pain, muscle wear, and cardiomyopathy.

A dietary excess ($>400\ \mu g\ day^{-1}$) can cause selenosis, which manifests by hair loss, halitosis, gastrointestinal disorders, fatigue, nail disorders, irritability, and neurological damage. Extreme cases of selenosis can result in liver cirrhosis, pulmonary edema, and death.

Fluorine (F)

This mineral is involved in Ca uptake, prevents tooth decay, and aorta calcification, is part of dental enamel, and reduces osteoporosis due to its beneficial effects on bone tissue.

The main dietary source is drinking water, especially fluoridated drinking water; among food, seafood, tea, and infant formulas (prepared with fluoridated water) also represent a good source of this mineral. Fluoride dental products can sometimes exceed the dietary intake, especially in young children with poor control of swallowing reflex.

Fluoride deficiency usually occurs in people living in areas where drinking water supplies less than $1\ mg\ ml^{-1}$ of fluoride, which manifests as dental caries.

The chronic toxicity due to its overconsumption is called fluorosis, which affects bone health, producing teeth yellowing, and bones and enamel weakness; this can lead to decalcification, osteoporosis, and tooth decay. It can also affect kidney, muscle, and nerve functions.

Chromium (Cr)

The trivalent chromium (Cr^{+3}) is an essential nutrient in the metabolism of carbohydrates (enhancer of insulin action), lipids, and proteins (involved in phosphorylation and dephosphorylation mechanisms of insulin receptor proteins). Brewer's yeast, black pepper, and grape must are good dietary sources of this mineral.

Cr deficiency can be observed in populations of developed countries that consume refined food, as well as in subjects undergoing long-term total parenteral nutrition with low Cr levels. Symptoms include glucose intolerance, hyperglycemia, hypoglycemia, glycosuria, insulin resistance, and hypercholesterolemia.

If orally administered, it is slightly toxic due to the low absorption of Cr^{+3} (predominant form in food); it can induce a chronic renal failure. On the contrary, the toxicity of the mineral in its hexavalent (Cr^{+6}) form is common in industrial environments and can cause dermatosis and lung cancer.

Molybdenum (Mo)

This mineral may exist in various oxidation states (+3, +4, +5, and +6) and, therefore, it functions as a facilitator of electron transfer reactions, being a cofactor of iron- and flavin-containing enzymes in the metabolism of pyrimidines, purines, pteridines, and sulfur-containing amino acids.

Milk, dairy products, legumes, liver, kidney, cereals, cereal products, and nuts are good sources of this mineral.

Mo deficiency is uncommon, but, in case it occurs, it produces acidosis, mouth and gums disorders, rapid heart rate, mental and metabolic alterations, and male sexual dysfunctions.

Regarding its toxicity, oral doses of 10–15 mg day^{-1} may lead to a gout-like syndrome. Higher Mo intakes can cause anemia, anorexia, diarrhea, joint abnormalities, osteoporosis, hair bleaching, and death.

Manganese (Mn)

This mineral is an important catalytic cofactor for some metalloenzymes such as superoxide dismutase (cellular antioxidant system), arginase (urea cycle), and pyruvate decarboxylase (gluconeogenesis).

It is mainly found in cereals, black bread, nuts, ginger, and tea. The latter contain tannins that bind to this mineral, forming insoluble complexes that decrease Mn absorption at intestinal level.

Mn deficiency data in humans are scarce, but it can cause weight loss, dermatitis, delayed growth of hair and nails, hair color change from black to red, and decreased blood lipids. It may be more common in infants due to the low concentration of this mineral in breast milk.

It is not toxic if orally administered, but it may lead to psychiatric disorders in persons overexposed to high Mn levels present in the atmosphere and smoke.

Other Elements

There are other microminerals (such as lithium, silicon, vanadium, nickel, and boron) that can also be considered essential, based on data obtained from animal models or biochemical reactions that suggest a beneficial biological role in humans. In addition, other minerals (such as lead, arsenic, cadmium, and mercury) have no beneficial effects in humans and these are considered as food contaminants that may derive from agricultural technology, industrial emissions, geological sources, and certain processing technologies.

Vitamins

Vitamins are essential micronutrients, which are necessary for every living cell function, growth, and development. Because human endogenous metabolism is not able to produce them or generates negligible amounts, they should be regularly introduced through the diet. Vitamins are involved in multiple functions of cell metabolism as catalysts and enzyme prosthetic groups. As biocatalysts, vitamins activate food oxidation and facilitate energy release and utilization, so that human body can take advantage of the plastic and energetic elements deriving from redox reactions.

Hydrosoluble

Vitamin B_1 (Thiamine)

This vitamin plays a fundamental role in the carbohydrate metabolism by means of the formation of its coenzyme derivative (thiamine pyrophosphate (TPP)).

It is mainly found in pork meat, pork meat products, viscera, dried beans, wheat germ, breakfast cereals, sunflower seeds, and oleaginous nuts.

Deficiency of this vitamin causes beriberi, which can manifest itself in two different ways (wet or dry) depending on whether it is edematous or neurological. In general, patients exhibit weakness, poor coordination, and functional impairment of the cardiovascular, muscular, nervous, and gastrointestinal systems.

When a mother has thiamine deficiency, the newborn may develop a serious, acute form of beriberi, which is one of the most important causes of perinatal mortality in Southeast Asia. In industrialized countries, thiamine deficiency can be developed under the following conditions: high consumption of refined food, anorexia, gastrointestinal disturbances, excess of carbohydrates in the diet (athletes), and total parenteral nutrition based on glucose.

In people with alcohol abuse problems, Wernicke–Korsakoff syndrome can occur, where thiamine absorption is decreased, whereas its elimination is increased. This causes vision changes (double vision, crossed eyes, and rapid eye movement), staggering gait, and impaired mental functions.

A dietary excess of thiamine generally does not produce harmful effects and, because it is easily eliminated, it is not possible to speak about B_1 hypervitaminosis. However, sensitization phenomena have been observed in some subjects after repeated intravenous administration of thiamin.

Vitamin B_2 (Riboflavin)

This micronutrient is easily absorbed, plays an important role in the energy utilization, and is required for the metabolism of fats, carbohydrates, and proteins. Once absorbed, especially in the small intestine, heart, liver, and kidney, it is converted into its two coenzyme forms (flavin mononucleotide and flavin adenine dinucleotide).

It is widely distributed in foods, but it is especially found in viscera, breakfast cereals, meat (beef and duck), blue fish, milk powder, cheese, fresh yeast, pate, foie gras, wheat germ, egg yolk, and almonds.

Isolated riboflavin deficiency is rare, given its abundance in natural food products. Reported cases usually correspond to generalized hypovitaminosis, being characteristic of the one produced by excessive alcohol consumption as it interferes with vitamin digestion and absorption. The observed signs and symptoms usually affect mucous membranes, leading to cracked and red lips, tongue inflammation (glossitis), cracking of mouth corners (cheilitis), mouth ulcers, and sore throat. The deficiency can also cause dry skin, fluid in mucous membranes, and anemia. At eye level, burning sensation and ocular pruritus are detected, as well as photosensitivity.

No toxicity has been established for an excessive dietary intake of riboflavin.

Vitamin B_3 (Niacin, Vitamin PP)

The generic name 'niacin' is used for nicotinic acid, its amide (nicotinamide), and all derivatives that can be transformed into biologically active compounds. This vitamin is the precursor of two coenzymes involved in almost all redox reactions (nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate), which can act as coenzymes or participate in anabolic and catabolic reactions of carbohydrates, proteins, and lipids.

Liver can synthesize niacin from tryptophan, but it is very inefficient as 60-mg tryptophan is required to synthesize 1 mg of niacin.

The best sources of niacin include blue fish, liver, meat (beef, rabbit, and chicken), breakfast cereals, mushrooms, seafood (squid and cuttlefish), meat products, peanuts, and peanut butter.

Niacin deficiency causes pellagra, a disease characterized by skin lesions (scaly skin sores), gastrointestinal disorders (diarrhea), and mental disorders (mental confusion and hallucinations).

A large niacin intake may lead to decreased levels of low-density lipoprotein-cholesterol and to an increased content of high-density lipoprotein-cholesterol. In fact, very high amounts of niacin (approximately 100 times higher than the actual recommendations for its dietary intake) have been used as drugs.

Vitamin B_5 (Pantothenic Acid)

This vitamin has two coenzyme derivatives of great biological importance. The acyl carrier protein is part of the enzymatic complex used for fatty acid synthesis and it is, therefore, essential for lipogenesis. The coenzyme A is required for metabolical activation of acyl groups, including fatty acids and acid metabolites arising from catabolism of carbohydrates and some amino acids. Thus, it is essential for an efficient utilization of all types of macronutrients, from an energy standpoint.

Pantothenic acid is practically found in all foods. Meat, cereals, and legumes are rich sources of this vitamin, whereas fruits and vegetables contain a lower amount.

Given its abundance, serious deficiency of this vitamin is very uncommon. Deficiencies occur only when there is a generalized malnutrition with lack of other vitamins. Signs and symptoms include general malaise, gastrointestinal disorders, and muscle and neurological disorders.

No toxicity cases have been reported for high doses of pantothenic acid.

Vitamin B_6 (Pyridoxal)

Vitamin B_6 is actually a group of three chemical compounds called pyridoxine (or pyridoxol), pyridoxal, and pyridoxamine. Their phosphorylated derivatives (pyridoxal phosphate and pyridoxamine phosphate) act as coenzymes. They are involved in many enzyme reactions of the amino acid metabolism and their main function is to transfer the amino group.

They are all abundant in foods, especially in the liver, legumes, nuts, and bananas. Pyridoxine and pyridoxamine are predominant in plants, whereas pyridoxal prevails in food from animal origin.

Pyridoxine deficiencies are rare in industrialized countries due to food abundance. In addition, a nonnegligible stored amount of vitamin is also available in human body besides the contribution of intestinal biota. The vitamin deficiencies give rise to neurological problems, growth retardation, hypochromic anemia, seborrheic dermatitis, glossitis, depression, and seizures. Some pyridoxine deficiencies often lead to a secondary deficiency of niacin, as its endogenous synthesis from tryptophan does not work properly.

It is one of the vitamins with greater therapeutic use, given its metabolic implications, particularly in the synthesis of biogenic amines. Excessive use of pyridoxine (2–4 g day^{-1}) may produce toxic effects, particularly peripheral neuropathy.

Vitamin B_8 (Biotin)

It is an essential vitamin for various animal species and humans, is found in different food products, and is synthesized by bacteria from the lower gastrointestinal tract and some fungi. Liver, egg yolk, soy flour, cereals, and yeast are rich sources of biotin.

It is the only vitamin that acts as coenzyme without structural modifications and, when covalently bonded to the enzymatic proteins, it is involved in carboxylation reactions that affect the Krebs cycle, lithogenesis, and the degradation of some amino acids.

Biotin deficiencies are uncommon, given its broad occurrence in most foods and additional endogenous contribution of intestinal microbiota. However, deficiency can arise when abundant raw eggs are ingested due to the presence of avidin (a thermolabile glycoprotein), which specifically binds biotin and prevents its intestinal absorption. The clinical consequences of this deficiency are primarily metabolic acidosis, digestive disorders, hypotonia, alopecia, skin and mucous membranes lesions, and general tiredness.

No toxicity cases have been reported for high doses of this vitamin.

Vitamin B_9 (Folic Acid)

Folate or folates are used as a generic name for this vitamin and refers to the different forms that naturally occur in foods. It is known that intestinal bacteria are able to synthesize folate, which is probably lost in the feces. The term folic acid refers to vitamin form found in supplements and enriched food.

The best dietary sources from both quantitative and bioavailability standpoints are liver, foie gras, legumes, green leafy vegetables, breakfast cereals, fresh yeast, wheat germ, soybean flour, peanut, chestnut, and hen eggs.

Vitamin deficiencies can occur due to insufficient ingestion, alcoholism (interferes with enterohepatic absorption), or increased requirements during certain life stage (such as pregnancy). The major disease associated with folate deficiency is megaloblastic anemia, with a consequent decrease of immunological functions. If the deficiency occurs during pregnancy, it can result in neural tube defects in the fetus (spina bifida and anencephaly (the absence of brain)). Moreover, the involvement of folate in the metabolism of homocysteine (a nonproteinogenic, cytotoxic amino acid produced during methionine catabolism) leads to a vitamin deficiency that may represent an independent risk factor for cardiovascular diseases and stroke. A low-folate nutritional status is a risk factor in cancer development, particularly colorectal one.

No vitamin toxicity has been recorded even when large amounts are ingested, as the excess amount is easily eliminated due to its hydrosoluble nature.

Vitamin B_{12} (Cyanocobalamin)

This vitamin is also known as cyanocobalamin, antipernicious, and extrinsic factor. It consists of a class of chemically related compounds (such as its two coenzyme forms, methylcobalamin and 5-deoxyadenosylcobalamin) that display vitamin activity. This vitamin can be synthesized by bacteria, fungi, and algae; some ruminants (cattle and sheep) can obtain it from endogenous bacterial activity.

Viscera (such as the liver and kidneys), meats, eggs, dairy products, and fish are good sources of this vitamin. It naturally

occurs in the animal kingdom only, and its presence is negligible in plants and vegetables; for this reason, vegans usually have vitamin deficiency and therefore they need to increase their dietary intake with vitamin supplements. At present, there are some enriched-plant products available, such as fortified cereals.

The lack of cobalamin or its derivatives leads to a shortfall in the transport of methyl groups, which adversely affects purine synthesis (deoxyribonucleic acid component) and therefore causes a deficiency in the cell multiplication process. This deficiency mainly affects the bone marrow where erythropoiesis (formation of blood cells) occurs, so it may result in a serious medical condition called pernicious anemia.

Another important alteration is a neuropathy that can be fatal due to a discontinuous, diffuse, and progressive demyelination. This produces sensory disturbances in the legs (tingling and numbness), loss of concentration or memory, disorientation, and dementia. Unlike other water soluble vitamins, vitamin B_{12} is stored in the liver and therefore its deficiency may arise months after a low or very low dietary intake.

As to their toxicity, it is advised to avoid it under myeloproliferative conditions, especially leukemia. Nevertheless, no toxicity cases have been reported for high doses of this vitamin.

Vitamin C (Ascorbic Acid)

The word vitamin C is used for L-ascorbic acid (or anti-scorbutic vitamin), other molecules having vitamin activity (such as ascorbic acid and its salts), and some of their oxidized forms (such as dehydroascorbic acid).

All the fruits and vegetables provide vitamin C, even though there are some of them that are richer sources, such as red and green peppers, Brussels sprouts, broccoli, fruit, and raw vegetables. Many food products are fortified with vitamin C, but several technological precautions must be taken as it is highly thermolabile and prone to oxidation.

Lack of this vitamin causes scurvy (<0.2 mg dl^{-1}), a disease that begins with symptoms of fatigue, pinpoint hemorrhages in the back part of arms, legs, joints, and gums, due to alterations of collagen synthesis. Several studies have linked vitamin C deficiency with increased risk of cardiovascular disease and preeclampsia; it also favors the development of lung infections.

In terms of toxicity, the main adverse effects are gastrointestinal disturbances, nausea, cramps, and diarrhea; the formation of kidney stones has also been reported.

Liposoluble

Vitamin A

Vitamin A is also known as the antixerophthalmic vitamin. It can be found as retinoids (retinol, retinal, and retinoic acid) and provitamin A carotenoids (mainly β-carotenoid); other natural carotenoids do not exhibit vitamin activity in humans.

The main dietary sources of total vitamin A are liver, cod liver oil, carrots, spinach, sweet potatoes, pate, foie gras, margarine, butter, egg yolk, mango, and cheese. In the case of carotenoids, they can be found in vegetables, milk caps, and fruits.

Deficiency of this vitamin is one of the key public health problems in developing countries. The most important disease deriving from this dietary deficiency is night blindness, which can end up in permanent blindness. It may also produce xerophthalmia (keratinization of ophthalmic tissue), and this is related to macular degeneration associated with age, xerosis, and diverse skin and immunocompetence problems.

The toxicity of this vitamin is called hypervitaminosis A and can be of three types: acute (due to very excessive intake), chronic (long-term high consumption), and teratogenic. During pregnancy, this can cause spontaneous abortion or birth defects. Toxic status only occurs when retinoids are used, whereas carotenoids cannot generate such symptoms. In severe cases, it manifests with muscle and bone pain, appetite loss, skin disorders, headache, dry skin, hair loss, liver damage, double vision, bleeding, vomiting, hip fracture, and even coma. Acute toxicity can cause gastrointestinal disturbs, headache, blurred vision, and muscle incoordination.

Vitamin D (Cholecalciferol)

It is also known as antirachitic vitamin, as its deficiency causes rickets. In the presence of sunlight, the epithelial cells are able to synthesize it from 7-dehydrocholesterol (produced in the liver and transferred to the skin). Both animals and humans are, therefore, able to synthesize vitamin D_3, and an adequate exposure to sunlight or ultraviolet radiation can avoid its deficiency.

The main food sources of vitamin D are cod liver oil, blue fish, caviar, milk caps, mushrooms, eggs, margarine, and breakfast cereals. There are also several fortified food products available in the market (such as milk, ready-to-eat breakfast cereals, and orange juice), which in some countries represent the main dietary source of vitamin D.

When the sunlight exposure is not enough to produce the required amount of this vitamin, dietary intake is essential. If the latter also fails, children rickets can thus occur, producing abnormal growth of head, thorax, and joints with curved pelvis and legs. Adults can present osteomalacia (weak bones) and even osteoporosis. Vitamin D deficiency has been epidemiologically linked with an increased risk of various types of cancer, hypertension, autoimmune, and infectious diseases.

Vitamin D is a very toxic substance, so an excessive intake of vitamin D can cause hypercalcemia, thirst, anorexia, risk of soft tissue calcification, and stone formation in the urinary tract.

Vitamin E

This vitamin is a family of eight natural compounds (four tocopherols (α, β, γ, and δ-) and four tocotrienols (α, β, γ, and δ-)) with different biological activity and antioxidant capacity. The most active form of this vitamin is α-tocopherol, which is widely distributed in nature and it is often used in vitamin supplements.

The main food sources are vegetable oils, nuts, oil seeds, wheat germ, and food products prepared with any of these ingredients (such as margarine).

The deficiency of this vitamin is rare, but it may occur in smokers (tobacco easily destroys it), people with a low dietary intake of lipids, or with a poor lipid absorption; it is also present when there is a genetic abnormality in lipoprotein synthesis. In animals, it has been demonstrated that the effects of this deficiency are associated with the lack of antioxidant protection provided by this vitamin; however, such effects have not been thoroughly proved in humans, even though they seem to be related to the development of anemia.

Vitamin E is practically classified as a nontoxic substance. Hypervitaminosis is not observed even after a high dose administration over a long time period.

Vitamin K

This vitamin prevents bleeding as it helps maintaining the blood clotting system. The natural forms of this vitamin are K_1 (phylloquinone or phytomenadione) and K_2 (menaquinone), which are of vegetable and microbial origin, respectively. Vitamin K_3 (menadione and menadiol) is a synthetic molecule, which displays an activity that is twice as much as those of the natural forms.

Different levels of vitamin K are found in milk, dairy products, meat, eggs, liver, whole grains, fruits, green vegetables, and vegetable oils.

Vitamin K deficiencies are rare because of the vitamin abundance in food and the endogenous synthesis by intestinal microbiota. However, when they occur, they result in a poor blood clotting that causes spontaneous bleeding or extended bleeding time. The deficiency may arise in the following situations: total parenteral nutrition without vitamin supplementation, presence of absorption problems (intestinal resection, biliary obstruction, etc.), medication intake of vitamin K antagonists, antibiotic treatment that destroys intestinal microbiota, laxatives, and vitamin A or E overdose (which inhibits the absorption of vitamin K).

Its deficiency is more likely to occur in newborns, especially premature ones, those who only breastfed (because breast milk contains a lower amount of vitamin K as compared with infant formula), or those whose mothers took anticonvulsant medication. In addition, the newborn intestines have not been yet colonized with bacteria that synthesize menaquinones, and vitamin K cycle may not work correctly in premature babies. Vitamin K deficiency in infants can cause a condition called hemorrhagic disease of the newborn, which occurs within the first days after birth (2–5 days) and manifests with bleeding in the baby's stool, urine, and also around the umbilical cord. Sometimes intracranial bleeding may also occur, which happens suddenly, thus causing serious injuries or even infant's death.

Despite being a fat soluble vitamin, no toxicity cases have been reported for high doses of the natural forms of this vitamin. Menadione, however, can be more dangerous, especially in neonates, as it can produce hemolytic anemia and hyperbilirubinemia.

See also: Food Additives: Antioxidants; Food Additives – General; Natural Preservatives; Preservatives. Institutions Involved in Food Safety: FAO/WHO Codex Alimentarius Commission (CAC); Food and Agriculture Organization of the United Nations (FAO); World Health Organization (WHO). Public Health Measures: Fundamentals of Food Legislation. Safety of Food and Beverages: Cereals and Derived Products; Coffee, Tea and Herbals, Cocoa and Derived Products; Fruits and Vegetables; Meat and Meat Products; Milk and Dairy Products; Nuts; Oils and Fats; Poultry and Eggs; Safety Consideration in Developing Functional Foods; Seafood; Spices and Seasonings

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Macronutrients: Essential Fatty Acids

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Glossary

Elongase (ELOVL) Any enzyme that catalyzes the elongation of an aliphatic chain, preferentially fatty acyl chains.

Fatty acid desaturase (FADS) An enzyme that removes two hydrogen atoms from a fatty acid, creating a carbon–carbon double bond.

Lipid Organic substance that is relatively insoluble in water and soluble in organic solvents.

Monounsaturated fatty acid (MUFA) A fatty acid that contains one carbon–carbon double bond in its carbon chain length structure.

Omega-3 (ω3) fatty acid A fatty acid in which the first double bond is located between the third and fourth carbons from the methyl or omega (ω) end.

Omega-6 (ω6) fatty acid A fatty acid in which the first double bond is located between the sixth and the seventh carbons from the methyl or omega (ω) end.

Polyunsaturated fatty acid (PUFA) It is a fatty acid that contains more than one carbon–carbon double bond in its carbon chain length structure.

Prostaglandins A group of physiologically active substances derived from essential fatty acids. They are present in many tissues to perform functions such as the constriction or dilation of blood vessels and stimulation of smooth muscles and the uterus.

Saturated fatty acid (SFA) A fatty acid that contains only carbon–carbon single bonds in its carbon chain length structure.

Thromboxanes Biologically active substances produced in platelets that increase platelet aggregation (and therefore promote blood clotting), constrict blood vessels, and elevate hypertension.

Introduction

Essential fatty acids (EFAs) are needed for normal bodily function and development, but cannot be synthesized in sufficient quantities to meet human needs. Thus, humans must obtain EFAs through dietary consumption. Owing to this innate inability to synthesize EFAs *de novo*, these fatty acids have been termed 'essential' as this refers to their requirement in maintaining biological processes. Besides acting as fuel sources for the body, EFAs play a role in many physiological systems in metabolism, inflammation, and energy homeostasis. Only two EFAs are known for humans: alpha-linolenic acid (ALA, 18:3ω3), an omega-3 (ω3) fatty acid; and linoleic acid (LA, 18:2ω6), an omega-6 (ω6) fatty acid. These two EFAs were discovered in 1923 and were designated as 'vitamin F' due to their necessary role in the diet of rats for the sake of standard health. However, subsequent studies during the 1930s by Burr as well as Burr and Miller have determined that ALA and LA are suitable for being classified as fats rather than as vitamins.

LA and ALA serve as the building blocks to make other ω3 and ω6 families of long chain polyunsaturated fatty acids (LC-PUFAs). These EFA LC-PUFA derivatives are coined as only being 'conditionally essential,' because EFA precursors are needed for their production. LA is the parent of the ω6 fatty acid family. One of the main derivatives of LA is the LC-PUFA arachidonic acid (AA, 20:4ω6), which is known to play a role in many physiological functions concerning cell signaling and the inflammatory response progression. In addition, AA has been implicated in serving its role as a structural constituent of the central nervous system. In westernized diets, most vegetable oils, human milk, and meat products contain high amounts of LA, which tend to be excessively higher than that required for normal biological activity. As a result, this surplus of fatty acids is often stored by the body as excess fat.

ALA is the parent of the ω3 fatty acid family. ALA can be found in a variety of dark green vegetation, vegetable oils, and flaxseed. Main derivatives of ALA include the LC-PUFAs docosahexaenoic acid (DHA, 22:6ω3) and eicosapentanoic acid (EPA, 20:5ω3). The human body produces relatively low levels of EPA and DHA, because the conversion rate of these fatty acids from ALA is rather slow. However, EPA and DHA can enter the body directly through dietary consumption of foods rich in ω3 fatty acids, such as cold-water fish, shellfish, human milk, or supplements like fish oil. EPA and DHA have been implicated to play a pivotal role in the resolution of inflammation by displaying antiinflammatory properties that promote health benefits. If fish high in ω3 fatty acids (salmon, tuna, herring) is consumed in two or more servings weekly, it has been shown to protect against abnormal heartbeat,

hypertension, atherosclerotic plaque formation in arteries, stroke, and even sudden death. Particularly, DHA is concentrated within organs and systems that are vital for human life, such as the central nervous system (including the brain and retina) and the male reproductive organs.

Derivatives of ω3 and ω6 family LC-PUFAs include prostaglandins (PGs), leukotrienes (LTs), and thromboxanes (TXs). These highly biologically active derivatives, which are not themselves fatty acids, serve as potent signaling molecules in a variety of physiological systems. Moreover, these molecules tend to have somewhat combating effects. The ω6 derivatives often lead to inflammatory processes and vasoconstriction, whereas ω3 derivatives stimulate a less inflammatory response, vasodilation, and have also been implicated in preventing the formation of blood clots. Regardless of the antagonizing events already mentioned, both families of LC-PUFA derivatives have profound hormone-like effects that aid in the regulation of the immune system and inflammatory response. Collectively, the derivatives of ω6 and ω3 fatty acids are important for health and the overall energy balance of the human body.

The body's shift in ω6/ω3 fatty acid ratio can be influenced solely by the diet alone. The ratio of ω6/ω3 fatty acid intake is important because the actions and dietary influx of one family of fatty acids may adversely modify the concentration of the other in a disproportionate manner. Despite this fact, it has been generally accepted that the ideal ω6/ω3 ratio for optimal human health is the consumption of no more than 4 times as much ω6 fatty acids as ω3 fatty acids. In a westernized diet, although vegetable oils are consumed regularly by people, fish or fish products are also eaten but infrequently. Subsequently, the ω6/ω3 fatty acid intake ratio exceeds the ideal limit, indicating that it is necessary for individuals to consume more ω3 fatty acids.

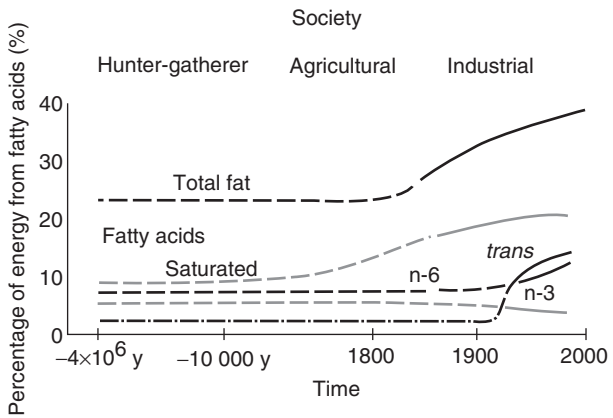

Figure 1 Hypothetical scheme of the relative percentages of fat and different fatty acid families in human nutrition as extrapolated from cross-sectional analyses of contemporary hunter-gatherer populations and from longitudinal observations and their putative changes during the preceding 100 years. Adapted from Simopoulos AP (1995) Evolutionary aspects of diet: Fatty acids, insulin resistance and obesity. In: VanItallie TB and Simopoulos AP (eds.) *Obesity: New Directions in Assessment and Management*, pp. 241–261. Philadelphia: Charles Press.

Dietary Evolution of PUFAs

Humans have undergone intense evolutionary selective pressures in order to optimize their capacity to obtain/store energy and utilize fatty acids – particularly PUFAs, for the purpose of growth, development, and survival. Conversely, dramatic dietary changes in LC-PUFA consumption over the past 100 years have probably created mal-adaptations for our ancient genome as humans have drastically changed the composition of their intake of fatty acids (Figure 1). These dramatic dietary changes are probably driving the onset of human diseases that relate almost exclusively to our current dietary consumption through interactions with human genomic adaptations over many generations when such adaptations have undergone development.

Moreover, the prevalence of disease within westernized countries has disproportionately affected individuals of different populational groups. For example, in the United States, remarkable differences are seen in the disease status between individuals of African and European descent. As a result, health disparities run rapid as populations of African ancestry are disproportionately impacted by inflammatory diseases when exposed to a westernized diet. Both genetic and metabolic changes are perhaps driven by the existing dietary patterns. Recent studies have revealed that the adaptive genetic changes are probably found within genes coding for enzymes by which LC-PUFA biosynthesis are regulated and increased risk for certain populational groups has thus been conferred. Identifying these specific gene-diet mismatches in different human populations will probably lead to both a better understanding of health disparities and effective strategies for preventing and treating human disease.

The topic of recent investigations have upheld the analysis of gene networks that code for enzymes and transporters necessary for the synthesis and incorporation of LC-PUFAs into human blood and tissue (LC-PUFA PATHWAY), Besides diet, active LC-PUFA content may be influenced by genetic fluctuation. LC-PUFAs and their downstream derivatives have been implicated in a variety of health outcomes including brain development, cardiovascular disease (CVD), and pyschological disorders. LC-PUFAs are highly enriched in the brain, being critical for its development and function. It is hypothesized that improvements in the efficiency of LC-PUFA synthesis enabled humans to convert short chain (SC) PUFAs for the synthesis of LC-PUFAs more efficiently. These implications have facilitated the use of the LC-PUFA DHA for its critical role in brain expansion and function. Moreover, one of the most striking dietary changes of the 20th century has been the massive increase in plant-based SC ω6 PUFA consumption that is coupled with lower ω3 PUFA ingestion, creating a dramatic shift in the ω3/ω6 PUFA ratio. This paradigm shift in dietary PUFA consumption has probably the potential to impact different populations at varying levels. In addition, recent studies are beginning to demonstrate the effects of secondary factors, such as genetics and racial ethnicity, on the predisposal of certain populational groups with higher/lower frequency of genetic-derived efficiencies in the LC-PUFA pathway. Thus, certain individuals within a population may be at a high/low risk of acquiring devastating mismatches between their genes and current dietary PUFA profile. This is

especially important because LC-PUFAs like DHA play a critical role during fetal and early infant development. Also, the development of chronic inflammatory diseases such as type-2 diabetes and metabolic syndrome are related almost exclusively to diet alone in adults and is associated with a shift in $\omega6/\omega3$ LC-PUFA content.

Dietary Sources and Recommended Intake of PUFAs

Consumption of 20–35% of total calories from fat has been currently recommended. However, much recent evidence has revealed that the 'type' of fat consumed may be more important to overall health rather than the total fat intake. Fats that elevate low-density lipoprotein (LDL) cholesterol levels (increased risk of heart disease) are attributed as being 'unhealthful,' whereas those that can raise levels of high-density lipoprotein cholesterol (displays opposite actions of LDL and helps eliminate excess cholesterol in the blood) are considered 'healthful'. Unhealthy fats include trans fats, saturated fats, and cholesterol. Healthy fats include monounsaturated fatty acids (MUFAs) and PUFAs, particularly ALA, DHA, and EPA. In an effort to combat the potential harmful effects of adding saturated fatty acids in processed food production, companies in the 1960s started utilizing unsaturated fatty acid oils such as soybean oil. The historical event that preceded the increase in soybean oil consumption in the United States was the 1961 American Heart Association (AHA) Central Committee Advisory Statement by which Americans were replace their saturated fat intake with polyunsaturated fats (Figure 2). Consequently, vegetable oils, shortening, and margarine were recommended as substitutes for animal fats such as butter, cream, and cheese. Unlike the increase in $\omega6$ consumption, dietary intake of $\omega3$ LC-PUFAs has declined, with a $\omega6/\omega3$ ratio now being as high as 15:1.

LA is found in high concentrations in nuts, seeds, and certain vegetable oils made from soybean, safflower, or corn. LA is also abundant in some oils such as those made from canola or flaxseed, even though these oils have an abundance of ALA. Since foods containing oils are very common in the westernized diet, getting sufficient amounts of LA can be easily achieved. Conversely, $\omega3$ PUFAs such as EPA and DHA are

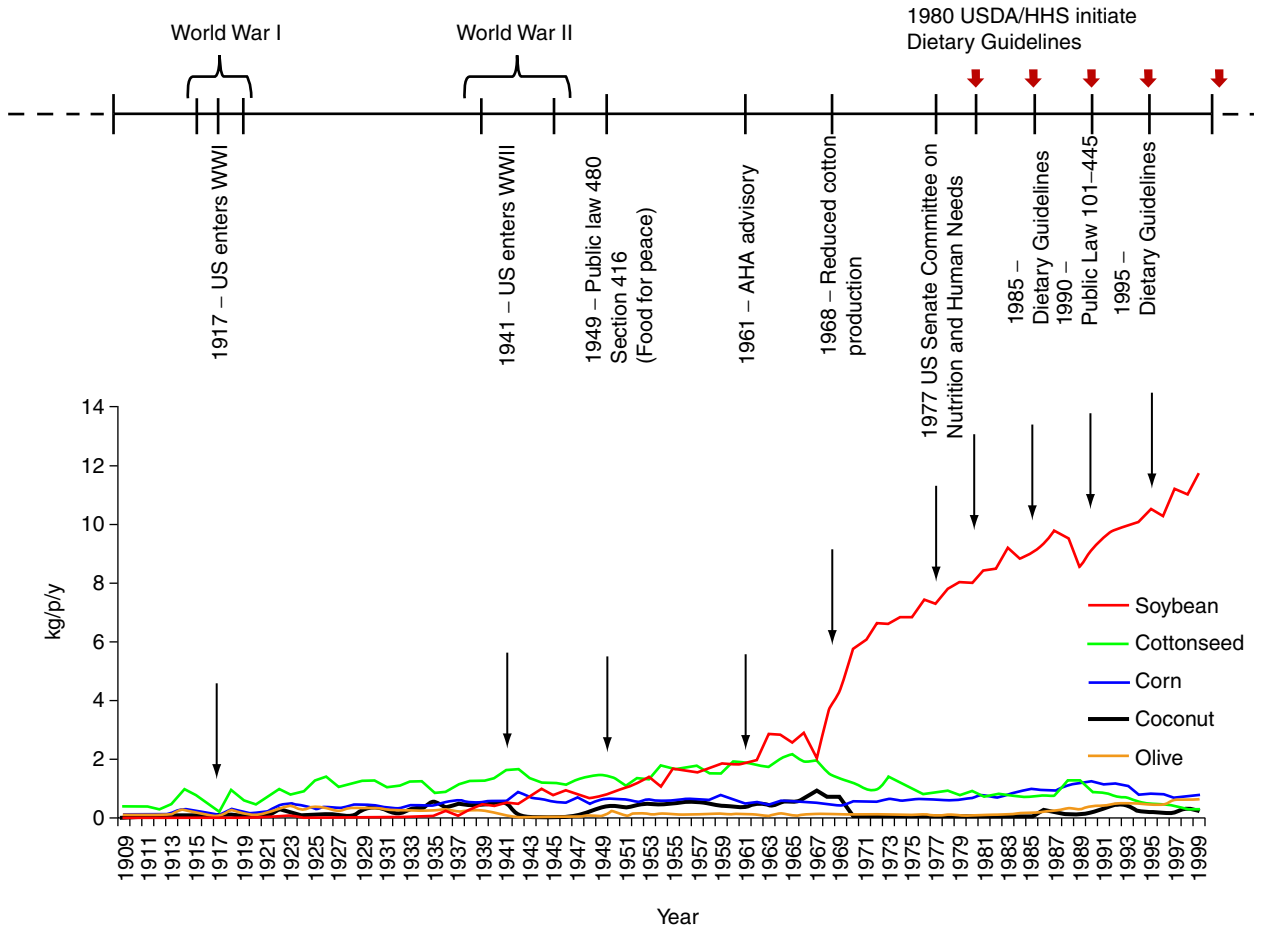

Figure 2 The historical event immediately preceding the largest increase in apparent consumption of soy oil in the United States was the 1961 AHA Central Committee Advisory Statement that advised Americans to replace their saturated fat intake with polyunsaturated fats. Vegetable oils and, to a lesser extent, shortening, and margarine were recommended as replacements for animal fats such as butter, cream, and cheese. Adapted from Blasbalg TL, Hibbeln JR, Ramsden CE, *et al.* (2011) Changes in consumption of omega-3 and omega-6 fatty acids in the United States during the 20th century. *American Journal of Clinical Nutrition* 93(5): 950–962.

found in smaller amounts of the westernized diet because fish and seafood consumption is very minimal when compared to unsaturated oil consumption. However, small amounts of the LC ω3 fatty acid DHA can be found in some meat and egg products.

Dietary Reference Intakes, or DRIs, for both ω3 and ω6 essential fatty acids may also be impacted by the age factor. Adequate intake levels (AIs) for LA are 17 and 12 g day^{-1} for adult males and females, respectively. For ALA, the AIs are 1.6 and 1.1 g day^{-1} for adult males and females, respectively. The Acceptable Macronutrient Distribution Ranges for LA and ALA are 5–10% and 0.6–1.2% of calories, respectively. Furthermore, one need to consume weekly at least two servings of fatty fish high in ω-3 fatty acids in addition to oils rich in ALA such as canola or flaxseed according to the recommendation of health agencies like the AHA and the US Department of Agriculture (USDA). Supplemental recommendations are also encouraged for patients with CVD, as a meta-analysis on the doses of EPA and DHA in fish oil necessary for optimal cardioprotection has demonstrated that a daily intake of 250 mg is an absolute minimum requirement to reduce the risk of some coronary heart diseases. The meta-analysis had revealed that when the intake of EPA and DHA exceeded 250 mg, the risk of sudden cardiac death in a healthy population was reduced by 35% as well as that of nonfatal coronary events by 16%. Further supplementation of EPA and DHA in amounts as much as 1000 mg in fish oil daily has been currently recommended by the AHA for those who already have heart issues.

Metabolism of PUFAs

Fats (lipids) are grouped into two basic subtypes: saturated and unsaturated. To determine whether a fat is saturated or not, there must be one or more double bonds between carbon atoms within the fatty acid carbon chain length. If only one double bond is present, then the fat is called a MUFA. However, if the fat molecule contains two or more double bonds in its carbon chain length, these fats are PUFAs. Saturated fatty acids (SFAs) are fats that do not contain any double bonds within their carbon chain length and are typically solid at room temperature. Many animal products such as butter, certain meats, cheese, and plant oils (coconut and palm) are rich in SFAs. In a westernized diet, most foods contain both SFAs and PUFAs; however, animal products probably contain more SFAs than PUFAs when compared to the products from plant oils.

LC-PUFA precursors are 'essential,' meaning that mammals lack the desaturase enzymes (delta-(Δ)12 and delta-(Δ)15), which add the carbon–carbon double bond to generate LA (ω6) and ALA (ω3). Thus, mammals must obtain the parent fatty acids LA and ALA through the diet, primarily from plant/botanical sources. The enzymes responsible for the subsequent desaturation (fatty acid desaturase (FADS)) and elongation (elongases; ELOVL) steps utilize ω9, ω6, and ω3 substrates (Figure 3). The omega number refers to the position of the carbon at the latest site of desaturation from the terminal methyl carbon atom (omega) of the fatty acid carbon chain. For example, in ω3 PUFAs, the last double bond is located three carbon atoms from the terminal methyl carbon of the molecule.

PUFAs are classified according to their degree of unsaturation and length of the hydrocarbon chain, and can be further subdivided into SC and LC classes. PUFAs that contain 20 or more carbon atoms are classified as LC-PUFAs. There are three PUFA subtypes: the ω9, ω6, and ω3 series. Once obtained through the diet, these parent unsaturated fatty acids can be further converted into fatty acids that are longer in chain length and higher in unsaturation by a series of elongation and desaturation steps, respectively. Through the unsaturated fatty acid pathway, ω9, ω6, and ω3 fatty acids may share similar enzymes for desaturation and elongation.

Oleic acid, the ω9 parent fatty acid, can be obtained through the diet in addition to being synthesized *de-novo* by the body. Palmitic acid (16:0,ω9) is elongated to form stearic acid (18:0,ω9), which is then further desaturated to produce oleic acid (18:1,ω9). The final major product of the ω9 series is mead acid (20:3,ω9), which is synthesized from oleic acid by Δ6 desaturation (FADS2). LA and ALA serve as substrates for other critical LC-PUFAs as well. Depending on which PUFA precursor is consumed, the metabolic fate leads to the production of either ω6 or ω3 LC-PUFAs. Because both fatty acids share comparable enzymes within their reaction pathways, competition may exist between both the ω3 and ω6 fatty acid subsets for metabolic conversion. LA and ALA can both be converted by the Δ6 FADS enzyme (FADS2) to gamma(γ)-linolenic acid (GLA, 18:3ω6) and stearidonic acid (SDA, 18:4ω3), respectively. This metabolic step is rate limiting and is subsequently followed by the elongation of GLA to dihomo-γ-linolenic acid (DGLA, 20:3ω6) as well as of SDA to eicosatetraenoic acid (ETA, 20:4ω3). Another key rate-limiting step is the desaturation of DGLA and ETA by the Δ5 desaturase (FADS1), which leads to the production of arachidonic acid (AA) and EPA, respectively. However, both AA and EPA can be further elongated on the ω3 branch of the PUFA pathway, and the very important LC-PUFA, DHA, is produced by the occurrence of several subsequent elongation and desaturation steps of EPA, followed by beta (β)-oxidation. This β-oxidation step is known as the Sprecher's Shunt and is essential for the retroconversion of EPA into DHA. The desaturation and elongation of fatty acids takes place in the endoplasmic reticulum, but the translocation of PUFAs into peroxisomes is necessitated by the β-oxidation step. Recent studies have revealed that besides peroxisome, mitochondrial β-oxidation may also serve as a secondary pathway for the production of DHA.

The Role of PUFAs in Chronic Disease and Inflammation

Chronic diseases such as CVD, diabetes, obesity, allergy, asthma, and arthritis have a strong underlying inflammatory component. With its normal operation to respond appropriately (pro-inflammatory events) to offending pathogens, the immune system encompasses and destroys these pathogens. After a pathogen is cleared, the immune system reverts back to the preexisting state by breaking the immune response and promoting tissue repair events (anti-inflammatory and

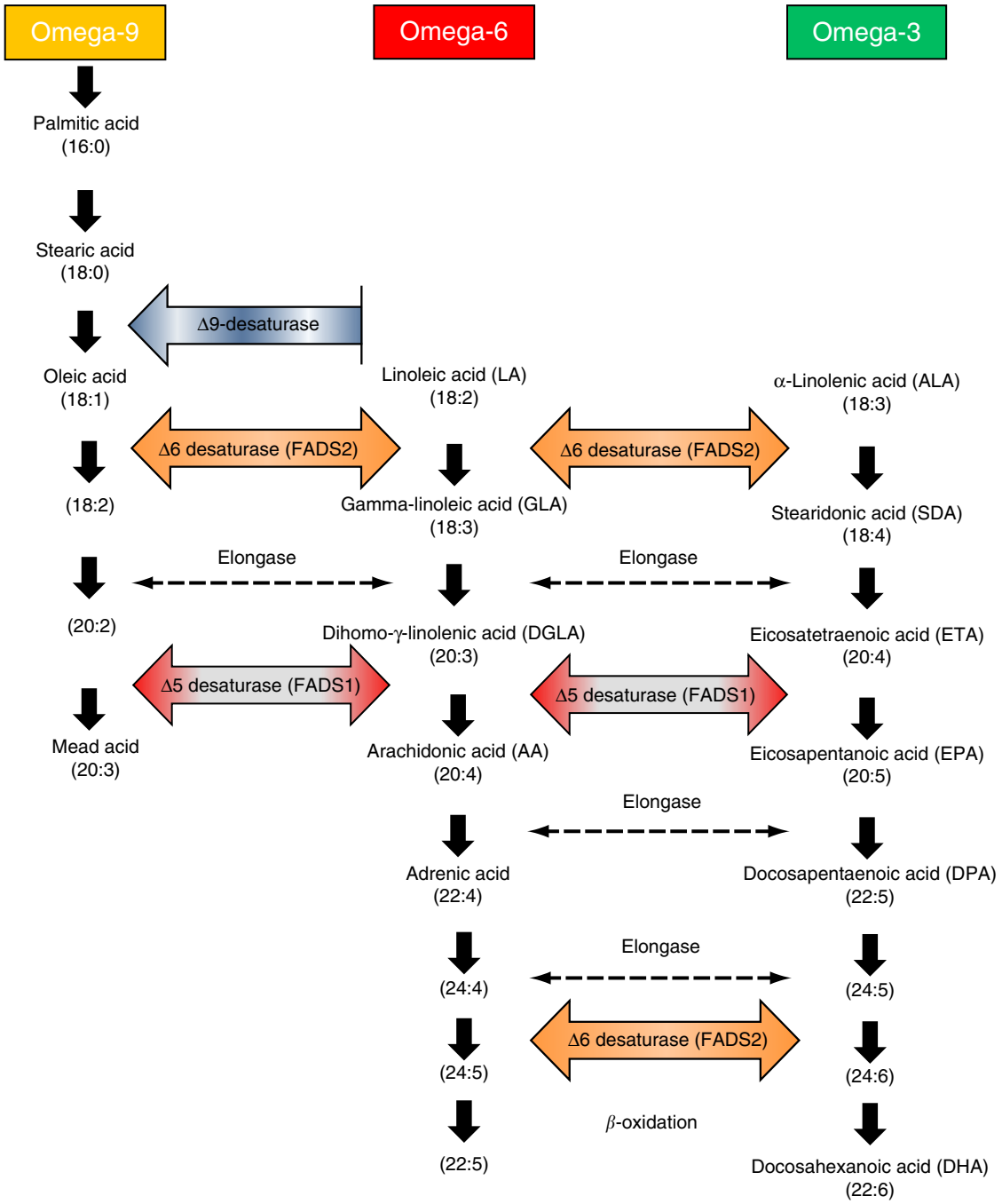

Figure 3 The ω9, ω6 and ω3 fatty acid metabolism pathways.

resolution). The immune system's homeostatic cycle is vital for health and is uniquely integrated with metabolic processesas immune responses are quite metabolically demanding. Metabolic dysregulation can therefore communicate inappropriately with the immune system, which leads to a state of unresolved chronic inflammation. Owing to decreased inflammatory resolution, the onset of chronic diseases can thrive. Despite the erstwhile notion of tissues being strictly in the metabolic realm (adipose, liver, and muscle), they are now recognized to generate a battery of mediators to which immune cells can respond.

Numerous inflammatory mediators are derived from LC-PUFAs. LA and ALA along with their derivatives (AA, EPA, and DHA) may serve as precursors to pro- and anti-inflammatory mediators. With their participation in numerous physiological systems, the ω3 and ω6 series LC-PUFAs have generally been shown to exert opposite actions in these systems (Figure 4). AA gives rise to a variety of lipid mediators (PGs, TXs, and LTs) that are potent stimuli of immune cells, platelets, and the vasculature. In general, pro-inflammatory actions are exhibited by AA-derived mediators. In contrast, anti-inflammatory properties are exhibited by both

the series-3 lipid mediators, which are derived from EPA, and the series-1 lipid mediators, which are derived from the AA precursor DGLA. The resolvin and protectin derivatives of DHA promote tissue repair as an immune response subsides. Thus, an imbalance between the intake and synthesis of the $\omega 3$ and $\omega 6$ LC-PUFAs has the potential to perturb immune system responses as well as the homeostatic processes associating with the system.

LC-PUFAs, especially AA and DHA, are critical for the proper development of the immune system during the neonatal period and neuronal development, respectively, and this role of LC-PUFAs is in addition to their role in immune responses, homeostasis, and energy storage. Fat constitutes almost 60% of the human brain mass and DHA comprises the PUFAs for the rest of the brain mass. Given the prominent roles of PUFA metabolism in both immune and nervous system development and maintenance, dramatic changes in dietary PUFA availability and/or metabolic capability can be predicted to have important health effects.

LC-PUFAs, Populational Differences, and Nutrigenomics

The major regulating enzymes of LC-PUFA biosynthesis are FADS1 and FADS2 (Figure 3). These desaturases are located in the plasma membrane of cellular surface bilayers. Both are located in a variety of tissues throughout the body, with the liver displaying the greatest level of enzymatic activity. Other major tissues displaying FADS1 and FADS2 activity include adipose, brain, heart, and lung. Three members of the FADS gene family have been identified in mammals. Located on chromosome 11, FADS1, 2 and 3 are speculated to have arisen through the mechanism of gene duplication as evidenced by their high degree of sequence identity (62–70%) and almost identical intron/exon organization (Figure 5).

There is strong evidence that FADS1 and FADS2 support the association of PUFA metabolism with circulating and cellular levels of PUFAs but only little evidence exists for loci outside of chromosome 11 being significant. Associations between genetic variants in the FADS gene cluster exist with CVD, cardiovascular mortality, insulin resistance, metabolic syndrome, breast milk fatty acid profile, and childhood IQ. Among FADS gene variants, many investigators have identified single nucleotide polymorphisms (SNPs). These SNPs are mapped to the FADS gene family on chromosome 11, and on observation the SNPs have strong genome-wide association signals that are associated with PUFA levels. Although these genetic findings are valuable, most have been conducted only in populations of individuals with European descent and there is limited data available for other ethnic populations. In contrast, when examining comparative studies involving American subjects of European and African descent, striking populational-based differences have been observed between circulating AA levels and estimated FADS1 activity. On average, African American subjects have exhibited significantly higher fasting AA levels than European Americans. Earlier comparisons of circulating fatty acids by population had revealed that AA levels in subjects of Zimbabwe Africa were almost twice that of Europeans.

Importantly, it was observed that such AA levels were strongly correlated with the genetic variations near the FADS gene cluster. When comparing the circulating AA levels between European and African Americans in the case of diabetes, metabolic syndrome, or healthy subjects, significantly higher levels of AA are found among African Americans. An inverse relationship was seen for DGLA, the immediate precursor of AA, suggesting that genotypic and populational differences in the enzymatic efficiency of FADS1 exist, as estimated from the AA/DGLA product–precursor ratio. Importantly, genetic

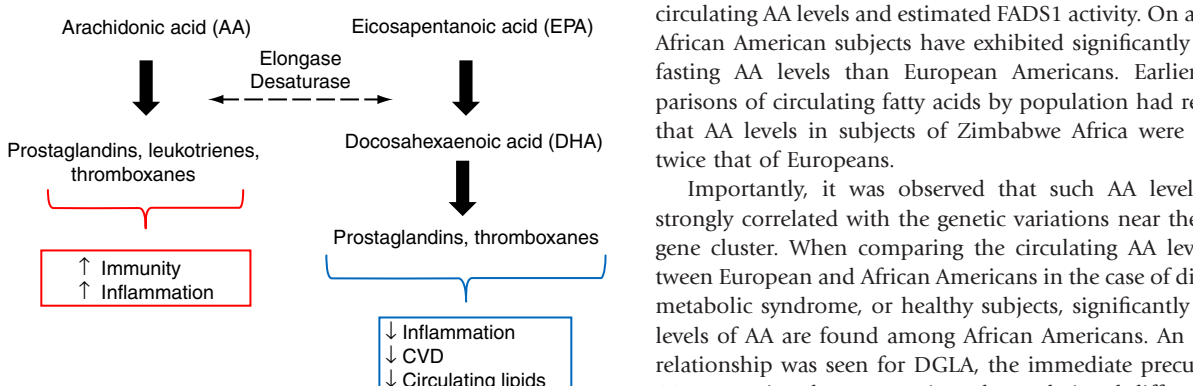

Figure 4 Derivatives and inflammatory impact of arachidonic and eicosapentaenoic acid metabolism.

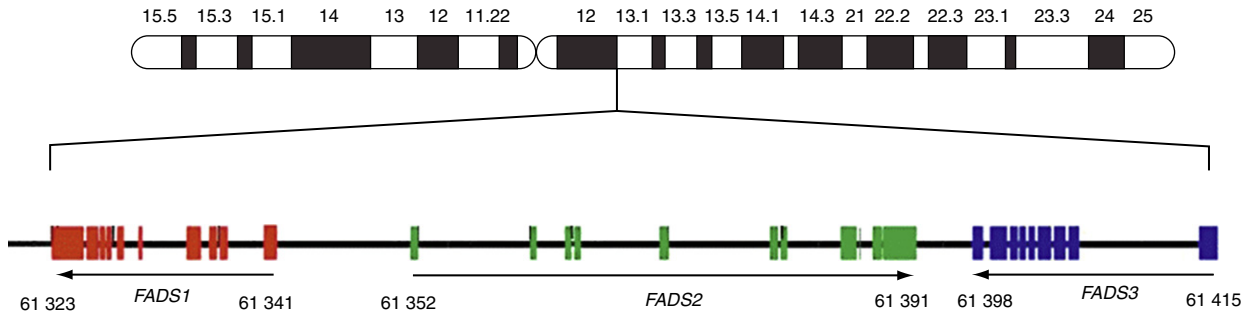

Figure 5 The human *FADS* gene cluster located on chromosome 11 with exon/intron organization; FADS1, fatty acid desaturase 1; FADS2, fatty acid desaturase 2; FADS3, fatty acid desaturase 3. Adapted and modified from Glaser C, Lattka E, Rzehak P, *et al.* (2011) Genetic variation in polyunsaturated fatty acid metabolism and its potential relevance for human development and health. *Maternal and Child Nutrition* 7: 27–40.

variants in the FADS gene cluster associated with high FADS1 activity were found to be nearly twice as prevalent in African Americans (~81%) than in European Americans (46%). In contrast, genetic variants associated with low FADS1 activity were observed as being rare within the sample of African American study participants. Collectively, these findings suggest that African Americans are genetically predisposed for more efficient conversion of dietary precursors to LC-PUFAs. Mapping these different genetic variants near the FADS gene cluster suggests that the fixation of PUFA metabolic variants has evolved throughout the African continent. In contrast, populations beyond Africa show increased frequency of genetic variants due to a lower rate of PUFA metabolism, and future studies are being focused on them.

Safety Considerations of PUFAs

Although optimal daily intake of PUFAs still remains unknown, additional questions regarding other dietary constituents need to be addressed. High dietary consumption of PUFAs and PUFA supplements may entail a potential risk of adverse effects and it is important to examine the experimental and clinical data regarding the safety of PUFA intake. When PUFAs are exposed to oxidative stress, they may be attacked by free radicals (reactive oxygen species) and are oxidized into lipid peroxides. The downstream derivatives of lipid peroxide metabolism include molecules such as ketones, aldehydes, and cyclic peroxides, which may potentially modify other lipids and proteins within the cell membrane that contain PUFAs. Lipid peroxidation is involved in the progression and pathogenesis of inflammation, various cancers, and atherosclerotic plaque formation.

Food products containing PUFAs that have been oxidized into lipid peroxides can be toxic; however, this is easily detectable. When PUFAs within food products are oxidized, such food products become rancid and develop an unpalatable taste. This often prevents the intake of large amounts of products containing lipid peroxide derivatives. Nonetheless, lipid peroxidation still remains a potential health hazard within industrial food settings because many products are manufactured and processed under conditions that allow oxidation. In a westernized diet, where the intake of processed foods using PUFA oils is high, it may be reasonable to consider supplementing the diet with antioxidants. Although dietary recommendations of antioxidants for diets rich in PUFAs have not yet been defined, their habitual incorporation may remedy the potential effects of lipid peroxide derivatives.

Derivates of ω3 PUFAs, which have been implicated in preventing the formation of blood clots, may also have undesirable effects when consumed in excess. The incidence of increased bleeding (from the nose, urinary tract, and/or obstetric bleedings) was associated with the unique diet of Greenland Eskimos. These individuals have ω3 PUFA intakes that are 7–10 g day^{-1}. This high ω3 consumption has been implicated in the inhibition of platelet blood clotting processes, causing a shift in eicosanoid metabolism when AA is replaced by EPA in platelet membranes. The increasing shift in PUFA intake leads to the generation of thromboxanes and prostacyclins that in totality leads to a more vasodilatory and antiaggregatory state. Additionally, very high ω3 intakes have been implicated in decreasing platelet counts.

Commercial PUFA supplements have recently become too popular. Drug stores, wellness clinics, and physicians are all working to determine the clinical efficacy of PUFA supplements or that of foods fortified with PUFAs. It should be noted that PUFA intake should be at the expense of saturated fat intake because SFAs are generally accepted as being less healthy than PUFAs. However, a habitual diet with high amounts of PUFAs can result in weight gain and possibly potentiate the progression of metabolic disorders. In regards to PUFA products, it is of critical importance that consumers are informed properly of potentially toxic substances such as those found in heavy metals, pesticides, fat soluble vitamins, and lipid peroxides.

The shift in consuming PUFAs instead of SFAs has been associated in promoting health benefits, especially in CVD. However, the current recommendation that at least 10% of daily energy intake must come from ω6 fatty acids may not be as practical for a variety of populational groups because studies to derive these current values have been performed predominantly on populations of European decent. Thus, further research is required to demonstrate populational differences in the capacity of separate ethnic groups to metabolize PUFAs. Defining differences in PUFA metabolism among ethnic groups allows for the potential to elucidate novel alternatives toward understanding undefined functions of lipid metabolism while deriving more sound recommendations for these nutrients.

Conclusions

Dietary fat consumption has undergone dramatic changes over the past five decades. The evolutionary discordance proposes a new set of interactions of how the human genome responds to its environment with respect to diet. Diets maintained by our ancestors, all the way to modern man, have been fairly constant, with minor changes occurring over long periods of time. The hunter-gatherer phenotype, seen by our ancestors, allowed for the stabilization of genomic alterations, which allowed for man to adapt to a changing environment and diet. However, due to the dramatic change in diets of westernized populations today, many diseases have emerged that are attributable to diet alone. In many westernized countries, such as the United States, sixty-five percent of adults over the age of twenty are either overweight or obese. In addition, recent studies have started revealing that there may be distinct ethnic differences in the efficiency of certain populational groups to synthesize LC-PUFAs. Previous studies have demonstrated increased levels of AA within the fasting serum of diabetic/metabolic syndrome patients of African descent when compared to those of European descent. Recent investigations have also explored genetic variations in FADS genes, using genetic tools like the International HAPMAP database (hapmap.ncbi.nlm.nih.gov/), and have identified many genetic polymorphisms that associate with PUFA metabolism.

The study of the genetic and physiological response to diet is known as nutrigenomics, which is a relatively new field of science, yet more commonly, it is personalized nutrition. The rapidly emerging field of personalized medicine will be important for identifying those at risk, for a given human disease. Nonetheless, the impact of personalized medicine is likely to be dwarfed by the significance of understanding the basis of chronic inflammatory diseases being observed epidemically, especially by certain racial/ethnic groups in developed countries. For these previously mentioned reasons, it is critical to study additional ethnic populations, which rather tend to be highly under-studied and neglected in the context of human genetics.

Genetic analyses may show a trend toward FADS gene variants effecting FADS1 activity and ω6 PUFA metabolism. However, direct evidence is not yet available for demonstrating a correlation between genetic changes at the FADS gene locus impacting LC-PUFA metabolism and FADS1 activity. More importantly, these deoxyribonucleic acid (DNA) variations could confer different phenotypic outcomes among individuals from different populational groups. Besides direct changes in genomic composition, DNA variations may have the ability to impact disease onset or susceptibility by altering other components such as messenger ribonucleic acid transcription, protein expression, protein stability, and levels of metabolites. Although numerous genetic markers and metabolic changes are probably contributing to health disparities, certain genetic polymorphisms could confer increased risk for particular populations with western-type diet when the enzymes regulating PUFA biosynthesis (FADS) contain such genetic polymorphisms. Consequently, the examination of genetic differences, and whether they translate into functional differences in fatty acid metabolism may be critical toward understanding the potential role of populational-specific phenotypes/genotypes in health disparities. By identifying DNA variations in those populations that are associated with the pandemic of chronic inflammatory diseases, which are exacerbated by consumption of a western-type diet, it will be possible to detect and prevent disease progression by diet alterations in at-risk groups. Preventing chronic disease development will significantly decrease the burden on our healthcare system, and most importantly, the quality of life for patients and their families will improve.

See also: Food Additives: Antioxidants. Public Health Measures: Fundamentals of Food Legislation. Safety of Food and Beverages: Oils and Fats. Toxic Metals: Mercury

Further Reading

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Relevant Websites

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Catalog of Published Genome-Wide Association Studies.

http://hapmap.ncbi.nlm.nih.gov/
International HapMap Project.

OTHER SIGNIFICANT HAZARDS

Contents

Food Allergies and Intolerances

SM Gendel, Food and Drug Administration, College Park, MD, USA

Glossary

Basophils Basophils are circulating immune-system cells that bind IgE through a surface receptor and release effector molecules by degranulation after activation.

Eosinophilic esophagitis A condition characterized by infiltration of esophageal tissue by eosinophils. This can result in difficulty in swallowing, heart burn, and food impaction.

Eosinophilic gastroenteritis A condition characterized by infiltration of gastrointestinal tissues by eosinophils. This can result in a range of symptoms, including nausea, vomiting, diarrhea, and malabsorption.

Major histocompatibility complex The major histocompatibility complex (MHC) is a gene complex that produces a number of cell surface proteins that have a place in a number of roles in the immune system.

Mast cells Mast cells are noncirculating immune-system cells that bind IgE and release effector molecules by degranulation after activation.

Oral tolerance Also known as immune tolerance, oral tolerance is a state in which the immune system does not respond to a particular external antigen.

Introduction

Consumers experience adverse reactions to foods for a variety of reasons. Generally, these have been described as being immune-mediated (sometimes called hypersensitivities), nonimmune-mediated (also known as intolerances), or of unknown (or idiopathic) origin (Figure 1). Within each of these broad categories, there are multiple biological mechanisms that produce the final reaction.

Nonimmune-Mediated Adverse Reactions

The nonimmune-mediated intolerances can be broadly thought of as either being metabolic or toxicological in origin; that is, they derive primarily from causes that are either internal or external to the consumer. The most common forms of metabolic intolerance are associated with enzyme deficiencies. These include problems such as lactose intolerance, aldehyde dehydrogenase deficiency, and favism. Lactose intolerance is a common metabolic deficiency caused by a lack of the lactase enzyme, which cleaves the lactose in dairy products into glucose and galactose. This enzyme is needed during infancy and early childhood, when milk is the major (or only) form of nutrition. However, lactase production declines or stops as part of the normal maturation process after infancy in most of the population. It has been estimated that up to 70% of adults worldwide have reduced lactase activity. When lactase-negative individuals eat lactose-containing products, generally dairy foods, the sugar passes into the large intestine, where it is fermented by the gut microbiota. This can lead to abdominal pain or discomfort, flatulence, and diarrhea. A deficiency of mitochondrial aldehyde dehydrogenase results in reduced ability to oxidize acetaldehyde produced during the metabolism of alcohol. This leads to symptoms of alcohol-flush syndrome, erythema, or flushing, when alcoholic beverages are consumed. This deficiency is found primarily in Asian populations. Favism is the result of a deficiency in glucose-6-phosphate dehydrogenase (G6PDH), although not everyone with low G6PDH manifests favism. Favism is a hemolytic response (an abnormal breakdown and loss of red blood cells) that occurs after consumption of fava beans (broad beans) by individuals with low G6PDH. Although the mechanism of the response has not been fully described, it appears to involve oxidative damage linked to exposure to the glycosides vicine and covicine in fava beans.

The second form of nonimmune-mediated intolerance is caused by toxicological or pharmacological reactions to

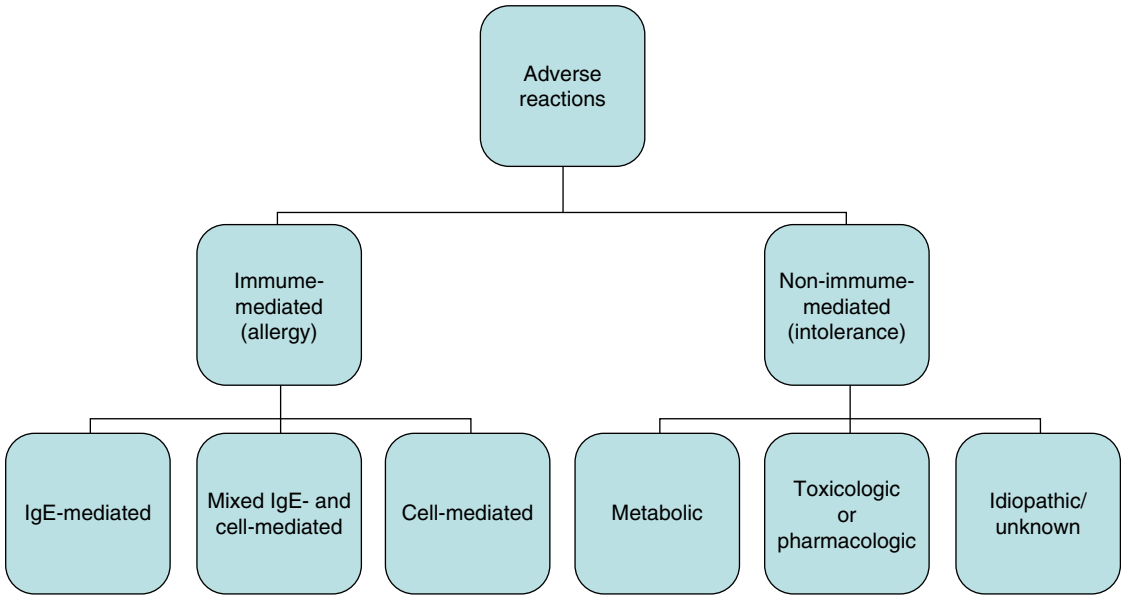

Figure 1 Types of adverse reactions to foods.

foodborne chemicals. These chemicals may include normal components of foods such as caffeine, chemicals added to foods such as monosodium glutamate or sulfites, chemicals formed by biological processes such as histamine, or chemical contaminants such as melamine. In many cases, the underlying metabolic process that leads to an adverse reaction is unknown. In other cases (e.g., caffeine) the pharmacology of the substance is well known although it may not be clear why certain individuals are more sensitive than others. These reactions generally occur after consumption of a relatively large amount of these chemicals in the food. Caffeine is the most widely consumed example of the methylxanthines. These compounds can affect a number of organ systems, including the nervous, respiratory, and cardiovascular systems. The most commonly observed adverse reactions are generally similar to panic or anxiety disorders. Histamine is a vasoactive amine produced by decarboxylation of a naturally occurring amino acid (histidine), generally by microbial action. Related compounds also produced in foods include tyramine, dopamine, and serotonin. Normally these compounds are rapidly converted to inactive derivatives. However, ingestion of large amounts can exceed the capacity of the metabolic system and can lead to clinical effects. For example, ingestion of large amounts of histamine (scombroid poisoning) leads to an adverse reaction that resembles anaphylaxis.

Immune-Mediated Adverse Reactions

Immune-mediated adverse reactions can occur through IgE-mediated, mixed IgE- and cell-mediated, or cell-mediated mechanisms. The IgE-mediated reactions represent the classic type of food allergy and are differentiated from the other forms of immune-mediated reactions in that they generally have a rapid onset and may be severe.

Food Allergy

The manifestation of an IgE-mediated food allergy is a two-stage process involving sensitization and elicitation (Figure 2). Sensitization triggers the production of IgE antibodies that recognize epitopes on food proteins. These antibodies bind to effectors cells, either mast cells or basophils. Re-exposure to the target food protein leads to crosslinking of IgE molecules on the surfaces of the effector cells, triggering release of effector molecules such as histamine and tryptase. These effector molecules interact with other cellular receptors to trigger the physiological processes that manifest as an allergic reaction.

Little is known about the overall mechanism of allergic sensitization in humans, although the general outline of the process can be inferred from detailed studies *in vitro* and from animal model systems. One important point that emerges from these studies is that sensitization can be viewed as the inverse of oral tolerance. Sensitization involves uptake of food proteins, intracellular processing of these proteins, and presentation of peptide fragments by major histocompatibility complex (MHC) proteins on the surface of antigen-presenting cells (APCs). The APCs then interact with CD^{4+} T-lymphocytes. Depending on a number of factors that are not yet fully understood, this interaction stimulates differentiation of the T-lymphocytes into Th2 type cells. The Th2 cells interact with B-cells to stimulate production of IgE antibodies to the proteins. These antibodies bind to the surfaces of mast cells and basophils through an IgE-specific receptor.

In some cases, sensitization to food proteins may occur through nonoral routes of exposure. For example, in oral allergy syndrome (OAS) sensitization occurs through inhalant exposure to pollen proteins. Immunologic crossreactivity between some pollen proteins and similar food proteins can result in a reaction involving pruritis and angiodema of the lips, tongue, palate, and throat in a sensitized individual. It

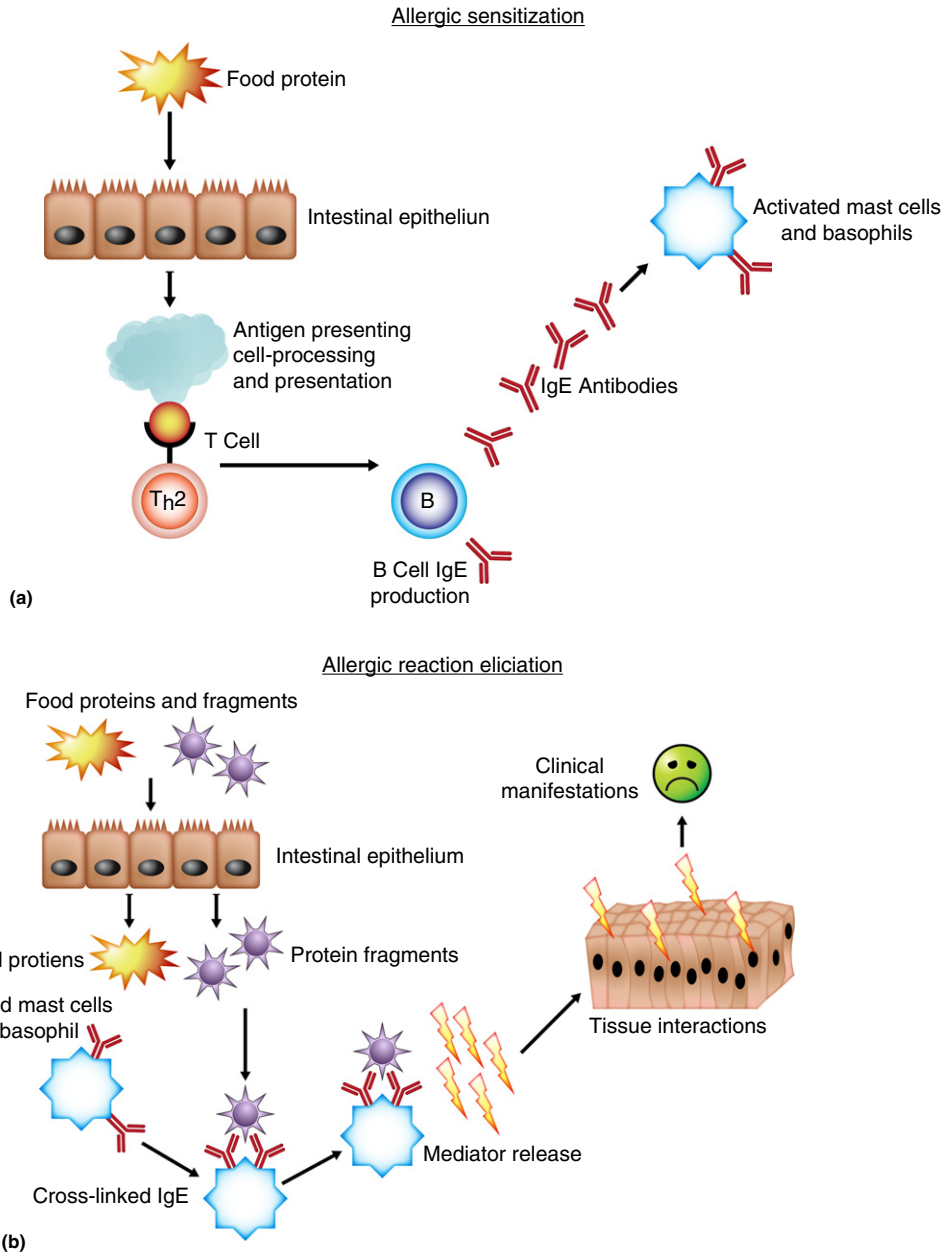

Figure 2 Mechanisms of an allergic reaction: (a) allergic sensitization and (b) elicitation of an allergic reaction.

may also be possible for sensitizing exposure to occur through skin contact.

Elicitation of an allergic reaction occurs on subsequent exposure to the same (or similar) proteins through crosslinking of IgE molecules on the surface of the effector cells. This crosslinking triggers a degranulation process that releases a suite of active molecules, including histamine and cell-type-specific cytokines and chemokines. It is the interaction of these signal molecules with tissue receptors that leads to manifestation of the signs and symptoms of an allergic reaction. The need to crosslink two IgE molecules to initiate the degranulation process suggests that relatively large peptide fragments must survive digestion and uptake in the gut in order to provoke a reaction. However, it is not known if this requires a single polypeptide or whether hybrid molecules produced by aggregation during food processing or consumption can trigger reactions.

IgE-mediated allergic reactions are usually rapid in onset, occurring within minutes of exposure. However, biphasic reactions are also seen in which the initial rapid manifestations subside or are controlled, followed by more severe signs several hours later. The signs and symptoms of an allergic reaction can range in severity from mild (that is, tingling of the lips) to life-threatening (anaphylactic shock; Table 1). Reactions can involve one or more organ systems and can be either localized or diffuse. The most severe reactions involve

Table 1 Signs and symptoms of allergic reactions

Organ system	*Signs and symptoms*
Cutaneous	
Skin	Itching Flushing Pilor erection ('goosebumps') Urticria (hives) Angioedema (swelling)
Oral cavity	Itching Numbness Dryness Edema
Eyes/conjunctiva	Itching Edema (periorbital) Redness of conjunctiva Tearing
Gastrointestinal	Nausea Abdominal pain Vomiting Diarrhea
Respiratory	
Nose	Itching Congestion or runniness Sneezing
Throat	Itching Dryness Tightness Hoarseness Wheeze Cough Swelling
Lungs	Respiratory distress Wheeze Cough
Cardiovascular	Chest pain Tightness Faintness/fainting Dizziness Hypotension Dysrhythmia Shock

multiple organ systems, particularly the respiratory and circulatory systems. Extrinsic factors such as exercise, alcohol consumption, and viral infection can affect both the likelihood and severity of reaction in some individuals. Severe reactions are also more likely to occur in individuals with uncontrolled asthma. There are no treatments to prevent a food allergic reaction. The medical response to a food allergic reaction focuses on preventing the progression of symptoms by administering epinephrine followed by antihistamines and other agents such as corticosteroids to minimize effector molecule responses and to maintain fluid balance.

There are few data related to why some individuals develop food allergies whereas most others do not. There appears to be a genetic component to susceptibility because the odds of developing an allergy are greater for individuals with close relatives who are allergic than for the general population. However, this increased susceptibility is not predictive of whether an individual will develop a food allergy. There is also no indication that susceptibility is related to either the type of food allergy that might develop or to the level of individual sensitivity.

Although individual foods may contain hundreds of unique proteins and peptides, only a small percent of these appear to provoke sensitization. It is widely believed that highly expressed proteins that are resistant to processing and digestion are more likely to be allergens. Binding studies using sera from allergic patients show that an individual might be sensitized to one or more proteins in a single food, and that the spectrum of specific proteins varies between individuals. However, these studies have also shown that certain proteins are more likely to be allergens than others. Those proteins that are recognized by IgE antibodies from more than half of the individuals who are sensitive to a specific food are called the 'major allergens.'

Although any food can be allergenic, a limited number of foods are responsible for the bulk of clinically significant allergies and allergic reactions. The most common allergenic foods worldwide include milk, eggs, peanuts, tree nuts, wheat, soy, fish, and shellfish. In addition, in some countries allergies to sesame, mustard, and buckwheat are considered common. It is not clear why these particular foods are the most common allergenic foods, although patterns of exposure probably play a role in determining relative risk. Many allergies develop in early childhood, but some (e.g., fish and shellfish allergies) often develop in adults.

There are limited data on the prevalence of food allergies, either in aggregate or to specific foods. It is difficult to measure the prevalence of food allergies through general health surveys because different surveys have used different methods and diagnostic criteria. Attempts to measure rates of allergic reactions through review of hospital records are similarly difficult because of inconsistent recording practices. Nevertheless, it is generally estimated that 2–3% of adults and up to 5–6% of children have true IgE-mediated food allergies. Although most allergic individuals react to a single food, some individuals react to multiple foods. Evidence exists that the prevalence of food allergy (and respiratory allergy) has increased significantly over the last two decades. Although many allergies are lifelong, a significant fraction of children will outgrow milk and egg allergies.

The diagnosis of food allergies involves the use of both a medical history and clinical tests. The history is used to establish a temporal association between consumption and reaction and to distinguish food allergies from intolerances or foodborne illness. The most useful clinical tests are skin prick tests, *in-vitro* assays that measure IgE titers, and tests that detect the presence of IgE specific for a particular food. Negative results in these clinical tests are considered to have a high predictive value for the absence of a food allergy. Positive results have a lower predictive value for the presence of clinically significant allergies because individuals may be sensitized (that is, they may have food-specific IgE) without showing clinical reactions. The 'gold standard' for allergy diagnosis and for understanding levels of sensitivity is the double blind placebo controlled food challenge (DBPCFC) using the first objective sign of a reaction as the endpoint.

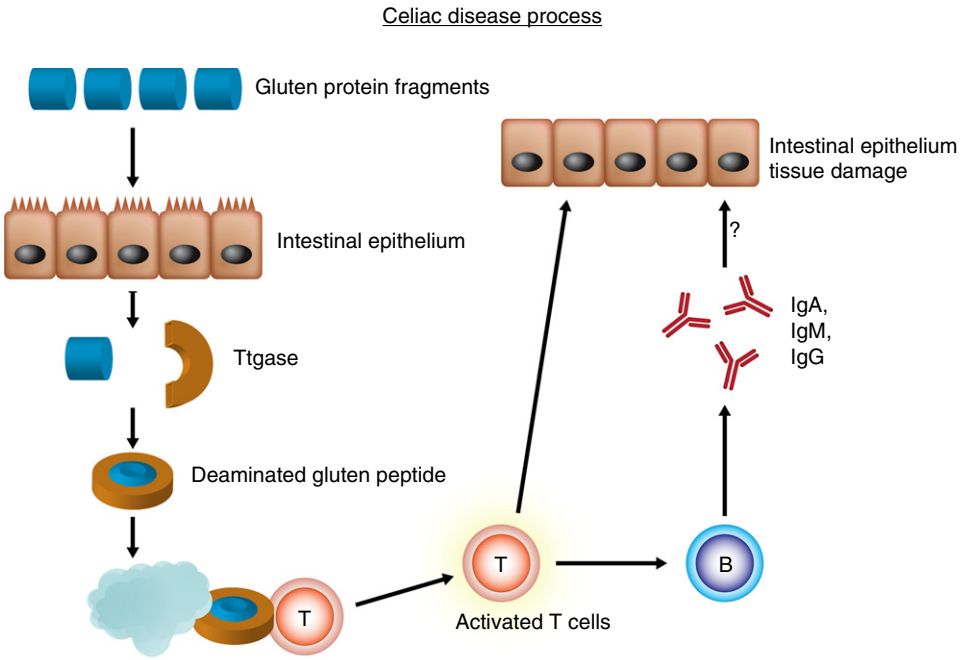

Figure 3 Mechanism of celiac disease.

The level of exposure required to elicit a reaction in a food allergic individual varies tremendously in the sensitive population. DBPCFC studies have found minimal eliciting doses for individuals over ranges as large as 6 orders of magnitude. The most sensitive individuals may react to less than 1 mg of protein. These studies also suggest that the specific food, the form of protein, the dose escalation pattern, and the nature of any masking agents used in a DBPCFC study may affect the observed minimal eliciting doses.

Because there is no cure for food allergy at this time, allergic patients must avoid eating those foods that provoke reactions. This includes avoiding both whole foods and any derivatives or ingredients that contain proteins from those foods. Practising avoidance is difficult when food labels use complex or technical terms to describe ingredients. In the last several years, a number of jurisdictions have implemented food allergen labeling laws or regulations that mandate the use of clear language to indicate the presence of ingredients derived from defined sets of food allergens. This improved clarity has not been extended to food service or retail environments.

Several approaches to eliminating or mitigating food allergies are currently undergoing preliminary clinical evaluation. These include desensitization, the use of anti-IgE antibodies, and herbal medications. Preliminary results indicate that some or all of these approaches may be effective for some sensitive individuals, although a number of questions must be resolved before these treatments can be applied more broadly.

IgE antibodies are also involved in 'mixed' reactions that involve both antibodies and cell-mediated processes. The two most common forms of these mixed reactions are eosinophilic esophagitis and eosinophilic gastroenteritis. Both of these syndromes involve eosinophilic infiltration of sections of the digestive tract. Food-specific IgE can be detected in individuals with these disorders and symptom resolution may occur after avoidance of these specific foods. However, reactions are typically localized to the intestinal tract and do not result in typical IgE-mediated symptoms.

Celiac Disease

Non-IgE-mediated reactions include protein-induced enteropathies, cell-mediated reactions, and celiac disease. Celiac disease is a hypersensitivity associated with consumption of gluten-containing grains (Figure 3). Celiac disease can have a wide range of clinical manifestations. The most distinctive effect is damage to the villi of the small intestine leading to gastrointestinal illness (including diarrhea, abdominal pain, nausea, and vomiting), and nutrient malabsorption. Celiac disease can also manifest through a wide range of symptoms that are less obviously linked to the digestive system such as anemia, fatigue, a skin disorder called dermatitis herpetiformis, or neurological problems. Individuals with celiac disease are also more likely to develop certain autoimmune diseases and malignancies than the general population. Definitive diagnosis of celiac disease requires histological examination of intestinal biopsy specimens. However, not all individuals with morphological changes have clinical symptoms, a condition that is sometimes called 'silent celiac disease.' It is not know what proportion of these individuals will eventually develop clinical manifestations.

Celiac disease occurs in genetically predisposed individuals who carry histocompatibility haplotyes HLA-DQ2 and HLA-DQ8. Although these haplotypes are necessary for development of celiac disease they are not sufficient. Other genetic and environmental factors determine which susceptible individuals will develop either 'silent' or frank celiac disease. These factors probably also determine the age at which symptoms

develop, the severity of those symptoms, and the progression of symptoms over time.

In susceptible individuals, a cascade of events occurs when the enzyme tissue transglutaminase (tTG) binds to gluten, which potentiates uptake and presentation by APC in the intestinal lamina propria. This triggers a T cell response leading to production of antibodies directed at gluten and to tTG as well as to direct T cell-mediated tissue damage that leads to disease manifestation.

As with food allergy, there is no treatment or cure for celiac disease. Individuals with celiac disease must avoid consumption of 'gluten,' which includes wheat, rye, and barley, and ingredients containing proteins from these grains. A fraction of individuals with celiac disease may also be sensitive to the homologous proteins from oats.

Idiopathic Adverse Reactions

Adverse reactions can also be idiopathic. Although the mechanism of reaction is unknown, such reactions are not necessarily rare. For example, sulfite sensitivity has been estimated to occur in as many as 10% of the asthmatic population. Although the mechanism of this sensitivity is unknown, regulatory agencies have been able to establish labeling and usage limits to protect sensitive consumers, which have greatly reduced the number of reported reactions each year. Other idiopathic reactions can be considered as 'emerging' issues. For example, the syndrome known as 'pine mouth' (a transient metallic taste disturbance after eating pine nuts) has been reported at an increasing rate in the last several years.

Although the mechanisms behind idiopathic reactions such as sulfite sensitivity and 'pine mouth' are unknown, the fact that many individuals experience similar reactions to a single food or food ingredient suggests that the common biological mechanisms exist. In other cases, idiopathic adverse reactions appear to be unique to an individual. Such individual reactions can manifest through virtually any sign or symptom, but can usually be prevented through simple avoidance.

The Future

The application of advanced genomic and molecular technologies promises to have a significant impact on our understanding of the biological mechanisms that underlay all types of adverse reactions. This includes understanding both differences in consumers and differences in the foods that lead to such a variety of sensitivities and range of responses. Further, changes in food labeling regulations have made it easier for sensitive consumers (particularly allergic and gluten-sensitive consumers) to identify and avoid those foods that trigger reactions. Increased awareness should improve our ability to measure and characterize the public health impact of the sensitivities.

Risk Management Measures

Because there are currently no treatments or cures for food allergies, allergic individuals must practice complete avoidance to prevent adverse reactions. This means that these individuals and their care givers become avid readers of food labels. Under the food labeling laws and regulations of many jurisdictions, manufacturers are responsible for providing labels that are complete and accurate, listing all the food allergens that are present in plain language. Manufacturers can insure that their labels meet this standard using Good Manufacturing Practices, supplier controls, and allergen control programs. See Management of Allergens in the Food Industry. Sensitive consumers must also rely on information supplied by restaurants, food service sites (such as schools and hospitals), and retail establishments. See Management of Food Safety in the Food Service Sector. It is also important for physicians to educate allergic consumers on how to respond to the first signs of an allergic reaction, and how to use rescue medications such as epinephrine autoinjectors epi-pens.

The importances of these measures are illustrated regularly in news stories and medical reports of allergic individuals having severe reactions when exposed to hidden allergens. For example, in late 2010 a 13-year-old student with peanut allergy in a Chicago public school died after eating take-out Chinese food with hidden peanut protein at a school function. Because some allergic individuals are sensitive to very small low levels of protein even small exposures, such as from the use of shared kitchen utensils or through carry-over in frying oil, can lead to severe reactions.

See also: Food Safety Assurance Systems: Management of Allergens in Food Industry. Processing Contaminants: Biogenic Amines

Further Reading

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Relevant Websites

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Celiac Sprue Association.

http://www.foodallergy.org/
Food Allergy and Anaphylaxis Network.

http://www.foodintoleranceawareness.org/
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Medical Information

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http://www.eaaci.net/
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http://www.niaid.nih.gov/
National Institute of Allergy and Infectious Disease.
http://www2.niddk.nih.gov/
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Academic Sites

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http://www.celiaccenter.org/celiac/default.asp
University of Maryland Center for Celiac Research.

OTHER SIGNIFICANT HAZARDS

Food-Related Choking

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Glossary

Asphyxia Oxygen deprivation caused by inability to breathe normally.

Asphyxia by ingestion Asphyxia caused by ingestion of food or a foreign body is the result of the distension of the anterior wall of the esophagus into the tracheal volume.

Aspiration Inhalation of food or other matter into the airway rather than the esophagus.

Bolus A mass of food that has been chewed or otherwise prepared for swallowing.

Choking Asphyxia caused by an object in the oral cavity or oropharynx.

Introduction

Foods are among the most common objects involved in choking and related injuries. Children constitute the largest susceptible subgroup for food-related choking, although other subgroups such as elderly adults and persons with certain psychiatric disorders experience elevated levels of risk. Risk of airway obstruction injury and fatality caused by food is a product of the potential hazard level of the foods, multiplied by exposure to these foods. The risks to the consumer are injury or death, and to the producer and distributer loss of brand value and costly litigation. The potential airway obstruction hazards of food are related to both the design and manufacturing quality of the products. The choking hazard level of many natural foods is inherent; however, the hazard potential of manufactured food products may be controlled and reduced through design. Exposure to choking hazard relates to the physical and cognitive developmental level of the consumer as well as marketing and societal factors. Exposure to foods that present a choking hazard may be mitigated through caregiver vigilance, labeling describing hazards, or packaging, which may mitigate or aggravate hazard manifestation.

Identification of Hazard Factors for Airway Obstruction by Food

The etiology of airway obstruction injuries from foods includes three components: exposure (to the hazardous characteristics), consequences (of contact with the hazard), and mitigation (of the effects of the hazard).

Injury and fatality data related to food and foreign objects may be analyzed to identify benchmark levels of hazard. Cognitive and behavioral foreseeable use and potentially hazardous characteristics should be assessed qualitatively or, preferably, quantified and subjected to comparison and analysis in order to determine potential risk of injury from food objects.

Creation of a Hazardous Condition

Consumer exposure to a food product with hazardous characteristics may create a hazardous condition for airway obstruction. The probability of this event must be determined. For example, food that is hard or elastic may be difficult to process into a bolus. Slippery food may be swallowed inadvertently before it has been masticated. Excessive amount of time (e.g., hard food) or insufficient amount of time (e.g., slippery food) taken to process a food in the oral cavity can increase the likelihood of a possible adverse event.

Probable exposure to the hazard may be determined using anthropometric data (e.g., the number of teeth), pediatric biomechanics, and foreseeable use analysis. The potential for the creation of a hazardous condition includes the evaluation of the impact of physical and cognitive development including the behaviors and capabilities of consumers on their abilities to assess hazardous product characteristics.

Direct Hazard

If a consumer is exposed to hazardous product characteristics, the severity level or potential consequence of this exposure must be evaluated. For example, consider aspiration of a food fragment. Analysis of the interaction of the size, shape, and consistency of the food with the anatomical characteristics of the airway may be conducted to determine the consequences, i.e., potential product-related injuries, based on the foreseeable behaviors consumers will use when interacting with products. To understand the severity of the direct hazard an understanding of the human anatomy may be used to effectively diagnose and demonstrate hazardous product characteristics.

To determine the level of hazard represented by a food involved in a potential choking, aspiration or esophageal obstruction incident, both product characteristics and

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anatomical characteristics of likely consumers should be examined.

Mitigation of Hazard

The severity level of an airway obstruction hazard caused by food may be reduced by design characteristics that lead to reduced consequence or decreased time to effective treatment. For example, a food engineered to dissolve very rapidly will represent a reduced hazard for serious or fatal airway obstruction injury.

Injury and Fatality Data

Foods are the most common cause of choking, aspiration, and ingestion injuries throughout the world. Three demographic subgroups have particularly high incidence of food-choking injuries and fatalities: neurologically impaired persons, the elderly, and children. The first group includes persons with both permanent and temporary neurological impairment. Elderly persons may be at increased risk of food choking due to loss of motor control or loss of dentition. Children constitute the largest and best-studied susceptible subpopulation and reasons for their increased risk are discussed here in detail.

Approximately 75 children die from choking on food each year in the US, and approximately 10 000 children are seen for food choking incidents in US emergency rooms annually. Toys or consumer products, for example, can be designed to prevent choking by being too large to fit in the oral cavity and tough enough to resist releasing small parts. By contrast, foods must by definition be small enough to fit at least partially into the mouth and lend themselves to maceration or dissolution and subsequent swallowing. The specific foods associated with choking-related injury and fatality exhibit international variation according to local diet. However, the physical and rheological characteristics of foods appearing in injury and fatality studies remain remarkably constant, as do the demographics of persons involved in the incidents. In general, children less than 4 years of age represent most of the injuries and fatalities caused by food foreign object airway obstruction. This trend is demonstrated in Figure 1, which includes injury data collected from 48 children hospitals and fatality data from a 1984 paper by Harris *et al*.

Factors influencing the likelihood and severity of a choking injury include the physical properties of the food and the characteristics of the person consuming the food. Certain foods, such as nuts and seeds, appear with great regularity in injury data, but rarely cause fatalities. Others, including pliable cylindrical foods that can effectively plug the airway, are often associated with fatalities. However, the nature of the food is not the sole predictor of hazard. Anatomical and psychological factors can predispose population subgroups to injury or fatality from specific types of foods. For example, infants and young children have immature dentition that prevents them from grinding food into a smooth homogeneous bolus.

A study examining data related to foreign object injuries treated at 48 children hospitals throughout the world found nuts, meat, sunflower seeds, carrots, chicken (and chicken bones), popcorn, hot dogs, fish (and fish bones), hard candy, and apples to be the 10 foods most frequently associated with injury (see Table 1). Fatality data compiled from a 1984 paper by Harris *et al.* indicate a somewhat different trend (Table 2). Hotdogs, hard candy, nuts, grapes, meat, cookies and biscuits, carrots, apples, popcorn, and peanut butter were found to be the 10 foods most commonly associated with fatal airway obstruction.

Although the data in Tables 1 and 2 provide epidemiological information on the risk associated with foods, they do not provide guidance on inherent hazard. The latter

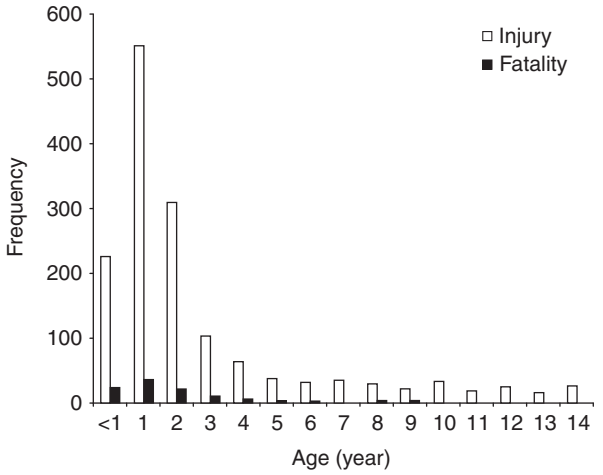

Figure 1 Airway obstruction by food injuries and fatalities by age (fatality data ends at 9 years).

Table 1 Foods associated with choking-related injury (1989–1998)

Food	*Number of injuries*
Nuts and peanuts	406
Meat	93
Sunflower seeds	66
Carrots	57
Chicken and chicken bones	54
Popcorn	44
Hot dog	41
Fish bones and fish	34
Candy	34
Apple	31

Table 2 Foods associated with choking-related fatality (1979–1981)

Food	*Number of fatalities*
Hot dog	17
Candy	10
Nuts and peanuts	9
Grapes	8
Meat	7
Cookies/biscuits	7
Carrots	6
Apple	5
Popcorn	5
Peanut butter	5

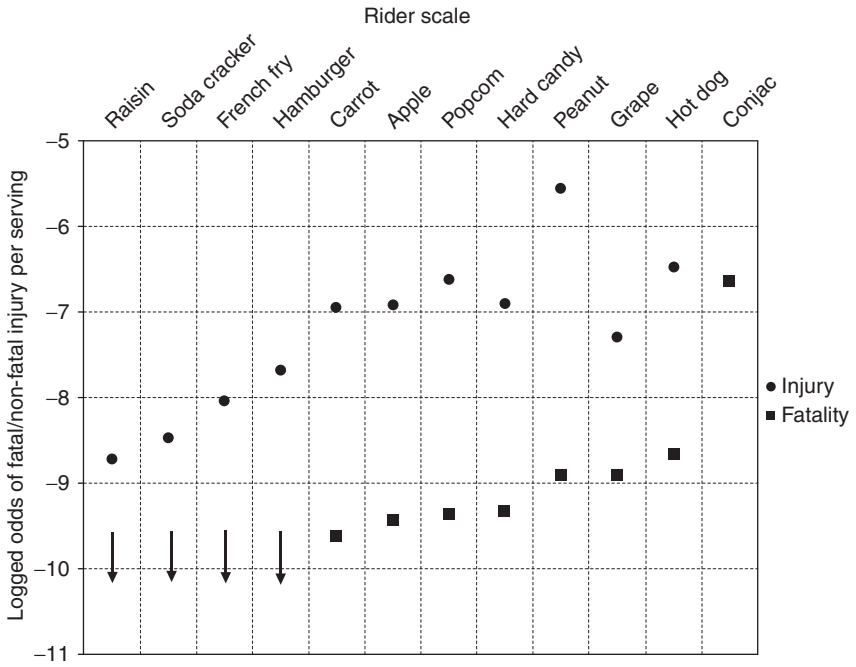

Figure 2 Rider scale: injury and fatality hazard levels of foods.

requires correction of the data for exposure to the foods in question. Knowing the percentage of individuals eating a certain type of food, and the typical serving size of that food when children eat, the annual number of servings can be estimated by dividing the product of percentage of individuals consuming, the population size, the daily consumption amount, and the number of days in 1 year by the typical serving size. Using data from the US, injury and fatality data were projected to serve as a national estimate on an annual basis. Finally, the ratio of injuries and fatalities to the number of annual servings was calculated. The results are plotted in Figure 2.

To create Figure 2, food intake information was acquired for children less than 3 years of age. It is presented in terms of the percentage of individuals consuming a certain type of food, and the daily consumption amount among the individuals who consume. In conjunction with the demographic information, the annual number of servings was estimated.

The ratio of injury and fatality statistics to the annual number of servings of a certain food was calculated as a risk indicator for that food. Raisins, soda crackers, and French fries were associated with risk indicators with low values, regardless of their high exposure level. In contrast, apples and grapes exhibited relatively high likelihood of injuries per serving. This may result from the hazardous characteristics associated with apples and grapes, which demand more advanced oral xmanipulation skills for young children.

Cognitive Development

Potential for airway obstruction is profoundly influenced by the feeding abilities developed at different ages. Newborn children cannot eat solid foods. Their oral cavities are a compact vertically moving structure and are instinctually capable of suckling. Indeed, ultrasound images of late-term fetus show suckling behaviors before gestation. These behaviors are innate or instinctual and are governed by robust hindbrain reaction to stimuli. Consumption of more solid foods is a learned behavior. As children age, they develop muscular coordination and oral fluency with more diverse textures, and their lower jaws gain transverse motility.

As children begin to feed themselves, personality and situational factors affect feeding. The feeding behavior of a child sitting quietly at a table with caregiver vigilance does not create the same hazardous condition as a child eating while running, laughing, or playing with a low level of caregiver vigilance. One child may nibble at food whereas another may prefer to shove large amounts of food into their mouth.

Adults may create a hazardous condition for airway obstruction injury related to food by consuming food while under the influence of drugs or alcohol, or in conjunction with other tasks such as watching television or driving. The elderly may suffer diminished oral perception and cognitive difficulty, leading to the creation of a hazardous condition for airway obstruction. In addition, those members of the population suffering from neurological deficits or developmental delay may have difficulty in handling any but the most benign food textures.

Injury Location and its Effect on Severity

The hazards associated with different types of foods are related to the anatomy of the human upper airway (Figure 3).

Of the majority of foreign objects which are not immediately fatal, approximately 65% lodge in the esophagus

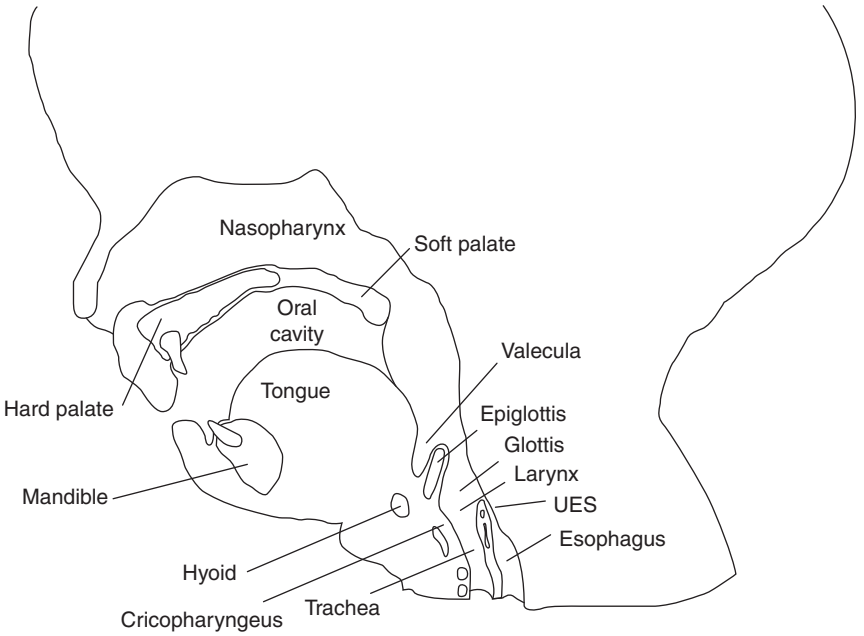

Figure 3 Anatomy of the upper airway.

(ingestion), usually at the level of the crico-pharyngeus. Foreign objects aspirated below the epiglottis make up 26% of injuries (aspiration). Objects lodged in the posterior oral cavity or orophaynx are referred to as choking incidents and make up 5% of incidents (choking). Objects inserted into the nasal passages make up 4% on foreign object incidents (insertion). These locations have a profound effect on severity of injury. Although choking incidents make up only 5% of all incidents, they account for 25% of foreign body incidents resulting in severe injuries including permanent brain damage.

Physical Development

Newborns suffer relatively few airway obstruction events, both because any objects introduced into their oral cavities are likely placed there by a caregiver, and because the laryngeal inlet has not yet lowered into the mature position. Newborn humans can suckle and breathe simultaneously due to this structure.

Dental Development

Incisors erupt at approximately 12 months of age. Incisors are teeth at the front of the upper and lower jaws used to scrape or tear food objects into smaller particles. Canine teeth erupt at 16–21 months of age. Canines are adjacent to the incisors and are used to tear or cut food objects into smaller particles. Molars erupt at 15–24 months of age. Molars are used to crush food objects into smaller particles.

Primary dentition is replaced in the same order by secondary dentition beginning at approximately 4 years of age for incisors, through maturity and full skull development for molars. As indicated in Figure 1, food injuries and fatalities begin before the eruption of incisors but peak at ages 1 and 2 years. This is consistent with a picture in which incisors and canines allow children to tear relatively large pieces from foods but young children lack the molars necessary to process these pieces into easily manageable boluses.

The elderly may be edentulous, and may employ dental appliances. Consequently, this segment of the population may have greater difficulty processing foods for proper bolus formation.

The Swallowing Process

Most of the process of eating is a learned skill. Although a child readily puts most things in its mouth, the ability to distinguish food from any other material has to be taught, and even then it is several years before the whole range of foods can be consumed. Our mastery of the complex set of mechanical actions involving tongue, teeth, and swallowing is so complete that we do not consciously note what we are doing or how we do it. Being able to bite and chew food efficiently is influenced by the sensory properties of the food and its placement in the mouth. Biting and chewing food is a multisensory task that requires a high level of coordination of all parts of the mouth. The degree of skill is determined by sensory awareness and discrimination and integrated coordination of the jaw, lips, cheeks, and tongue.

Airway obstruction from food objects may be caused by premature or inadvertent swallowing. To understand how airway obstruction from food objects occurs, we must first understand the mechanism of successful swallowing.

Swallowing can be roughly divided into four phases: oral preparatory, oral, pharyngeal, and esophageal. The first phase and first half of the second phase are voluntary. If a problem is sensed during these phases there is a possibility of aborting the swallow and preventing potential injury. The second half of the oral phase is involuntary, as are the pharyngeal and

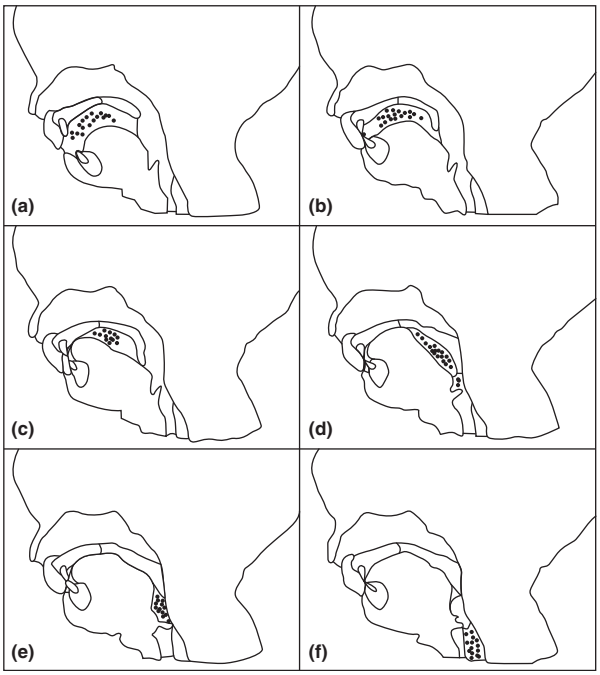

Figure 4 Phases of swallowing: (a) oral preparatory, (b) oral, (c) oral, (d) pharyngeal, (e) pharyngeal, (f) esophageal.

esophageal phases. Once these stages are reached it is impossible to stop or alter the swallow. Figure 4 illustrates the swallowing process, and the swallowing phases are explained in more detail below.

Oral Preparatory Phase

The oral preparatory phase is the initial stage of oral food manipulation in which food is chewed and manipulated into a bolus suitable for swallowing. The length and quality of this phase vary dramatically with the physical properties of the food and the quantity of food taken into the mouth. For infants consuming liquid food no chewing is involved and the oral preparatory phase occurs quickly. As children mature they begin eating thicker liquids and solid foods, and the oral preparatory phase requires more time. For these children and adults, the oral preparatory phase requires execution and synchronization of several highly complex motions. The tongue must effectively detect and sort food particles according to size and move the larger food particles toward the teeth where they can be processed into smaller pieces. In some cases, this task is made even more complicated if additional food is added to the mouth before swallowing. Once the food has been adequately chewed and mixed with saliva, the bolus is moved into a position between the tongue and hard palate. In this position, it is possible to voluntarily initiate a swallow. During the oral preparatory phase, the soft palate is moved into a lowered position by the palatoglossus muscle to help prevent parts of the bolus from prematurely entering the pharynx. The larynx and pharynx are relaxed during this phase and breathing through the nose is possible.

Oral Phase

The oral phase begins with a voluntary peristaltic motion of the tongue that moves the bolus toward the back of the oral cavity. As this phase progresses, the bolus stimulates nerves at the back of the oral cavity that trigger involuntary actions including movement of the bolus into the pharynx and closing of the nasopharynx to prevent reflux of the bolus into the nose. The oral swallowing phase is rapid, generally requiring less than a second.

Pharyngeal Phase

The pharyngeal phase begins as the soft palate elevates to close off the nasopharynx. This phase involves a number of complex coordinated motions. As the bolus enters the pharynx, the pharyngeal muscles constrict to force the bolus through the pharynx. At the same time the larynx is closed to keep food from entering the airway. The epiglottis is brought down over the glottis and the bolus moved toward the upper esophageal sphincter (UES), which is the opening to the esophagus.

In a normal pharyngeal swallow the entire bolus must proceed through the pharynx and the UES whereas the airway is protected against aspiration of the swallowed material. This requires precise coordination between motions of the bolus laryngeal closure that protects the airway.

Esophageal Phase

The esophageal phase consists of a weak involuntary peristaltic wave that moves the bolus through the esophagus and into the stomach. The peristaltic wave propagates at approximately 2–4 cm s^{-1} and terminates when the food passes through the gastroesophageal junction.

At birth, the greater pressure in the esophagus is the principal means of preventing reflux of stomach contents. However in the first few weeks after a term birth, closure at the gastroesophageal junction takes over in preventing reflux. At this point, esophageal swallow peristalsis is much the same in infants, children, and adults.

Mechanism of Injuries from Food

Several characteristics influence a foreign object's chance of penetrating the defenses of the mouth and pharynx (Figure 5).

Foreign objects that are small, thin, smooth, or slick when wet may prematurely slip through the oral cavity and enter the pharynx. Foreign objects that are round or cylindrical and pliable or compressible most effectively form a plug in the airway. Highly viscous foreign objects will also mold to the airway. A large bolus or foreign objects mass is more likely to block the airway at the pharynx and cause asphyxia. Foods that are ingested and lodge beyond the UES may distend the wall of the esophagus into the volume of the airway along the length of the trachea. When premature or inadvertent foreign object penetration occurs, a gag and or cough reflex may be triggered.

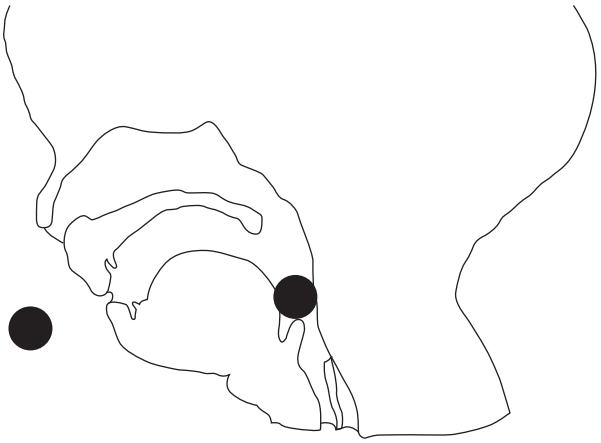

Figure 5 Obstruction of the oropharynx.

Smooth, slick, and pliable food-like objects are less likely to trigger a timely gag reflex than textured or sharp objects.

Gagging and coughing is frequently followed by rapid deep inhalation as the victims attempt to regain their breath. This action may draw the object downward leading to physical impaction and obstruction. This consequence is facilitated by the temporary expansion of the pharyngeal and laryngeal chambers that occurs during vigorous inspiratory effort. This reaction is more intense in infants than older children or adults. Pliable conforming objects are less likely to be expelled from the airway than rigid objects.

Protective Mechanisms

The body has several protective mechanisms to prevent choking-related injury. Gagging and productive coughing are vigorous hindbrain reflexes that may remove foreign objects from the airway. The gag reflex includes anterior and inferior jaw movement, tongue extension, and spasm of the stomach and esophagus. The purpose of these actions is to expel food objects sensed as noxious by the nervous system. A productive cough may expel foreign objects which enter the trachea or bronchial tree. Very small foreign objects or particles may be removed from the airway by muco-cilliary action. The cilia or hair cells lining the lung passages carry particles upward to the laryngeal inlet, where a productive cough can carry them out of the airway. These reflexes come into play after a foreign object has penetrated the defenses of the oral airway.

Evaluation of the Hazard Levels of Foods

The choking hazard associated with a given food is largely associated with its physical properties. Physical property measurement can be approached in two ways: sensory and mechanical evaluation. Sensory evaluation uses the human senses of taste, smell, touch, and sight to measure food properties. These evaluations can range from informal quality checks to trained test panels to very sophisticated consumer tests. Sensory evaluations have proven to be difficult to maintain consistency; therefore, these evaluations are often correlated and compared to objective physical measurements. Few physical property measurements have ever been shown to be correlated with sensory evaluations. Complete assessment of the physical properties of foods involves interactions between the material properties of the food and its structure, complex stress states, and interactions with lubricated oral surfaces. It is, therefore, hardly surprising that attempts to correlate sensory assessments and physical property measurements are often inconclusive.

It is also possible to use rheological techniques to measure quantities that contribute to food hazard. Commonly measured quantities include the following:

- Lubricity or 'slipperiness': Slippery objects are more difficult to control in the mouth, potentially creating a hazardous condition. Very slippery objects may initiate a premature swallow before the food is processed into a bolus that is safe to swallow.
- Hardness: Hard objects are difficult to process into a bolus. The longer a food takes to process into a bolus that is safe to swallow, the greater the potential that the food will prematurely enter in to the pharyngeal phase of the swallow cycle. Foods that are hard generally require more developed dentition and oral fluency to process. Therefore, the softer a food, the more suitable it will be for children who have not yet developed their full dentition.
- Dissolvability: Objects which rapidly break down in the presence of heat or moisture, and particularly in saliva with the presence of enzymes, may be easily formed into a bolus that is safe to swallow. Objects which dissolve slowly create a hazardous condition due to the greater amount of time required for bolus formation, and a direct hazard if they enter the airway and remain in a solid state.
- Surface roughness or texture: Roughness and increased surface area provide improved oral manipulability. Smoother foods may be more difficult to manipulate in the oral cavity.
- Friability: Objects which easily break into small soft pieces are more easily formed into a swallow-safe bolus. The development of teeth is critical to determination of age appropriateness of foods. In general, multiple fragments increase mouthing difficulty. This is especially true for fragments that have diverse material properties. Multiple fragments which are homogenous in characteristic pose a lower level of difficulty, but may still become scattered in the oral cavity and potentially provoke a gag reflex.
- Size: Oral sensors can discriminate the size of solid materials, but children may make poor decisions as to what they can successfully swallow. Objects that pass into the airway have a direct hazard in proportion to the area of the airway they obstruct.
- Shape: Spherical or cylindrical shapes will occlude the airway if they are the diameter of the airway. Analysis of injury and fatality data indicates that foods that retain these characteristics in the environment of the oral cavity and oral airway such as hot dogs, hard candy, and grapes are particularly hazardous, especially for young children and infants. By contrast, 'one dimensional' objects such as coins are less likely to effect a complete airway blockage.

- Packaging: In some cases, packaging can increase the risk associated with food products. In particular, package designs that promote the transit of foods to the rear of the oral cavity rapidly may create a hazardous condition for premature or inadvertent swallow, leading to the potential for airway obstruction injury.

Prevention of Food-Related Choking

Choking is an inherent hazard associated with food, although food-choking incidents are highly preventable. In the case of un- or minimally-processed foods such as fruits, nuts, seeds, fish, or vegetables, caregiver discretion and vigilance are needed to reduce choking risks. Caregivers can limit access to hazardous foods or modify their physical properties to reduce hazard. In the case of manufactured food products, manufacturers can design or redesign foods to avoid shapes, sizes, textures, and other characteristics that increase choking risk. Furthermore, developmental appropriateness needs to be taken into greater consideration. A food of benign hazard to adults may be challenging to young children. Appropriate warning labels can aid in proper product selection and safe consumption.

Public education is another key component of choking prevention. Pediatricians, dentists, and other healthcare providers should provide choking-prevention counseling to caregivers and consumers. Enactment and enforcement of safety legislation can also serve to reduce risk by eliminating particularly hazardous foods or mandating labeling that increases hazard awareness.

See also: Other Significant Hazards: Physical Hazards in Foods

Further Reading

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OTHER SIGNIFICANT HAZARDS

Physical Hazards in Foods

M Edwards, Campden BRI, Chipping Campden, UK

Glossary

Container glass Jars and bottles used to pack food and beverage products, made from soda lime glass.

Extraneous vegetable matter (EVM) Intrinsic (qv) plant material such as fragments of pea pod or pea leaf included in peas.

Extrinsic foreign material Derived from any origin other than the plant or animal product that has been contaminated; *cf.* intrinsic foreign material.

Heat-resistant glass Sometimes known as borosilicate glass, it is resistant to cracking as a result of heat shock because its coefficient of expansion is much less than that of ordinary soda lime glass. Originally developed for railway signal lamps and typically used for domestic glassware such as casseroles for use in ovens and microwaves.

Intrinsic foreign material Derived from another part of the plant or animal product that has been contaminated; *cf.* extrinsic foreign material.

Lead glass A type of glass in which lead replaces the calcium content of a potash glass. Lead glasses may contain 18–40% lead oxide. To qualify as lead crystal, the glass must contain at least 24% lead oxide. Many cheaper items of glassware are available with lower lead oxide contents.

Potash glass A variety of soda lime glass types with an increased potassium content, resulting in greater clarity. Used for higher quality domestic glassware.

Soda lime glass Used to make ordinary domestic glassware such as drinking glasses and sweet bowls, also container items such as jars and bottles, and sheet glass used for windows, picture framing, horticultural glasshouses, vehicle windows, etc.

Struvite Magnesium ammonium phosphate, a clear, colorless crystalline material occasionally found in canned fish products such as salmon, tuna, or crab. The material is harmless and can be dissolved in dilute acid. Because of its appearance, it is sometimes mistaken for glass, giving rise to consumer complaints.

Toughened glass Glass that has undergone a controlled heat treatment to prestress the glass, such that when it breaks, instead of forming shards with sharp points, it shatters into many small fragments. Used for some windows and domestic glassware.

X-ray microanalysis A laboratory analytical technique where the sample is bombarded with electrons, typically in an electron microscope, and the energies of the X-rays released can be analyzed to determine the elements present in the sample.

Foreign Matter Complaints in the Food Industry

Foreign matter incidents constitute the biggest single cause of consumer complaints received by many food manufacturers, retailers, and enforcement authorities. The accidental inclusion of unwanted items can occur in even the best-managed processes. Foreign matter in foods is therefore a matter of concern to all food manufacturers and retailers. The publicity given to a range of food issues, including contamination incidents of glass in food products, concerns about *Salmonella* and other microorganisms in a range of foods, and more recent issues such as bovine spongiform encephalopathy (BSE) and genetically modified (GM) foods, have left consumers very aware of the safety of what they eat. This has been encouraged by increased media emphasis on consumer rights, and, unfortunately, a burgeoning culture of personal injury claims. Therefore, any measures that can be taken to lessen the incidence of foreign matter in foods will be of importance to food manufacturers, retailers, and enforcement authorities and will benefit consumers.

Foreign matter may be defined as something that the consumer perceives as being alien to the food. The perception of the consumer is important, because not all foreign matter is, in fact, alien to the food, though all may have the potential to result in a consumer complaint. Hence, foreign matter can range from items that are clearly alien to the food, such as fragments of glass, metal, or plastic through items that are related to the food, such as fragments of bone in meat products, to part of the food itself, such as crystals of sugar or salt that are mistaken for glass.

It follows from this definition that the range of possible foreign matter is virtually limitless. One distinction which can be made is between intrinsic and extrinsic foreign matter. Intrinsic foreign matter includes items that are related either to the raw materials used in the food product itself or to packaging materials used with the product. Examples of these might include fragments of bone in a meat product, fruit stalks in dried fruit, or pieces of plastic from the food packaging. Extrinsic foreign matter includes items that are not so related, which become incorporated in the product flow from an external source. Examples of these might include stones

accidentally harvested with a field crop or metal nuts lost from food processing machinery.

Hazards Caused by Foreign Matter

Foreign matter may be unpleasant for the consumer to find, but the vast majority of incidents do not pose any real hazard. Of the few that do, potential hazards can be grouped into several classes:

Physical Hazards

Hard objects such as glass fragments, metal, and bone pose the biggest food safety concern because they can cause injuries such as cuts, broken teeth, choking, and intestinal perforation. There is a risk of cuts or lacerations to the hands during food preparation and to the mouth, esophagus, stomach, or intestines. There is also a risk of chipped teeth, broken dental fillings, and damage to prosthetics (dentures).

The possibility of choking due to obstruction of the upper airway is greatest in young children up to 3 years old. Objects causing such choking are common items such as toys, coins, or food, but very rarely foreign matter in food as such. Objects may become lodged in the upper esophagus and cause choking by compression of the trachea. Aspiration – the inhalation of foreign matter into the lungs – may result in partial lung collapse, secondary infection, or destruction of lung tissue from retained foreign material.

From American studies, approximately 80% of reported foreign matter ingestions occur in children, and 80–90% of such ingested foreign objects will pass through spontaneously over the following 4–7 days. It was estimated that only 1–5% of ingested foreign objects resulted in actual injury. Sharp objects accounted for approximately 10% of ingested foreign matter, but a disproportionate number of injuries. Of foreign objects which had to be removed surgically, 37% were in the airway and 63% were in the upper digestive tract. In a review of United States Food & Drug Administration (FDA) consumer complaints of foreign materials in food, the most frequently reported injury was mouth or throat laceration. In the FDA review, glass was the foreign material most frequently reported as causing illness or injury.

There are three main factors which determine whether or not an object may be hazardous. The most obvious factor is probably the size, but opinions differ on precisely what size of object may present a hazard. For example, the United States Department of Agriculture (USDA) Food Safety Inspection Service indicated that bone particles of less than 10 mm are unlikely to pose a food safety hazard. Bone particles from 10 to 20 mm may cause discomfort, but would be a low risk for a food safety hazard, whereas bone particles greater than 20 mm have the potential to be a food safety hazard and may cause injury to consumers. However, they also stated that each instance should be considered on a case-by-case basis, irrespective of size.

The Consumer Product Safety Commission (CPSC) concluded in 1995 that spherical objects less than 1.75 in. in diameter are dangerous to children under 3 years old due to the risk of choking, ingestion, or aspiration. CPSC uses a small-parts test fixture (a cylinder) to judge other nonspherical objects for choking hazard.

The FDA Health Hazard Evaluation Board concluded that in cases of foreign materials examined over a period of 25 years (1972–97), 56% of objects measuring 1–6 mm might pose a limited acute hazard. For objects greater than 6 mm, only 2.9% were judged to present no hazard.

The FDA/Office of Regulatory Affairs (ORA) Compliance Policy Guide gives some criteria for direct reference seizure:

- Hard or sharp objects measuring 7–25 mm across in ready-to-eat products not requiring additional preparation.
- Criteria are also given for recommending legal action:
  - objects 7–25 mm across in products requiring additional preparation,
  - objects less than 7 mm and intended for special-risk groups, and
  - objects greater than 25 mm in length in all products.

The second factor is shape. Spherical or cylindrical objects present a greater risk for choking, whereas slender and sharp or pointed objects (e.g., fish bones) present a greater risk of laceration or perforation.

The third factor to consider is the consistency of the object. Rigid objects such as coins caused most choking deaths in 3 years and older children, whereas conforming objects such as balloons caused more choking deaths in children under the age of 3 years.

Illness Associated with Foreign Matter

Illness complaints from the ingestion of foreign matter include nausea and vomiting, diarrhea, headache, fever and dizziness, and chest pain. These may be due to chemical hazards, microbiological contamination, or a psychosomatic response to the belief that foreign matter has been ingested.

Chemical Hazards

A very few foreign objects may represent possible chemical hazards to a person consuming them. Examples include certain berries such as those of members of the nightshade family, which may be accidentally harvested with field crops such as peas, or ergots accidentally harvested with cereal crops.

Concern is sometimes expressed by complainants finding medical tablets or capsules in their food, but there are very few tablets or capsules that would cause serious or lasting harm to anyone accidentally consuming a single tablet or capsule. Similarly, concern is sometimes expressed regarding items such as dead rodents or slugs reported from food products, which may have been killed by commercial pesticides. However, the toxicity of such materials is expressed in milligram per kilogram body weight, and so the absolute amount of pesticide required to kill a rodent or a slug is many times smaller than that required to harm a human being, simply by virtue of the large difference in body weight.

Contamination of food with ingredients from other recipes, such as nuts, may also present a risk of causing an allergic reaction. Most food companies take great care to prevent cross contamination of this type in the factory, but accidental

cross contamination by consumers in self-service outlets may be a significant risk.

Biological Hazards

Again, some consumers finding potential biological hazards in food products, such as blood, used wound dressings, or condoms, have expressed fears that diseases such as AIDS may be transmitted to them by such means. In fact, most of these diseases are quite difficult to transmit indirectly by such means, and if the offending item has been subjected to heat processing along with the food, any infective agent is in any case likely to have been killed.

The Control of Foreign Matter

The control of foreign matter in food products has to be seen within the commercial environment in which it takes place. The approach to foreign matter control must, therefore, be chosen in relation to the risks and costs involved. The choice of method will be influenced both by the size of the enterprise and the impact the cost will have on the commercial viability of the business. For example, a low cost manual system may be the correct solution for a small operation, whereas a larger company might install some form of an automated machine. A low cost manual system may be the appropriate solution when the problem is short term. Control methods may include physical barriers like screens on windows and doors and a stream of air (curtain) at entrances. Pest exterminators should be employed when it is considered that there is a risk of an invasion of insects, birds, or rodents. Metal detectors are often installed at food production plants, but are not always turned on or are not sensitive enough to detect small fragments. The choice of equipment will depend on the technical problem to be solved, the cost of the equipment, the particular foreign matter hazard, and the risk involved. Assessment of the risk will involve not only the legal position but also the publicity risk to the business of a foreign matter incident.

Sources of Foreign Matter

It is important to be able to identify foreign objects and to locate the source as far as possible, in order to determine when, where, how, and why the object got into the food. This can help to identify patterns in the incidence of foreign matter, which can then be used as part of a strategy to reduce the numbers of occurrences. If the source is shown to be in the raw materials, measures can be taken to prevent the incorporation of foreign matter, or means of removing them may be introduced. Alternatively, a different supplier may be sought. Results may identify where manufacturing practice could be improved, in which case they should be incorporated in a hazard analysis and critical control points (HACCP) system. The identification may indicate that the problem is with the consumer, but even in this case it may be possible to make improvements, such as modifying the pack design or amending the instructions for opening a pack to reduce the chance of packaging materials becoming incorporated with the product. Foreign matter discovered during food processing frequently becomes the subject of dispute between the company finding the foreign matter and their suppliers, and production may also be held up or product quarantined until the source of the foreign matter is identified. Rapid and accurate identification of the foreign matter in such a case is vital to resolution of the problem.

Insects and Other Invertebrates

Many food companies dealing in a broad range of food products, but particularly those involved with fresh produce, regard complaints about insects and other invertebrates as one of the most important foreign matter problems. One of the main reasons for this is undoubtedly the difficulty of detecting and removing these creatures on a production line, particularly in fresh produce.

Insects reported as foreign matter can be divided into two broad groups: those that have some specific connection with the particular food product and those that do not. The former group generally consists of insects that feed either on the food product or on something closely connected with it. It may include field pests such as caterpillars, which feed either directly on the crop itself or on its leaves, or storage pests, which infest the harvested or fully manufactured product during storage. It may also include insects such as flies that lay their eggs directly on the surface of meat during preparation. The second group of insects includes a wide range of species that have no particular interest in the food concerned, and have become associated with it quite accidentally. This may include insects harvested inadvertently with a field crop or those alighting on the food while it is being prepared or consumed.

In all of these cases, the accurate identification of the insect is crucial to the correct identification of the source of the problem. Insect fragments include heads or parts of legs, rarely whole insects, and can only be identified through referring to an identification key. Field pests are generally well studied and documented, and so their habits and requirements are well known, as are suitable control measures. Similarly, storage pests and those insects that feed on fresh meat are well understood and their requirements in terms of access to food, temperature, and so on are fully documented. For example, some adult female flies will actively seek out meat on which to lay their eggs, whereas males of the same species, which feed on plant sugars, will have no interest in meat. However, figs are fertilized by a small wasp that is considered to be a part of the fruit and is not extraneous, although a consumer finding the insect may not share this view! Control measures for storage pests should be implemented in all well-run storage facilities, but this may not always be the case in domestic kitchens. An infestation of psocids, which are very small insects that are relatives of booklice, may begin in a bag of flour and rapidly spread to the rest of a domestic pantry. A discovery of maggots feeding on meat may be particularly revolting. However, accurate identification of the growth stage reached by the larvae, together with knowledge of the temperature at which the meat has been stored, should enable the analyst to estimate the age of the larvae and hence the approximate time at which the eggs were laid.

The most widely reported insects in food products appear to be those which become associated with the food by accident. These include a wide range of insects that live within field crops and are accidentally harvested with them, and those that live around food processing sites which either fly in accidentally or are attracted to lights at night. Many moths become associated with foods in this way, but other insects are also attracted to windows, such as the aptly named window gnats. These can be controlled by suitable insect screens and insectocutors in factories. However, many insects appear to become associated with food products while being prepared or eaten by the consumer, particularly with the increasing popularity of snacking and eating on the move. Many day-flying insects such as flies, shield bugs, and others have been reported in this way. The source can sometimes be confirmed either by their day-flying habits or by relating the time of year when the insect is on the wing to either the time of discovery or the time of manufacture of the product.

Very few insects or other invertebrates represent a real hazard to the consumer. Larger insects may present a potential choking hazard, but very few will cause any chemical or biological hazard. The presence of pest species may cause significant losses of harvested or stored product, representing an economic loss to the owner, and may also be an indication of a breakdown in hygiene.

Plastic Materials

With the increasing use of plastics in everyday applications, it is not surprising that many food companies regard plastics as one of the most important causes of foreign matter complaints. Their common use is exacerbated by the technical difficulty of detecting plastics on the food production line. Although fragments of the final packaging materials of a food product can sometimes form the source of a foreign matter complaint, items such as plastic gloves, plastic sacking, or twine used in packing the raw materials appear to be a more common source. Similarly, cardboard boxes in which fish is frozen on ship can be a source of complaints of pieces of card in fish products. Plastics used on the production line such as parts of piping or machinery can sometimes find their way into the products as a result of breakages. However, fragments of consumer equipment are also common sources, pieces of kitchen equipment being especially common. These can include fragments of plastic pepper grinders, pieces of casing from food mixers and blenders, and fragments broken from domestic electrical plugs. Complainants often mistake clear colorless plastic fragments for glass, and in some cases these result from the substitution of plastics such as Perspex for glass, for safety reasons.

The main hazard caused by plastic materials is a physical one, sharp edges presenting a risk of cuts or lacerations, and larger fragments being a potential choking hazard.

Metals

A wide range of metal fragments is still reported from food products, despite the widespread use of metal detectors and magnets on food production lines. However, the efficiency of these measures usually means that most of the reported foreign bodies consist of quite small pieces of metal which have somehow evaded detection. The type of metal involved is usually an important indication of the source of the problem. Pieces of wire often form the cause of complaint. In flour and bakery products, pieces of broken sieve may be the origin, whereas in other products, the cause may be fragments of electrical wire from repairs, either in the factory or the consumer's home, or pieces from a wire brush. Pieces of tinplate, aluminum, or tin-free steel used in the manufacture of cans or more particularly can ends are sometimes found. Can ends are a particular problem because they are stamped out of a flat sheet of metal, leaving behind a relatively fragile fretwork of waste metal, but a key feature in their identification is often the type and distribution of lacquer on the surface(s). Pieces of aluminum from baking trays, scraped from the surface by mechanical contact, may be the cause of complaints in bakery products. Similarly, fragments of stainless steel from food machinery are sometimes reported, and the precise composition of these steels can be helpful in tracing the source of the problem. Deteriorating bearings for moving parts may also shed minute fragments of metal coated with oil, and in cases like this the complaint will usually be of oil spots on the product rather than of metal. The detection of tiny metal fragments in the center of the oil spot, and their subsequent identification as metals suitable for bearings such as brass or bronze, will confirm the source of the problem. Parts of knives used on the production line are also sometimes found.

A source of metal fragments that is related strongly to either hard foods such as biscuits or crusty bread, or to sticky foods such as toffee, is dental fillings. This relationship with a particular type of food suggests strongly that, in most cases, the complainants themselves are the source of the problem. Experience shows that these are generally from badly diseased teeth or from poorly mixed fillings, both of which are more likely to lead to failure of the filling while the food is being eaten. Metal dental posts, used to secure large fillings or crowns, are also found, with or without the relevant filling or crown. Dental bridges have also been reported occasionally. Other dental samples, such as fragments of human teeth, or, surprisingly commonly, temporary crowns made of polycarbonate or polymethylmethacrylate, also occur.

As with plastics, the main hazard caused by metal fragments is a physical one, sharp edges presenting a risk of cuts or lacerations, and larger objects being a potential choking hazard.

Glass

Fragments of glass reported from food products are amongst the most important foreign objects, because of the emotive impact on the finder, the reputation of glass fragments for causing injury, and hence the potential for bad publicity. Indeed, although glass fragments can and do occasionally cause cuts to the mouth and throat, in many cases the actual risk is less than the consumer's perception of the risk. Larger fragments may present a choking risk.

Glass fragments reported from ready-to-eat/heat prepared meals often include a high proportion of heat-resistant glass from the rims of domestic items such as casseroles. The rims of

casseroles often have a characteristic chunky profile, and frequently carry scratch marks as a result of contact with the lid of the casserole. There is usually a groove caused by a mold parting line during manufacture of the casserole, which is difficult to clean and in a well-used item may carry a line of burnt-on food material from repeated use in an oven. Finally, the identification can be confirmed by X-ray microanalysis, which will show the characteristic elemental composition of a heat-resistant glass. In the case of heat-resistant glasses, it is often possible to identify the brand of glassware involved, because each type has its own characteristic composition. Similarly, some products, such as frozen peas, are sometimes microwaved in heat-resistant glass bowls. These items are occasionally subject to similar damage to that suffered by the rims of casseroles. Rim fragments fall into the bowl or casserole, and remain unnoticed when food is added. Bowls are often damaged by the use of a metal spoon or fork to scrape food away from the side of the bowl. This results in characteristic scratch marks in a line on or just below the rim, depending on its exact profile, and in further random single lines of scratching lower down on the inside of the bowl.

Ordinary domestic glassware made from soda lime glass, such as sweet dishes, may occasionally be broken in use, and sometimes fragments from these breakages may be found as glass fragments in food products. Glass fragments of this type may typically be found in products used in desserts such as canned fruit products, whipped toppings, and other products often served in ordinary domestic glassware. Although pieces from the rims of such items may be found, some fragments have been identified that clearly result from a completely smashed item, such as a piece of glass rod forming the stem of a sweet dish.

Container glassware such as jars and bottles is also made of soda lime glass. A common domestic source of fragments from glass jars is the scraping of the last remnants of a product such as jam or peanut butter from the bottom corner of a jar, using a metal implement such as a spoon or knife. If excessive pressure is applied, the spoon or knife can punch a small hole in the corner of the jar, producing a fragment of characteristic shape. This has a large area of external convex surface with sharp edges all round, presenting a risk of cuts to the consumer. The reverse side has large tapering fracture surfaces, with a very small central area corresponding to the internal manufactured surface of the jar. The fragment falls onto whatever is immediately beneath the jar at the time. The jar, being empty, is discarded without the consumer even being aware of the breakage. The fragment, when found, is then associated with whatever it fell onto, and the true origin is forgotten.

Fragments of glass are sometimes reported from cans of beer or other drinks products. Because a metal beverage can is not transparent, the glass fragment is generally discovered in the glass into which the product is poured, rather than in the can. Nevertheless, the complainant is often quite certain that the glass must have come from the can. In most cases, examination of the glass fragment indicates that it came from an ordinary domestic drinking glass, and it would appear that most of such instances are the result of a breakage in the home resulting in a glass fragment resting in the bottom of the glass, and only being discovered after the drink has been poured onto it. Often these pieces of glass include part of the rim, with its characteristic beaded edge.

From time to time, fragments of glass from lead potash or lead crystal glassware are reported from food products. These are often associated with the more expensive kinds of ready-made desserts, such as might be served up at a dinner party, when the best glassware is also being used.

Manufacturing Problems

The experience of analysts at Campden BRI over the years suggests that the vast majority of glass fragments, unlike many other types of foreign matter, originate from the consumer's own home. A particular exception to this is glass fragments reported from food products containing rice. These fragments have a characteristic appearance. They are of roughly the same size and shape as a rice grain, and they have heavily abraded surfaces due it having been tumbled with the rice grains in the polishing process, giving them an almost opaque appearance. This makes such glass fragments very difficult to separate from the rice by either automatic sorting machines or by hand. However, because of their rounded shape these glass fragments present no hazard in terms of cuts or lacerations, and are too small to cause a choking hazard. The only real cause for concern from a safety viewpoint might be the slight possibility of damage to teeth by biting on a hard object.

Another type of manufacturing problem is the breakage of glass jars or bottles on a filling line. This can result in fragments of glass flying some distance, with the consequent risk of glass getting into neighboring, unbroken jars or bottles. Good operating practices in factories should prevent potentially contaminated jars or bottles leaving the factory as a result of such breakages. Analysis of the glass fragments by X-ray microanalysis, and comparison of the results with data from the jar from which the fragment was reported, enables analysts to determine whether or not the glass fragment came from the same batch as the jar, and hence whether a breakage on a filling line is likely to be the cause of the problem. Experience from analysis of complaint samples suggests that the numbers of such incidents are much less than they used to be, indicating that good operating practices are having their effect.

Glass Introduced at Point of Sale

Glass fragments may be accidentally introduced into food products at the point of sale, particularly in sandwich bars and restaurants. Food products such as sandwiches sold from glass display cabinets are occasionally contaminated with glass fragments from the edge of the glass shelves used in these cabinets. These fragments frequently are part of a frosted edge of the shelf. Food served in restaurants may occasionally be contaminated with glass fragments from chips of the glassware used in the restaurant. These include items such as drinking glasses and sweet dishes, which inevitably become chipped as a result of repeated use. Most of the chips come from the rim, and the rim carries good evidence of having come from an article subjected to repeated use in the form of a series of fine scratch marks, caused when the glass is placed upside down on a storage shelf.

Objects Frequently Mistaken for Glass

Probably the most frequently encountered materials mistaken for glass are clear colorless plastics such as Perspex, polycarbonate, or polystyrene. Examples of salt and also sugar crystals being mistaken for glass are surprisingly common. Another material often mistaken for glass is struvite or magnesium ammonium phosphate, a clear, colorless crystalline material occasionally found in canned fish products such as salmon, tuna, or crab. The material is harmless and can in fact be dissolved in dilute acid. Other objects frequently mistaken for glass include glass-like minerals such as quartz and other silicates.

Plant Material

Plant material may be reported as foreign matter in many different forms. Extraneous vegetable matter, such as bits of leaf or stalk, is frequently found to be the cause of complaints in vegetables such as peas or beans, or fruits such as raisins or sultanas. Woody material from the center of roots of carrot or parsnip, or from the stem of cabbage, can result from overwintering root crops or from the use of older cabbage than the ideal. In the case of overwintering root crops, good crop husbandry, including roguing of older plants, has been found to be a very effective cure for the problem. Parts of other plants inadvertently harvested with crops can sometimes give rise to complaints: for example, the fruits of weeds such as woody nightshade are sometimes harvested with peas, and if still green and immature, are extremely difficult to separate from the crop. Fragments of true wood can occur from broken boxes or pallets, and are often identifiable by their softwood structure and sometimes the presence of paint. Fragments of hardwood may be pieces of manufactured articles such as furniture, but are more frequently found to be pieces of a branch or twig, probably also inadvertently harvested from the hedgerow at the edge of a field. Again, examination of the wood structure under the microscope can often help to identify the species and thereby isolate the cause of the problem. Most plant material is too soft to present a physical hazard, but pieces of wood may cause choking, and berries from deadly nightshade (*Atropa belladonna*), sometimes accidentally harvested with crops like peas, may be a poisoning hazard.

Other Foreign Matter

The possible range of foreign matter is almost limitless, and only a small range can be dealt with here. Stones, sand, and soil may be incorporated when soil-based crops are harvested, as well as tramp metal and other debris in the soil, and these can sometimes cause damage to teeth. Creatures living in fields of crops may also be inadvertently caught up in harvesting machinery, identifying them or their parts, to species level can sometimes be helpful in identifying their source. Similarly, the droppings of animals such as rabbits, mice, and rats need to be positively identified; sometimes agglomerations of plant material caught during harvesting in processing machinery can take on the appearance of droppings. Animal parts and droppings are important more in terms of the revulsion they induce in the consumer than any real safety concern. Many parts of food processing machinery and packaging have the potential to become foreign objects, as do a wide range of ordinary household items.

Holes in food containers such as cans or pouches caused either by accidental puncturing or by the consumer starting to open the product before changing their mind and returning the product to the store cupboard may result in the product becoming contaminated by mold spores or insect eggs. This can result in the development of a mold pellicle, a tough leathery layer on the product surface, or the hatching of insect eggs into larvae, both of these foreign matter types being discovered when the product is finally opened.

Deliberate Contamination

Although relatively rare, the deliberate contamination of food can be a serious problem, whether perpetrated within the factory, through the distribution chain or by the consumer. Deliberate contamination within the factory can often be identified by demonstrating a positive relationship between foreign objects retrieved from different packs carrying the same or similar batch codes, and these batch codes can also be used to identify the factory personnel who might be responsible for the deliberate contamination. Deliberate contamination through the distribution chain or by the consumer may often be more difficult to prove because the incident is often an isolated one. Examples of deliberate contamination for fraud purposes may include broken glass or a mouse in a bottle, and there may be factors that demonstrate clearly that the contamination must have occurred after the product was opened, such as an allegation of a live insect in a sealed pack of a fully heat-processed product. However, it may sometimes be possible to demonstrate a link between different occurrences if multiple attempts have been made. In one case, it was possible to show that the pieces of glass contaminating two own-brand products from different retailers were identical. They were produced in the same factory at two quite different times, but further investigation revealed that the complainant was the same person in both cases.

Conclusions

An analysis of the occurrence of different types of foreign objects in a variety of food products shows that the types of foreign matter can be related to the way in which the product is manufactured, packed, sold, or used by the consumer. It demonstrates that some foreign matter can be quite specifically linked to specific types of food product. In particular, it demonstrates the reason why the great majority of glass fragments do originate from consumers' homes. This is not surprising because most food companies operate strict "no glass" policies in their factories. In contrast, not only does the consumer have glassware in the kitchen where the food product is being used, but also in many cases the glassware is actually being used in conjunction with the food product. In these circumstances, it is not surprising that most of the glass fragments originate from end-use of the product rather than from its manufacture. This fact also explains why the number of complaints of glass fragments in food products remains stubbornly high. Although a

food manufacturer can reasonably be expected to take measures to prevent glass fragments getting into food in the factory, he cannot control the way the product is used by the consumer. In contrast, many other foreign matters can be very effectively controlled by suitable measures within a food processing system, including metal detectors, X-ray machines and magnets, mechanical separation methods such as sieving, flotation and gravity separation, automatic vision systems, and even visual inspection by factory staff.

See also: Food Safety Assurance Systems: Personal Hygiene and Employee Health. Other Significant Hazards: Food-Related Choking

Further Reading

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Relevant Website

http://www.campden.co.uk
Campden BRI.

FOOD TECHNOLOGIES

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Aseptic Packaging

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Glossary

Cleaning-in-place (CIP) A method for cleaning purposes, whereby the plant is cleaned automatically and without opening of pipes or other machine parts, prevents human failures, and allows reproducible cleaning results.

Colony-forming units (CFUs) A unit for direct quantification of microorganisms.

Critical control point (CCP) A point or step in a process that represents a critical point for hazard, which should be controlled with special care.

Hazard analysis and critical control points (HACCP) A concept for determination, elimination, prevention, and control of critical control points, helping to guarantee food safety.

Sterilization-in-place (SIP) It is a method for sterilization purposes, which is similar to CIP and commonly follows on CIP.

Ultra-high pressure treatment (UHP) Preservation method that uses high pressure (up to 600 MPa in industrial facilities and up to 1400 MPa in research facilities) instead of temperature.

Ultra-high temperature treatment (UHT) A sterilization method used for liquid and semi-solid foodstuffs using extremely short processing time (1–2 s) as a result of the used ultra high temperatures (>135 °C).

Introduction

Consumer demand for shelf-stable, high-quality food with a maximum degree of safety has been a focus in processing of liquid and semisolid foodstuffs. Food products should also meet the sensory and nutritional parameters, as specified by the consumer and legislative authorities. Thermal processing is one of the reliable methods to preserve the food. Aseptic processing and packaging is an established technique used for decades to process suitable low-acid food (pH > 4.5) products like milk and milk products, for example, puddings as well as nondairy desserts, fruits and vegetables juices, soups, sauces, and particulate foods. The word aseptic is derived from the Greek word *septicos*, which means the absence or

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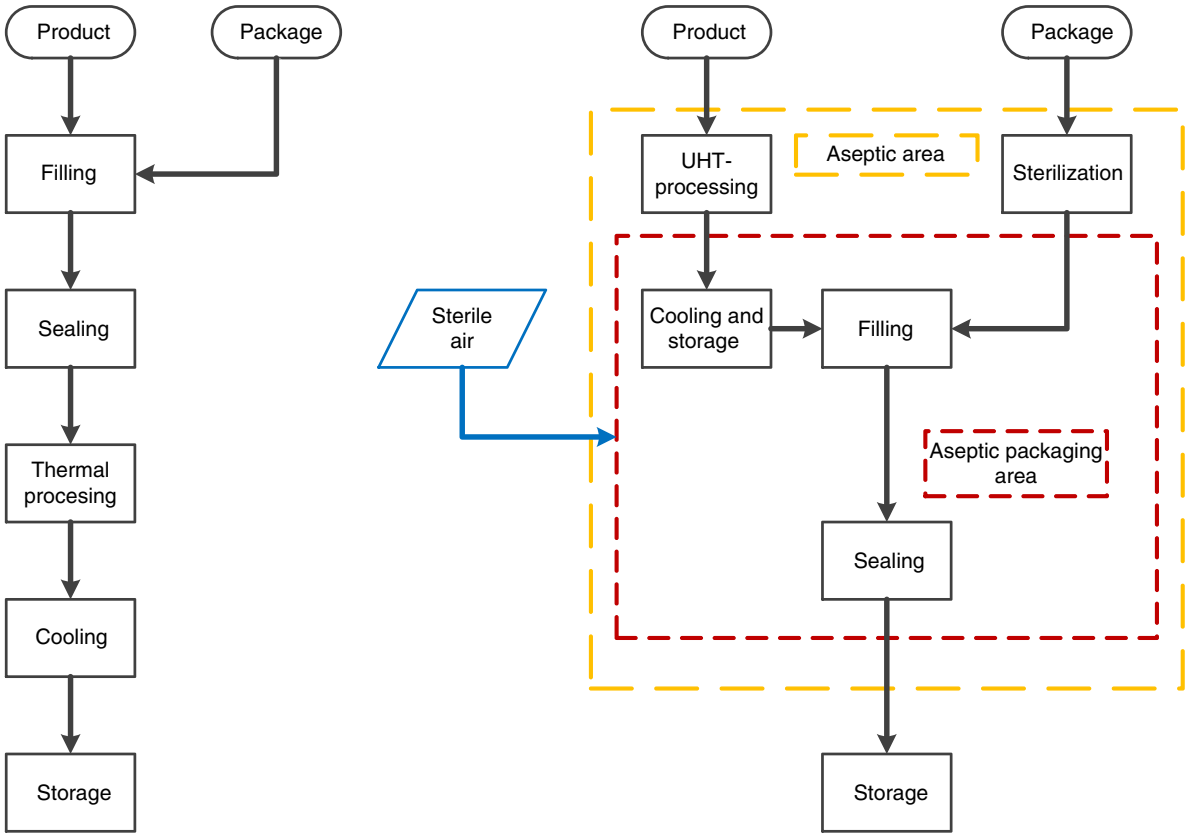

Figure 1 General comparison of common packaging technology for sterilized products and modern aseptic packaging technology. UHT, ultra-high temperature.

exclusion/elimination of microorganisms that cause spoilage. Aseptic packaging can generally be defined as the filling of a commercially sterile product, with its sterilization being achieved through adequate thermal treatment into a pre-sterilized container under aseptic conditions, followed by its hermetic sealing to prevent recontamination. The word 'hermetic' is a synonym for strictly 'air tight' and is the designation for the mechanical properties of the used sealing to prevent any kind of mass transport between the packed product and the environment like microorganisms, water vapor, or any further gas.

Typical examples of packaging of presterilized products are milk and other dairy products without a fermentation flora, like cream, puddings, and deserts as well as juices, soups, sauces, and foods with both small discrete particles and a minor amount of larger particles. Packaging of pasteurized products is a method for the packaging of products in which spore-forming bacteria cannot grow (as consequence of pH and/or water activity (a_w)); therefore, sterilization is not required for the microbiological stability of such products.

Aseptic packaging is absolutely different from conventional packaging technology, which allows a similar shelf life like retort processing or canning. In conventional packaging process, the product is filled into the previously cleaned packages, followed by extensive heat processing to achieve the desired commercial sterility. This is limited to packaging materials like tinplate, glass, and aluminum (Al)-foil laminates that can tolerate high temperatures (up to and higher than 127 °C), high pressures, and acidic conditions too. The containers are commonly metallic, making them expensive and prone to corrosion. More importantly, the duration of the heat treatment, dictated by the rate of heat transfer, leads to degradation of nutritional and organoleptic properties. A comparison of the two technologies can be observed in Figure 1.

Significance and Advantages

Strict regulations on food safety by regulatory and advisory bodies in the area of food process hygiene have compelled the food industry to maintain the uniformity in the quality standards. The main reason for framing such regulations was the reports of outbreaks of foodborne disease due to poor hygiene during the processing. Aseptic packaging has been around for 100 years, with the first aseptically packaged food (specifically milk in metal cans) dating back to 1913 in Denmark markets. Over the years, new techniques like heat–cool filling systems in 1933 and can sterilization with superheated steam at 210 °C in 1940 were developed. The first commercial aseptic packaging machine developed in 1950 was based on similar principles but with limited market due to high cost of the equipment and cans. The establishment of laminate paperboard cartons by Tetra Pak®, Sweden led to the first commercial successful product in 1961, with a huge market to

date. The main focus was to develop a system that allows the economical production of products with long shelf life at ambient conditions. The progress in processing technology in the past decades has made aseptic processing a commercial viable and successful technique – UHT short-time processing is a specific example.

The numerous advantages of aseptic packaging, which will be discussed in the following text, can be summarized in three main aspects:

- The usual food containers are unsuitable for in-package sterilization; this method uses economical, light and highly protective packaging materials like paper and special plastic foils, the preferential container design in terms of material choice for optimized protection of liquid foods.
- Extension of product shelf life at ambient conditions by aseptic packaging. For example, commonly pasteurized milk is stable for approximately 5–8 days under refrigerated conditions, whereas aseptically processed and packed milk is stable for up to 6 months at ambient conditions.
- Possibility to use high-temperature-short-time (HTST) processing for heat-sensitive products in order to preserve nutritional, sensory, and other quality attributes without increased costs.

All these aspects clearly reflect the significance of aseptic packaging for contemporary foods. High consumer acceptance for aseptic products may be demonstrated in terms of shelf life extension without both refrigeration and addition of chemical preservatives. More than 90 billion liters (2009) are aseptically packed with an estimated increase of 30% (113 billion liters) by 2013. The main markets are Western Europe wherein dairy products, especially milk, have been the main product.

Basics of Sterilization

Sterilization is a process that should eliminate virtually all colony-forming units (CFUs) of any kind of microbe present in the food product. Thus, a sterile product should be free of any CFU, and microbial spoilage may be caused only by re-infection or recontamination from the surrounding environment. Autoclaving in hermetically sealed containers at 121.1 °C and 2.013×10^5 Pa pressure for longer than 15 min is the only technique that is considered to guarantee sterile conditions. This results in the virtually complete destruction of all CFUs in the initial product, but also leads to a significant reduction of its nutritional and sensory properties. For aseptic packaging, high-temperature short-time (HTST) processing is used, especially UHT, to preserve the nutritional and sensory properties besides achieving a commercially sterile product. In such a case, the reduction of CFUs follows a logarithmic function as shown in eqn [1].

$$\log S(t) = \log\left(\frac{N_t}{N_0}\right) \qquad [1]$$

The equation shows the time-dependent survival rate $S(t)$ as a function of cell count after a defined treatment time t (N_t) in comparison with the cell count exactly at initial time $t=0$ (N_0). This shows that S cannot exactly reach zero level, an acceptable low number of CFU of relevant microorganisms (bacteria, yeasts, and molds) being able to contaminate, multiply or survive in the product and being harmful to the consumer or product quality) is achievable, which ensures the microbial stability of the closed product over a defined period of storage time (in general, approximately 3–6 months at ambient conditions). Hence, the number of active cells is reduced to an amount of 1 CFU per 1000 l or lower. This means that the risk per package to include one CFU equals 1:1000. If, sometimes required, the risk for a microbiological failure of package should be less than 10**5 to achieve higher food safety. But the advantages first become clear by looking on the z values of microbes and desirable quality factors. Typical z values for microbes have been approximately 10 °C until the proposal of typical values for quality factors C_0 to compare the heating effect on appropriate compounds. It is similar to the F_0 value, with the change that the reference temperature is 100 °C:

$$C_0 = 10^{(T-100)/z}\, T \qquad [2]$$

Equation [2] reflects that the high temperatures show less effect on the degradation of quality factors due to their increased z values. UHT processing at temperatures of 130–150 °C for very short heating times of 2–6 s has very low thermal effect on food but it is quite effective for microbial degradation. Owing to this fact, such methods are often described as 'mild' methods. The only problem with UHT processing is that several heat resistant enzymes like proteases, lipases, and peroxidases are still active after high heat treatment, which tends to deteriorate the food quality. This may lead to quality defects like off-flavors and age gelation during storage.

Aseptic Processing

The aseptic process comprises of several steps, which must be fulfilled to achieve a shelf-stable product (Figure 1). The critical cleaning and safety concerns may be briefly described as:

- Sterilization of all installations before their operation in the aseptic section of the product processing. This usually includes the sterilization of product and the package, pipes and tubing for product, sterile air and gas, as well as filler, and other relevant machine zones. The industry premises are frequently disinfected to minimize contamination risks.
- Sterilization of all media entering the system. They are generally air, gases, and water for cleaning, heating, and pneumatic applications.
- Sterilization during closure of packaging materials as well as containers.
- Sterilization and cooling of the product before filling in the package.
- Securing a sterile environment in the whole system during filling and sealing.
- Guaranteed sealing quality to obtain hermetic packages.

It is clear by this above enumeration that aseptic processing is complex due to interactions between many compounds in

the process line. Modern processing plants allow a high level of control and automation, making aseptic processing highly cost efficient and handling of food much easier.

Product Processing

In the aseptic packaging process, the plant design is based on the principles of sterilization as already discussed. UHT processing is commonly carried out in four major steps for the presterilization of the product.

1. Preheating of the product to a defined temperature.
2. Final heating to sterilization temperature.
3. Temperature holding over a defined period of time.
4. Instant adequate cooling at ambient or chilled conditions to avoid product damage.

The processing steps should be held as short as possible to reduce the quality losses through thermal processing. Different types of plants have been developed that fall into two classes. Class one is the indirect heating approach, whereas the second type is direct heating through steam infusion into the product.

1. Indirect heating in closed heat exchangers, with separate product and heat exchange by a heat-conducting surface. This category has three main types available in the market:
   a. Scraped surface heat exchangers.
   b. Plate heat exchangers.
   c. Tubular heat exchangers.
2. Direct heating by steam as heating media rapidly heats the product to the desired temperature. The steam is rapidly injected to achieve the desired consistency in the 'sterile' product. The product is precooled before being filled into an aseptic package. It can be of two types:
   a. Steam infusion (product is injected into the steam flow).
   b. Steam injection (steam is injected into the product flow).

Both systems have their advantages and disadvantages. Direct methods deliver better product quality through high temperature and short heating time. Indirect methods require longer heating time, which may also affect the food quality and material buildup on the heating surface. Their advantage lies in the enormous cost efficiency through energy saving and lower installation as well as repair costs together with a higher throughput than direct processing plants. In addition, they are easier to handle because dilution by condensing steam is not problematic. Today, most companies use indirect heating methods and direct systems have almost disappeared from the market.

New Technologies for Product Sterilization

Several new potential techniques had come up in the past in which heat is not used for killing of microorganisms. Owing to the physiological properties of microorganisms, factors like pressure, electricity, and radiation can also lead to their cellular death. Some of them are:

- Radio heating frequency.
- Microwave heating.
- Ohmic heating.
- Pulsed electric fields (PEFs).
- Ultraviolet (UV)-radiation.
- Pulsed light.
- Ultrahigh pressure (UHP).

Some of these processing technologies (UHP and PEF) are already in use and show promising results in terms of product quality. They are limited in use due to high operational costs in comparison to UHT processing. Moreover, sterility issues are still a concern, with the use of such technologies.

Packaging Technology

For aseptic packaging, the package has to fulfill a series of criteria that are mandatory to ensure the safety and quality of the packaged product:

- Food safety: The most important function of aseptic packages is to ensure that the product sterility is maintained over extended period of time at ambient temperatures; especially, the package should avoid or prevent recontamination. In addition to this, the packaging material must be free of any substances that may raise safety concerns and must be stable under the product conditions, for example, pH, temperature, oxygen, etc.
- Shelf life: The shelf life of a product can be defined as the time span after which a consumer does not accept the product. This might be due to the product's color or smell as well as its taste. Technologically, such products are still consumable as the degradation of appropriate compounds is commonly caused by light or atmospheric oxygen but not always in aseptic packaging.
- Physical protection: A package must be strong enough to ensure the protection of the product, even after minor physical damages like drops or hits. This is very important to guarantee the safety of the product. It also makes transportation and handling easier, faster, and cheaper.
- Product containment: This is the main function of a package to prevent the hazards of contamination. Liquid foods must be contained in packages with adequate tensile properties to avoid leakages.
- Nutritional protection: Standing in direct relation with the shelf life of a product, nutritional protection is of major concern today for the consumer acceptance of a product. The effects that also lead to color degradation reflect the depletion of nutritionally important substances like vitamins, omega-fatty acids, and antioxidants. Carotenoids are one of the best examples; through oxidation, they degrade from deep red bioactive pigments to colorless and biological inactive substances.

All these examples together reflect the importance of packaging and the difficulties, which have to be realized by an aseptic package. Therefore, the right packaging material, sterilization method, and sealing are required, together with an intelligent package design to accomplish all requirements.

Packaging Materials

Packaging materials for aseptic packages must fulfill a series of requirements before they are commercially used:

- It must withstand sterilization conditions.
- It must be inert to the product and should not allow the migration of any substances from the package to the product.
- It must protect the product from environmental influences like light and oxygen.
- The physical integrity must be ensured.
- It must be cheap and easy to handle.

A brief overview about the materials used in aseptic packaging is presented in Table 1. A series of packaging systems consisting of such materials are available on the market. They can be segregated into five major groups, which can be further classified into subgroups (Table 2). Laminated paperboard cartons are the most widespread ones, being composed of several materials with diverse properties.

Composite Materials

Most of the packaging systems used for aseptic packaging consist of several materials with different functions. The improvement in bonding and development of vapor deposition technique had led to the development of composite materials. These composites unify the properties of several materials, thereby forming an effective and cost efficient barrier against environmental influences. Figure 2 shows a composite material such as the Tetra Pak-laminated paperboard cartons wherein up to six layers provide total protection against factors like microorganisms, light, moisture, oxygen, and smell from outside and the loss of liquid and flavors from inside. The most famous ones are Tetra Pak aseptic cartons, which consist of three basic materials, forming a six-layer sandwich. The use of composite structures allows the use of cheap packaging materials on the outside and the high quality film in contact with the liquid food. A very good example is the paperboard layer, which helps in the mechanical stability of the package but has no other protective properties. The paperboard layer (providing high stability at low cost) is often combined with very thin layers of PE (sealability, liquid and flavor barrier) and Al foil (light, odor, gas and microorganism (MO) barrier), so that all the desired packaging properties can be imparted.

Package Sterilization

Package is also presterilized in the similar fashion as described above to achieve the sterility necessary to prevent the microbial contamination from package. Therefore, the package has to fulfill a series of requirements before it is considered for aseptic packaging:

- Should be compatible with the aseptic treatment or sterilization.
- The residues should not pose any health hazard.
- Should have no adverse effects to the product.
- Safe for personnel working with the method.
- Reliable and economical.

Several techniques can be used in package sterilization. The sterilization technique depends on the requirements of the packaging material and product as shown in Table 2. The techniques can be separated into three main groups: (1) sterilization by heat; (2) sterilization by chemical agents; and (3) sterilization by radiation (Table 3).

Sterilization by Heat

Heat sterilization is performed mainly by 'moist' or 'dry' heat. For 'moist' heat, temperatures of approximately 121–129 °C with pressure are used, whereas 'dry' heat requires temperatures from 176 to 232 °C for longer duration. The high temperatures with long heating times limit this technique to metallic packages or packages out of plastic laminates that can withstand much heat. The sterile air used for this process is commonly sterilized at temperatures ranging from 260 to

Table 1 List of functional attributes of several packaging materials being used for aseptic packaging

Material	*Barrier property*				*Durability*		
	Oxygen	*Moisture*	*Light*	*Seal quality and adhesion*	*Stiffness*	*Tear*	*Puncture*
Linear-low density polyethylene		X		X		X	X
Low-density polyethylene		X		X			
High-density polyethylene		X	X	X	X	X	X
Polystyrene					X		
Polypropylene		X				X	
Polyvinylidene chloride	X	X					
Ethylene vinyl alcohol	X						
Polyamide (Nylon)						X	X
Ethylenacrylic acid				X			X
Paperboard			X		X		
Aluminum foil	X	X	X				
Metalized film	X		X				

Source: Adapted from Singh RK and Singh N (2005) Quality of packaged foods. In: Han JH (ed.) *Innovations in Food Packaging*, pp. 24–40. Oxford: Academic Press.

Table 2 Available aseptic package systems in the market

Packaging system	*Materials*	*Subcategories*	*Properties*
Cartons	Laminate of polyethylene, paperboard, and aluminum foil (for detailed construction seal, see **Figure 3**)	Form-fill-seal	● Commonly sterilized by hydrogen peroxide (H_2O_2) in deep bath, wetting systems as H_2O_2 aerosol, H_2O_2-steam or in combination with UV. ● Directly formed in the filling machine ● Continuous filling of product until sealing ● Filling without headspace possible
		Prefabricated	● Folding is carried out under nonsterile conditions ● Filling machine must consists of several separate areas ● Commonly sterilized by hydrogen peroxide (H_2O_2) in deep bath, wetting systems, as H_2O_2 aerosol, H_2O_2-steam or in combination with UV ● Residual peroxide might be a problem ● Filling only with headspace
Cans	Tinplate; electrolytically chromium-coated steel and aluminum; and composites of foil, plastic, and paper with metal ends	Metal cans	● Available in volumes from 125 ml to 22 l ● Superheated steam is used to guarantee aseptic conditions during filling ● Filling with headspace ● Resistant against nearly all heat conditions
		Composite cans	● Similar to metal cans ● Sterilization by hot air at 143 °C for 3 min
Bottles	Glass, high-density polyethylene, and polypropylene (PP)	Glass	● Can be sterilized with all methods ● Risk of breaking ● No more commercially used today ● Weak or no light protection
		Plastic	● Material can be mixed with pigments for light protection ● Available as nonsterile, and single station systems (blowing, filling, and sealing in one step) ● H_2O_2 or peracetic acid (PAA) for sterilization ● Only headspace filling possible
Sachets and pouches	Laminate of low-density polyethylene, a center layer of ethylene vinyl alcohol (EVOH), and carbon black	Form-fill-seal	● Production is similar to laminated cartons ● Material is presterilized by bathing in H_2O_2
		Lay-flat tubing	● Blown polymer film tubes ● Only transversal seal required
Cups	High impact polystyrene, PP, polyvinylidene chloride, and EVOH	Preformed	● Sterilization by H_2O_2 or a combination of both
		Form-fill-seal	● One step process ● Sterilization by H_2O_2, saturated steam ● Often used for puddings

320 °C. The advantage of this technique is that it is simple in use, effective, easy to control, and cost effective. However, the application field is limited to packages consisting of heat sensitive materials. This process is also compatible with multilayer laminated packages. The heat that is required to melt the raw materials is high enough to guarantee the sterilization of the material. To prevent recontamination, the final forming is carried out in the aseptic packaging machine.

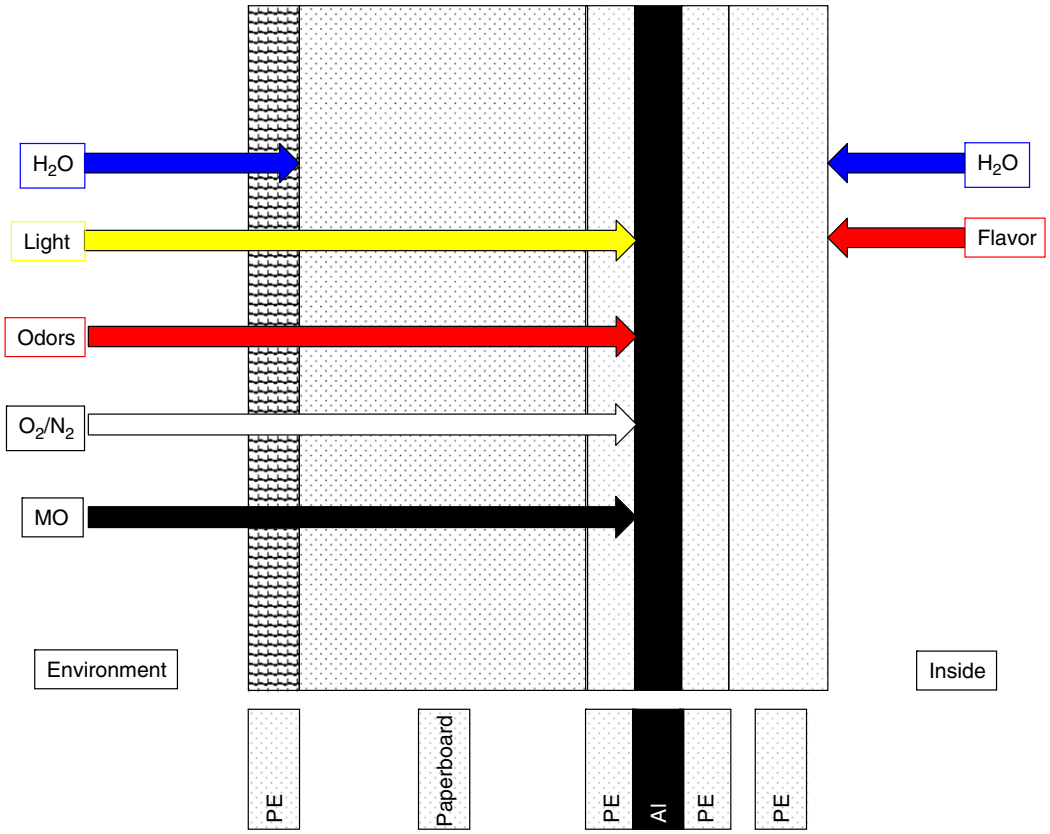

Figure 2 Schematic material stack like that used in laminated paperboard cartoons (Tetra Pak). PE, polyethylene.

Sterilization by Chemical Agents

Chemical agents are often used for package sterilization. The type of chemicals being used depends largely on the product meant to be packaged. Hydrogen peroxide (H_2O_2) at concentrations of 30–35% and peracetic acid (PAA) is are widely used. The typical temperatures for sterilization with H_2O_2 are from 60 to 90 °C for fluid H_2O_2 and above 90 °C for H_2O_2-steam, being much lower than 'dry' heat and still lower than 'moist' heat. All materials are not resistant against all chemicals, so it is very important to pay attention as to which chemical is suitable for the packaging material. Three different techniques are available for wetting, depending on whether prefabricated containers or raw material is used, as well as shape in case of prefabricated containers.

- Dipping/rinsing: The package is passed through a bath of H_2O_2 that contains a wetting agent to ensure a uniform wetting of the surface. After the bath, the liquid is mechanically removed and the package is dried and activated by hot air.
- Spraying/aerosol: H_2O_2 is sprayed on prefabricated packages. Hot air is used as carrier gas by which the sterilization effect of H_2O_2 is enhanced. New technique uses vaporized H_2O_2 mixed with hot air of 130 °C instead of liquid droplets.
- Rinsing: This process is quite similar to the dipping but it is much more suitable for prefabricated containers with an intricate shape. H_2O_2 or a mixture of H_2O_2 and PAA are sprayed in the container. In this process, the container is drained and then dried with hot air.
- Steam/aerosol: This is also applied to the packaging materials to achieve the desired sterility.

Sterilization by Radiation

Sterilization with radiation has the advantage of not involving the use of heat or chemicals, thereby not only offering chemical-free materials but also allowing the usage of heat-sensitive materials for packaging. But depending on the type of radiation, the material penetration must be considered so that shadowing effects may result in a worse sterilization. The disadvantage with the use of radiation is discoloration of the packages. Two types of radiations are used: γ-Radiation: This technique has been in use for a long time. Owing to the high penetration of γ-rays, packages are treated in bulk with doses of approximately 1.5 Mrad. The disadvantage with this technique is its low consumer acceptance.

A relatively new approach is electron beams. They have good penetration, together with good consumer acceptance and high sterilization effects. Until now, equipment size and cost have limited the use of such systems, but appropriate plants are in development and with time these problems may be solved. Some of the prototypes have already appeared in the market.

Table 3 Overview of different methods for package sterilization

Method	*Application*	*Advantages*	*Disadvantages*
Superheated steam	Metal containers	High temperature at atmospheric pressure	Less effective than saturated steam due to microorganisms higher resistance
Dry hot air	Metal or composite containers	Same like superheated steam	Same like superheated steam
Hot hydrogen peroxide	Plastic containers and laminated foils	Fast and efficient	Highly depending on used temperature and chemical reagent
Hydrogen peroxide/ultraviolet (UV) light combination	Plastic containers (preformed cartons)	Increased effectiveness of hydrogen peroxide through UV light	Chemical reagent
Ethylene oxide	Glass and plastic containers		Cannot be used where chlorides are present or where residuals would remain
Process heat from coextrusion	Plastic containers	No chemicals used and environmental friendly through energy recovery	
Radiation	Heat-sensitive plastic containers	Can be used to sterilize heat-sensitive packaging materials	Expensive, risk of radiation source, and requires specially trained staff

Source: Adapted and modified from Ansari MIA and Datta AK (2003) An overview of sterilization methods for packaging materials used in aseptic packaging systems. *Food and Bioproducts Processing* 81: 57–65.

Filling Systems

Filling is the most critical part in aseptic packaging process. It combines the sterilized product with sterile package. Before the product is filled, the system is commonly degased to prevent any kind of undesirable oxidative reactions. This process commonly takes place in a tube, in which the product is exposed to vacuum until it flows through the tube. The degased product is then stored in a cooled tank. This tank system allows both sterilization and filling processes to be carried out independently of each other. For the purpose of filling, the product is initiated into the filling machine by sterile air, which expels the product out of the tank. The whole filling process is carried out in a hermetically sealed sterile environment. This is required to avoid product contact with the surrounding air during the filling process in the package. If no sterile air is used, microbes will be 'filled' in the package, thereby destroying the achieved sterilization.

Sealing

Sealing is the last step in the aseptic packaging process. It has to guarantee the package integrity by sealing it hermetically to maintain sterility during appropriate storage time. The seal must be strong enough to survive physical stress during handling and distribution in order to maintain the sterility of the product. The most commonly used technique is heat sealing. The package material is heated to an appropriate temperature; the sealing material begins to melt and easily flows in all cavities, forming a total closing. This is supported by the application of pressure on the sealing by a pair of jaws, which is usually fitted with a heater. The most widely used sealing material is PE, which constitutes the outer and inner layer of composite packages. It is melted by sterile hot air or by a short electrical impulse as indicated in the Figure 2. PE is easy to melt, very strong, and has better sealing properties than other polymers in forming the hermetical seal, an important requirement for the package integrity. The only disadvantage of PE is its permeability to gases, which may lead to oxidation and water loss.

Safety Aspects

In the light of the previous sections, it is apparent that aseptic packaging is a highly complex process. The process is prone to a series of contamination risks, the most important ones being schematically represented in Figure 3. For better understanding, the risks may be separated into three different groups as listed below in enumeration, whereby all can be viewed in their relationship:

1. Risks associated with prepackaging.
2. Risks with packaging.
3. Postpackaging risks.

Each group can be further divided into several subgroups for characterization of individual risks. The sum of all individual risks can be used as an indicator for the safety of the process. Thereby it can establish a factor R_{con} (eqn [3]), which allows the estimation of risks going out from the process.

$$R_{con} = \sum_{i=1}^{n} RSG_i \qquad [3]$$

where, risk per subgroup (RSG) is percentage of the associated risks from one subgroup. These values need to be summarized due to safety concerns and R_{con} should be as low as possible. A similar approach is the calculation of the microbial contamination rate (CR) from various sources.

$$\sum_{i=1}^{n} CR_i = CR \leq x \qquad [4]$$

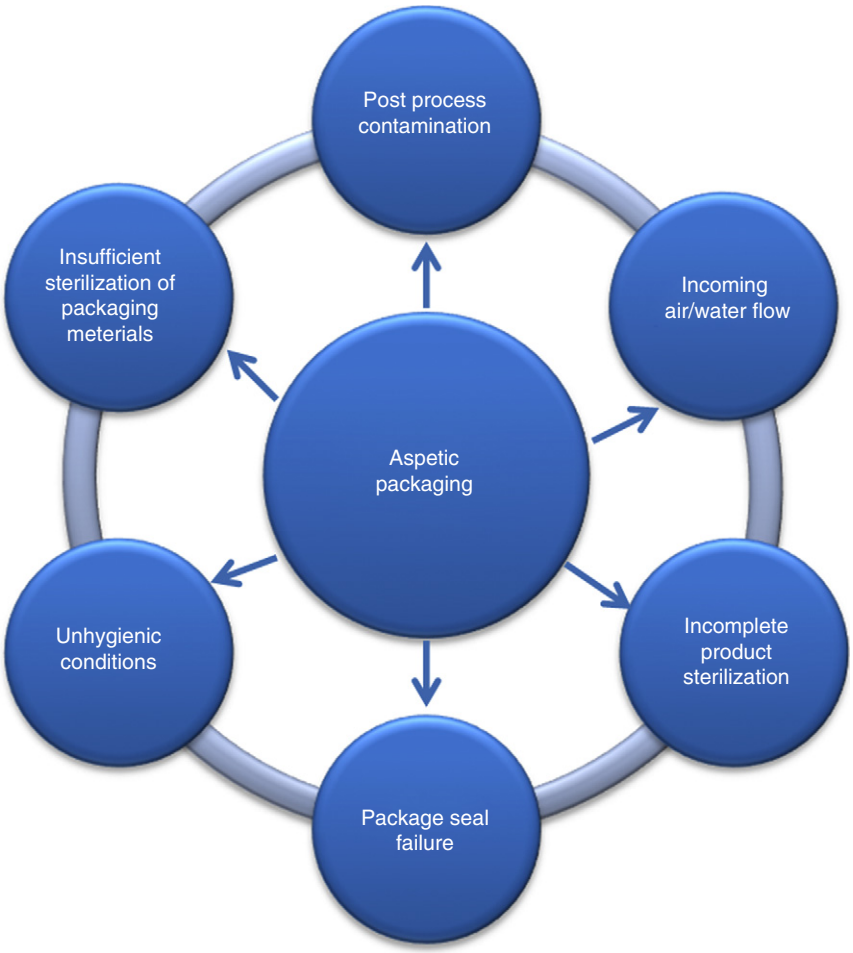

Figure 3 General risks affecting aseptic packaging processing.

where CR_i is the contamination risk resulting from an individual source and x represents the acceptable CR. Like R_{con}, the total CR is the sum of the individual CRs, which are contributed by various sources. Overviews of various sources for RSG as well as CR are listed in Table 4. The advantage of R is that this factor is much more segregated than CR in not being limited to considering the microbial contamination risk alone, and this segregation is important for extremely sensitive products like baby nutrition wherein heat sterilization is used to reduce the allergic risks resulting from several proteins like β-lactoglobulin. Separating appropriate risks into small subgroups helps to detect critical control points (CCPs) and establish an effective hazard analysis and CCP (HACCP) concept, so that contamination risks can be held in an acceptable range.

Table 4 List of various contamination sources for RSG and contamination rate (CR)

RSG	*CR*
Air	Air
Cleaning	Pack integrity
Flow diversity	Packaging material
Machine sterilization	Product adhering to the filling nozzle
Packaging machine	Product
Packaging material	Others (not nearer defined)
Remaining chemicals	
Staff	
Staff training	
Under processing of product	
Under sterilization of package	
Product composition	
Redundant of sensory	
Storage	

Insufficient Plant Sterilization

Methodical cleaning and sterilization of processing plants provide the basis for aseptic packaging. Cleaning-in-place (CIP) and sterilization-in-place (SIP) allow for reproducible results with a highest degree of reliability. The application of these techniques depends largely on plant design. Poor plant design results in product deposition in the plant, providing a nutrient for microbes, which can contaminate the product. Moreover, biofilms cause a problem in being hard to be removed by CIP, so they form a perfect and protected environment for pathogen microbes. The practice shows that CIP

followed by SIP is often used with plants that are not completely suitable for such processes.

Under Processing

Extended processing can result in quality and nutrient loss, whereas under processing is associated with high food safety risk. To maintain product quality at low price, both heat processing and package sterilization times are held as short as possible. This procedure requires a lot of time for process optimization in order to obtain the required commercial sterility. Therefore, it is important to specifically adjust the processing conditions for each product due to minor differences in viscosity, flow rate, and particle concentration, which may otherwise cause major differences in the sterilization process. Also, factors such as temperature differences in heat exchange media, concentration of sterilization chemicals, and the age of radiation sources may result in risks during aseptic processing.

Filling and Sealing

The storage of the presterilized product in storage tanks before filling is associated with a high level of risks. Low number of heat-shocked microorganisms survive, as could be the case in commercial sterile products, can start to grow with storage time. This may also happen due to insufficient product heating. They may completely contaminate whole product in the tank. In this perspective, the thermocouple in the flow diversion value needs frequent check on its sensitivity for recording the desired product temperature so as to avoid the microbial survival in the product. Until filling, the requirement of a hermetically sealed packaging plant is difficult to implement, but is done in all aseptic systems. The incoming flows like product, sterile air, and packages or packaging material must be brought through an airlock or hermetically closed pipes in the plant. Especially, the sterile air might cause problems due to air filter damage. If unnoticed, this will allow spores/microbes to survive in the pipes behind the filtering and sterilization system. They would grow and spores may be transported by the airflow in the plant to enable their entry into the packages. The final point in the aseptic chain of risks is the sealing step. If the sealing temperature is not high enough or the applied pressure is too low, the sealing will not be strong enough to prevent microbial migration to the product, thereby leading to postpackaging contamination. Additionally, it will not be strong enough to survive physical state use during transportation and storage, thereby leading to damaged packages.

Risk Prevention

The ways to prevent hazards or risks in the aseptic packaging processes are widespread, ranging from automation and plant design to employee trainings. In case of plant design, the European Hygienic Engineering and Design Group provide a series of guidelines that are quite helpful for the right design of processing plants. An additional approach is establishing a good working HACCP concept, together with routine testing. Table 5 provides a series of CCPs and possible methods to evaluate and prevent any kind of risks. A variety of destructive as well as nondestructive methods are available in the market, especially for testing. Package integrity is one of the most

Table 5 Overview of several critical control points requiring to be monitored in an aseptic packaging process

Process	*Processing steps*	*Parameter that should be monitored*
Product sterilization	Processing plant, pipes, and storage tanks	Sufficient cleaning and sterilization (refer Table 3), well functioning, sensor are correctly working, and no leakages
	Standardized product	Product parameters (viscosity, composition, density, particle size, etc.) are into specified ranges and microbial contamination is not too high
	Sterilization process	Humidity, temperature, concentration, pressure are correct, and depending on the used method
	Cooling	Temperature of chilled product
Package decontamination	Processing plant	Equal to product processing
	Decontamination step	Temperature, time, quantity dosed, concentration, residual amount energy, etc. depending on the used method
	Decontaminated material	Remaining colony-forming unit of relevant microorganisms on material surface, damages of material surface, recontamination until storage in packaging machine
Cooling and storage	Storage of sterilized product	Sterilization of storage tanks, contamination of stored product, temperature, and storage time
Filling	Processing plant	Equal to all others, sterility of incoming process flows and air, and hermitical sealing
	Package filling	Needle contamination, pressure, filling height, speed, and temperature
	Sealing	Time, temperature, pressure, positioning, and pack seal integrity

Source: Adapted and modified from Mostert M, Buteux G, and Harvey P (1993) Microbiologically safe aseptic packing of food products. *Trends in Food Science and Technology* 4(1): 21–25.

critical issues. All factors affecting aseptic packaging need to be evaluated before the product is available on the market, to ensure the product safety.

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: Multivac: Manufacturer of innovative packaging machines for aseptic packaging and UHP treatment.

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Biopreservation

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Glossary

Bacteriocins Peptides, ribosomally synthetized by bacteria, that specifically inactivate (inhibit or kill) other (mostly close related) bacteria.

Biopreservation Use of microorganisms, enzymes, or natural antimicrobials to protect foods against deterioration caused by microorganisms and/or against growth of pathogenic microorganisms, without major changes in their sensory properties.

Endolysins Bacteriophage-encoded proteins attacking bacterial cell walls.

European Food Safety Authority (EFSA) European reference body for risk assessment on food and feed safety, animal health and welfare, nutrition, plant protection, and plant health.

Generally recognized as safe (GRAS) Status of a substance that is generally recognized, among qualified experts, as having been adequately shown to be safe under the conditions of its intended use, or unless the use of the substance is otherwise excluded from the definition of a food additive (quoted from the US Food and Drug Administration (FDA)).

Qualified presumption of safety (QPS) A tool for safety assessment tool for microorganisms added to food and feed (quoted from EFSA).

Definitions and Scope

In the broad sense, biopreservation may be defined as the use of biological systems (microorganismss and their metabolic products, enzymes) to improve food safety and to extend the shelf life of food and feed. In this sense, biopreservation has a long tradition. For example, milk is preserved by conversion into sour milk products and cheeses, and meat is processed into shelf-stable sausages by a combination of microbial fermentation and drying. Likewise, animal feed may be preserved by ensiling. Obviously, the resulting products are completely different from the original raw materials. In the strict sense, the term 'biopreservation' is used for the use of biological processes that change the sensory properties of a food as little as possible.

The term 'biopreservation' may also be extended to the use of biological systems to protect humans, livestock, and plant crops from colonization by pathogens. This aspect is not discussed further in this article. Biopreservation of animal feed, and possible use of bacteriophages, are also not covered. For an extensive treatment of these aspects, the reader is referred to the book edited by Lacroix and the literature on probiotics.

It should also be remembered that frequently, mechanisms other than microbial activity, limit the shelf life of food. These factors include the activity of food enzymes, the action of oxygen, recrystallization of starch in bread, and loss of turgor in fruit and vegetables. For example, use of oxygen-scavenging enzymes to extend shelf life may also be termed as 'biopreservation.' However, in this article, the term 'preservation' is limited to inhibition of microorganisms, following the definition of 'preservatives' as 'substances which prolong the shelf life of foods by protecting them against deterioration caused by microorganisms and/or which protect against growth of pathogenic microorganisms' (European Union (EU): Regulation No. 1333/2008).

Interest in biopreservation is triggered by various factors:

- Increased awareness of regulatory authorities and consumers on the importance of food safety.
- Increased recognition of the responsibility of food industry to ensure safety of its products.
- Increase in the number of susceptible individuals in the population, especially in industrialized countries, and thus the need for more stringent hygiene standards.
- The need for increased stability in food quality in order to prevent food loss due to spoilage.
- Consumer demand for increased convenience, for example, less frequent shopping, while still expecting 'fresh' and 'natural' food without chemical additives. Hence, retailers urge their suppliers to extend their shelf life and to cut the list of additives on the label ('clean labeling').

Generally, current food regulations do not require the use of enzymes and microorganisms to be labeled. Hence, it is tempting to add microorganisms or enzymes that form antimicrobial compounds *in situ*. However, the regulations were set up with the assumption that the consumer knows that,

for example, yogurt and cheese are made by the action of microorganisms. Whether this holds true for novel use of biological systems is, at best, doubtful. Current trends in legislation will be treated in a separate section of this article.

Mechanisms of Biopreservation

Viable microorganisms may be added to the food to be preserved. In contrast to 'starter cultures', these 'protective cultures' should only minimally change the sensory properties of the food. They may act by competitive exclusion (occupying binding sites, competing for nutrients) or by excreting antimicrobial compounds. Such compounds may be main products of energy metabolism (usually organic acids), other low-molecular mass compounds, or ribosomally synthesized peptides, namely, bacteriocins.

Lactic and acetic acids are the main metabolic products of lactic acid bacteria and essential for the safety and stability of various fermented foods. Other organic acids (such as propionic acid, phenyllactic, and *p*-hydroxyphenyllactic acids, 3-hydroxy fatty acid, 2-pyrrolidone-5-carboxylic acid) are formed in low amounts but have some inhibitory activity, particularly against fungi. This could be exploited to extend the mold-free shelf life of certain cheeses and of bread. Some neutral metabolites (e.g., diacetyl, hydrogen peroxide, reuterin, certain cyclic dipeptides) have a wide spectrum of antimicrobial activity. However, most of the compounds mentioned have an impact on the sensory properties, and it is difficult to select strains that form a 'cocktail' of these compounds that optimally suits the food to be preserved. For example, the aroma of diacetyl may be tolerated in some dairy products but definitely not in meats or juices.

Bacteriocins of interest to food preservation include small, heat stable, hydrophobic cationic peptides formed by Gram-positive organisms. Class I bacteriocins are also called lantibiotics and comprise posttranslationally modified peptides making pores into the cytoplasmic membrane. Of these, nisin (formed by strains of *Lactococcus lactis*) has been approved as an additive to certain foods (see section Legal Aspects). Within the Class II bacteriocins (not modified after translation), compounds with effect against *Listeria* are of interest. Of these, pediocin PA-1 is formed by commercially available cultures of *Pediococcus acidilactici* and *Lactobacillus sakei*, and leucocin A/B are formed by a commercial culture of *Leuconostoc carnosum*. Their main mode of action is also the permeabilization of the membranes. Application of bacteriocins or cultures producing them appears attractive because they have no effect on sensory properties and are readily destroyed in the upper intestinal tract, which may eliminate concerns about spread of resistance genes and resistant organisms. Moreover, it may be argued that bacteriocin-forming bacteria are present in traditional fermented foods. However, use of bacteriocins in food preservation has limitations: Their inhibitory spectrum is small and restricted to Gram-positive organisms, unless sublethally injured by other preservation techniques, Gram-negative bacteria are protected against these bacteriocins by their outer membrane. Another consequence of this specificity is that the susceptibility of the target strains may vary. Moreover, bacteriocins may be inactivated by food components such as membrane lipids or proteases.

Search for protective cultures was usually focused on lactic acid bacteria isolated from foods because these organisms have a favorable reputation for safety, and are competitive in many foods. However, for foods that have to be stored in the presence of oxygen (such as shredded vegetables), or other foods in with lactic acid bacteria are poor competitors, use of other microorganisms is also considered. However, this has limitations because it will be easier to obtain a generally recognized as safe (GRAS) or qualified presumption of safety (QPS) status for lactic acid bacteria than, for example, Gram-negative bacteria dominating the flora of ready-to-eat vegetables and fresh unpackaged meat.

Alternatively, one may cultivate food-grade microorganisms outside the food, in suitable food-grade media such as milk, isolate the compounds and add them to food. The advantages of this approach are that the producer organism need not to be competitive in the food to be preserved, and control of the levels of the antimicrobial compounds in the food is easier. For example, *Lactobacillus reuteri* forms a broad-spectrum antimicrobial compound, reuterin, with potential as a food preservative, but the bacterium is found in the gastrointestinal tract and in sourdoughs and competes poorly in foods like meats and cheeses. However, legal approval is generally required before these antimicrobials compounds can be used as food additives. Hence, much research and development are under way on the antimicrobial effect of natural food components such as essential oils from herbs or lactoferrin from milk, and the isolation and application of them. However, this is not covered by the term 'biopreservation' as used in this article, and the reader is referred to recent reviews.

Certain enzymes may also be used for biopreservation. Lysozyme, a muraminidase degrading bacterial cell walls, is already being used as a substitute for nitrate in some cheeses, in order to inactivate clostridia. Specific bacteriophages or their endolysins acting on the cell wall of the target organisms may be used to inactivate specific microbial species, for example, *Listeria monocytogenes*, or to eliminate certain biofilms. Lactoperoxidase is naturally present in raw milk and can oxidize thiocyanate ions in the presence of hydrogen peroxide generated by lactic acid bacteria (or added as such). The antimicrobial effect of the reaction product (hypothiocyanate) can be used to stabilize raw milk during transport under poor refrigeration.

Antimicrobial peptides are also part of the host – defense system of higher organisms and raised much interest in pharmacy. Whether or not they are useful in food preservation remains to be shown.

Applications

Meat, Poultry, and Fish

Raw Unprocessed Meat, Poultry, and Fish

When unprocessed or minced meat, poultry, and fish are stored in air or in oxygen-permeable packaging material, it is spoiled by Gram-negative bacteria, and relevant pathogens are Gram-negative, too. Lactic acid bacteria compete only poorly.

Therefore, very high levels of protective cultures or biopreservatives would have to be added to obtain a relevant effect. This is not a realistic option. However, lactic acid bacteria are favored by reduced oxygen, elevated CO_2 tension, and the presence of fermentable carbohydrates. On chilled red meat packaged under vacuum or under modified atmosphere (20–30% CO_2), use of selected strains of well-adapted psychrotrophic lactic acid bacteria may extend shelf-life by suppressing other lactic acid bacteria and *Brochothrix thermosphacta* that cause off-odors.

Fermented Meats

The main microbial hazards associated with fermented meats are salmonellae and shigatoxin-forming *Escherichia coli* (STEC). Growth of these bacteria is well under control by appropriate formulations (salt, curing agents, sugar, starter cultures, ripening temperature). The same applies to *Li. monocytogenes*; however, no outbreaks of listeriosis could hitherto be traced to the consumption of fermented sausages, so the risk should not be overestimated. The growth of *Staphylococcus aureus* is also well controlled by present technology.

Sausage fermentation usually reduces the counts of salmonellae, STEC, and *Li. monocytogenes* by 1–3 log cycles, depending on the strain, the pH, and the duration of drying and aging. It is desirable to accelerate the death rate of these pathogens further. Use of bacteriocin-forming lactic acid bacteria has been shown to reduce *Li. monocytogenes* counts by 1–2 additional log cycles (as compared to a non-bacteriocinogenic control culture with similar acidification rates) in various types of sausages, and anti-*Listeria* protective cultures (containing bacteriocin-producing *Pediococcus* and/or *Lactobacillus* strains) are commercially available. Cell-free preparations of bacteriocins (e.g., enterocins) have also been used successfully to inactivate listeriae. However, use of bacteriocins (including nisin) is not permitted for meats in the EU.

As outlined above, bacteriocins have some effect on Gram-negative bacteria only if the outer membrane of these organisms is damaged by additional stress factors. Some effect against these organisms may only be expected if a bacteriocin-forming starter is combined with other preservation methods.

For fermented sausages developing a surface flora (e.g., Italian-style salami or French 'saucissons'), surface inoculants (molds, yeasts) antagonistic against mycotoxigenic molds and/or *S. aureus* are useful.

Other (Nonfermented) Processed Meat, Poultry, and Fish Products

The main microbial hazard to be controlled in nonfermented ready-to-eat meat, poultry, and fish products is *Li. monocytogenes* because this organism grows at refrigeration temperatures, even in the range of water activity (usually 0.96 and above) and pH values (usually 5.8 and above) typical for these products. Heat treatment such as hot smoking of fish normally inactivates listeriae but also spoilage organisms which would otherwise compete with these organisms. *Li. monocytogenes* may be present in the processing environment, especially for fish, and it is difficult to avoid recontamination of the products during further processing. Hence, the 're-addition' of food-grade bacteria should be considered that inhibit (or even kill) listeriae while not causing spoilage. The feasibility of this approach has been demonstrated by various research groups, using selected strains of *La. sakei* or *Le. carnosum* that only minimally change the sensory properties of the products, provided that the content of fermentable sugar is below 0.1% (which means that no sugar should be added to the product). The antagonistic effects may be increased by using bacteriocin-forming strains and/or of bacteriophages specific for *Li. monocytogenes*. The inoculum size is critical for the desired effect. Preparations of *La. sakei* (with and without ability to form bacteriocin) and *Le. carnosum* (bacteriocin-former) are commercially available. Nevertheless, the prevention of recontamination by listeriae during handling, slicing, and packaging operations should have priority, and growth potential of *Li. monocytogenes* may also be reduced by the addition of small amounts of sodium acetate or other acidulants.

In lightly preserved fish and seafood, *Carnobacterium* species contribute to spoilage. However, some strains of carnobacteria have little spoilage potential and an antagonistic effect against other spoilage bacteria and *Li. monocytogenes*. Bacteriocinogenic *Carnobacterium* strains have an even stronger effect and have potential in suppressing *Li. monocytogenes* in particular on cold-smoked salmon and similar products, which are frequently contaminated by this organism. An antagonistic strain of *L. lactis* has also been patented.

For lightly preserved fish and seafood, nonproteolytic strains of *Clostridium botulinum* are a hazard to be controlled, especially if fish has been grown in poorly managed aquaculture or caught in areas with high prevalence of spores of this organism (such as the Baltic Sea). For control of spore formers, the 'fail-safe' approach could be an option.

Milk and Dairy Products

To improve the safety of dairy products, biopreservation systems inactivating *Li. monocytogenes* are of interest. Under insufficient hygienic conditions, this organism may contaminate the surface of cheeses, especially those ripened by a surface smear applied after lactic fermentation. Many bacteriocins are effective against listeriae, but adding bacteriocins or bacteriocin-forming lactic acid bacteria to the milk may interfere with the fermentation of the milk. Hence, a more promising approach is to test microbial consortia in surface smear for anti-*Listeria* properties, to select the strains (staphylococci, coryneforms or yeasts) responsible for this effect, and to develop strongly antagonistic smear cultures. In the future, use of endolysins specific to listeriae may also be an option.

There are cultures on the market that have been selected for antagonistic activity against lactate-fermenting clostridia during cheese ripening. Lysozyme, an enzyme degrading bacterial peptidoglycan, also falls within the definition of 'biopreservatives' and is a permitted additive to prevent 'late-blowing' of cheese by clostridia. Commercial antifungal cultures combine strains of *Lactobacillus rhamnosus* or *Lactobacillus paracasei* with a strain of *Propionibacterium freudenreichii*, and propionate as well as various other low-molecular mass compounds contributes to the inhibitory effect. Cell-free preparations

with metabolic products of *P. freudenreichii* ssp. *shermanii* have been on the US market since decades under the brand name MicroGARD®.

Vegetables and Fruit

There is an increasing market for minimally processed vegetables and fruit, either ready to eat (for salad bars etc.) or ready to cook. Hazards to be controlled include Gram-negative pathogens (salmonellae, STEC) and *Li. monocytogenes*. Shredding of vegetables and fruit, damages the mechanical barriers against microbiological colonization and also causes enzymatic browning and texture changes resulting in a very short shelf life. Moreover, these products cannot be packaged and stored in the absence of oxygen. The safety of sprouts is especially difficult to ascertain because conditions needed for seed germination also favor the growth of bacteria. For all these reasons, use of protective cultures and biopreservatives is considered as an option. It was shown that certain Gram-negative aerobic bacteria isolated from fresh produce can inhibit the growth of pathogens on iceberg salad and sprouts. Application of bacteriocins to these products also had some effect against listeriae. However, it will be difficult to get approval of Gram-negative bacteria as protective cultures under the GRAS/QPS scheme, and, generally, it is difficult to preserve them, which is essential for developing cultures for commercial use.

Vegetables (especially cabbage and cucumbers), can be fermented by lactic acid bacteria to give products like sauerkraut, Korean-style kimchi, and pickles. Also, most table olives are produced by a lactic fermentation. Even though starter cultures are not generally used, the resulting products have a good record of safety. However, lactic acid bacteria with strong antagonism against microorganisms causing sensory deficiencies or spoilage, or forming biogenic amines could be useful. This applies particularly to table olives because the lye treatment for debittering also reduces the levels of 'spontaneous' lactic acid bacteria on the olives, which leads to poor fermentation and to problems due to growth of certain *Propionibacterium* and *Clostridium* species. Bacteriocin-forming lactic acid bacteria have been isolated from fermented vegetables and olives, and shown to be useful in pilot-scale experiments.

Other Foods

In food service operations, there is a trend to uncouple cooking from serving. Meals are normally not stabilized by low pH and/or low water activity, and, for culinary reasons, the times and temperatures of cooking are usually not sufficient to inactivate spores of psychrotrophic spore formers such as nonproteolytic *C. botulinum*. To inhibit this bacterium, a strict maintenance of the chill chain is needed, and the storage temperature must be below 3 °C if the storage time exceeds 5 days. This is not always ascertained, especially if cook-chill meals are marketed through retails. One option to improve the safety of these products is to add protective cultures that grow rapidly and acidify the product under conditions of temperature abuse. This 'fail-safe' approach has also been suggested for inhibition of *C. botulinum* in bacon, and for delicatessen salads.

Since most bacteriocins of Gram-positive bacteria are heat-stable, and some of them – including nisin – are also effective against spore-forming bacteria, it was considered to add them to food to be canned, in order to reduce the intensity of the heat treatment. However, there are problems with stability of bacteriocins especially in meats and other foods rich in fat and membrane constituents. Hence, a 'botulinum cook' is still necessary for low-acid shelf-stable canned foods, even with bacteriocins added. Rather, it may be considered to use heat stable bacteriocins to aid inactivation of thermophilic (e.g., *Geobacillus thermophilus*) and acidophilic (e.g., *Alicyclobacillus* spp.) spore formers in canned vegetables and fruit products, respectively. These organisms may cause spoilage, but are no threat to human health.

Legal Aspects

In general, additives are defined as food ingredients that are not consumed as food, and must be included into a positive list (as provided in the annexes of official regulations). This applies – with certain exceptions – also to metabolic products of microorganisms such as bacteriocins. Nisin has been approved as a food additive in the EU (for semolina and tapioca puddings, clotted cream and certain cheeses), and in the US, nisin and pediocin PA-1 have GRAS status and are permitted to be used in various foods. The required wordings in the list of additives differ between countries. This is the main reason why poorly defined preparations of metabolic products from food-grade microorganisms such as MicroGARD® are being used in the US but not to any extent in the EU.

As indicated above, there is a trend in food legislation to cut certain 'privileges' of microorganisms and enzymes as food additives. Positive lists of food-grade microorganisms (e.g., the QPS list of the European Food Safety Authority (EFSA), GRAS list of the Food and Drug Administration (FDA)) and enzymes (as stipulated in Regulation 1332/2008 of the EU) are being developed, and, for example, the EU regulation on provision of food information to consumers (as of July 2011) generally requires labeling unless the culture and/or enzymes are just 'carried over' with some ingredient, and/or are no longer active in the final product. Extensive approval procedures and labeling requirements will hamper the implementation of biopreservation procedures in commercial practice. Moreover, use of protective cultures for unfermented food will result in higher microbial counts and their possible misinterpretation by food inspectors.

It should also be mentioned that the interest in systems killing *Li. monocytogenes* is, to a great extent, powered by regulations interpreted to require 'zero-tolerance' for *Li. monocytogenes* in ready-to-eat food, and specify decimal reductions of pathogens, which are difficult to achieve by conventional fermentation methods. The EU (in its Regulation No. 2073/2005) and the Codex Alimentarius Commission standard CAC/GL 61-2007 (amended in 2009 in ALINORM 09/32/13) require zero-tolerance (i.e., absence in 25 g) for *Li. monocytogenes*, only in food that support growth of this bacterium, and in infant food, whereas in other foods not

supporting growth of *Li. monocytogenes*, a maximum level of 100 g^{-1} is tolerated.

Concluding Remarks

Use of biopreservation techniques may contribute to the safety and quality of perishable food but cannot replace good hygienic practice in food processing. For example, avoiding cross-contamination is the most important measure to avoid problems with *Li. monocytogenes* in meat and cheeses. In many fermented products (including dry sausages), *Li. monocytogenes* does not grow, and use of listericidal cultures for such products does not reduce the risk to public health but merely the risk that viable *Li. monocytogenes* cells are found in the final product, with subsequent problems in explaining this to customers and public health authorities.

Although there is plenty of scientific information on bacteriocins formed by food-grade bacteria (more than 11 000 hits for 'bacteriocins and food' in the SCIRUS® database, accessed July 2011), there are to date, rather few examples for commercialization of bacteriocins and cultures producing them. More attention should therefore be given to combining the effects of bacteriocin-forming cultures and other intrinsic and extrinsic factors affecting microorganisms, and on other mechanisms of biopreservation.

See also: Food Technologies: Fermentation. Public Health Measures: Fundamentals of Food Legislation. Safety of Food and Beverages: Fruits and Vegetables; Meat and Meat Products; Milk and Dairy Products; Probiotics and Prebiotics; Seafood

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FOOD TECHNOLOGIES

Chilling

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Glossary

Conduction, thermal Mechanism for heat transfer. The process of heat transfer through a solid material/medium in which kinetic energy is transmitted by the particles of the material from particle to particle without gross displacement of the particles.

Convection, thermal Mechanism for heat transfer. The process of heat transfer through a liquid or gas by means of circulating currents caused by changes in density.

Hazard A biological, chemical, or physical agent in, or condition of, food with the potential to cause an adverse health effect.

Heat transfer coefficient Coefficient used in thermodynamics to calculate heat transfer, typically by convection or phase change, between a fluid and a solid.

Pasteurization A form of heat treatment that kills certain vegetative bacteria and/or spoilage organisms in milk and other foods. Temperatures below 100 °C are used.

Radiation, thermal Mechanism for heat transfer. Electromagnetic radiation generated by the thermal motion of charged particles in matter. All matter with a temperature greater than absolute zero emits thermal radiation.

Refrigeration May be defined as the process of removing heat from any substance to: (1) render colder – reduce temperature, (2) change its state – for example, water to ice, (3) maintain its state – preserving foods, storing ice.

Retail An operation that stores, prepares, packages, serves, or otherwise provides food directly to the consumer for preparation by the consumer for human consumption. This may be freestanding markets, sections in grocery or department stores, packaged, chilled, or frozen and/or full service.

Shelf-life The period during which the product maintains its microbiological and chemical safety and sensory qualities at a specific storage temperature. It is based on identified hazards for the product, heat or other preservation treatments, packaging method, and other hurdles or inhibiting factors that may be used.

Introduction

There is no strict definition of what constitutes a chilled food, although some countries provide legislative requirements for particular chilled foods. In general, a chilled food is any food in which the temperature of the food is reduced to, and maintained at, a temperature below that of the ambient temperature, but above the temperature where any of its water content will change from a liquid to a solid (i.e., begin to freeze). With fish and meat the maximum chilled shelf-life will be achieved at a temperature close to their initial freezing points. However, for some foods, such as bananas and other tropical fruit, the optimum temperature can be as high as + 14 °C.

Chilling is often preferred over other food preservation methods, such as smoking, drying, salting, or canning, because it produces no significant changes in the texture, taste, smell, or appearance of the food and maintains the original 'fresh' quality characteristics of the food.

Temperature is the principal controlling factor for the safety of chilled foods. Temperature is particularly important in such foods in slowing, or inhibiting, the growth of pathogenic bacteria.

Chilling Process

Chilling is a process of removing heat from a food and can only be achieved by four basic mechanisms: radiation, conduction, convection, or evaporation.

Conduction requires a good physical contact between the food to be chilled and the cooling medium, and this is generally achieved only with foods that can be shaped into regular shapes, such as blocks of meat or fish.

Radiation does not require any physical contact but a large temperature difference is required between the surface of the food being cooled and that of surrounding surfaces to achieve significant heat flow. In primary chilling, radiation is only important in the initial stages of the process in a system where the food is not surrounded by other products. Again, in the initial stages of the chilling of cooked food products (e.g., pies and other pastry products, meat joints, baked cakes, etc.), radiant heat loss can be substantial if the products are surrounded by cold surfaces.

Evaporation from a food surface reduces yield and is not desirable in most food refrigeration operations but can be useful again in the initial cooling of cooked food products and

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Table 1 Generation times for foodborne bacteria in raw meat

Bacteria	*Temperature (°C)*	*Time (h)*		*References*
		Lag	*Generation*	
Salmonella spp.	12.5		6.79	Mackey *et al.* (1980)
Clostridium perfringens	12		11.5	Lund *et al.* (2000)
Escherichia coli O157:H7, pH 5.7	12	16.2	6.0	Walls and Scott (1996)
E. coli O157:H7, pH 6.3	12	2.78	3.9	Walls and Scott (1996)
Salmonella spp.	10		13.87	Mackey *et al.* (1980)
Salmonella Typhimurium	10	45	9.65	Smith (1985)
Yersinia enterocolitica	10		12.73	Logue *et al.* (1998)
E. coli SF	8.2	40	6.9	Smith (1985)
B. cereus	5		8.3	Lund *et al.* (2000)
Y. enterocolitica	5		16.53	Logue *et al.* (1998); Lund *et al.* (2000)
Listeria monocytogenes	4		22.8	Lund *et al.* (2000)
L. monocytogenes	4		9.3	Pawar *et al.* (2000)
E. coli O157:H7	2		No growth	Ansay *et al.* (1999)

is used in the immediate postharvest cooling of many fruits and vegetables. However, as soon as the surface of the food is close to that of the cooling medium then any heat loss due to evaporation is minimal.

Convection is by far the most important heat transfer mechanism employed in the majority of food chilling systems. In most cases, refrigerated air is the transfer medium; however, in some cases brine or a cryogenic gas can be used. The rate of heat removal depends on the following:

1. Surface area available for heat flow.
2. Temperature difference between the surface and the medium.
3. Surface heat transfer coefficient.

Each combination of product and cooling system can be characterized by a specific surface heat transfer coefficient whose value depends principally on the thermophysical properties and velocity of the medium. Typical values range from 5 W m^{-2} K^{-1} for slow moving air to 500 W m^{-2} K^{-1} for immersion in an agitated refrigerant.

Heat must also be transferred from within the food to its surface before it can be removed. In a solid food this will be via conduction; whereas in a liquid this will mainly be via convection. Most foodstuffs are poor conductors of heat and this imposes a severe limitation on attainable chilling times for either large individual items or small items cooled in bulk.

Effect of Chilling on Food Safety

The principle of chilling as a preservation process is the basis that all biological systems are controlled by enzymatic reactions including those that control microorganisms and cause quality degradation and that the rate of these reactions is directly related to temperature. Reducing temperatures below the optimum growth range of a microorganism increases its generation time. The main group of microorganisms of concern in chilled foods are psychrophilic pathogens. These organisms can grow at temperatures below 0 °C, and some reportedly have an optimum growth temperature as low as 10 °C. The optimum temperature growth range of mesophiles is 25–30 °C and with many the minimum growth temperature is approximately 10 °C. Because most chilled food is kept below this temperature mesophiles are not usually of concern in chilled distribution. However, mesophilic pathogens, such as *Staphylococcus aureus* and *Bacillus cereus*, are also of concern when food handlers and producers fail to chill foods properly or when adequate temperatures are not maintained during storage and handling. In addition, some mesophilic microorganisms are psychrotrophic and can grow on refrigerated foods.

Although microorganisms can grow at low temperatures, they grow more slowly as the temperature is reduced. Thus the generation time for a pseudomonad (a common form of spoilage organism) might be 1 h at 20 °C, 2.5 h at 10 °C, 5 h at 5 °C, 8 h at 2 °C, or 11 h at 0 °C. As temperatures are reduced below 10 °C, fewer strains can grow and cause spoilage. In general, food will spoil approximately four times as fast at 10 °C and twice as fast at 5 °C, as at 0 °C. In the usual temperature range used for the storage of chilled meat and meat products, −1.5 to 5 °C, there can be as much as an eight-fold increase in growth rate between the lower and upper temperatures used (Table 1). Storage of chilled meat and meat products at -1.5 ± 0.5 °C would attain the maximum storage life without any surface freezing of the product. Chill temperatures also have a marked effect on the type of spoilage microflora present on food by altering the microbial community. For example, raw milk stored at temperatures close to 0 °C tends to putrefy because of the activity of pseudomonads, rather than to sour due to the activity of lactic acid bacteria.

The essential characteristics of pathogenic microorganisms can be found in numerous texts. There is a certain degree of conflicting data concerning the importance of various pathogens with regard to safety of specific foods and the effect of specific temperatures or packaging atmospheres on their growth or inhibition. Pathogenic bacteria such as *Salmonella* spp., *L. monocytogenes*, *Clostridium botulinum* type E, psychrotrophic *B. cereus*, *Aeromonas hydrophila*, and *Y. enterocolitica* are of particular concern in chilled foods because they are capable of growth at low temperatures (Table 2). Many of the organisms that compete with pathogens at ambient temperatures will not grow at low temperatures, thus low temperatures may

Table 2 Minimum and optimum growth temperatures for pathogens associated with foods

	Minimum temperature (°C)	*Optimum temperature (°C)*
Campylobacter spp.	30–32	42–45
Arcobacter spp.	15	37
C. perfringens	12	43–47
B. cereus mesophilic	10–15	35–40
C. botulinum proteolytic	10	35
Shigella spp.	7–10	37
S. aureus	7	35–40
Pathogenic *E. coli* strains	7	35–40
E. coli O157:H7	6–7	42
Salmonella spp.	5	35–43
B. cereus psychrotrophic	4–5	28–35
Vibrio parahaemolyticus	5–10	30–37
C. botulinum non-proteolytic	3	30
A. hydrophila	−0.1 to 1.2	15–20
L. monocytogenes	−1 to 0	30–37
Y. enterocolitica	−2	22–30

preferentially favor the growth of pathogenic organisms. Pasteurization may also favor the outgrowth of surviving spores of psychrotrophic strains of *C. botulinum* and *B. cereus* by destroying competing microflora, a particular concern in vacuum packaged pasteurized foods ('sous vide' products), and ready-to-eat (RTE) products. However, most will not grow, or produce toxins, below 4 °C, with the exception of *L. monocytogenes*, *C. botulinum* type E, psychrotrophic *B. cereus*, *A. hydrophila*, and *Y. enterocolitica*.

Investigations into the effect of different storage atmospheres on pathogenic growth at low temperatures appear to show that carbon dioxide (CO_2)-enriched atmosphere produce the greatest inhibitory effect on psychrotrophic pathogens (*Y. enterocolitica*, *A. hydrophila*, and *L. monocytogenes*). García de Fernando *et al.* (1995) concluded that "at a normal meat pH (i.e., 5.5) and at a low temperature (e.g., 1 °C) the growth of psychrotrophic pathogens is stopped when the CO_2 concentration is 40%." However, high pH meat (≥ 6) and/or higher storage temperatures will support growth of such pathogens.

The bacterial safety and rate of spoilage depends on the numbers and types of microorganisms initially present, the rate of growth of those microorganisms, the conditions of storage (temperature and gaseous atmosphere), and characteristics (pH, a_w) of the food. Of these factors, temperature is by far the most important. In the context of food safety the safest practical chilled temperature will be the lowest that can be used without significantly affecting the quality of the food. In many cases this will be as close to the freezing point of the food as practically possible. Maintaining temperatures and the integrity of the whole of the chill chain is vital to ensure the safety and quality of chilled foods.

Effect of Chilling Rate

Whether 'rapid' chilling offers any clear advantages to product safety will depend on what biological hazards (pathogens, etc.) are present, at what numbers they are present, and whether they are on, or in, the food in question, and how 'rapid' the rate is in comparison with other rates. There is no definition of 'rapid' and 'slow's rates. Size of product will also have a big effect on relative rates of chilling, because conduction through the product will become the rate-limiting factor as product size increases.

There are instances where excessively rapid chilling rates, or too low a chilling temperature, can cause quality problems in foods. For example, a serious defect known as 'woolly texture' can be produced in rapidly cooled peaches. Substantial textural problems due to a phenomenon known as 'cold shortening' can occur in rapidly chilled meats (particularly beef and lamb), although electrical stimulation before rapid chilling will mitigate this problem.

Few countries provide legislative requirements for chilling foods that specify chilling rates. For example, European legislation requires that red meat carcasses be chilled to a maximum temperature of 7 °C and poultry carcasses to a maximum temperature of 4 °C. No time limits on achieving these times have been set. There are very little data on the effect of current commercial chilling rates and conditions on changes in bacterial numbers during the process. In most cases no change or a small reduction (0.5 to 1 $\log_{10}$ cfu cm^{-2}) in number of organisms on the surface has been measured. Current European legislation for foods of animal origin places importance on core meat temperatures, because microbial contamination is primarily a surface phenomenon there is an argument to be made that surface temperatures are far more important than deep temperatures. This is the basis behind the controls in the Australian Export Control (Meat and Meat Products) Orders 2005 and their Refrigeration Index (RI) model. The central idea of the RI model is to measure the performance of the chilling process until all the sites of microbiological interest are at or below 7 °C and calculate the risk of *E. coli* growth from temperatures measured during chilling.

Although there are few legislative requirements, there are many guidelines and recommendations for chilling cooked/pasteurized food products (Table 3), particularly RTE products that can be eaten cold. Inadequate cooling of such products has been identified as potential safety risk, as there is always the possibility that some microorganisms, particularly spores, will not be killed by the cooking/pasteurization process. Therefore most guidelines recommend that cooked products should be cooled as quickly as possible through the temperature range 63 to 5 °C or less to minimize risk of spore germination and outgrowth. There is particular concern with vacuum packaged pasteurized foods (termed by some as refrigerated processed foods of extended durability, and including 'sous vide' products) that conditions (low oxygen and destruction of competing microflora) may favor the outgrowth of surviving spores of psychrotrophic strains of *C. botulinum* and *B. cereus*. Consequentially lower storage temperatures (<3 °C) are recommended for these products and/or more severe heat processes. With RTE products, there have been concerns over the possible presence and outgrowth of spores of psychrotrophic *B. cereus*. Although many of these guidelines were initially produced specifically for cook-chill catering operations, in many countries, the producers of chilled ready meals for retail sale use them.

Table 3 International chilling time guidelines/recommendations for the cooling of cooked foods

Country	*Chilling range (°C)*	*Time (h)*	*Chilling rate (°C min^{-1})*	*Storage temperature (°C)*	*References*
Australia	60–21	≤2	0.33	5	De Jong *et al.* (2004)
	21–5	≤4	0.07		
Canada	60–20	≤2	0.33	4	Canadian Food Inspection System Implementation Group (CFISIG) (2004)
	20–4	≤4	0.07		
Codex Alimentarius	60–10	≤2	0.42	–	Codex Alimentarius Commission (1999)
Denmark	65–10	≤3	0.31	<5	Evans *et al.* (1996)
France	70–10	≤2	0.50	0–3	Evans *et al.* (1996)
Germany	80–15	≤2	0.54	2	Evans *et al.* (1996)
	(15–2)	≤24			
Ireland	70–3	≤2.5	0.45	3	Food Safety Authority of Ireland (FSAI) (2004)
The Netherlands	60–7	≤5	0.18	–	De Jong *et al.* (2004)
	7–4	≤24			
Sweden	80–8	≤4	0.30	3	Evans *et al.* (1996)
UK	70–3	≤1.5	0.74	3	UK Department of Health (1989)
USA	60–5	4–6	0.23–0.15	–	De Jong *et al.* (2004)

Because prolific histamine-forming bacteria are predominately mesophilic, rapid cooling of at-risk fish species (such as tuna and mackerel) immediately after catching is recognized as the key control for reducing histamine formation in such fish, as is the maintenance of adequate temperature control during the rest of the cold-chain.

Similarly prompt and rapid cooling of other seafood, particularly fish and shellfish that are eaten raw, such as oysters, is an important control for *V. parahaemolyticus*. The maintenance of tight temperature control during the rest of the cold-chain is also very important with *V. parahaemolyticus* because it is capable of growth at temperatures as low as 5 °C, and is known for rapid growth and generation times as short as 12–18 min.

Chilling Operations

Chilling systems for foodstuffs will contain many, if not all, of the following unit operations:

- Preparatory treatment; conditioning, waxing, cooking, pasteurizing, blanching, etc.
- Chilling; primary or secondary.
- Storage.
- Transportation.
- Retail display.
- Domestic storage.

During the preparatory treatment there can be a range of temperature responses, from a large gain to a small decrease in the temperature of the product.

During chilling there is a substantial decrease in the mean temperature of the product. Within a correctly designed cold-chain there should be no significant change in mean product temperature during storage, transport, retail display, or domestic storage.

From a food safety-based approach, prepacking the food before chilling will lower the risk of contamination/cross-contamination during the chilling process, however, it will significantly reduce the rate of cooling, and this may allow the growth of any microorganisms present. Provided the cooling media (air, water, etc.) and refrigeration equipment used are kept clean, no one cooling method can be said to be intrinsically more hygienic, or safe, than any other. For example, although there has been much debate concerning the safety of chilled water-cooling versus blast air chilling of poultry carcasses, reviews of published data have found little evidence of any difference microbially between poultry cooled by either method. Similarly there appears to be no evidence of a difference between spray chilling versus blast air chilling of red meat carcasses. Although some pathogens, such as campylobacters and arcobacters, may show sensitivity to air chilling; immersion and spray chilling may result in some physical removal (washing off) of microorganisms. In addition the use of antimicrobials, such as chlorine or ozone, in chilled water immersion/spray systems has been shown in some studies to reduce microbial contamination and subsequently may improve food safety. Such substances, however, are not permitted in all countries (notably the European Union) at present.

The potential for the fans used in air chilling to disseminate molds and bacteria has been identified in a number of reviews as a potential hazard, but very little work has been carried out to evaluate whether this is in fact the case. Similarly, condensation in the chiller has also been identified as a possible source of cross-contamination during chilling and storage (particularly of unpackaged foods). EU legislation regarding the chilling of foods of animal origin specifies that "during the chilling operations, there must be adequate ventilation to prevent condensation on the surface of the meat." However, there appears to be little published data to support such a control. It is however prudent that chillers should be properly constructed and maintained. The design of chillers should be such as to provide for effective cleansing and disinfection.

Chilling Systems

Moving Air

Air is by far the most widely used method of chilling food as it is economical, hygienic, and relatively noncorrosive to equipment. Systems range from the most basic in which a fan draws air through a refrigerated coil and blows the cooled air around an insulated room, to purpose-built conveyerized blast chilling tunnels or spirals. Air chilling is slow but versatile especially when there is a requirement to cool a variety of products.

The cooling time of the product is reduced as the air speed is increased, thus air distribution is very important. Conveying the product through the cooling system overcomes the problem of uneven air distribution because each item is subjected to the same velocity/time profile. The refrigeration capacity and air conditions can also be varied throughout the length of the tunnel. Using higher air temperatures in the latter stages can avoid surface freezing.

Immersion/Spray

As their names imply, these involve dipping product into a cold liquid, or spraying a cold liquid onto the food. When water is used as the heat transfer medium the process is often called 'hydrocooling.' This produces high rates of heat transfer due to the intimate contact between product and cooling medium. Both offer several inherent advantages over air cooling in terms of reduced dehydration and coil frosting problems. Clearly if the food is unwrapped the liquid has to be 'food safe.' Any uptake of the cooling medium, whether 'food safe' or not, by the product may present problems both in terms of flavor changes and the requirement for periodical replacement of the medium.

Hydrocooling is probably the least expensive method of achieving rapid cooling in small products. The product to be cooled is immersed in, or sprayed with, cool water, either at ambient or near 0 °C. Where permitted, the water is often treated with a mild disinfectant, such as chlorine. Practical systems vary from simple stirred or unstirred tanks to plants where the product is conveyed through agitated tanks or under banks of sprays. Hydrocooling is very effective for chilling fruit and vegetables, however, not all crops can tolerate wetting.

Spraying with ambient or chilled water is also an effective method for initially cooling cooked products that can withstand wetting, i.e., hams, sausages, chubs, etc. Spray bars are either fitted in the batch cookers or in separate cooling cabinets. Spray chilling of meat carcasses is widely practiced in the USA and used for poultry in Europe.

Chilling with crushed ice or an ice/water mixture is simple, effective, and commonly used for fish cooling. Cooling is more attributable to the contact between the produce and the cold melted water percolating through it than with the ice itself. Ice has the advantage of being able to deliver a large amount of refrigeration in a short time as well as maintaining a very constant temperature, 0 to -0.5 °C (where sea water is present). Ice may also be used for cooling fruit and vegetables, however, as with hydrocooling, ice is only applicable to vegetable and fruit produce that can tolerate wetting, such as watercress, broccoli, and some other brassicas. Large poultry carcasses, such as turkeys, are also often initially cooled by immersion in an ice/water mixture, with the carcasses being conveyed through a tank of ice/water using Archimedean screws.

Vacuum

Solid products having a large surface area to volume ratio and an ability to readily release internal water are amenable to vacuum cooling. The products are placed in a vacuum chamber (typically operating at between 530 and 670 N m^{-2}) and the resultant evaporative cooling removes heat from the food. Evaporative cooling is quite significant, the amount of heat released through the evaporation of 1 g of water is equivalent to that released in cooling 548 g of water by 1 °C. Suitable products, such as lettuce, can be vacuum cooled in less than 1 h. In general terms, a 5 °C reduction in product temperature is achieved for every 1% of water that is evaporated. Because vacuum cooling requires the removal of water from the product prewetting is commonly applied to prevent the removal of water from the tissue of the product.

Plate Heat Exchangers

In a plate cooling system product is essentially pressed between hollow (horizontal or vertical) metal plates containing a circulating refrigerant. Cooling rate depends on product thickness, good contact, and the conductivity of the product. Thus the need for regularly shaped products with large flat surfaces is a major hindrance. Air spaces in packaging and fouling of the plates can have a significant effect on cooling time.

Jacketed Heat Exchangers

Batch coolers for liquid foods can range in capacity from 100 to 10 000 l with the foodstuff usually contained in a stainless steel vessel. The cooling medium may circulate through the jacket of the vessel, through a coil immersed in the liquid, or both. Most vessels are provided with agitators to improve the rate of convective heat transfer and stop temperature stratification. One common method used to decrease cooling times of liquid products in a closed vessel is to apply a vacuum to produce evaporative cooling. Temperature stratification is a problem in unstirred vats whilst in agitated vessels the design and operation of stirrers is critical if breakdown of delicate solid product is to be avoided.

Belt Heat Exchangers

Belt systems consist of an endless steel belt (approximately 1 mm in thickness), the underside of which is cooled either directly with water, brine, or glycol sprays or by sliding over a stationary cold surface. Because only one side of the product is in contact with the cooling surface relatively thin products are required, such as hamburgers, fish fillets, or liquid and semi-liquid products such as purées and sauces. The main advantages of belt systems are: (1) continuous processing, (2) easy continuous cleaning and sanitation, (3) reduced evaporative losses, in comparison with air systems, and (4) the possibility of operating with several temperature zones.

Continuous Heat Exchangers

The majority of liquid foodstuffs require cooling after a heat processing operation such as cooking of sauces and soups, and pasteurization or sterilization of fruit juices, milk, and other dairy products. Milk is also cooled at the point of collection to maintain its quality. Unpasteurized 'freshly squeezed' fruit juices are cooled immediately after production. Fermented beverages are often cooled during primary and secondary fermentation, and before storage. Multiplate coolers are extensively used for liquid foods. They have the highest available heat transfer surface, lowest material requirements, maximum efficiency (up to 90% heat recovery in counter current mode) and are very flexible in operation and easy to clean. Scraped surface heat exchangers can have advantages in the cooling of very viscous liquid foods and where surface fouling is a potential problem.

Cryogenic

Cryogenic cooling uses refrigerants, such as liquid nitrogen or solid carbon dioxide, directly. Owing to very low operating temperatures and high surface heat transfer coefficients between product and medium, cooling rates of cryogenic systems are often substantially higher than other refrigeration systems. Avoiding surface freezing of the product is the main problem in using cryogens for chilling. Forced gas cooling is the only method that can be employed when surface freezing has to be avoided. However, the system tends to be inefficient.

Chilled Storage

Most unwrapped meat, poultry, fish, fruit and vegetables, and all types of wrapped foods are stored in large rooms with circulating air. To minimize weight loss and appearance changes associated with desiccation air movement around the unwrapped product should be the minimum required to maintain a constant temperature. With wrapped products low air velocities are also desirable to minimize energy consumption. However, many storage rooms are designed and constructed with little regard to air distribution and localized velocities over products. Horizontal throw refrigeration coils are often mounted in the free space above the racks or rails of product and no attempt is made to distribute the air around the products. Using a false ceiling or other forms of ducting to distribute the air throughout the storage room can substantially reduce variations in velocity and temperature.

Controlled atmosphere storage rooms were developed for specialized fruit stores. In addition to the normal temperature control plant these stores also include special gas-tight seals to maintain an atmosphere that is normally lower in oxygen and higher in nitrogen and carbon dioxide than air. An additional plant is required to control the carbon dioxide concentration, generate nitrogen, and consume oxygen. The optimum atmosphere must be determined experimentally for the specific product being stored. There is growing use of controlled atmosphere and modified atmosphere retail packs to extend the chilled storage and display life of red meats, poultry, fish, and vegetables. Because the packs tend to be large and insulate the products efficient precooling before packaging is especially important if product quality is to be maintained. Provided that temperatures during chilled storage are sufficient to prevent or inhibit the growth of any pathogens present on the food in question, in general the food will spoil before there is significant growth in pathogens would be a cause of concern.

The safe storage life of chilled foods depends on the control of all the preceding factors, but must be validated by challenge testing and/or modeling for each product and process under defined storage conditions. However, from a practical point of view, the lowest storage temperature that can be used without affecting the quality of the food will be the safest.

Transportation

Chilled foods are transported around the world and locally via a range of transportation systems. All these transportation systems are expected to maintain the temperature of the food within close limits to ensure its optimum safety and high quality shelf-life. It is particularly important that the food is at the correct temperature before loading because the refrigeration systems used in most transport containers are not designed to extract heat from the load but to maintain the temperature of the load. In the large containers used for long distance transportation food temperatures can be kept within $\pm 0.5\ ^{\circ}C$ of the set point.

Control of the oxygen and carbon dioxide levels in shipboard containers has allowed fruits and vegetables, such as apples, pears, avocados, melons, mangoes, nectarines, blueberries, and asparagus, to be shipped (typically 40 days in the container) from Australia and New Zealand to markets in the USA, Europe, Middle East, and Japan. Even longer shelf-lives (over 20 weeks) can now be achieved for meats, particularly beef and lamb.

Air-freighting is increasingly being used for high value perishable products, such as strawberries, asparagus, and live lobsters. Although air-freighting of foods offers a rapid method of serving distant markets, there are many problems because the product is usually unprotected by refrigeration for much of its journey. Up to 80% of the total journey time is made up of waiting on the tarmac and transport to and from the airport. During the flight the hold is normally between 15 and $20\ ^{\circ}C$. Perishable cargo is usually carried in standard containers, sometimes with an insulating lining and/or dry ice but is often unprotected on aircraft pallets.

Overland transportation systems range from 12 m refrigerated containers for long distance road or rail movement of bulk chilled or frozen products to small uninsulated vans supplying food to local retail outlets or even directly to the consumer. The rise in supermarket home delivery services where there are requirements for mixed loads of products that may each require different storage temperatures is introducing a new complexity to local overland delivery.

Retail Display

The temperature of individual consumer packs, small individual items, and especially thin sliced products responds very quickly to small amounts of added heat. All these products are

commonly found in retail display cabinets and marketing constraints require that they have maximum visibility. Maintaining the temperature of products below set limits while they are on open display in a heated store will always be a difficult task.

The required display life and consequent environmental conditions for wrapped chilled products differ from those for unwrapped products. The desired chilled display life for wrapped meat, fish, vegetables, and processed foods ranges from a few days to many weeks and is primarily limited by microbiological considerations. Retailers of unwrapped fish, meat, and delicatessen products, for example, sliced meats, pate, cheese, and prepared salads, normally require a display life of one working day. The introduction of humidification systems can significantly improve display life of unwrapped products.

Average temperatures in chilled retail display cabinets can be varied considerably from cabinet to cabinet, with inlet and outlet values ranging from -6.7 to $+6.0\,^{\circ}C$, and -0.3 to $+7.8\,^{\circ}C$, respectively, in one survey. The temperature performance of an individual display cabinet not only depends on its design but also its position within a store and the way the products are positioned within the display area significantly influence product temperatures. External factors such as the store ambient temperature, the position of the cabinet, and poor pretreatment and placement of products substantially affect cabinet performance. Warm and humid ambient air and loading with insufficiently cooled products can also overload the refrigeration system. Even if the food is at its correct temperature, uneven loading or too much product can disturb the airflow patterns and destroy the insulating layer of cooled air surrounding the product. An in-store survey of 299 prepackaged meat products in chilled retail displays found product temperatures in the range -8.0 to $14.0\,^{\circ}C$, with a mean of $5.3\,^{\circ}C$ and 18% above $9\,^{\circ}C$. Other surveys have shown that temperatures of packs from the top of stacks were appreciably higher than those from below due to radiant heat pickup from store and cabinet lighting. It has also been stated that products in transparent film overwrapped packs can achieve temperatures above that of the surrounding refrigerated air due to radiant heat trapped in the package by the 'greenhouse' effect. However, specific investigations have failed to demonstrate this effect.

The display life of chilled foods depends on the control of all the preceding factors, but must be validated by challenge testing and/or modeling for each product and process under defined chill display conditions. It must be recognized that the integrity of the whole of the chill chain is vital to ensure the safety and quality of chilled foods.

Domestic Handling

Recommended refrigerator temperatures are in general below $8\,^{\circ}C$ throughout the world, with many countries (including the UK) recommending below $5\,^{\circ}C$. The numerous surveys on the domestic storage of refrigerated foods show remarkable similarities in consumer attitudes and handling of chilled foods and the performance of their refrigerators. Perhaps even more remarkable is that despite numerous recommendations on handling and storage temperat'ures, consumer use and the performance of refrigerators remain remarkably unchanged throughout the world over the past 30 years. Numerous surveys (Figure 1) show that mean temperatures range between 5 and $7\,^{\circ}C$, with 50–70% of domestic refrigerators operating at temperatures above $5\,^{\circ}C$. It is clear that many refrigerators throughout the world are running at higher than recommended temperatures. Because even these recommended temperatures are higher than the 0–$1\,^{\circ}C$ that is usually the recommended temperature range for storing fish and seafood, meat, and many chilled products the current situation is even more detrimental to maintaining the high quality life of chilled foods. Although there have been many surveys of refrigerators, how fridge temperatures, cleanliness, and consumer practices (e.g., over filling with warm products, use for cooling hot cooked foods) impact on consumer health remains to be fully assessed. The few studies that have assessed these factors do not appear to have demonstrated clear links between incidences of food poisoning and operating temperatures of cleanliness.

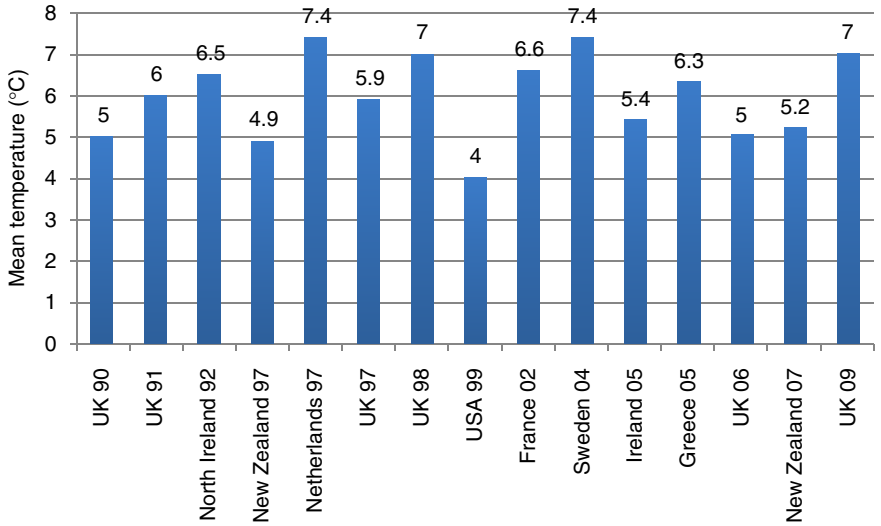

Figure 1 Reported mean temperatures recorded in surveys of domestic refrigerators throughout the world since 1990.

Temperature Measurement and Monitoring

Temperature measurement and monitoring is an integral part of any food cold-chain management system; as well as being, in many areas of the cold-chain, a legislative requirement. Monitoring the cold-chain requires detailed information on food product temperatures. Temperature monitoring includes both measurement and recording. Temperature measurement can be achieved using a variety of instrumentation such as bimetal style thermometers, thermistors, thermocouples, infrared thermometers, etc. Typically, in the food industry, temperature measurement is achieved using calibrated thermocouples and data loggers. Owing to the variety of available equipment, manufacturers and suppliers are best positioned to give advice to the food business on the choice of temperature measurement equipment for specific purposes and food products. Advice can also be found in numerous International and National recommendations and guidelines for chilled foods.

One possible aid in the future may be the widespread use of time temperature indicators (TTI) or integrators throughout the cold-chain. TTIs are simple devices that are capable of reporting a visual and straightforward summary of either the temperature (indicators) or time-temperature exposure history (integrators) of the product. Indicators show that a product has exceeded, positively or negatively, a given temperature Whereas Integrators monitor both time and temperature during a given period and show the cumulative effect of temperature fluctuations during the history of the product.

Conclusions

The safety of a chilled food depends on the numbers and types of microorganisms initially present, the rate of growth of those microorganisms, the conditions of storage (temperature and gaseous atmosphere), and characteristics (pH, a_w) of the food. Of these factors, temperature is by far the most important. In the context of food safety, the safest practical chilled temperature will be the lowest temperature that can be used without significantly affecting the quality of the food. In many cases this will be as close to the freezing point of the food as practically possible, i.e., between 0 and $-1\,^{\circ}C$. Maintaining temperatures and the integrity of the whole of the chill chain is vital to ensure the safety, and quality, of chilled foods.

In general, after initial chilling, as a chilled product moves along the cold-chain it becomes increasingly difficult to control and maintain its temperature. Temperatures of bulk packs of chilled product in large storerooms are far less sensitive to small heat inputs than single consumer packs in transport or open display cases.

If primary and secondary cooling operations are efficiently carried out then the food will be reduced below its required temperature before it is placed in storage. In this situation the cold-store's refrigeration system is only required to extract extraneous heat that enters through the walls, door openings, etc.

Even when temperature controlled dispatch bays are used there is a slight heat pickup during loading. In bulk transportation the resulting temperature rise is small and the vehicle's refrigeration system rapidly returns the product to the required temperature. Larger problems exist in local multidrop distribution to individual stores. There is a large heat input every time the doors are opened and product unloaded, small packs rapidly rise in temperature and the vehicle often lacks the refrigeration capacity or time to recool the food.

Temperature control during retail display is often poor due to the retailers' need to display as much product as possible in a way that is easily assessable to the consumer. Increasing energy costs may be the key factor that persuades retailers to reduce consumer access, i.e., install doors on chilled retail display cabinets, and hence improve temperature control.

At present domestic storage of chilled foods would appear to be the weakest link in the entire chill chain. However, despite all the many published surveys on refrigerator temperatures carried out in the past 30 years, how fridge temperatures, cleanliness, and consumer practices (e.g., over filling with warm products, use for cooling hot cooked foods) impact on consumer health remains to be fully assessed.

See also: Foodborne Diseases: Overview of Biological Hazards and Foodborne Diseases

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Relevant Websites

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http://www.chilledfood.org/
UK Chilled Food Association.

Cleaning and Disinfection Technologies (Clean-In-Place, Clean-Out-of-Place)

R Ryther, Ecolab, Eagan, MN, USA

Glossary

Chemical activity Cleaning factor using chemical agents to optimize cleaning time, temperature, and need for mechanical action.

Cleaning factors The use of time, temperature, chemical activity, and mechanical action in a balanced manner to ensure an optimized cleaning program.

Clean-in-place Clean-in-place is the automated cleaning of equipment with minimal dismantling of food production equipment before the cleaning and sanitizing operation.

Clean-out-of-place Clean-out-of-place is the removal of food production equipment or portions of the equipment as well as related food production tools to an external area for cleaning, sanitizing, and drying before reassembly.

Environmental surfaces Environmental surfaces are those external to food processing equipment within the food production facility. Cleaning and sanitizing of all environmental systems is generally accomplished manually but in some cases automated cleaning systems have been utilized.

Line circuit cleaning Automated cleaning process for transfer piping used to deliver raw materials and transfer out final food product in a food processing system.

Mechanical action Cleaning factor involving automated or manual application of physical force against a soil to be removed.

Sanitation standard operating procedure A protocol describing the sequence of steps for cleaning and sanitizing within a food processing facility including required quality inspections, corrective actions as well as required microbiological and allergenic verification testing.

Tank circuit cleaning Automated cleaning process for food processing tanks – usually performed in conjunction with a line cleaning circuit.

Cleaning and Sanitizing Operations in Food Processing Facilities

Effective cleaning and sanitizing, whether it is automated or manual requires an understanding of where soil and microbial cross-contact can occur in a food processing system. Cleaning and sanitizing procedures that inadvertently fail to remove soil from food contact surfaces can result in sources of microbial growth and the potential to contaminate subsequent food production. Developing effective cleaning and sanitizing procedures using optimized cleaning and sanitizing technology is critical to achieving a safe food production environment.

Food Production Facility Cleaning Based on Sanitary Design Principles

Despite the very broad range of food systems and very specialized equipment developed for many food production and packaging operations, cleaning and sanitizing systems should be designed using standard principles. An example of these principles adapted from the American Meat Institute is shown in Table 1.

Cleaning methods, manual or automated, cannot overcome poorly designed production equipment and facilities. For example, even sealed hollow areas in support structures or walls have been known to develop cracks and become microbial harborage points.

Types of Cleaning and Sanitizing Systems: Clean-In-Place (CIP), Clean-Out-of-Place (COP), and Environmental Cleaning

Cleaning systems for food plants are generally separated into three categories, CIP, COP, and environmental cleaning:

1. CIP is the automated cleaning of equipment with minimal dismantling of food production equipment before the cleaning and sanitizing operation.

Table 1 Ten principles of Sanitary Design

1.	Cleanable
2.	Made of compatible materials
3.	Accessible for inspection, maintenance, cleaning, and sanitation
4.	No liquid collection
5.	Hollow areas eliminated or sealed
6.	No niches
7.	Sanitary operational performance
8.	Validate cleaning and sanitizing protocols
9.	Separate processes wherever possible
10.	Meet personnel hygiene and sanitation requirements

Source: Adapted from American Meat Institute.

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2. COP is the removal of food production equipment or portions of the equipment as well as related food production tools to an external area for cleaning, sanitizing, and drying before reassembly.
3. Environmental surfaces are those external to food processing equipment within the food production facility. Cleaning and sanitizing of all environmental systems is generally accomplished manually but in some cases automated cleaning systems have been utilized.

In all three categories, cleaning is usually followed by sanitizing although sanitizer chemistry and procedures will differ based on regional regulatory requirements. Methods used for cleaning and sanitizing can also vary significantly depending on the food type, food additives, and processing temperature used to make the food.

Cleaning Factors

Four factors are generally accepted as being important to ensure effective cleaning and sanitizing. Cleaning time, temperature, chemical activity, and mechanical energy all need to be defined for all cleaning and sanitizing programs and are described below:

1. Time to clean and sanitize is often misunderstood, especially when chemical cleaning is involved. Optimizing the time for a cleaning operation to ensure effective soil dissolution and emulsification (tying up soil in solution to avoid redeposition) is generally a high priority for food producers. Rushing a cleaning operation can result in poor cleaning and residual food cross-contact occurring on equipment. Improper use of cleaning chemistry, temperature, or mechanical action can result in an inordinately long cleaning time.
2. Temperature effects on cleaning and sanitizing will vary depending on soil type and water quality. A rule of thumb is that for every 10 °C increase, cleaning chemical activity doubles resulting in fatty soils, sugars and starches and many other types of food soils being more easily removed with increased temperature. High temperatures (>145 °F/65 °C) will kill microbes but if used properly, lower temperature cleaning and sanitizing programs can be used to achieve effective microbial kill. Increasing cleaning temperature in some cases will precipitate proteins or hardness ions (calcium or magnesium) and make it difficult to remove scale deposits.
3. Chemical activity is important as cleaning chemistry is built to dissolve soils from the surfaces to be cleaned and emulsify to avoid redeposition. A sanitizing step will kill or inhibit microbial cross-contact with surfaces after the cleaning step. Chemical activity is impeded when:
   a. Cleaning or sanitizing solutions do not reach the soils because of a lack of solution flow (dead zones).
   b. Chemical concentrations are too low (cannot dissolve soils) or too high (precipitate out or react with soils).
   c. Inappropriate chemical systems are used and are not effective at cleaning or sanitizing the food processing equipment.
4. Mechanical action is required to move soils away from a surface. In the absence of manual cleaning, automated cleaning systems generally rely on pressurized water or air to provide mechanical force for soil removal. The need for mechanical force can be minimized if temperature, time and/or chemical activity can be optimized to permit better soil dissolution but some force is always required to move the soil away from a surface to prevent soil redeposition.

Figure 1 CIP cleaning solution and sanitizing solution tanks: This single use CIP system includes two medium-sized tanks for detergent and rinse/sanitizer, a steam heat exchanger, and programmable logic controllers to automate the cleaning process. Image courtesy of Ecolab.

CIP Cleaning Background

In practice, a standard CIP system will recirculate cleaning solution automatically through enclosed food processing equipment such as tanks, ovens, fryers, conveyors, and cooling systems and the associated food transfer piping. Recirculating a cleaning solution permits measurement and control of temperature, flow rate, and chemical concentrations. Such a CIP operation, runs for a time period sufficient to ensure all surfaces are free of contaminants, is generally completely automated to (1) ensure consistent cleaning results, (2) minimize labor compared to manual cleaning, and (3) provide electronic documentation that a cleaning program was run as desired (Figure 1).

For a CIP system, the mechanical energy is provided by circulation systems. Liquid impingement on surfaces or turbulent flow through piping generally cannot match the mechanical energy provided by manual scraping and scrubbing. To overcome this mechanical energy deficit, the other cleaning factors, temperature, chemical activity, and time must be emphasized. As an automated, enclosed CIP system does not expose operators to chemical mixtures, stronger chemical activity and higher temperatures can be safely used in cleaning and sanitizing. Cleaning times can also be lengthened as labor can be distributed to other tasks while an automated CIP is in operation.

Food CIP systems and beverage (such as milk, beer, or soft drinks) CIP systems use essentially the same cleaning and

sanitizing solution transfer and spray technology but can vary greatly in the required chemical strength, water temperature, and cleaning time for removing light versus heavy, aged and/or burnt on food soils. Use of an automated cleaning and sanitizing CIP systems have advantages over manual cleaning in the following ways:

1. Reduces the amount of time and labor spent on sanitation operations (increasing food production run times).
2. Decreases the impact of sanitation operations on water consumption, energy utilization, and wastewater processing.
3. Reduces overall wear on process equipment because of decreased manual cleaning.

CIP – Line Circuit Cleaning

The line circuit portion of CIP cleaning is focused on ensuring proper turbulent flow at rates to provide mechanical 'scrubbing' by the cleaning solution. Critical to the success of cleaning line circuits is the removal of all 'dead zones' where cleaning solution cannot flow and residual food product can buildup and result in microbial cross-contact occurring within the equipment. CIP circuits can often be extremely complicated, especially when using a single CIP system to clean numerous circuits (sometimes simultaneously). Careful design of each CIP piping circuit, especially when new food processes are added to existing equipment is suggested to ensure elimination of these dead zones.

Optimizing time, temperature, and chemical concentration is important after ensuring optimum mechanical action based on solution flow rates. Flow rates will need to go up with increasing pipe diameter (see Table 2). Care must be taken where line circuits contain multiple pipe sizes as pressure/velocity drops will occur going from smaller to larger diameter piping and inadequate cleaning of the larger piping can result. It is common for tank and lines to be cleaned in the same CIP operation and optimizing both types of CIP programs in conjunction is required.

CIP – Tank Circuit Cleaning

The tank circuit CIP program relies on a spray device or spray ball to achieve mechanical action. At a minimum a tank cleaning spray device must be designed to ensure all tanks surfaces are reached by the cleaning solution. Fixtures within tanks such as mixing blades or drain valves on tank surfaces can block spray from reaching soiled surfaces. Multiple overlapping spray devices are often required to overcome such blockages. Flow rates out of a spray device typically are on the order of 3 gallons per minute per foot of circumference of a cylindrical tank.

Table 2 Minimum required clean-in-place (CIP) flow (various line sizes)

Line size[a]	*Desired velocity*	*Minimum flow rate*	*Drain capacity*[b]
1″	5 ft sec^{-1}	15 GPM	22 GPM
1.5″	5 ft sec^{-1}	24 GPM	40 GPM
2″	5 ft sec^{-1}	43 GPM	75 GPM
2.5″	5 ft sec^{-1}	69 GPM	115 GPM
3″	8 ft sec^{-1}	163 GPM	190 GPM
4″	8 ft sec^{-1}	288 GPM	350 GPM

[a]Assumes standard sanitary pipe.
[b]Maximum drainage through the pipe.
Table 2 shows flow rates in feet second^{-1} (ft sec^{-1}) and gallons minute^{-1} (GPM) for different CIP line sizes; courtesy of Ecolab Inc.

Single versus Multiuse CIP Designs

CIP circuits can be designed to be (1) single use with cleaning solution dumped directly to drain after completion of the CIP cycle or (2) reuse or multiuse systems. Multiuse CIP systems are often designed to recover final rinse water for use to make up subsequent cleaning solutions. Some or all of the cleaning solution itself can also be saved and reused to minimize chemical usage. In all CIP designs, any final rinse or sanitizing rinse would not be reused in that function but fresh final steps would generally be required by food processing regulations in most regions.

Single use systems, where all cleaning, sanitizing, and rinse solutions are used once, are simple to design and result in the highest level of sanitation. These single use systems would be required for food production equipment having very high soil loads or allergenic material. These single use systems avoid the potential for cross-contact of these soils types on equipment that would be possible with CIP solution reuse. Facilities with very limited space for additional equipment would also benefit from a single use CIP.

Recovery of rinse water is a popular choice for CIP as there is little chance of soil redeposition. Multiuse systems in which the cleaning chemicals are reused require a fairly complicated CIP design and a high level of understanding of the CIP circuit to avoid soil redeposition. There are many of these systems in use and some where automated make up water and cleaning chemical additions are balanced to achieve equilibrium and can run for weeks or months without the need to dump the cleaning solution.

COP Cleaning

COP is the cleaning of removable parts of food processing equipment after disassembly or any ancillary food production tools. Typically COP systems are open tanks where a given cleaning solution can be heated and recirculated. As with CIP systems, automation is possible with recirculation step (permitting the monitoring of solution temperature, chemical concentration, and flow rate) so that 'push button' COP systems are common. The use of numerous distribution headers in COP tanks are used to create turbulence to aid in soil removal via mechanical action.

COP cleaning and sanitizing programs require:

1. Appropriate tank design:
   a. Sized for application.
   b. That can contain shelves or hangers for parts to maximize number of parts loaded while still maintaining separation between parts to ensure full access to cleaning solutions.
   c. With a recirculation system and associated headers sized to ensure tank turbulence.

2. Cleaning solution chemistry, temperature, and cleaning time balanced to ensure full cleaning of the toughest to remove soils.
3. A rinse step to ensure removal of residual soil and cleaning solution.
4. A sanitizer step effective for kill of any residual microbial cross-contact on equipment.
5. Proper parts storage program to ensure complete drying and avoidance of cross-contact of microbial or allergenic soils onto these parts before reassembly.

Environmental Cleaning

Cleaning and sanitizing environmental surfaces (sometimes termed 'open plant cleaning') in a food production facility is a critical part of a full food safety program. Poor design of a cleaning program for environmental surfaces can result in microbial cross-contact that can migrate into food products.

Environmental cleaning in a food processing environment is the cleaning of equipment's external surfaces, walls, floors, ceilings, elevated walkways, drains, piping, and conduit in addition to ancillary equipment (such as motors, electrical boxes etc.) that generally cannot be cleaned by CIP or COP methods.

Typically, cleaning environmental areas of a food production facility is done manually by first removing food debris followed by wet cleaning and sanitizing steps. Emphasis is on removing as much soil as possible before a wet cleaning to limit the biological load on the wastewater treatment system. Environmental cleaning programs for food processing facilities should remove as much water as possible after the completion of cleaning and final rinse steps and return to as dry a state as possible before resuming food production. Focusing even wet cleaned areas on maintaining as dry a state as possible will help limit microbial growth in the environment, thereby reducing the potential for microbial cross-contact with food product.

Systems used to support environmental cleaning include:

Foaming or Gelling Systems

Cleaning and sanitizing with foam or gel based chemistry increases the dwell time of chemical on the surface to help dissolve soils for cleaners or provide increased microbial kill efficacy for sanitizers. Foam generators mix a chemical solution with air to create a foam or gel. This solution is then sprayed under pressure onto the surface to be cleaned. Often entire rooms and all accessible surfaces of the food production equipment are foamed (with chemically sensitive equipment wrapped before foaming).

High Pressure Cleaning Systems

High pressure air, water, steam, or particle blasting systems can be used for cleaning the exterior parts of equipment, floors, and some building surfaces when mechanical action is required for soil removal. Soil types dictate which type of mechanical action will be most effective. For example, particle blasting works best on brittle food soils whereas hot steam can clean by liquefying soils. Steam can also be used to sanitize surfaces (care must be taken that all surfaces are heated to an appropriate temperature for a reproducible time period to ensure the desired microbial kill).

Pressurized air or steam injection systems generally operate with nozzle pressures between 60–170 psi. Cleaning effectiveness is dependent largely on the force of the cleaning system against the surface which will be a function of operating pressure and nozzle design. It will be emphasized that high pressure air or water systems (especially centralized systems) must be free of microbial containing residues that could be dispersed into a food production environment. Additionally, high pressure cleaning procedures should never be used in rooms where food production is occurring and great care must be taken to avoid the transfer of residues from high pressure cleaning onto equipment already considered cleaned and sanitized.

Ancillary Cleaning Equipment

Programs should be developed for cleaning ancillary food production tools as well as ensuring that cleaning tools themselves have appropriate cleaning procedures to avoid the potential for microbial or allergenic cross-contact with these tools into the food product. Strategies often involve use of tool carts to carry cleaning equipment and are specific to areas and/or types of equipment to be cleaned. Carts and the cleaning tools they contain are then cleaned and stored in separate areas with appropriate drying before the next cleaning operation. Often cleaning tools are color coded for use specific to cleaning food contact versus nonfood contact equipment and are stored separately when not in use for cleaning operations.

Cleaning of Allergens

Food allergies affects as many as 6% of young children and 3–4% of adults. Exposure of some individuals to very small amounts of allergenic proteins can be life threatening. Moreover, food recalls because of cross-contact with allergens make up a significant portion of all food recalls. Control of allergens in any food facility that produces both allergen and nonallergen containing foods must be accompanied by a stringent allergen control program. Potential for allergen cross-contact with allergen free foods can include (1) mislabeled raw material entering the food process facility, (2) mislabeling of materials by facility staff, (3) allergen residues remaining on food production equipment or tools cross-contacting nonallergen containing foods and (4) mislabeling of a final product containing allergens. There are many manufacturers who have separate production equipment or even separate manufacturing facilities to avoid the potential for such cross-contact.

Cleaning of Facilities Producing Dry or Low Moisture Foods

Low moisture or dry food production areas are usually cleaned without water for the purpose of minimizing growth of microbial pathogens. Some of these low moisture foods include milled grains, bakery goods, cereals, chocolate, dry dairy, nuts, spices, and fried or baked chips. Food facilities that have dry

processing areas (in some cases the entire production facility will be dry) have been historically more concerned about pest issues than cross-contact of foods with microbial residues.

Unfortunately, recent food recalls involving food contaminated in dry food production facilities have increased the need to identify ways to provide sanitation breaks by these food producers in their facilities. *Salmonella* is the main concern in these environments because of its persistence on dry foods and in food manufacturing facilities. *Escherichia coli* O157:H7 is also of major concern in these dry areas.

For most dry food facilities, dry raw material delivery, storage, and internal facility transport systems are rarely cleaned completely and almost never sanitized. These businesses rely on low moisture to eliminate microbial growth and appropriate testing to ensure absence of any pest issues.

The presence of some residual food raw material or final dry product on production equipment has generally been considered acceptable especially as these residuals are usually 'fresh' because of continual 'push through' of new raw material or final dry product on those equipment surfaces. Yet, validation of a dry cleaning process can prove difficult as it must demonstrate an ability to remove detectable levels of microbial residues from the surfaces cleaned (much easier in wet cleaning using a wet sanitizer already proven to provide a sanitizing microbial kill if applied according to the manufacturers recommendations). Adding water these to environments, even with the use of heavy duty cleaners and water based sanitizers may still have a higher potential for cross-contact with microbial residues than if cleaned dry if residual water remains in hard to dry areas of the facility.

Cleaning and sanitizing in such dry cleaning include traditional sweeping, scraping, and vacuuming coupled with sanitizing with quick drying alcohol solution applied directly to surfaces or using sanitizing wipes. Sanitizing with steam and heat also provides low water alternatives to water based sanitizers. More unique sanitizing methods include flushing the systems with the new lot of the food product itself to remove any traces of the older product. Nonallergenic food material such as rice or salt might also be used to rinse out systems and then tested to demonstrate removal of microbial residues from food processing equipment. This flushing step effectively provides what would be termed a rinse in a wet cleaning operation and material used to flush can be sampled at different points in the flushing step for confirmation that potential for cross-contact from microbial and/or allergenic residues has been eliminated from the process. Efficacy of such a procedure must be validated to ensure complete elimination old food product and/or microbial residues from all surfaces being cleaned and/or sanitized.

Cleaning and Sanitizing Chemistry

Cleaning Chemistry

Chemical cleaners are required where dissolving and emulsifying soils are more efficient at cleaning food processing equipment than manual cleaning. Food soils of concern to most food processing facilities will vary depending on food type and additives, temperature and type of food processing as well the condition of the water used in food production and cleaning. To effectively clean these soils requires an understanding of the functions of different chemical components in a cleaning system. A comprehensive review of these technologies can be found in the work by Stenga (2010). The pH of a cleaning solution (i.e., the relative level of hydrogen cation, acidity or hydroxyl anion, alkalinity, in a solution) is a key factor in the cleaning ability for most food soils. The chemical structures of food soils such as fats and oils, proteins, sugars and starches as well as minerals all have some ionic features under some or all conditions. A cleaning solution must be built from components that maximize the breakdown of soil residues on food facility equipment and associated environmental surfaces whereas minimizing the amount of chemical used for cleaning. The goal of an optimized chemical cleaning system is to use the cleaning solution to wet the soil, dislodge it from a surface to be cleaned and then emulsify it (hold the soil in solution) so it will not redeposit.

When choosing the chemical cleaning system for a given food soil the cleaning time, cost, compatibility with equipment, safety of facility operators as well as regulatory requirements for wastewater discharge and environmental sustainability are all factors that must be considered to ensure an optimum system.

Sanitizing Chemistry

The US Environmental Protection Agency (EPA) defines antimicrobial agents as substances or mixtures of substances used to destroy or suppress the growth of harmful microorganisms whether bacteria, viruses, or fungi on inanimate objects and surfaces. Definitions of each type of antimicrobial agent are described in the EPA Fact Sheet on their website:

1. Sterilizers (also Sporicides) 'will destroy or eliminate all forms of microbial life including fungi, viruses, and all forms of bacteria and their spores.'
2. Disinfectants are used 'on hard inanimate surfaces and objects to destroy or irreversibly inactivate infectious fungi and bacteria but not necessarily their spores' (these require a final rinse if used on food contact surfaces).
3. Sanitizers will 'reduce, but not necessarily eliminate, microorganisms from the inanimate environment to levels considered safe as determined by public health codes or regulations.' Sanitizers used in food processing plants in the US generally are nonrinse agents, safe for food contact surfaces when used according to the product label requirements.

It is important to understand that for different food processing facilities, government regulations will differ based on region. An operator using sanitizers and disinfectants for direct and indirect food contact surfaces needs to follow regulations for both sanitizer and disinfectant products. For example, in the US, any antimicrobial or chemical sanitizer used on food or for treating food contact or other surfaces in a food production facility must be registered at the EPA. The US Food and Drug Administration (FDA) regulates the use of antimicrobial agents used on food or food contact surfaces. All

approved no rinse food contact surface formulations and associated usage levels used in the US are listed in the FDA 21 CFR 178.1010 Code of Federal Regulations. In some regions or applications may require a final rinse for all sanitizer chemistry applications.

Chemical sanitizers fall into a number of categories based on whether they are oxidative or nonoxidative. These chemical sanitizers can be delivered as liquids, gases, or in vapor/mist form depending on the application. A comparison of the different chemical sanitizers for stability, foaming, corrosivity, pH stability, and efficacy against microorganisms can be found in a review by Richter and Cords (2001).

Application of Sanitizers in Food Processing Facilities

A sanitizing operation is generally performed after a thorough cleaning operation for CIP and COP systems as well as for all environmental areas. Automated CIP/COP sanitizing would use a nonfoaming sanitizer whereas a foaming sanitizer would be used for environmental surfaces (see Sections CIP Cleaning Background and COP Cleaning) as foam provides contact time for vertical surfaces as well as a visual confirmation of sanitizer application. (In the case of sanitizers that are approved for no rinse applications, such foam would break and leave no visible residue.)

Any manual sanitizer application should not rely on mechanical action or scrubbing to assist the sanitizing operation (using the cleaning operation for such removal). If applicators, such as fabric or mop systems, are used for sanitizer application, care must be taken to ensure that these applicators are themselves thoroughly cleaned, sanitized, and stored separately from equipment used for cleaning operations to avoid microbial or allergenic residue cross-contact onto food production equipment during a sanitizing operation.

Cleaning Validation and Verification Technology

There are three main components to developing a cleaning program for any food production area:

1. A cleaning and sanitizing protocol usually termed a sanitation standard operating procedure needs to be developed that is based on the specific legal and safety requirements for the food processing business.
2. Validation of that cleaning and sanitizing protocol requires development of tests for every point in the cleaning process to prove that the process meets regulatory standards.
3. A Verification program needs to be instituted (usually consisting of a subset of the validation test methods) that will provide proof that the cleaning program continues to be effective over time.

There are numerous verification tests to determine the presence of allergen and microbial residues on food production equipment. The Association of Analytical Communities Research Institute provides a wide array of validated methods for both allergens and pathogens. These verification tests or similar validated tests should be used in a validation program to ensure elimination of the desired contaminant.

Sampling for microbial or allergenic residues is typically done using swabbing techniques on hard surfaces and analyzing the swabs using the various applicable allergenic or microbial detection methods. Other techniques include testing rinse water or using air sampling techniques.

Some allergen verification tests include highly sensitive tests that can detect allergenic proteins from a variety of allergens but especially focused on the top eight (peanuts, tree nuts, milk, eggs, soy, fish, crustaceans, and wheat) which represent at least 90% of the global cases of allergic reactions in humans.

The common bacterial tests include measurements of general bacterial populations such as total plate count, total viable count, standard plate count, aerobic bacteria, gram positive cultures, thermoduric count, coliforms, gram negative bacilli as well as more specific tests such as *E. coli* O157:H7, *Salmonella*, *Listeria*, *Cronobacter*, *Staphylococcus*, and *Campylobacters*. In addition yeast and mold tests are commonly used in food processing verification, especially for air quality assessments.

Verification tests can be general or specific. Common general tests demonstrate the presence of soil in areas of food production facilities and can be very sensitive. Use of general verification tests are based on the understanding that if no soil is detected, no microbial or allergenic residue is present. Such sensitive tests include total organic carbon which will detect any type of organic soil. Adenosine triphosphate (ATP) or ATP-based detection technology will detect any type of food or microbial residue (from cell ATP content) but is not specific to the sources of the ATP. The ATP from cells (living or dead) reacting with the luciferase enzyme is the basis of swabbing tests that are quick and semiquantitative, providing a light density based numeric output that can be used for comparative purposes for hard surface soils.

Numerous other fluorescence technologies have been employed for identifying pathogens, living from dead cells, at high sensitivity. Enzyme-linked immunoassays and enzyme-linked fluorescent techniques have been commonly used for automated detection of specific pathogens. There are numerous techniques for automated testing for specific pathogens and the technologies are getting better and more rapid. The ultimate goal for food processing facilities is obtaining test results rapidly enough to permit release of food product from a production facility with minimal need for keeping food product on hold.

Conclusions

Automated or manual cleaning and sanitizing requires an understanding of where soil and microbial residue might be present in a food processing system. CIP, COP and environmental cleaning systems and the associated cleaning and sanitizing chemistry and procedures for food production facilities is discussed. Developing effective cleaning and sanitizing procedures using optimized cleaning and sanitizing technology is critical to achieving a safe food production environment.

See also: Food Safety Assurance Systems: Cleaning and Disinfection; Hazard Analysis and Critical Control Point System (HACCP): Principles and Practice; Management of Allergens in Food Industry; Management of Biofilm Risk

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FOOD TECHNOLOGIES

Drying

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Glossary

Dryer A unit operation that is used to remove water from solid substance.
Drying A process that applies thermal energy to remove water from solid substance.
Heat treatment A process where thermal energy is applied to food material.
Microbial inactivation Retard the growth of microorganisms.
Microwave A type of electromagnetic wave.
Photocatalyst A chemical substance that acts as catalyst after exposure to sufficient light.
Pretreatment Applying treatment such as osmotic dehydration and blanching before thermal drying.
Pulse combustor A chamber that combusts periodically at high frequency.
Superheated steam The steam whose temperature is higher than its saturation point.
Water activity Ratio of water vapor pressure in a substance to the water vapor pressure of pure water. It is used to indicate the amount of water available to microorganisms.

Introduction

Drying is one of the most important unit operations in food processing. It is applied to reduce the water content of food products including grains, fruits, vegetables, spices, meat, and marine products, biotechnological products, and agricultural products. Table 1 shows some typical food examples of these food categories which have been reported to undergo dehydration for the production of dehydrated food. Drying is performed in order to prolong the shelf-life of the products mentioned above by reducing the water activity to a level low enough to inhibit the growth of microorganisms, enzymatic reactions, and other deteriorative reactions. Figure 1 shows the critical water activity for the growth of various types of microorganisms. Low value of water activity inhibits the growth of microorganisms and prevents oxidation and enzymatic reactions. With reference to Figure 1, a dehydrated product with water activity lower than 0.6 is safe for long period of storage. Inhibition of microorganisms and prevention of undesirable reactions in turn minimize or avoid food wastage. In addition, drying is also applied to add value to the food products owing to some special characteristics that are only present in dried form, for instance, taste, texture, color, medicinal value, etc. Processing of the products mentioned above is usually subjected to various quality constraints, which are related to food safety, textural and sensory attributes, retention of bio-active ingredients, trade regulation, and consumer acceptance.

Table 1 Examples of dried food products available in the market

Category	*Examples of food products*
Fruits	Mango, guava, banana, longan, apricot, apple, strawberry, papaya, figs, grape, peach, plum, date, sapota, kiwi fruit, avocado, pineapple, prune, pear, persimmon, citrus peels, cherry, raspberry
Vegetables	Carrot, potatoes, pumpkin, garlic, tomato, chili, cassava, cauliflower, asparagus, bell pepper, eggplant, mushroom, spinach, onion, garlic, green beans, broccoli, celery, bamboo shoot, ginger, okra, artichoke, beet, cabbage, horseradish, japaleno, leek, peas, nuts, zucchini
Grains	Rice, wheat, maize, barley
Marine products	Fish, shrimp, squid, sea cucumber, jellyfish, cockles, mussels, sharkfin, fish maw, fish crackers, scallop
Meat products	Ham, beef, pork, sausages, meat jerky
Biotechnological products	Yeast, bacteria, proteins, amino acids, enzymes
Others	Pasta, noodles, cheeses, flavoring powders, juice mixtures, beverage extracts, soup mixtures

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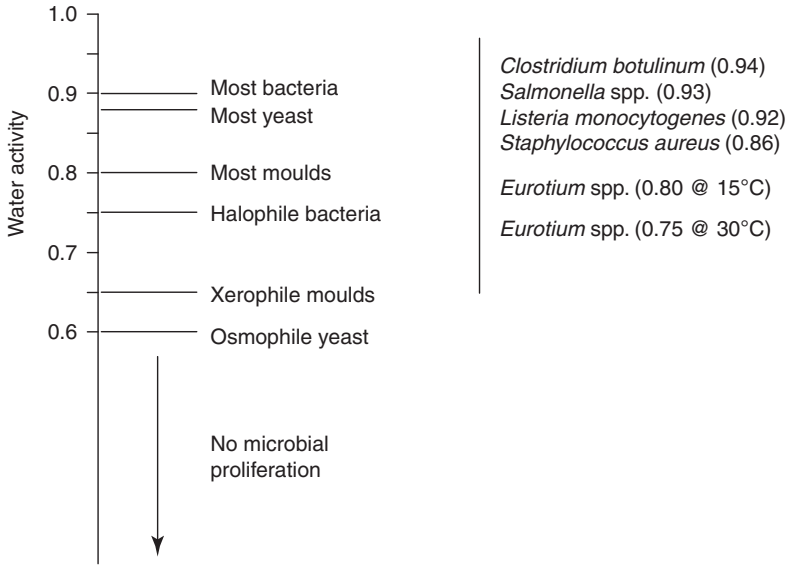

Figure 1 Water activity chart and susceptibility of food to spoilage by microorganisms.

Spoilage of food products due to microbial activities is a main concern. Further, degradation and spoilage of food products that are caused by microbial activity may produce microbial toxins and mycotoxins which bring adverse effect to human health. Contamination of microbial toxins and mycotoxins in food products is a serious concern with regard to food safety. Mycotoxins have been shown to be potent carcinogens, mutagens, and tetratogens.

Because the removal of moisture can only inhibit the growth of microorganisms and undesirable reactions, it cannot ensure food safety as rehydration of food may let the food product regain water which increases its water activity and thus makes it susceptible to spoilage by microorganisms and deteriorative reactions. As such, methods of eliminating contamination by microorganism are vital in processing of food products, including predrying, during drying, and postdrying.

Drying kinetics is seldom the sole parameter that affects product safety and guarantees low microbial count in the dehydrated products. This is due to the fact that the final microbial count is also dependent on the initial microbial count and drying temperature as well as the material being dried. Presence of nutrients like sugar will encourage rapid growth of microorganisms. Of course, shorter drying time is desirable in this aspect, but rapid drying may deteriorate product quality and cause problems such as cracking, surface hardening, surface discoloration, etc.

It should be noted that drying for a long period of time and at temperature of approximately 40 °C may lead to significant microbial proliferation and multiplication. In addition, initial microbial load of the drying material may also affect the final product safety and hygiene if improper processing method is used. Generally, drying at the shortest drying time may minimize the microbial proliferation; drying at high temperature may eliminate the microorganisms; however, drying at subzero temperature may inhibit microbial growth. Furthermore, pretreatment as well as practicing good manufacturing practice (GMP) during the predrying process may give a low initial microbial load which in turn ensures relative low final microbial load in the final product after drying.

There are more than 500 types of dryers reported in the literature and approximately 100 types are being used in various processing industries. Certain types of dryers permit elimination of microbial contamination during the process but many dryers do not. As such, they require pretreatment or posttreatment in order to eliminate the hazard and to meet stringent food quality requirement. In addition, dryers must be operated in a hygienic manner in order to avoid recontamination during the process.

This article elaborates drying from the food safety point of view in four main scopes namely:

- Classification of dryers.
- Description of dryers that have the inherent characteristics to permit sterilization, inactivation of microbial activity, and elimination of contamination during the process.
- Pretreatments that can be applied before drying, in order to minimize microbial load.
- Techniques that can be applied in a dryer to inactivate microbial activity.

Classification of Dryers

Dehydration of food products can be carried out by natural drying or in a mechanical dryer. Natural drying uses readily available sunlight and wind energy to realize the drying process.

Sun drying is a traditional drying method. It is applied in the drying of agricultural products, especially in many developing countries. Although the energy cost is free, its labor cost and requirement for space is high. A large space is

required to expose the drying material to sunlight, and the material needs to be mixed and spread from time to time to ensure uniformity and to avoid over-drying. Moreover, weather condition is a main constraint to sun drying; its availability is dependent on seasonal weather and thus not reliable. Further, the drying material is exposed to contaminating agents and insects thus posing hygienic issues.

Sun drying is often regarded as a method in eliminating microbial activity. This is a misconception. Drying is generally applied to remove water from drying material. If water activity of the dried material is below 0.6, it is safe for longer period of storage as most microorganisms cannot multiply or grow at this water activity level. However, drying does not eliminate or kill the microorganisms or spores. Some drying methods which are discussed in this article do have the potential to eliminate microorganisms, for example, superheated steam drying, pulse combustion drying, microwave drying, high temperature drying, etc., but sun drying does not. As a matter of fact, sun drying has some limitations with regard to product safety and hygiene. Sun drying is carried out in an open system, thus the drying materials are exposed to contaminants, microbes, and spores in the ambient air. It is highly dependent on the solar radiation intensity and local weather; thus, drying kinetics and product quality are not easy to control. Sun drying at temperatures of approximately 35–40 °C, in fact, provides an environment conducive to microbial growth. In fact, solar radiation does contain ultraviolet (UV) component in its spectrum which can kill microorganisms. However, this effect is confined to the line-of-sight surface only. Thus, microbes not directly exposed to solar radiation for long enough cannot be destroyed.

In addition, various pretreatments may eliminate initial microbial load which leads to low microbial count in the dehydrated material. These drying methods and pretreatments are discussed in detail in this article as well. Other drying methods which do not have the potential of eliminating microorganisms are discussed briefly in Tables 2–5. This is mainly because they are carried out at lower temperatures so as to safeguard quality parameters of the heat-sensitive material.

Solar dryer is an alternative to harness solar energy which eliminates the problems mentioned above. In a solar drying system, solar energy is transferred to heat storage agents such as water (medium which has high heat capacity), phase-change materials, etc. A heat exchanger is required where solar energy is transferred from the heat-storage agent to drying medium, normally air. Hot air is then supplied to a drying chamber. External heater is installed to supply supplement heat in case solar energy is not sufficient or not available.

In temperate regions, atmospheric air is dry (relative humidity is low). The air is a good drying medium for wind drying. However, this technique is not possible in tropical regions. It should be noted that a dryer or a drying system that works well in one place might not give the same drying performance in another area. Thus, selection of dryer is a not a simple task as the drying performance of a dryer is also dependent on weather condition and geographical location.

However, mechanical dryer has a wide range of dryers classified under different criteria. Figure 2 shows the classification of drying techniques. Generally, the mechanical drying system can be classified into four categories namely drying strategy, drying medium, handling of drying material, and mode of heat input. For the first classification which is with reference to drying strategy, dryers can be classified into subcategories in terms of the mode of operating parameters profile which include continuous mode (most common), intermittent mode, cyclic mode, stepwise mode, etc. For the second classification, drying medium such as hot air (most common), dehumidified air, superheated steam, combustor exhaust gas, inert gas, osmotic solution, etc., can be used in a drying system. With reference to the third classification, dryers are classified into different categories based on how the drying material is handled in a dryer. For instance, stationary (common), packed in a bed (common), conveyed on a conveyor (common), agitated in a rotary drum (common), fluidized, spouted, vibrated, pulsated in dryer, sprayed into fine droplets, or frozen and subjected to sublimation. Finally, dryers are also classified according to the mode of heat input namely convective, conductive, by radiation heat transfer, in microwave field, and high electric field.

Table 2 Description of dryer based on the first classification – drying strategy

Dryer/drying system	*Brief description*
Continuous	● Drying medium is supplied continuously throughout the process ● The most common strategy; most industrial dryers are continuous dryer, for example, oven, tray dryer, tunnel dryer, fluidized bed dryer, spray dryer, etc.
Intermittent	● Active drying and tempering (drying is stopped for a period of time) are carried out interchangeably and repeatedly in order to minimize product cracking; reduce operating cost; and enhance product quality ● This strategy can be applied to most dryers that are operated in continuous mode
Cyclic	● Operating conditions such as airflow rate, temperature, humidity, or operating pressure is varied individually or in tandem in cyclic mode ● It can be applied to most dryers that are possible in intermittent mode
Stepwise	● Operating conditions such as airflow rate, temperature, humidity, or operating pressure is varied individually or in tandem in two modes, namely step-up and step-down ● In step-down mode, operating condition is gradually decreased toward the end of drying and the reverse in step-up mode ● It can be applied to most dryers that are possible in intermittent

Table 3 Description of dryer based on the second classification – drying medium

Dryer/drying system	*Brief description*
Hot air	● Hot air is generated by contacting the air with heating element; burned with fuel in a burner ● Hot air generated from burning air–fuel mixture may pose contamination issue, thus it is generally not used in food processing. Instead, heating element is used ● Hot air makes contact with drying material, transfers heat to the material, and carries away water vapor ● The most common drying medium used in industrial drying which include oven, tray dryer, tunnel dryer, fluidized bed dryer, spray dryer, etc.
Superheated steam	● Superheated steam is generated by heating up steam generated from a boiler using a heat exchanger ● As it does not contain oxygen, oxidative or combustion reactions can be avoided, thus eliminating the risk of fire and explosion hazards ● Superheated steam can be used to replace hot air in almost all direct dryers such as, for example, oven, tray dryer, tunnel dryer, fluidized bed dryer, etc.
Dehumidified air	● Dehumidified air at low temperature can be generated from a heat pump system ● It is suitable for drying of heat-sensitive products and eliminates shortcomings in product quality that are caused by high temperature, for example, over-drying ● However, final moisture content is relatively high and drying duration is typically long unless combine with volumetric heating ● Low temperature processing environment can minimize microbial activity but not eliminate the hazard; may combine with other treatment processes ● Likewise, it can be used to replace hot air in almost all direct dryer
Exhaust gas	● Combustor exhaust gas is generated from high frequency combustor such as pulse combustor ● Complete combustion is normally achieved and the combustion is completed at extremely high temperature (700–800 °C) ● Drying time is within seconds or minutes ● Effective in eliminating pathogenic microorganisms such as *E. coli*, pseudomonas, coliform bacteria
Inert gas	● CO_2, N_2, organic vapor such as ethanol vapor can be used as a drying medium ● They are mainly used to eliminate oxidative reactions and to promote moisture removal rate
Osmotic solution	● Hypertonic solutions are used to remove moisture from drying material as well as microorganisms ● Sometimes it is applied as a pretreatment process before thermal dehydration

In addition, hybrid drying system can be introduced by combining variants in two different categories, for instance, combining superheated steam drying in the second classification with fluidized bed in the third classification gives superheated steam fluidized bed dryer; combining dehumidified air (heat pump drying) and microwave gives microwave heat pump drying.

Table 2 shows brief description about the different types of dryers with reference to the first classification in Figure 2 – drying strategy. The most common drying strategy is continuous drying where drying medium (can be any medium stated in Figure 2) is supplied to the drying system continuously and at constant profile throughout the entire process. Recent advancement reveals that the process can be conducted in intermittent, cyclic, or stepwise modes, mainly aims toreduce operating cost or enhance product quality especially in reducing product cracking and avoiding over-drying.

Table 3 provides brief description of dryers under the second classification – drying medium. The most common drying medium is hot air. Owing to the fact that hot air is detrimental to heat-sensitive product and may cause severe oxidative degradation, recent development in drying technology has introduced various types of drying media such as superheated steam, low temperature dehumidified air (heat pump drying), and inert gas to avoid thermal and oxidative degradation of bioactive compounds. In addition, extremely high temperature combustor exhaust air can also be used for rapid drying, which may eliminate the undesirable degradations that normally require relatively long period of time to cause significant deteriorative effect to the product quality. Further, osmotic solution such as sugar and salt solutions are excellent hypertonic solutions that can be applied to retard growth of microorganisms.

Drying medium at relatively high temperature (60–80 °C) may retard the growth of some microorganisms and enzymatic reactions. Nevertheless, certain types of microorganisms are resistant to heat and therefore hot air cannot eliminate the hazard completely. In this regard, precautionary actions should be taken in order to minimize the initial microbial load before drying. This can be achieved in two aspects namely pretreatment and hygienic drying practice. Both aspects will be discussed in the subsequent sections.

However, drying media at extremely high temperature such as superheated steam and combustor exhaust air are suitable for eliminating the hazard. Both superheated steam drying and pulse combustor drying are discussed separately in subsequent sections. In addition, osmotic solutions can also be applied to retard microbial growth.

Table 4 gives the description and illustration of types of dryers that handle drying material in different modes and states. Generally, thermal efficiency is low in conventional dryers, mainly due to poor contacting efficiency between solids and the drying medium. This results in low drying rate and long drying duration. If the drying material is stirred, mixed, agitated, fluidized, vibrated, pulsated, etc., intermittently during the drying process, the contacting efficiency between the material and drying medium is improved appreciably. This in turn enhances the drying efficiency.

Table 4 Description of dryer based on the third classification – handling of drying material

Dryer/drying system	*Brief description*
Stationary	● Drying material is placed on a tray in the dryer and subjected to drying medium ● Many industrial dryers are stationary dryers, for example, oven, tray dryer, tunnel dryer, freeze dryer (solids), etc.
Packed bed	● Particulate solids are packed in a drying column and form a bed of solids ● Drying medium is charged into the column from the bottom of the bed ● Solids are packed and in contact with each other. Certain solids surface is not exposed to the drying medium
Conveyor	● Drying material is placed on a conveyor and transported from inlet to outlet while contact with drying medium ● Impingement drying can be considered where a high speed drying medium is impinged to the material on the conveyor. Drying rate is typically high in impingement drying ● The drying system is operated in continuous mode and the material is stationary while it is being transported on the conveyor
Rotary	● Drying material is agitated in a rotary drum with the assistance of louvers ● Heat transfer can be achieved in two modes, namely convective and conductive ● Agitation of solids enhances the heat and mass transfer
Fluidized bed	● Particulate solids are placed in a fluidized bed column and forms a bed of particles ● Fluidizing gas stream is charged into the column from the bottom ● Gas passes through the bed of solids and suspends the particles in the gas stream, which expends the bed volume ● Sometimes bubbles are formed and therefore gives bubbling fluidized bed ● Good fluidization enhances contacting efficiency between solid and fluidizing gas which in turn enhances rate of removal of moisture
Spouted bed	● A powerful gas stream is charged into a bed of solids at the center region and therefore thrusting the solids into space above the bed of solids ● The solids falls on the bed surface after losing its momentum ● Thrusting and falling of particulate solids in a spouted bed gives a unique solids circulation and hence good contacting efficiency between solids and gas ● Suitable to dry coarse particles which are difficult to fluidized in conventional fluidized bed
Vibrated bed	● Vibration is applied to a bed of solids which gives pseudo-fluidization to coarse particles ● Suitable for fine and coarse particles which are difficult to fluidized in a conventional fluidized bed
Pulsated bed	● Bed of solids is divided into several parts ● Part of the bed of solids is fluidized bed intermittently and periodically ● It is also regarded as an intermittent dryer
Spray (liquid)	● Drying material in the form of liquid is sprayed into droplet and dried in the dryer ● Fine powder is formed and discharged from the bottom ● It may be combined with other types of dryers, for example, fluidized bed dryer
Freeze (liquid)	● Drying material in the form of liquid is frozen and subjected to sublimation for the removal water/solvent vapor ● A thin film is formed at the end of the process

Table 5 Description of dryer based on mode of heat input

Dryer/drying system	*Brief description*
Convection	● Drying medium makes contact with the drying material, transfers heat to the material, and removes moisture vapor ● This mode of heat input is the most common in industrial dryer as long as drying medium is applied ● Convective heat input may combine with conductive heat input such as heating plate/surface and volumetric heating such as microwave
Conduction	● Conductive heat transfer can be achieved by using hot surface, heating plate/rod, or inert solids that has high thermal inertia ● It can be applied in vacuum and freeze dryers where convective heat transfer is not possible or minimal. Some direct dryers also apply this mode to enhance heat transfer, for instance, internal tube fluidized bed dryer
Radiant (infrared)	● Infrared radiation transfer heat to the material surface by radiant heat transfer ● It can be combined with convective drying
Dielectric (microwave, radio frequency)	● Microwave generates rapid volumetric heating of the material by altering the electromagnetic field to interact primarily with the water molecules and ions in food materials ● It enables rapid removal of moisture and allows removal of internal moisture at initial stage of drying
High electric field	● Unlike microwave or radio frequency, heat is not generated in the material ● High electric field is applied across the drying material; particulate solids are usually negatively charged ● Water flows toward the direction of cathode

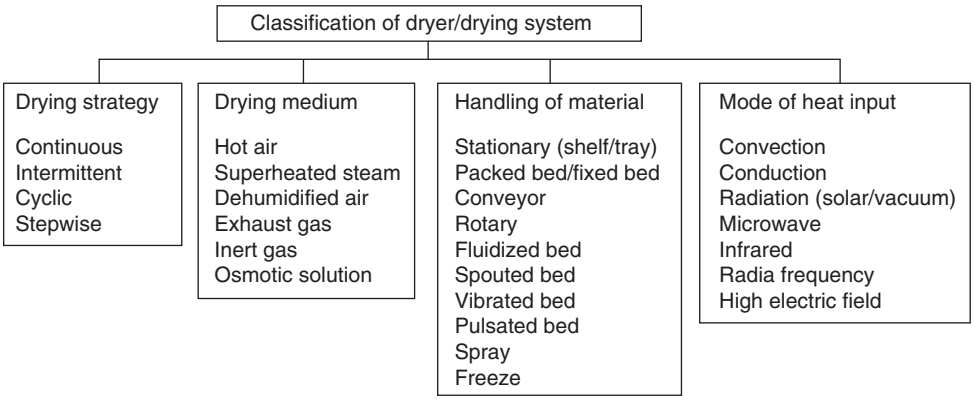

Figure 2 Classification of dryers.

Stationary dryers such as oven, tray dryer, tunnel dryer, freeze dryer, etc., are the most common dryers applied in the processing industry. Packed bed drying is common for drying of particulate solids. Further, the drying material can be conveyed on a belt dryer or agitated in a rotary dryer. Both conveyor and rotary dryers are also common in processing industry. To enhance solid–gas contacting efficiency, fluidized bed dryer can be applied to allow rapid heat and mass transfer and therefore higher rate of moisture removal. Further to this, the bed of solids may be spouted, vibrated, or agitated if the solids are difficult to fluidize in a fluidized bed. Pulsated bed is a variant of fluidized bed where part of the bed of solids is fluidized bed intermittently. It has the advantages of an intermittent dryer.

If the drying material is in a liquid form, it can be atomized and sprayed into droplets and dried in a spray dryer. Spray dryer is typically used to dry liquid, suspension, paste, etc., into dry powder. Alternatively, the material in the liquid form can be dried in a freeze dryer where the liquid is frozen and subjected to sublimation. Eventually, a thin layer of film is obtained. Both spray and freeze dryers are commonly used in processing industry.

Table 5 briefly explains the characteristics of dryers classified under the fourth classification – mode of heat input. Convective heat transfer is the most common mode in most industrial dryers. Conductive heat transfer is common in freeze dryer, rotary dryer, agitated fluidized bed dryer, and internal tube fluidized bed dryer and inert solids fluidized bed dryer. Infrared dryer transfers heat to the drying material in radiant heat transfer mode; dielectric drying (microwave and radio frequency drying) generates heat within the drying material using high-frequency electric fields. Both infrared and dielectric drying enable volumetric heating where the entire drying material is heated by the electromagnetic wave; this allows the removal of surface and internal moistures. Unlike convective drying, only the surface moisture is removed and the internal moisture has to migrate from the material core to the surface. One of the recent developments in drying technology is the development of hybrid drying where convective drying is combined with radiant heat transfer mode. For examples, convective microwave drying, microwave-assisted heat pump drying, etc.

Drying Techniques that have the Potential in Minimizing and Eliminating Microbial Load

Food contamination may occur in any process before drying. This may have an adverse effect on product hygiene and safety if effective microbial inactivation and decontamination method is not applied during drying or post drying. Therefore, the ability of dryers to inactivate bacteria may have considerable impact on the product hygiene and safety. In this regard, drying techniques that permit microbial inactivation and decontamination are worth to be considered when it comes to the selection of appropriate dryers. In General, factors that make microbial inactivation and decontamination in a dryer possible are high temperature processing, or low temperature processing, in a duration that is long enough to make the inactivation and decontamination effective. High temperature can denature proteins, thus eliminates microbes; low temperature inactivates microbial activity, hence stopping the microbes from multiplying. In addition, duration of operation must be long enough to realize the microbial inactivation and decontamination. Among the dryers that are described in Tables 2–5, superheated steam dryers, pulse combustor dryers, and microwave-assisted dryers have the inherent characteristics in eliminating microbial contamination during drying process.

Superheated Steam Drying

Superheated steam is steam heated to a temperature higher than its boiling point corresponding to the operating pressure. Figure 3 shows the schematic diagram of basic principle of a closed system superheated drying system. Saturated steam (100 °C, 1 bar) after heated to a superheated state at 110 °C, gaining 30 kJ kg^{-1} of energy, if it is used as a drying medium, absorbs moisture from drying material and transfers heat to the material until it reaches saturation. A major fraction of the exhaust steam must be recycled in order to maximize the energy efficiency. With reference to Figure 3, only 1% make-up steam is required and additional 30 kJ of heat is required to heat the saturated steam to superheated state. Thus, superheated steam drying system has high energy efficiency and it is normally conducted in closed system.

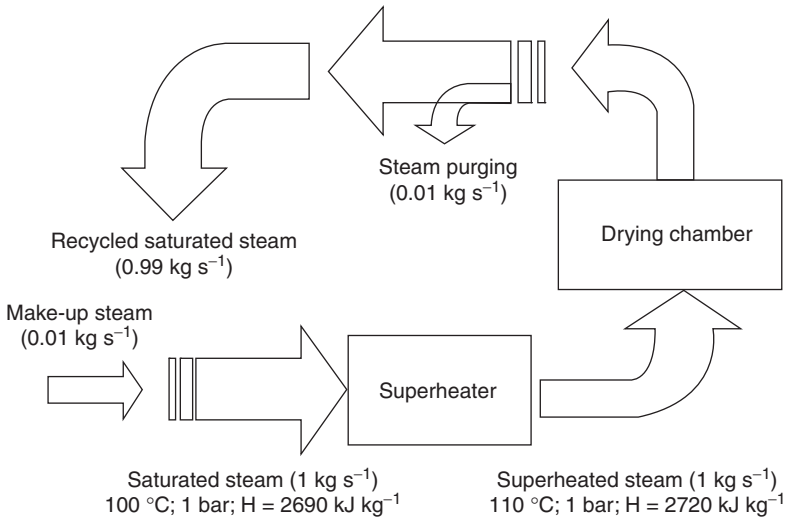

Figure 3 Schematic diagram basic principle of a closed system superheated steam drying.

Superheated steam can be used to replace hot air in direct dryers. As it does not contain oxygen, oxidative or combustion reactions can be avoided. Furthermore, it eliminates the risk of fire and explosion hazards. Superheated steam drying is a promising drying technique in processing industry as it also offers additional advantages such as low energy consumption and improved food hygiene. As the operating temperature of superheated steam drying is typically high, this drying technique permits pasteurization, sterilization, and deodorization of food products. In terms of microbial inactivation, moist heat is more effective than dry heat because proteins, which are important in maintaining cell viability, are more stable in the dry state. Therefore, superheated steam drying is effective in inactivating microbial activity. This is particularly important for food and pharmaceutical products that require high standard of hygienic processing.

It has been reported that surface sterilization could be achieved in fish products and cabbage after a short period of pretreatment with superheated steam. Abe and Miyashita (2006), Ono *et al.* (2006) and Cenkowski *et al.* (2007) after examining the effect of superheated steam drying on the vitality of *Fusarium* mycotoxin deoxynivalenol and with *Geobacillus stearothermophilus* spores concluded that the use of superheated steam drying is beneficial for reducing the contamination of foods.

However, superheated steam drying has deleterious effect on heat-sensitive compounds due to high operating temperature. As such, it is not suitable for the dehydration of biomaterials that contain high content of bioactive ingredients. Anyway, the operating pressure can be reduced in order to reduce the deleterious effect on bioactive ingredients.

Superheated steam has the ability to eliminate microbial activity, inactivate enzymatic reaction, and denature microbial proteins provided that the duration is sufficient to inactivate the microbial activity. This however gives drawbacks to product quality where long drying duration may result in problems such as over-dried, deteriorating product quality, etc. Constrain on both food safety and product quality poses a challenge to the processing industry if superheated steam drying is selected. Obviously, it requires optimization.

Like many other hot air dryers, the performance of superheated steam drying can be enhanced by combining it with other drying techniques such as microwave, fluidized bed drying, etc.

It has been reported that the exposure times required for 90% reduction in microbial population (D-values) of surrogate organisms *Clostridium sporogenes* (spores) and *Escherichia coli* at 300 °C were 0.33 and 0.10 min, respectively, in superheated steam fluidized bed dryer as compared to 54 and 1.12 min in hot air dryer. Whereas for the inactivation of the spores of thermophile *G.stearothermophilus*, 3.54 min in fluidized bed superheated steam drying compared to 228 min in boiling water.

Pulse Combustor Dryers

Combustible mixture of fuel and air is ignited and discharged periodically in a pulse combustor. The periodic combustion of fuel mixture and discharge of high-temperature exhaust gas results in periodic pressure oscillations. Liquid product is injected directly into the highly turbulent pulse combustor exhaust tailpipe; despite the ultra-high temperatures of the exhaust, rapid heat and mass transfer rates take place in the drying chamber. The highly turbulent flow in the chamber coupled with fine atomization allows drying to be accomplished within seconds or minutes. Although the operating temperature is extremely high, it is also suitable for highly heat-sensitive products because the duration is too short for thermal degradation to take effect.

Figure 4 shows the schematic diagram of typical pulse combustion dryer. The pulse combustor is mounted on a drying chamber. It can be a normal convective drying chamber or a spray dryer. The combustion gaseous product after being discharged from the combustor is mixed with product liquid and the mixture is then charged into the drying chamber. Rapid drying is carried out in the drying chamber

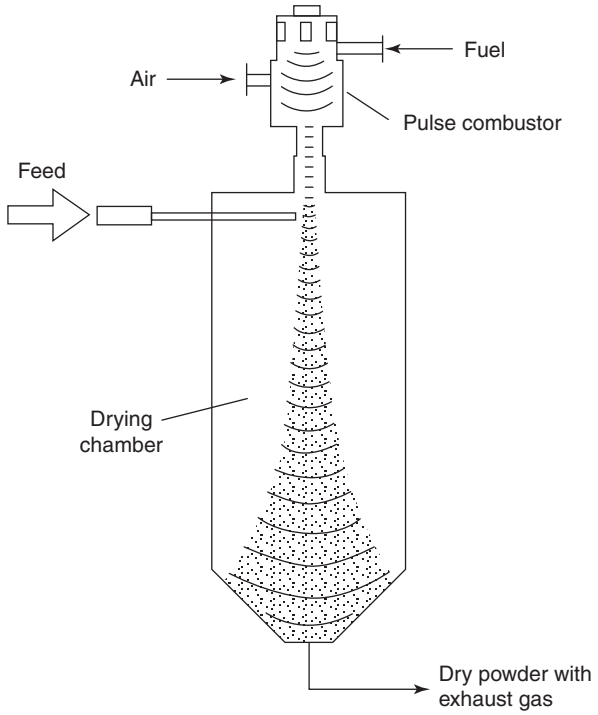

Figure 4 Schematic diagram of pulse combustion dryer.

where powders are formed and drops on the bottom of the chamber. The products together with the rapid moving gas stream exit the chamber and enter dust/powder separation system. The process has not been a commercial success yet, possibly due to problems of noise, scale-up, and capital cost.

Microwave Drying

Drying utilizing microwave energy is an attractive solution to many problems arising in drying of material that is based on the conventional, convective, and conductive heat transfer. Microwaves stimulate vibration and rotation of water and fat molecules as well as electrophoretic movement of ions for some depth inside the food, resulting in internal heat generation. Owing to the increase in vapor pressure, moisture from the interior of the product can be expelled. Microwave drying permits dielectric heating which heats volumetrically a drying material containing polar compound. When an alternating electromagnetic field is applied to a dielectric material, heat is generated. This allows the removal of internal moisture even at the initial stage of drying which is totally impossible for conventional convective drying that can only remove surface moisture. In conventional convective drying, the removal of internal moisture is dependent on the migration of internal moisture to the material surface and the evaporation of the moisture at the surface subsequently.

Microwave drying offers advantages which include rapid drying, high energy efficiency, uniform energy, and high thermal conductivity to the interior of the material, permits precise process control, space utilization, shorter drying time, short start-up and shut-down times, prevention of enzymatic reaction as well as microbial proliferation. However, it also gives some drawbacks which limit its application in food processing industry, for example, high start-up costs and relatively complicated technology compared with simple conventional convective drying, nonuniform heating patterns which are caused by focusing, corner and edge heating, inhomogeneous electromagnetic field, shape and composition of the drying material.

Daglioglu *et al.* (2002) dried tarhana dough (fermented product of yogurt–cereal mixture) inoculated with *Staphylococcus aureus* (10^4 CFU g^{-1}) in two types of dryers, namely hot air oven at 55 °C for 36 h and microwave oven (1500 W, 2450 MHz, 30% power level) for 10 min. It was found that microwave drying could completely eliminate *S. aureus* microbial population.

Because microwave offers the advantage of volumetric heating, as well as eliminating microbial proliferation, microwave drying is often combined with other drying techniques, for instance, microwave-assisted convective drying, microwave-assisted freeze drying, microwave-assisted vacuum drying, etc.

Microwave-Assisted Convective Drying

Microwave heating can, in principle, be coupled with any convective drying system such as fluidized bed or spouted bed. The drying time in a microwave assisted fluidized bed dryer can be 2–5 times shorter than in a conventional fluidized bed dryer, therefore resulting in higher drying efficiencies. However, the unique temperature leveling in microwave-assisted spouted bed helps to control product temperature and improve product quality as compared with microwave-assisted fixed-bed hot air drying methods.

Microwave-Assisted Freeze Drying

It is well known in the processing industry that vacuum freeze-drying can yield high-quality product, but its capital and operating costs are high and it requires a long processing time. Besides, microorganism count in freeze dried products sometimes exceed the permissible limit in food stuff. One of the ways to maintain freeze dried product quality and eliminate the drawbacks of freeze drying is to combine the freeze drying with microwave drying. In this regard, microwave does not only allow volumetric heating but also eliminates microbial activity during the drying process. This in turn improves the drying kinetics, meanwhile maintaining hygienic processing.

In an investigation on the sterilization effect of microwave-assisted freeze drying for the dehydration of cabbage, Duan *et al.* (2007) found that the change of standard plate count (SPC) treated by microwave-assisted freeze drying (MFD) is in the range of 2000 as compared to 14 000 in freeze dried (FD) cabbage and 15 000 in fresh cabbage. Further, it was reported that FD took 15 h whereas MFD required only 6 h to reach final moisture content of 6%.

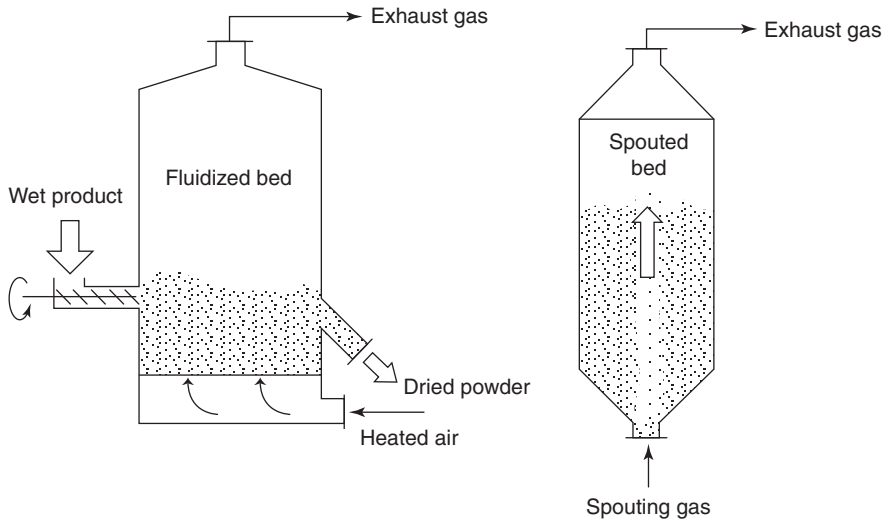

Figure 5 Schematic diagrams of a typical fluidized bed and a typical spouted bed.

Microwave Vacuum Drying

In vacuum drying, oxidative reaction is prevented as atmospheric air is not present in the dryer. Drying temperature is typically low as vacuum reduces the boiling point of water or solvent, thus reducing thermal stresses and overdrying that is caused by high temperature drying. This in turns improves color and texture of dried products. As drying medium is not present in the vacuum drying, convective heat transfer is not possible. This in turn results in relative long drying duration. Combination of vacuum drying and microwave drying gives the advantages offered by both vacuum and microwave drying, for example, minimization of oxidative reaction and rapid drying rate. Therefore, unwanted changes in sensory attributes and nutrient loss due to long drying times or high surface temperature can be prevented, and temperature-sensitive substances like vitamins, colors, and flavors may be retained. Vacuum microwave drying has been recognized as a good method for production of high-quality dry food materials.

It has been reported that vacuum microwave could reduce microbial population of fresh parsley leaves and grated carrots in a shorter time and at a lower final temperature as compared to traditional hot air drying. To further maximize the elimination of microbial load, it has been suggested to apply two-stage drying strategy, microwave drying in the initial stage of drying for a short period of time (e.g., 5 min) followed by vacuum microwave drying.

High Temperature Drying

Drying operated at high temperature (e.g., more than 80 °C) could minimize microbial activity but not eliminate the microbial activity entirely. In addition, the effect in minimizing the microbial activity is dependent on the duration of the application of high temperature drying. Often, the duration required to make the minimization of microbial activity to be effective tends to cause over-drying and therefore produces undesirable product quality. Hence, optimization is often required to maximize both aspects.

It (fixed constant maximum grain temperature 62 °C, retention time 10.5 min) of bread grain could reduce surface dwelling yeasts and filamentous fungi (especially the deoxynivalenol-, nivalenol-, and zearalenone-producing *Fusarium culmorum*) if compared to continuous hot air drying, albeit both drying methods gave the same drying kinetics and produced similar baking quality. This study demonstrated that temperature is not the only parameter that ensures effective minimization or elimination of microbial load. Contacting efficiency plays an equally important role in this respect. Therefore, if the drying material is in the form of a bed of solids, fluidized bed, spouted bed, vibrating bed, etc., may be considered. Figure 5 shows the schematic diagram of a typical fluidized bed and a spouted bed. In a fluidized bed, gas is charged into the fluidized bed column from the bottom, the fluidizing gas stream is powerful enough to suspend all particles in the gas stream, thus making the particles always in contact with gas stream. In a spouted bed, the gas stream penetrates the bed of solids at the center region, thus producing a fountain-like particle bursting at the center region. Particles after losing its momentum fall back on the bed surface and slowly move toward the bed center for another round of thrusting. In both systems, particle–gas contacting efficiency is enhanced if compared with conventional packed bed system.

Low Temperature Drying at Subzero Condition – Sublimation Drying

Drying at temperatures below ambient, including subzero temperatures, may offer significant advantages to materials that are sensitive to high temperatures, for example, biomaterials. Low temperature drying of grain has been reported in barley, corn, wheat, and rough rice. This technique is feasible for temperate regions where seasonal temperature falls

Table 6 Effect of washing pretreatment on microbial load

Technique	*Findings*	*References*
Washing shredded cabbage with acetic acid solution (0.5, 1.0, 1.5%) at different temperatures (5, 10, 30 °C)	• *Salmonella anatum* was destroyed rapidly during the first 5 min of soaking and decreased slightly thereafter • Higher temperature resulted in a higher elimination effect on *S. anatum*	Nanuam (2005)
Washing fresh and semi-dry tomatoes with chlorinated water (200 ppm) followed by hot air drying at 60 °C	• 1.7 log reduction for 5 min washing and 3.1 log reduction for 10 min washing in terms of yeast and mold count	May and Fickak (2003)
Washing cabbage slices with acetic acid solution (0.5, 1.0, 1.5% v/v) followed by hot drying (50–60 °C)	• Initial cell numbers of *Salmonella* was reduced approximately 1.5 log10 after soaking and a reduction of 3 log10 after drying • Higher acetic acid concentration resulted in a higher degree of acid injury and hence increased the susceptibility of *Salmonella* to heat during drying	Chiewchan and Morakotjinda (2009)
Beef slices inoculated with *L. monocytogenes* were treated with marinades with addition of acetic acid followed by hot air drying (60 °C for 10 h) and aerobic storage (25 °C for 60 days)	• Bacterial populations dropped below the detection limit (-0.4 log CFU cm^{-2}) as early as 4 h during drying • Acetic acid solution was found to be effective in inactivating *L. monocytogenes*,	Calicioglu *et al.* (2002)
Peach slices inoculated with *L. monocytogenes* were treated with sodium metabisulfite or acidic solutions followed by hot air drying (60 °C for 6 h)	• Bacterial populations were reduced by 4.3–5.1 log CFU g^{-1} (on TSAP agar) and 5.3–6.2 log CFU g^{-1} (on PALCAM agar), respectively	DiPersio *et al.* (2004)
Carp fillets treated with electrolyzed NaCl solutions and essential oil compounds followed by drying at 45 °C	• Inhibited microbial growth; reduction in 4 log (CFU g^{-1}) as compared with control sample • Gave good antioxidant effects and sensory attributes	Mahmoud *et al.* (2006)

after harvest and cooling can be achieved using ambient air. It has been reported that initial stage of low temperature drying of grain at subzero condition inhibits growth of molds, coupled with rapid drying (with the assistance of higher air flowrate) could reduce mold count as the molds have shorter time to multiply.

Pretreatment

To ensure the safety of dehydrated food products by drying techniques that do not eliminate microbial activity during the process, various pretreatment methods may be applied to reduce the initial numbers of spoilage and pathogenic organisms on the surfaces of food stuff. For food products that contain high initial microbial load, conventional drying techniques such as oven, convective hot air drying at moderate temperature (e.g., 60–80 °C) may not inactivate the microbial activity completely. Instead, the microorganisms could be incubated in the dryer. Pretreatment may be applied to reduce the initial microbial load to avoid incubation of microorganism during drying at moderate temperature. In this regard, pretreatment methods such as cooking, washing, radiation, etc., may be applied.

For washing pretreatment, chemicals such as ozone, chlorine, hypochlorite, and organic acids such as lactic acid and acetic acid have been suggested to sanitize the food stuff before drying. Proper washing of food stuff during the pretreatment stage can reduce microbial load by a factor of 10–100 although it may not remove pathogens completely from the product surface. Further to this, the efficiency of these chemicals on eliminating or reducing the initial microbial load is dependent on the chemical concentration, temperature, and treatment duration. It is noteworthy that some organic acids such as acetic acid have been shown to reduce enzymatic browning activity. Table 6 shows the effect of washing pretreatment using various chemicals on the microbial inactivation and elimination of microbial activity.

Coating of Photocatalyst

A thin coating of titanium dioxide (TiO_2) can be applied on the inside walls of a drying chamber. Titanium dioxide is a photocatalyst which generates strong oxidative potential that can oxidize water to create hydroxyl radicals (OH^-) if exposed to ultraviolet radiation or sunlight. It can also oxidize oxygen or organic materials including microorganisms that exist in the air or on the drying material surface. This in turn provides a hygienic environment to a drying process. Photocatalysis has been applied to many unit operations for bactericidal, deodorizing, and antifouling purposes but this technique is not common in commercial dryers yet.

In an investigation of the drying of pineapple slices in a solar-assisted heat pump dryer, it was reported that the average total counts of potato dextrose agar (PDA) and plate count agar (PCA) were reduced from 20 CFU per plate to less than 5 CFU per plate after the dryer was coated with a layer of titanium oxide photocatalyst.

In addition, exposure to UV, irradiation during drying can be an alternative to reduce microbial load and inactivate microbiological activity.

Good Manufacturing Practice in Drying to Avoid Contamination

Microbial activity in the final product may be derived from contamination sources located in the production line even preprocessing (such as predrying) is carried out in a hygienic condition. Contamination sources may establish at any site where temperature and moisture provide conditions that are conducive for the multiplication of microorganisms. This is more prevalent when the plant is idle. Recontamination therefore mainly occurs at the initial stage of production and in processes that have frequent production shutdowns.

With reference to drying system, microbial contamination and multiplication of microorganisms can occur in nozzle if it is not cleaned properly, and if dead ends and corners where flow of drying material is uneven or obstructed. Surface of components in a dryer which makes contact with product has to meet specific design standards to prevent occurrence of any internal sources of microbial contamination. In addition, contamination risks from external sources have to be avoided by applying good prevention system and GMP.

Concluding Remarks

Design of a good drying system needs to consider various aspects which include foods safety, product quality, energy efficiency, environmental impact, drying efficiency, and engineering aspect. Food safety aspect is definitely an important aspect and in this regard, drying techniques such as superheated steam drying, pulse combustion drying, microwave drying, and their hybrid drying such as microwave convective drying, superheated steam fluidized bed drying, etc., have the potential in minimizing microbial proliferation or eliminating microbial contamination hazards. Pretreatments such as washing may be applied before drying. During drying process, irradiation and exposure to ionized environment by photocatalyst may be applied to achieve this objective. Further it is important to practice good prevention system and GMP when operating a drying process.

See also: Food Safety Assurance Systems: Hygienic Design of Equipment; Microbiological Testing, Sampling Plans, and Microbiological Criteria. Food Technologies: Food Irradiation; Packaging; Pulsed Ultraviolet Radiation Processing. Public Health Measures: Monitoring of Contaminants

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Fermentation

MJR Nout, Wageningen University, Wageningen, The Netherlands

Glossary

Back-slopping A method to initiate fermentation by using a small quantity of previously fermented products as a starter.

Fermentation (1) Anaerobic metabolism for energy generation and (2) any microbiological process used to achieve desirable food product properties.

Food environment A combination of intrinsic (chemical composition) and extrinsic factors (external factors, such as temperature, relative humidity, and microbial contaminations) that influence the speed of biological and chemical reactions in the food.

Natural enrichment A fermentation starter with often complex and unknown microbiological composition, obtained by repeated natural fermentation with back-slopping.

Natural fermentation Fermentation in which no starter is added, but which relies on creating a favorable food environment to allow extrinsic microorganisms to multiply and establish desirable food properties.

Operation Technical actions, including physical (mixing, separating, and size reduction) and thermal (heating and cooling) treatments.

Process An organized assembly of operations and ingredients, aimed at producing a final product from its ingredient raw materials.

Starter An additive containing high numbers of living microorganisms used to initiate fermentation; starters may contain single or mixed pure-culture microbial strains, or may be natural enrichments.

Introduction

Fermentation (from *fermentare*, causing to rise) in the strict sense, refers to the anaerobic metabolism and generation of energy. This takes place, among others, in alcoholic fermentations caused by fermenting yeasts and in lactic acid fermentations caused by lactic acid bacteria (LAB). In the wider sense, the word 'fermentation' is used to refer to biological processes resulting in desirable modifications of ingredients. It also refers to the aerobic growth and metabolic activity of acetic acid bacteria in the oxidative production ('fermentation') of acetic acid, of bacilli in alkaline fermentations, and of filamentous fungi (molds) in food processing and enzyme production; the word fermentation is even used for some processes that are enzymatic rather than microbial, for example, the fermentation of tea leaves and the fermentation of certain Oriental fish sauces. In this article, the word 'fermentation' will be used in its broader sense.

A food manufacturing process is defined as the organized use of ingredients, equipments, and operations aimed at achieving a final product. The fermentation itself is only a part of the process, starting from the ingredients to the final product.

Ingredients for fermented foods may include practically all types of primary agricultural food items ranging from plant-derived foods (cereals, pulses, starchy roots and tubers, fruits, vegetables, herbs, and spices) to animal-derived products (meats, poultry, eggs, fish, and shellfish), but they always include water in order to enable biological activity and, thus, fermentation. Essential ingredients for fermentations are the microorganisms; these will be mentioned below.

Equipments used for fermentation may be simple or more sophisticated depending on the socioeconomic context. Equipment is needed to prepare ingredients by grading, removing unwanted parts, mincing, mixing, heating, cooling, etc. In addition, containers for soaking, cooking, and the actual fermentation are required. Other equipments may be needed to finish and distribute the final products to the consumer market.

Process operations can be categorized in groups, including physical and thermal operations. Physical operations include activities such as (1) grading, for example, sorting by color or size; (2) size reduction by grinding; (3) separation of unwanted parts by sieving; and (4) mixing, for example, by stirring. Thermal operations refer to all phenomena involving heat transfer, mainly heating and cooling. Heating may be (1) dry heating by contact with hot air or with hot surfaces as in roasting or frying; heating can also be (2) in wet conditions, such as steaming, immersion in hot water, or boiling under atmospheric or higher pressure. Cooling may be important at various stages in the process, for example, to adjust the temperature of previously heated ingredients to enable the optimum activity of added microorganisms for fermentation. Cooling may also be used to arrest fermentations at a level of

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optimum food composition reflected, for example, in the most attractive taste. Finally, cooling helps to lengthen the shelf life of foods.

Microorganisms are the driving force of fermentation. They need to be present in adequately large numbers and to be metabolically active, so as to exert their impact in changing the composition of the ingredients toward the desirable characteristics of the expected outcome (the fermented food). In principle, there are two approaches to ensure the presence of active microorganisms.

The first approach is called 'natural' or 'uncontrolled' fermentation; in principle, no microorganisms are added to the ingredients. Instead, the process conditions allow the survival and selective outgrowth of desirable microorganisms that were present in low numbers in one or more of the ingredients. This principle entails that – to allow microbial survival – ingredients are not heated before fermentation. In addition, process conditions may include the exclusion of air in sealed containers in order to favor the dominance of (facultative) anaerobic microorganisms. Also, certain ingredients, such as herbs, spices, and salt, may be used to favor desirable microbes, while restricting the development of potentially undesirable microorganisms or enzymatic activities.

The second approach is called 'starter-mediated' fermentation; in principle, a highly concentrated dose of desirable fermentation microorganisms is added to the ingredients, which may have been previously freed of potential competitive microbiota, and the fermentation is conducted under conditions that favor the added starter. Starters exist in various types, which could be categorized as (1) natural enrichments, (2) traditional multistrain starters, and (3) single or mixed pure-culture starters.

Natural enrichments are accumulations of groups of microorganisms that can be present in equipment, for instance, in porous textures of natural fermentation vessels, such as utensils, calabashes, leather bags, clay pots, etc. Accumulations may also be present at the processing site, for example, the ceiling, walls, and other surfaces of incubation rooms. It is well known that certain traditional Belgian beers can be made successfully only in specific brew houses, where the 'house flora' plays an essential role in the natural fermentation.

Traditional multistrain starters used for fermentation are concentrates of a mixed microbial flora in liquid or dry form. These starters are made with simple technologies without the use of selected pure cultures. In many oriental rice fermentations, such starters are used to initiate the enzymatic degradation of rice starch and the fermentation of the resulting sugars. The dry starters are sold as tablets in the open market; these have a shelf life of at least 6 months in ambient conditions.

Single or mixed pure-culture starters consist of laboratory-controlled pure strains of bacteria or fungi, which have been selected or created for their desirable fermentation outcomes. By combining diverse strains in one mixed culture, the objective is to enhance fermentation outcomes, such as combined acidification and flavor formation. The shelf life of pure-culture starters can be prolonged by freeze-drying (lyophilization) or by freezing. Usually, the viability of the strains needs to be supported by cryoprotectants, such as skimmed milk powder or glycerol.

Types of Food Fermentations

The worldwide diversity of fermented foods is amazing. Several authors observed similarities across national and continental borders and proposed categories of foods and beverages, according to the microorganisms that are responsible for the fermentation, and the kind of biochemical changes that take place, which are of relevance to the characteristic product qualities. In this article, five main categories of fermented foods will be discussed, namely (1) those acidified by the formation of organic acids, (2) alcoholic beverages, (3) those alkalized by the formation of ammonia from amino acids, (4) predigested food caused by the activity of filamentous fungi and their enzymes, and (5) those having undergone enzymatic processes in high-salt conditions.

Acidified by the Formation of Organic Acids

The major organic acids formed in fermented foods are lactic acid, acetic acid, and propionic acid. The most important microorganisms responsible for lactic acid production are LAB, although certain food-grade molds can also form lactic acid. Lactic acid is mainly produced from pyruvate, formed through glycolysis (degradation of monosaccharides). Although homofermentative LAB produce lactic acid as their only fermentation product, heterofermentative LAB may form acetic acid, ethanol, and CO_2 besides lactic acid. Lactic acid results in a decrease of pH and acidic taste; it can also affect the physical properties of food macromolecules, such as starch, and thereby influence food texture. The increased acidity may also have beneficial effects on nutritional aspects, for example, acidity increases the solubility of minerals, thereby facilitating their assimilation in the human gastrointestinal tract.

Thus, although the formation of acetic acid is mostly caused by acetic acid bacteria, it may also be formed by heterofermentative LAB. The major substrate for acetic acid production by acetic acid bacteria is ethanol, which is oxidized by acetic acid bacteria in a strictly aerobic, exothermic reaction. However, acetic acid is produced in a fermentative way by heterofermentative LAB. Acetic acid, like lactic acid, results in a pH decrease and acid taste. The internationally best-known acetic acid product is vinegar, which in principle is a watery solution of approximately 4% w/v of acetic acid. Vinegar is widely used as a natural food preservative and flavoring ingredient.

Propionic acid is formed as a result of the metabolism of lactic acid by propionic acid bacteria. Simultaneously with the formation of propionic acid, acetic acid and CO_2 gas are produced. This could lead to the characteristic taste and texture of certain Swiss cheeses.

Table 1 shows several examples of fermented foods in which organic acids dominate the quality of the product. Organic acids can be formed in a wide variety of food ingredients, such as cereals, vegetables, and dairy products. Combinations with animal protein are also possible, such as meat and fish products. However, in the latter cases, often an additional source of fermentable sugars is required for the LAB. This can be achieved by the incorporation of sugar or

Table 1 Fermented foods dominated by acidification

Name of food	*Origin*	*Main ingredients*	*Predominant microbiota*	*Typical metabolites*	*Typical pH values*
Pozol	Mexico	Maize	LAB (Lp, Lb, and S) and Y (C and T)	Lactic acid and vitamin B	3.8–4.0
Kenkey	Ghana	Maize	LAB (Lf) and Y (Ck and Sc)	Lactic acid, acetic acid, acetoin, and 2,3-butanediol	3.8–4.2
Sauerkraut	Europe	White cabbage and salt 1.5%	LAB (Lm, Lb, and Lp)	Lactic acid, acetic acid, and ethanol	3.8
Kimchi	Korea	Chinese cabbage, garlic, onion, ginger, chilli, and salt 3%	LAB (Lm, Wc, Ls, and Lc)	Lactic acid, acetic acid, and CO_2	3.9
Lassi	India	Cow/buffalo milk	LAB (Bb, Lec, Led, Lecr, St, La, and Pfs) and Y (Sc)	Lactic acid, acetic acid, ethyl alcohol, and CO_2	4–4.5
Yogurt	Europe	Cow milk	LAB (Lbg and St)	Lactic acid and acetaldehyde	4–4.5
Sik-hae	Korea	Fish, millet, and 6–7% salt	LAB (Lm and Lp)	Lactic acid and peptides	<5.0

Abbreviations: Bb, *Bifidobacterium bifidum*; C, *Candida* spp.; Ck, *Candida krusei*; La, *Lactobacillus acidophilus*; Lb, *Lactobacillus brevis*; Lc, *Lactobacillus curvatus*; Lf, *Lactobacillus fermentum*; Lm, *Leuconostoc mesenteroides*; Lp, *Lactobacillus plantarum*; Ls, *Lactobacillus saké*; Lbg, *Lactobacillus bulgaricus*; Lec, *Leuconostoc citrovorum*; Led, *Leuconostoc dextranicum*; Lecr, *Leuconostoc cremoris*; Pfs, *Propionibacterium freudenreichii* spp. *shermanii*; S, *Streptococcus* spp.; Sc, *Saccharomyces cerevisiae*; St, *Streptococcus thermophilus*; T, *Trichosporon* spp.; Wc, *Weissella confusa*; Y, yeast.

cereals, such as in the Korean product *sik-hae* in which millet provides the carbohydrates.

Pozol and *kenkey* are both made from whole-grain maize, which is processed by soaking in water, grinding, and cooking. The natural fermentation is uncontrolled, and the microbiota consists of mainly LAB, yeasts, and some minority organisms. LAB and yeasts can form rather stable communities, each group relying on metabolic outcomes of the other. In that sense, this kind of microbiota is similar to the 'sourdough fermentation communities' in which protocooperative growth of yeasts and LAB has been documented. When the fermentation is considered as completed – this depends on producers and on consumer demand – *pozol* balls are sold in the market, to be diluted with water or milk to make a beverage. *Kenkey* is wrapped in plant leaves and steam cooked, resulting in a compact sliceable mass, which is eaten with breakfast.

Sauerkraut and *kimchi* are occidental and oriental counterparts in the sense that both are made from cabbage and salt, and fermented predominantly by successive LAB without the use of added microbial starter. In *sauerkraut* making, the cabbage leaves are shredded, that is, cut to thin strips of approximately 1.5 mm width, which causes extensive damage to the plant cells. The addition of salt causes an osmotic gradient, which causes the expulsion of plant juice. Soon the cabbage is covered by its own juice, which helps to create anaerobic conditions, containing fermentable sugars and some allyl thiocyanates that all have a favorable effect on LAB. In *kimchi*, the cabbage is first wilted in salty water and then stuffed with a pasty mixture containing garlic, onion, chilli fermented fish, etc. The cabbage is left intact, and the stuffed cabbages are arranged in a fermentation vessel, which is hermetically closed. *Kimchi* is typically consumed in the raw (uncooked) state, in contrast to *sauerkraut*, which may be consumed raw or cooked.

Lassi and *yogurt* are also fermented dairy counterparts from India and Europe. *Lassi* is made by mesophilic LAB in milk from cows or buffaloes to which some salt may have been added. *Yogurt* is more solid and is obtained by cooking cow's milk and inoculating with thermophilic LAB.

Alcoholic Fermentation

Alcoholic fermentation refers to the production of ethanol. In foods, ethanol can be produced by several groups of microorganisms, including filamentous fungi, yeasts, and heterofermentative LAB.

Of these, yeasts are the major type of microorganisms that are used to produce alcoholic beverages and other alcohol-containing products, such as bread dough. In principle, two types of yeasts, that is, the oxidative and the fermenting yeasts, can be distinguished. Although the former group depends on aerobic metabolism to generate energy and thus does not produce ethanol, the latter group can derive energy under anaerobic conditions (the true fermentation) by reducing pyruvate that was formed from monosaccharides through the Embden–Meyerhof glycolysis pathway. Among these yeasts, the species *Saccharomyces cerevisiae* is the most important from an economic point of view. It is used in the fermentation of beers, wines, and other alcoholic beverages. Some major alcoholic fermented products are summarized in **Table 2**. *Saccharomyces cerevisiae* also plays an important role in the maturation (fermentation) of leavened bread dough. In dough, the main function of yeast is to produce CO_2 gas to extend the dough volume. As a favorable spin-off, yeast enzymes contribute to the bread dough texture by increasing its extensibility. In addition, the ethanol formed can react with acids to form esters, which contribute to the flavor of bread crumb.

As can be seen in **Table 2**, different principles of processing are in use to obtain alcoholic beverages. The simplest way is to harvest ripe fruit or palm sap, both of which contain high concentrations (10–30%) of mono- and disaccharides (glucose, fructose, and sucrose) that can be fermented directly by yeasts. The fruits are pressed, and the fresh juice is left to ferment naturally, that is, no yeast is added. The wide range of wines that are known worldwide are all made according to this simple principle. When all sugars have been assimilated, the fermentation will stop. Usually, grape wines will contain 10–14% v/v of ethanol.

Making of beer is more complicated. Because the main ingredient for beer is a cereal grain (barley, wheat, sorghum,

Table 2 Alcoholic fermentations

Name of product	*Origin*	*Main ingredients*	*Predominant microbiota*	*Typical alcohol content (% v/v)*	*Remarks*[a]
Beer	Europe	Barley and hops	Y (Sc)	4–8	1
Grape wine	Europe	Grapes	Y (Sc)	10–15	3
Sake	Japan	Rice	M (Ao) and Y (Ha and Ss)	15–16	2
Bai-jiu	China	Sorghum	M (R) and Y (Ef)	38–45	2 and 4
Palm wines	Africa–Asia	Palm sap	Y (Sc and Sp) and LAB (Lm and Lp)	2–4	3

[a]1, Brewed from starch with malt (germinated barley); 2, brewed from starch with fungal enzyme starter; 3, direct fermentation of fruit sugars; 4, distilled.
Abbreviations: Ao, *Aspergillus oryzae*; Ef, *Endomycopsis fibuligera*; Ha, *Hansenula anomala*; Lm, *Leuconostoc mesenteroides*; Lp, *Lactobacillus plantarum*; M, molds; R, *Rhizopus* spp.; Sc, *Saccharomyces cerevisiae*; Sp, *Saccharomyces pombe*; Ss, *Saccharomyces saké*; Y, yeast.

Table 3 Alkaline-fermented food products

Name of food	*Origin*	*Main ingredients*	*Predominant microbiota*	*Typical pH values*	*Remarks*
Kinema	Nepal–India	Soybeans	B (Bs) and LAB (Ef)	7–8.5	Curry base
Dawadawa and *Soumbala*	Nigeria and Burkina Faso	Locust bean	B (Bs)	7–8	Soup flavoring (condiment)
Tayohounta	Bénin	Baobab seed kernels	B (Bs, Bl, and Bt)	7–8	Soup flavoring
Iru	Bénin	Locust bean	B	7–8	Soup flavoring
Natto	Japan	Soybeans	B (Bn and Bs)	7–8	Poly-DL-glutamic acid causes elastic texture

Abbreviations: B, bacteria; Bl, *Bacillus licheniformis*; Bn, *Bacillus natto*; Bs, *Bacillus subtilis*; Bt, *Bacillus thermoamylovorans*; Ef, *Enterococcus faecalis*.

maize, or millet), the availability of freely available fermentable sugars is low. These will first have to be released by the degradation of starch, the bulk polysaccharide in cereal endosperm. It is interesting to note that worldwide, two fundamentally different strategies have evolved to achieve this aim.

The first strategy is to use starch-degrading enzymes, which are formed during the germination of cereal seeds. Germinated, and then dried, seed is called malt; it contains α-amylase (starch-liquefying enzyme), β-amylase (enzyme releasing maltose from starch and its dextrins), proteases, phosphatases, and other enzymes that are essential for the release of nutrients for the young growing seedling. The word 'brewing' refers to the transformation of starch into fermentable sugars, in this case mainly maltose. After brewing, the resulting sugar solution ('wort') is boiled with hops (providing desired bitter flavor) and inoculated with selected strains of brewer's yeast, usually *S. cerevisiae*. Two fermentation stages include the primary fermentation and the maturation, after which beer is bottled and pasteurized.

The second strategy to degrade cereal starch has evolved in Asia. Instead of using malt enzymes, fungal enzymes are used. In this principle, cereal grains are first soaked and steam cooked, either as whole kernels or as flour, to hydrate and gelatinize the starch. After cooling down, the cooked starch is inoculated with amylolytic starters consisting of mixed populations of starch-degrading molds and yeasts. During the first aerobic incubation stage, the growth of mycelial fungi is favored. These are producers of amyloglucosidase, an enzyme that splits single glucose molecules from starch and dextrin molecules. As a result, a strong accumulation of glucose (approximately 25% w/v) can result. As soon as fermentable sugars are available, the yeasts will start to assimilate them and grow. At the time when the starchy mass starts to exude liquid, the fermentation conditions are changed to favor anaerobic conditions in order to enhance the alcoholic yeast fermentation.

The fermented products resulting from both strategies can be distilled to obtain a variety of strong liquors, which can be matured subsequently. Worldwide, a diversity of strong liquors is known, including whisky and *bai-jiu* (see Table 2).

Alkaline Fermentation

The characteristic feature of alkaline fermentations is the increase of pH during fermentation, to values of approximately 7–8. This is usually caused by a decrease of organic acids in combination with the accumulation of NH_3, which tends to increase the pH. The kinds of products that undergo alkaline fermentation are mainly leguminous seeds with a relatively high protein content, such as soybeans, locust beans, baobab seed kernels, etc. To bring about the above-mentioned changes, the fermentation must be dominated by microorganisms that produce proteolytic and other lytic enzymes, in addition to enzymes acting on peptides and amino acids, such as various peptidases, deaminases, transaminases, etc. Although these kinds of enzymes are found in a range of microorganisms, the major microorganisms that have impact are filamentous fungi and *Bacillus* spp. Among the filamentous fungi, *Aspergillus* spp., for example, *Aspergillus oryzae*, as well as Phycomycetes such as *Rhizopus*, *Mucor*, and *Actinomucor* spp. are associated with alkaline fermentations. However, by far, the most important are the *Bacillus* spp. such as *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus pumilus*, etc. Some examples of alkaline-fermented foods are summarized in Table 3.

The main use of alkaline-fermented foods is as a flavoring agent. It is remarkable how very similar processing approaches have evolved as traditional fermentations in the Orient and in Africa. The common features are the use of plant seeds with a relatively high protein content (40–45% dm), a prolonged boiling of dehulled seeds in water, and a fermentation of 2–3 days at warm (30–35° C) temperatures. Prolonged cooking will not only kill all vegetative microbial cells, but also activate the heat-resistant *Bacillus* endospores to germinate. As a result, *Bacillus* spp. will germinate and already start growing during the cooling down phase; their high numbers and the metabolites formed restrict the growth of postprocessing contaminating microorganisms.

Fungal Fermentation

Fungi, especially filamentous fungi (molds), were mentioned already in the previous section. Although thousands of mold species are known, only a small number of species have a long history of safe use in (traditional) food fermentations. The most important are summarized in Table 4 along with examples of the foods and other products in which they are a functional part of the fermentation. There are some specific reasons for the use of molds, such as (1) they provide texture by forming a dense and edible mycelium, (2) they produce desirable color and flavor compounds, and (3) they are strong producers of enzymes that cause desirable modifications of the food ingredients. The latter aspect can have profound impact on the nutritional and health beneficial food properties.

Tou-shi is a fermented paste of black soybeans; the fermentation is dominated by *Aspergillus* and *Mucor* spp., which cause the partial degradation of soy protein. It is a type of product that could also be listed under alkaline-fermented foods, but the latter are dominated by *Bacillus* spp. The result is a typical zesty flavor, which makes *tou-shi* an appreciated ingredient in various Chinese dishes.

Angkak or *red kojic rice* is made by fermenting steamed whole-rice kernels with *Monascus* molds. These produce secondary metabolites, including pigments, which are used as bio-colorants in food, for example, *tou-fu-ru* and beverages. Recently, *angkak* has attracted much attention because it also contains antibiotic-like compounds, such as monacolin K, which have been associated with health benefits.

Oncom is a by-product of small-scale pressing of peanut oil. After pressing, the residue or press cake can be valorized by fungal fermentation, to provide texture and flavor to a meat replacer in Indonesia. Two different types of *oncom* are known. Red *oncom* is fermented with *Neurospora intermedia* and black *oncom* with *Rhizopus* spp.

Tapé ketan, known in Indonesian and Malaysian cuisine, is a delicious fermented snack made from glutinous rice. After steaming the rice, it is inoculated with ragi tape, an amylolytic starter (see section Alcoholic Fermentation), and incubated overnight. This will result in a partial liquefaction of the rice making it juicy, accumulation of glucose, and the production of traces of alcohol and lactic acid, all of which combine to give a sweet and sour taste with a tickle on the tongue.

Kochujang has some similarity with *tou-shi*. After fermentation, it is pounded into a paste. This is used as a side dish as well as an ingredient for *kimchi* (see Table 1).

Enzymatic Brine or Salty Fermentations

Brine is a solution of salt in water. The salt is usually NaCl, but other trace ions may be present, such as K^+, Mg^{2+}, SO_4^{2-}, CO_3^-, etc. The salinity or salt concentration depends on the product and processor's preferences. Probably, the historic reason for applying salt is its preservative effect. As it turned out, certain microbial species can grow (albeit slowly) and thus find a niche in brines. Such halophilic and halotolerant species may contribute to the flavor of salt-preserved foods. Another effect of salts is that they increase the ionic strength and improve the dissolution of, for example, enzymes. The latter effect plays a crucial role in many of the popular Asian fungal-fermented foods, such as soy sauce and fish sauces. Table 5 provides a summary of major brine fermentations.

Soy sauce is made in a two-stage process, starting with 3–5 days of fungal solid-state '*koji*' fermentation to favor the formation of fungal lytic enzymes. The molded mass is fermented in brine to be degraded to water-soluble components (amino acids, peptides, etc); during this '*moromi*' stage that may take several months; halophilic LAB and halotolerant yeasts grow slowly and contribute to flavor formation.

Tou-fu-ru is a flavorsome spreadable paste, which is a popular addition to the Chinese rice breakfast. It is made from soybeans by first producing soy milk, which is coagulated into *tofu*. *Tofu* is inoculated with a pure culture of *Actinomucor elegans*, which will cover pieces of *tofu* with its mycelium. The molded pieces ('*pehtze*') are matured in a vessel with brine containing salt, rice wine, and natural flavorings and pigments. Maturation may take 6 months and results in the accumulation of glutamic acid (taste enhancer) and the softening of the texture.

Salami and many other types of raw fermented sausages evolved from the need to preserve meat for the army. This was

Table 4 Fungal-fermented foods

Name of food	*Origin*	*Main ingredients*	*Predominant microbiota*	*Typical pH values*	*Remarks*
Tou-shi	China	Soybeans	M (Ao and M)	8	Condiment
Angkak red rice	China	Rice	M (Mp)	8	Biocolorant
Oncom	Indonesia	Peanut press cake	M (Ni)	6–7	Side dish
Tapé Ketan	Indonesia	Glutinous rice	M (Ar) and Y (Ef and Hb)	5	Snack
Kochujang	Korea	Soybeans	M (M and R)	6–7	Side dish

Abbreviations: Ao, *Aspergillus oryzae*; Ar, *Amylomyces rouxii*; Ef, *Endomycopsis fibuligera*; Hb, *Hyphopichia burtonii*; M, molds; Mp, *Monascus purpureus*; Ni, *Neurospora intermedia*; R, *Rhizopus* spp; Y, yeast.

Table 5 Brine food fermentations

Name of food	*Origin*	*Main ingredients*	*Predominant microbiota*	*Typical pH values*	*Remarks*
Soy sauce	East Asia	Soybeans and wheat	M (Ao and As), Y (Zr and H), and LAB (Th)	5–6	Condiment
Tou-fu-ru	China	Soybeans	M (Ae and M)	6–8	Soy paste
Salami sausage	Europe	Lean meat and fat (lard)	LAB (Lc, Lp, and Ls) and B (Sca and Sx)	5–6	Protein food
Cucumber pickles	Europe	Cucumbers and salt brine	LAB (Lp) and Y (Sc and Sr)	4	Side dish
Olives	Europe	Olives and salt brine	LAB (P, L, Lp, and Ld)	4–8	Side dish

Abbreviations: Ae, *Actinomucor elegans*; Ao, *Aspergillus oryzae*;; As, *Aspergillus sojae*; B, bacteria; H, *Hansenula* spp.; L, *Leuconostoc* spp.; Lc, *Lactobacillus curvatus*; Ld, *Lactobacillus delbrückii*; Lp, *Lactobacillus plantarum*; Ls, *Lactobacillus saké*; M, mold; P, *Pediococcus* spp.; Sca, *Staphylococcus carnosus*; Sr, *Saccharomyces rosei*; Sx, *Staphylococcus xylosus*; Th, *Tetragenococcus halophila*; Y, yeast; Zr, *Zygosaccharomyces rouxii*.

achieved by a combination of salting and drying. A good way to prepare a homogenous product that can be dried efficiently is to mince red muscle meat and lard (fat) with herbs, spices, starter microorganisms, and some salt, and stuffing the resulting 'dough' into pork intestines (natural casing). At present, China is the largest producer of natural casings. Nowadays, many meat processors use artificial casings made of edible fiber; these are more evenly shaped. The stuffed fresh sausages are first incubated in a warm brine bath or temperature-controlled ripening chamber to allow a rapid acidification (pH <5.5 within 12 h) by LAB, which is followed by a gradual drying process to achieve the required moisture content.

Cucumber pickles and fermented olives are preserved in brine for several months, during which an exchange of salt, sugars, and moisture will take place. At intervals, the brine concentration is adjusted. The combination of temperature, salt, fruit sugars, and anaerobic situation favors the dominance of LAB and some yeasts.

Antimicrobial Principles Formed During Fermentation, Contributing to Food Safety

In fermented foods, the presence of high numbers of microorganisms has a profound impact on the food environment, that is, the chemical and physical conditions that may influence the opportunities for survival and growth of minority microorganisms. The latter could include contaminants that could cause food spoilage, or that could be of pathogenic character.

Figure 1 explains the impact of fermentation on the food environment. It shows that, in principle, fermentations are dynamic and that environmental conditions keep changing. Table 6 summarizes the major microbial metabolites that restrict the growth of pathogenic contaminants. In addition to the accumulation of metabolites, a depletion of available substrates (sugars and amino acids) occurs, which causes intercellular competition. This results in a gradual termination of the fermentation and a competition with spoilage-causing microorganisms.

In acidified foods, the impact of the undissociated organic acids has been well documented. Other articles in this book deal with organic acids, bacteriocins, and reuterin, and the reader is referred to them.

The inhibitory effect of alcohols, ethanol in particular, is due to an increased permeability of the plasmalemma

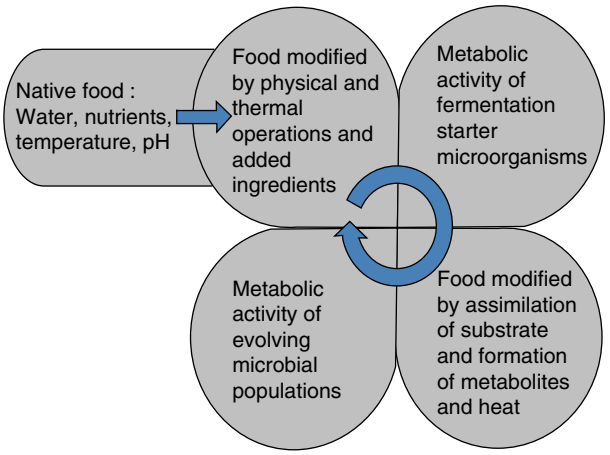


Figure 1 The impact of fermentation on the food environment.

resulting in the decrease of pH gradient and of membrane potential. Although C_2H_5OH is the most abundant alcohol, the C_6 alcohols have the highest impact on the plasmalemma.

Carbon dioxide is formed as a result of alcoholic and heterofermentative lactic acid fermentations, and decarboxylation reactions. In aqueous conditions, it is in equilibrium with HCO_3^-, which has an antimicrobial effect at pH ≤ 6 as its undissociated acid. However, at those pH ranges, the solubility of CO_2 is low, and in fermented foods, this substance has little antimicrobial impact. However, in closed packages, CO_2 in gas form has a significant antimicrobial effect when present at $>10\%$ of the gas atmosphere.

Although diacetyl has a broad antimicrobial activity, it is of little preservative importance because of its flavor, which has a low threshold value, thus making applications at higher concentrations unacceptable to the consumer.

Hydrogen peroxide is produced by microorganisms, but it is also broken down by catalase. Because of its unstable presence, there is little evidence that it has antimicrobial impact in fermented foods.

Ammonia when formed at significant levels in alkaline-fermented foods may also have an antimicrobial impact because it increases the pH to suboptimal levels. However, little evidence about the effect of ammonia has been published.

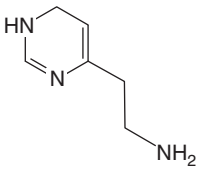
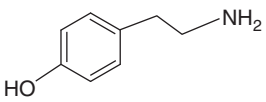
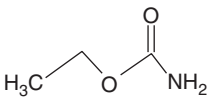
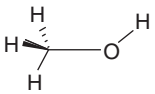
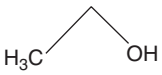
One other important effect of fermentation is the assimilation, by the functional starter microbiota, of substrates (see Figure 1), which thereby causes a competition with contaminant microorganisms. A well-documented example is

Table 6 Antimicrobial fermentation metabolites

Name	*Sensitive microbiota*	*MIC or pH levels*	*Remarks*
Lactic acid	All microorganisms	pH dependent; pK_a 5.2	Causes acidic taste
Acetic acid	All microorganisms	pH dependent; pK_a 4.75	Causes acidic taste
Ethanol	All microorganisms	At concentrations approximately $\geq$10%	Only in suitable products
CO_2	Most microorganisms	Aqueous at pH $\geq$6 Gas: at 20–50%	Can cause textural defects (bloating)
Diacetyl	Yeasts, Gram-negative bacteria Non-LAB, Gram-positive bacteria	At 200 ppm At 300 ppm	Butter flavor threshold: 2–4 ppm
Hydrogen peroxide	All microorganisms	500 ppm	Restricted use
Reuterin	Many bacteria and fungi		FDA approved
Bacteriocins, i.e., nisin	Gram-positive bacteria	50–100 ppm	Generally recognized as safe

Abbreviation: FDA, Food and Drug Administration.

Table 7 Hazardous compounds potentially encountered in fermented foods and beverages

Compounds	*Structure*	*Accepted level*	*Toxic effect*
Biogenic amines			
Histamine		100 ppm	Skin flushing, headache, abdominal cramps, and nausea
Tyramine		100–800 ppm	Tyramine pressor response (increased systolic blood pressure)
Ethyl carbamides			
Ethyl carbamate		Wines $<$15 ppb Strong liquor $<$125 ppb	Not acutely toxic, but potentially mildly carcinogenic
Alcohols			
Methyl alcohol			Highly toxic; depression, headache, and dizziness; and at 10 ml permanent blindness
Ethyl alcohol		40% strong liquor 10–15% wines to be consumed with moderation	At $>$0.1% blood alcohol content: nausea, vomiting, and intoxication; chronic: liver damage
Higher alcohols (propanol, butanol, pentanol, etc.)		Level of toxicity similar to ethyl alcohol	Headache, nausea, and hangover effect

the role of fermentative yeasts in cucumber pickle fermentation. The yeasts 'scavenge' all remaining fermentable sugars and thereby prevent growth of spoilage microorganisms.

Potential Hazardous Effects Related to Substandard Processing

Although food fermentation has a positive image because of its association with attractive sensory aspects, like all processes it also has its limitations. In this section, some of these will be shortly discussed.

Biogenic Amines

Biogenic amines (Table 7) are formed from amino acids by decarboxylation, or by amination and transamination of aldehydes and ketones. Because of the structure of their precursor amino acids, they can have either aliphatic, aromatic, or heterocyclic chemical structures. Although some play a role in

the physiology of the living cell, the formation of biogenic amines during fermentation is of most concern here. Some biogenic amines, such as histamine and tyramine, are mildly toxic. A total level of approximately 1000 ppm is associated with toxicity, and in good manufacturing practice, 100 ppm histamine, or a total of biogenic amines of 200 ppm are regarded as acceptable, no-effect levels. Prerequisites for the formation of biogenic amines include the presence of free amino acids (such as in meat, fish, cheese, and also in wine, beer, and cabbages), the ability to decarboxylate them, that is, decarboxylases (Enterobacteriaceae, Enterococci, and some heterofermentative LAB are the most relevant), and suitable conditions for the growth and metabolic activities of these bacteria (low pH and high NaCl concentrations are associated with higher levels of biogenic amines). Preventive measures include the pasteurization of cheese milk and good hygienic practice, including the use of starters selected for low decarboxylase activity.

Ethyl Carbamate

Ethyl carbamate (EC; Table 7) is formed from ethanol with urea or carbamic acid. It is found in several fermented foods, especially those in which yeasts and LAB have been active and some ethanol was formed. Urea and carbamic acid can be formed from the deamination of arginine, a pathway found in, for example, LAB. This pathway serves to generate energy and can be instrumental in the homeostasis of intracellular pH levels. The reaction leading to the formation of EC is favored by increased temperatures, and this is reflected in the higher EC levels in products such as distilled liquors (saké and whisky) and roasted products (toasted bread). Although in some countries maximum tolerated levels of EC are enforced, its toxicity is quite low compared with the health risks of the corresponding consumption of strong alcoholic liquors.

Alcohols

Methyl alcohol is highly toxic. It is formed by the enzymatic degradation of pectic polysaccharides, mainly originating from stone fruits, such as cherries, apples, plums, and pears. In pectins, the polygalacturonate backbone is methylated, and during processing, the endogenous enzymes, such as pectin methyl esterase, will release methanol from pectins. In regular fruit wines, the levels of methanol are too low to be of any concern. However, for a number of popular liquors distilled from these fermented fruits, such as kirsch, apple jack, slivovitch, and Williams, it may have safety consequences.

Although enjoyed by many, ethyl alcohol, formed by alcoholic fermentation through reduction of pyruvate, is also toxic. Excessive use leads to intoxications and if chronic, to liver cirrhosis.

Higher alcohols, such as propanols, butanols, pentanols, and their branched structures, are formed by the deamination and reduction of amino acids in yeast cells. Fermented products containing appreciable levels of free amino acids include beers from barley, maize, and other cereals in which proteins have been degraded. If such brews are used as an ingredient for distilling of, for example, whisky, their concentration may increase as a result of the distillation process. Although modest levels of higher alcohols contribute to the flavor of whiskies, in other distilled products, such as vodka, they are associated with unwanted off-flavors. In principle, the toxicity of higher alcohols is similar to that of ethyl alcohol, whereas in some reports they have been associated with the 'hangover effect.'

Hazards of Microbiological Nature and Control Measures

Inadequate processing, such as lack of pasteurization of vulnerable ingredients, poor hygienic practices, and use of low-grade ingredients, may result in fermented products that are contaminated with microbial toxins, or in which pathogenic microorganisms survive and may be the cause of foodborne infections.

Because the respective compounds and microorganisms are discussed in detail in other articles, a short summary of hazards and measures to control them will be presented here.

Raw milk, fish, and meat are known carriers of pathogenic bacteria, parasites, and viruses. Although some traditional fermented products are claimed to be of better quality when prepared from raw ingredients, experience has shown that it is wise to preheat (pasteurize) such ingredients before or after fermentation.

Poor hygiene may lead to postprocessing contamination, mostly with pathogenic and/or spoilage-causing bacteria. Although some fermented products have inherent antimicrobial properties (especially the low-pH products), many other products offer good chances for survival. In traditional fermented raw milk, the levels of Enterobacteriaceae are reduced only if pH values lower than 4.5 are obtained. Even despite this acid-sensitivity of most Enterobacteriaceae, it must be noted that some strains of *Escherichia coli*, for example, *E. coli* O157, as well as viruses are not adequately affected by acidity. Well known are the outbreaks caused by *E. coli* contaminated Brie cheese, among others. Also, several fatal cases of infections with *Listeria monocytogenes* and *E. coli* have been associated with the consumption of similar raw-milk white-mold ripened cheeses, such as Vacherin Mont d'Or. Pasteurization of cheese milk and strict hygiene are therefore control measures to minimize these hazards.

In addition to raw milk, other uncooked ingredients may cause safety problems. For instance, the custom of fermenting raw pork meat in South East Asia (e.g., Thailand and Vietnam) has caused infections with parasites, such as *Trichinella*. Such parasites are not or hardly killed by acid or salt: The only adequate way to eradicate them is heating. Therefore, the fermented meat must be cooked well before consumption. In a similar way, the consumption of raw (uncooked) fishery products may lead to foodborne infections with *Vibrio parahaemolyticus*, a typical seafood-associated pathogen.

Another hazard associated with raw (uncooked) water, shellfish, vegetables, etc. are viruses, such as norovirus and rotavirus. Cooking food before consumption is the only safe method to inactivate them.

Table 8 Major food safety hazards in fermented foods and beverages, with control measures

Hazards	*Control measures*
Chemical hazards	
Biogenic amines	Control lysine decarboxylase activity by, for example, blanching and avoidance of Enterobacteriaceae by GMP and hygiene
Ethyl carbamate	No measures
Methyl alcohol	Optimize separation of distilling units to avoid methyl alcohol collection from fermented stone fruits
Higher (fusel) alcohols	Optimize temperatures and free amino acid content in alcoholic fermentation to reduce formation, and in distilling optimize separation capacity of column
Microbiological hazards	
Botulin	Prevent outgrowth of *C. botulinum* spores
Staphylococcal enterotoxins	Prevent growth of *S. aureus*
Mycotoxins	Prevent growth of mycotoxigenic fungi and remove molded ingredients
Enterobacteriaceae (*E. coli*, *Salmonella*, *Shigella*, etc.)	Pasteurization; pH <4.4; a_w <0.95
L. monocytogenes	Pasteurization; pH <4.4; a_w <0.92
V. parahaemolyticus	Pasteurization; pH <4.8; a_w <0.94
S. aureus	Pasteurization; pH <4.0; a_w <0.83
C. botulinum	Botulinum 12D cook; pH <4.5 and/or apply sodium nitrite to avoid germination of surviving spores
Viruses	Cooking
Parasites	Freezing (not always adequate) and cooking

Abbreviations: a_w, water activity; GMP, good manufacturing practice.

In addition to the heat treatment of raw (uncooked) ingredients, some other process factors can contribute to the safety of fermented foods, such as:

- rapid (within 12 h) acidification to pH <4.5 by LAB – sometimes supported by acidulants, such as glucono-δ-lactone – supports their competition with other microbiota. In particular, the growth or survival of most Enterobacteriaceae is minimized. *Staphylococcus aureus* may still grow, although due to competition pressure from starter microbiota, it does not produce its enterotoxins;
- salt (NaCl) and nitrite used in meat curing are essential, not only for taste reasons but also to especially avoid the germination, outgrowth, and botulin formation by *Clostridium botulinum*;
- reducing the water activity (a_w) by dehydration, or by addition of sugar, salt, or other solutes; and
- hermetic sealing to prevent oxygen entry will reduce oxidative quality defects, but especially it will prevent mold growth and associated risks of mycotoxin formation in products that are cured or stored for prolonged periods of time.

Of special concern is the hazard of mycotoxins, mostly because these occur at low concentrations and lead to chronic mycotoxicoses only after long-term ingestion.

Mycotoxins may occur in fermented foods if the ingredients used were contaminated before processing. Most mycotoxins (in particular aflatoxins and ochratoxins) cannot sufficiently be degraded or detoxified during heating and fermentations to expect safe food from unsafe ingredients. However, patulin (frequently found in apple juice) has been shown to be degraded by lactic fermentation involving *Lactobacillus plantarum*.

With regard to the quality of starters used for fermentation, it has been observed that in some traditional mold-fermented products, such as sausages and country-cured hams, some strains of, for example, *Penicillium roqueforti* and *Penicillium camemberti* used as starters in the process, were actually able to produce mycotoxins when grown in the laboratory as pure cultures. Although these are very different cultivation conditions than in, for example, meat or cheese, it is an indication that much care should be taken with the selection of starter strains. Therefore, food legislation should require the use of selected starter strains selected for their inability to form mycotoxins or other toxic substances.

In Table 8, an overview is provided of the major hazards mentioned and measures to control them. Which measures will be feasible will depend on the type of food and the market preferences.

In conclusion, fermentation has much to offer as a technology that renders foods attractive, digestible, and safe. However, there remain several aspects that require research for further development, and the technology has its limitations. Research is required in order to (1) develop starter microorganisms that are both safe and beneficial for human health, (2) selection of starter microorganisms that can kill or compete with disease-causing microbes, and (3) adaptation of scientific knowledge to application in regions or situations that lack infrastructure in order to harness fermentation as a beneficial technology to public health.

See also: Disciplines Associated with Food Safety: Food Microbiology; Food Virology; Parasitology. Food Technologies: Biopreservation. Foodborne Diseases: Overview of Emerging Food Technologies. Mycotoxins: Mycotoxins – General. Processing Contaminants: Biogenic Amines

Further Reading

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FOOD TECHNOLOGIES

Food Irradiation

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Glossary

Dose (absorbed) The dose (absorbed) is the amount of energy absorbed per unit mass of irradiated ;material.
Dosimetry The measurement of radiation quantities, specifically absorbed dose, and absorbed dose rate.
D_{10}-value Amount of radiation required to reduce the population of specific (micro)organisms by 90% (one $\log_{10}$ cycle) under stated conditions.
Electron accelerator A device for imparting high kinetic energy to electrons.
Electron volt (eV) A unit of energy. One electron volt is the kinetic energy acquired by an electron in passing through a potential difference of one volt in a vacuum.
Ionizing radiation Such forms of radiation which have sufficiently high energy to cause ionization (in food processing: sgamma-rays, X-rays, and accelerated electrons). Ionization is the creation of ions by expulsion of orbital electrons from atoms.
Unit of absorbed dose The unit of absorbed dose is the Gray (Gy). (1 Gy is equal to 1 Joule kg^{-1} absorbed energy.)

Background

What is Food Irradiation?

Food irradiation is a process that exposes food to a prescribed amount of ionizing radiation. Three different kinds of ionizing radiation are applicable for food irradiation processes:

- gamma rays from the radionuclides cobalt-60 (^{60}Co) or, less commonly, cesium-137 (^{137}Cs),
- X-rays ('bremsstrahlung') generated from machine sources (electron accelerators with converters) operated at or below an energy level of 5 MeV (US 7.5 MeV), and
- electron beams produced by electron accelerators operated at or below an energy level of 10 MeV.

Even if these ionizing radiations are different, they have the same chemical and biological effects. None of these radiations has sufficient energy to induce radioactivity.

Whether every mass element of a food requires irradiation will depend on the purpose of the treatment. In some cases, irradiation of the surface will suffice. In other cases, the entire food must receive at least the minimum dose.

Because irradiation causes practically no temperature rise in the food processed, it is in effect a nonthermal technology that may even inactivate microorganisms in frozen food without thawing it. An effective radiation dose can be delivered through most standard food-packaging materials, including those that cannot withstand heat. This means that irradiation can be applied to hermetically sealed products without the risk of recontamination or reinfestation of properly packaged foods. Some food products may have to be irradiated under special conditions, for example, at low temperature or in an oxygen-free atmosphere. Others may undergo a combination treatment, using, for example, both radiation and heat.

Processing by irradiation, either alone or in combination with other treatments, offers some advantages over conventional methods of food processing. They are:

- Reduction of the need for some chemical treatments.
- Reducing contamination of foodborne pathogens or spoilage organisms in such a way and efficiency that may be difficult to achieve with other techniques.
- Preservation of food in the fresh state for extended periods in a quality-friendly way.
- Use of less energy for processing and storage when compared with other food-processing methods.
- Treatment without changing the identity of a food.

Main Biological Effects of Ionizing Radiation

One of the main reasons for the use of ionizing radiation is to inactivate living organisms that cause spoilage and other forms of quality deterioration or are a hazard to the health of the consumer. The application potential of ionizing radiation for food products is based mainly on the fact that ionizing radiation damages DNA and very effectively inhibits DNA synthesis and further cell division. Damage to the genetic material can also occur indirectly because the interaction of radiation with water molecules results in the production of reactive molecules such as hydroxyl radicals, hydrogen peroxide, and hydrogen atoms, which induce on the adjacent

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genetic material similar effects to those resulting directly by radiation. Therefore, not only microorganisms but also insect gametes and plant meristems can be prevented from reproducing, thus securing safety and quality attributes (e.g., stability) of foods. The amount of radiation energy used to bring about the control of these organisms varies according to the radiation resistance of the particular organism, which is often specific to the species level, and to the numbers or load of organisms present.

Applications of Food Irradiation

Typical Dose Requirements

The actual dose of radiation employed in any food-processing application represents a balance between the amount of radiation needed to produce a desired result and the amount of radiation the food can tolerate without suffering unwanted changes of sensorial or technological properties. Therefore, detailed requirements must be considered as specific for a given food. The main purposes of food irradiation and examples of recommended dose ranges are given in Table 1.

To facilitate regulation of food irradiation, it has been proposed to implement advisory technological dose limits.

After the concept of 'overall average dose' has been abolished, it would be prudent to provide a frame work for regulatory dose setting, collecting from practical experiences the minimum dose values (Figure 1) needed to achieve an effective treatment and the maximum dose values below which any deterioration of the food quality can be avoided. The regulatory authorities accepting such a table could be sure that any radiation processing of food within those advisory dose limits would be safe and wholesome for the consumer. Also in international trade, the adoption of such table could facilitate export and import of irradiated products.

Inhibition of Sprouting

Radiation treatment at low doses inhibits the sprouting of potatoes and yam tubers, onions, garlic, and ginger, because cell division, elongation of growing points, and thereby bud growth are inhibited. The dose required to inhibit sprouting of potatoes and yams is 0.08–0.14 kGy; for ginger is 0.04–0.10 kGy; and for onions, shallots, and garlic, 0.03–0.12 kGy. The appropriate dose within these ranges depends on the variety and other properties of the product.

For potatoes, mechanical damage occurred during harvest and other handlings may lead to increased rotting of potatoes, due to microbial invasion of the tissue at points of injury. Thus, careful sorting and a 2–3 weeks time delay after harvesting before application of radiation treatment is required to allow for healing of such mechanical damage. However, with some varieties, cooking darkens irradiated potatoes more as opposed to nonirradiated ones; these unwanted effects can again be largely prevented by careful handling of the product before and after radiation treatment, including careful sorting, curing, and proper storage temperature. It has also been shown that in some situations increased after-cooking darkening may be related to the use of certain fertilizers on the field and agricultural practices need to be adapted to avoid such effect.

Irradiation of onions is most effective as a sprout inhibitor when the treatment is carried out in the dormancy period, preferably within 1 month of harvest and no later than 2 months postharvest. When inner buds of bulbs have already started to grow before radiation treatment, their browning may still occur due to radiation damage.

Delay of Ripening and Aging of Fruits and Vegetables

Exposure to a low dose of radiation delays the ripening or senescence of some fruits and vegetables, thereby extending their shelf life. Such positive effects were discovered in the course of studies on the role of radiation in controlling microorganisms. A delay in postharvest ripening can occur only in climacteric fruit. Senescence, or physiological breakdown, may be delayed by irradiation in both climacteric and nonclimacteric fruit during postharvest storage. The magnitude and even the direction of such changes depend on the radiation dose and the state of ripeness at the time of treatment. A measurable extension of shelf life may be obtained with doses of 0.3–1.0 kGy. This level of exposure will increase the shelf life of mangoes by

Table 1 Main purposes of food irradiation and examples of recommended dose ranges

Purpose and effects	*Dose range (kGy)*
Inhibition of sprouting stored tubers, roots, and bulbs	0.05–0.15
Prevention of postharvest losses by destruction of insects in stored cereals, fresh and dried fruits, nuts, oilseeds, and pulses, or phytosanitary (quarantine) treatment for insect pests infesting fresh fruits and vegetables	0.15–1
Delay of afterripening and senescence of certain fruits and vegetables	0.2–1
Shelf-life extension of fruits and vegetables, meat, poultry, fish, and ready meals by reduction of microorganisms that cause spoilage	0.5–3
Inactivation/destruction of various foodborne parasites	0.3–6
Prevention of foodborne illness by destruction of nonsporeforming pathogenic bacteria (e.g., *Salmonella*, *Campylobacter*, *Listeria*) in fresh or frozen foods	3–7
Reduction in viable counts of microorganisms in spices and other dry ingredients to minimize contamination of food to which the ingredients are added	5–10
Production of microbiologically shelf-stable vacuum-packaged meat, poultry, and ready-to-eat meals by heat-inactivating of their tissue enzymes and destruction of microorganisms, including bacterial spores, by irradiation in deep-frozen state.	up to 50

Advisory technological dose limits (kGy)

Food classes

Purpose	Fresh fruits and vegetables Bulbs, roots, tubers 1:	Cereals, dried fruits etc. 2:	Fish and seafood 3:	Raw poultry and meat 4:	Dry vegetables, spices etc. 5:	Dried, of animal origin 6:	Miscellaneous 7:	8:
Sprout inhibition	0.2	-	-	-	-	-	-	-
Ripening delay	-	1.0	-	-	-	-	-	-
Insect disinfestation	-	1.0	1.0	-	-	1.0	1.0	-
Quarantine measures [1)]	-	1.0	-	-	-	-	-	-
Shelf-life extension	-	2.5	-	3.0	3.0	-	-	-
Pathogen reduction [2)]	-	-	-	5.0	7.0	>10	-	-
Parasite elimination	-	-	-	2.0	3.0	-	-	-
Other	-	-	-	-	-	-	-	>10

Class 8: Including honey, space food, hospital meals, military rations, liquid egg, spices, thickener

[1)] minimum doses for particular pests, fruit flies > 0.15 kGy

[2)] minimum doses to ensure hygienic quality

from:

FAO/IAEA Workshop on "Guidelines for preparing and adapting harmonized legislation on food irradiation", Accra, Ghana, 21–25 October 1996

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Federal Research Centre for Nutrition, Karlsruhe Institute of Process Engineering, 2002

Figure 1 Advisory technological dose limits (kGy) for different classes of food.

approximately 1 week and that of bananas by up to 2 weeks. Maturation of mushrooms and asparagus after harvesting can be retarded with doses in the range of 1.0–1.5 kGy. Irradiation of (field) mushroom had been the first commercial application in the Netherlands; later on this treatment was no longer used, as irradiation essentially retarded the cap-opening but did not significantly preserve the overall quality. Likewise, irradiation of asparagus did not become commercial as the consumers preferred to buy it immediately after harvest.

Insect Disinfestation and Phytosanitary/Quarantine Treatment

Radiation at relatively low doses (up to 0.5 kGy) inactivates or sterilizes all of the developmental stages of common insect pests of grain, including insect eggs deposited inside grains. The effective radiation dose must be determined for a specific insect species, food product, and set of conditions.

Fresh and dried fruits, vegetables, and nuts are also liable to insect attack, and some of these products, especially dried fruits, cannot be effectively disinfested by either chemical treatments or physical means other than irradiation. Application of 0.2–0.7 kGy to products that have been suitably packaged to prevent reinfestation eliminates the insect problem in dried fruits and vegetables and in nuts. Cocoa beans, which are traded in bulk quantities, can also be disinfested without impairing the technological or sensory quality. The same technique could drastically reduce losses of dried fish, an important source of protein in many developing countries.

Radiation disinfestation can contribute significantly to improving trade in certain tropical and subtropical fruits, such as citrus fruit, mangoes, and papayas. Irradiation could be used in place of chemical fumigation, and it affords a residue-free means of preventing the introduction of harmful insects. As such, radiation treatment may offer a viable alternative to fumigation to satisfy the quarantine regulations in a number of countries. Fruit flies, for example, and even the weevil that lodges deep inside the seed of mango can be controlled by irradiation. (Note: the mango weevil cannot be reached by chemical or heat treatment, there is no alternative to irradiation.) Irradiation is different from all other quarantine treatments that have been used commercially in one key aspect: irradiation does not provide significant acute mortality (within 48 h) at doses tolerated by fresh agricultural commodities. However, irradiation is the fastest quarantine treatment available. The measure of efficacy of irradiation quarantine treatments for fruit flies is prevention of emergence of adult flies.

Controlling Foodborne Parasites

Irradiation inactivates foodborne parasitic organisms that are responsible for both human and animal diseases. Such precautionary treatment would, in particular, be beneficial where such food is traditionally eaten raw. The parasitic roundworm, *Trichinella spiralis*, which causes trichinosis and may be found in pork, is inactivated by radiation at minimum dose of 0.15 kGy. Other parasites, including tapeworms in pork and beef, as well as the protozoon responsible for toxoplasmosis and various

flukes that infest fish are rendered noninfective by low-dose radiation treatment. The doses used to treat food to reduce microbial load is sufficiently high to control many types of parasite as well. However, the larvae of *Anisakis* spp. are exceptionally resistant to irradiation. For example, *Anisakis simplex* larvae require doses as much as 6–10 kGy for inactivation.

Control of Foodborne Microorganisms

Important uses of food irradiation are for inactivating microorganisms that cause spoilage or other aspects of quality deterioration of the food and to destroy foodborne nonsporeforming pathogenic bacteria, such as *Salmonella* spp., *Campylobacter* spp., *Listeria* spp., *Escherichia coli* O157:H7, and *Yersinia* spp.

The amount of radiation needed to inactivate these microorganisms depends on the radiation tolerance or resistance of the target microorganism, and their number in the particular volume or mass of food to be treated. Relatively low or medium doses are needed in this application as the target is to reduce the microbial load below the infective level. The effect of ionizing radiation on microorganisms is also affected by the environmental conditions under which they are irradiated. Such factors include among others: temperature, water activity, pH, chemical composition of food, and gaseous environment.

Irradiation, for instance, is beneficial in reducing the microbial contamination of food characterized by a low water activity such as dry food ingredients. For such ingredients radiation treatment may improve the safety and storage properties of foods prepared with them. Spices, dry vegetable seasonings, herbs, protein concentrates, and commercial enzyme preparations used in the food industry are very often heavily contaminated with spoilage and pathogenic microorganisms, and can be decontaminated with radiation doses of 3–10 kGy without adverse effect on their flavor, texture, or other properties. Dried commodities are more resistant than fresh foods to the organoleptic effects of ionizing radiation because their low water content suppresses most of the indirect effects of irradiation. Therefore, from the omnifold possibilities of food irradiation, radiation decontamination of dried food ingredients, particularly spices, herbs, and vegetable seasonings, has the most immediate application potential, and it has been commercially implemented in numerous countries. Notably, microorganisms that survive this treatment become more susceptible to subsequent processing.

Food irradiation may successfully be used for control of foodborne diseases by radiation decontamination of specific foods. Radicidation is the term for the reduction in the number of specific viable nonsporeforming pathogenic microorganisms (other than viruses), thereby improving the hygienic quality and reducing the microbiological risk of foods. Thus, radiation could play an equally important role in the processing of critically important solid foods as does heat treatment (pasteurization) in the processing of fluid milk and fruit juices.

Extensive experience has demonstrated that radiation treatment under normal industrial conditions, at a dose that does not produce unacceptable changes in the food product, will destroy pathogenic nonsporing bacteria in red meat, poultry, and fish. A dose of 2–7 kGy is sufficient to control such foodborne pathogens, for example, in frozen meats, poultry, egg pulp, shrimp, and frog legs.

Irradiation as a Critical Control Point in Risk Management

On the basis of the facts above, food irradiation could and should play a role as an effective critical control point in the framework of application of the hazard analysis critical control point (HACCP) risk management system. This HAACP-based approach of food safety is of particular importance in the production of foods which are to be marketed raw or minimally processed, such as poultry, meat, meat products, fish, seafood, and fruits and vegetables. In these products, pathogens must be eliminated or reduced to acceptable (i.e., below the infective) levels, at the primary processing and, therefore, not be allowed to reach the home or institutional kitchen. When integrated into an overall food safety management program that includes good agricultural, manufacturing, and hygienic practices and HACCP, depending on the dose applied, food irradiation can contribute to improved consumer safety.

Extension of Shelf Life of Foods by Reduction of Populations of Spoilage Microorganisms

Radurization is the term for substantial reduction in the number of spoilage microorganisms, thereby extending the shelf life of a food. Most food-spoilage microorganisms are killed at doses of less than 5 kGy. Thus, the shelf life of many fruits and vegetables, meat, poultry, and fish and other fishery products can be considerably prolonged – certainly doubled – by treatment with relatively low doses of radiation that do not alter flavor or texture combined with storage under refrigeration conditions.

With higher doses, approaching 5 kGy, the normal spoilage organisms are often eliminated, whereas some more resistant but metabolically less active species, for example, *Moraxella* spp., lactic acid bacteria, and yeasts, may remain. During chilled storage, this surviving microbiota grows less rapidly than the normal spoilage biota. As with unirradiated foods, the nature and behavior of the spoilage biota is strongly influenced by the nature of the packaging. In anaerobic packages, lactic acid bacteria and yeasts may predominate.

Radiation Resistance Parameters

Table 2 shows ranges of decimal reduction doses (D_{10} values) of the most important nonsporeforming pathogenic bacteria determined in various atmospheres and solid foods. The data have been summarized from a large amount of publications.

Bacterial spores are more resistant to irradiation than vegetative bacteria. *Clostridium botulinum* type A and B spores are particularly resistant. Owing to their usually nonlinear survival curve, estimating a lethality parameter such as the minimum required dose (MRD) to achieve n log cycles reduction can be more meaningfully calculated. This approach has been used to calculate the dose required to achieve a 10^{12} reduction in certain foods under specific conditions (see the section on Radiation Sterilization of Enzyme-Inactivated High-Moisture Foods).

Table 2 D_{10} values (kGy) of some foodborne nonsporeforming pathogenic bacteria[a]

Bacteria	*Nonfrozen food*	*Frozen food*
Vibrio spp.	0.02–0.14	0.04–0.44
Yersinia enterocolitica	0.04–0.0.21	0.20–0.39
Campylobacter jejuni	0.08–0.20	0.18–0.32
Aeromonas hydrophila	0.11–0.19	0.21–0.34
Shigella spp.	0.22–0.40	0.22–0.41
Escherichia coli (iactncl. O157:H7)	0.24–0.43	0.30–0.98
Staphylococcus aureus	0.26–0.57	0.29–0.45
Salmonella spp.	0.18–0.92	0.37–1.28
Listeria monocytogenes	0.20–1.0	0.52–1.4

[a]Courtesy of Wageningen Academic Publishers, The Netherlands.

Similarly, nonlinear survival curves with an initial shoulder may often be found when plotting $\log_{10}$ numbers of survivors of fungal propagulae against dose. For this reason, the radiation sensitivity of microscopic fungi may also be expressed as the minimum required or effective dose rather than as a D_{10} value. The effective dose is then related to the size of the initial population of these microorganisms.

D_{10} values for viruses generally tend to be high, typically 2–7 kGy. Thus, irradiation doses below 10 kGy often will not give an adequate reduction in their numbers depending on the numbers present in the food to be treated. However, viruses can be well inactivated by heat so the combination of heating with irradiation can possibly be used successfully (see the section on Combination Processes Involving Food Irradiation).

Preformed bacterial and fungal toxins are not affected at the doses normally used in food irradiation.

Combination Processes Involving Food Irradiation

The benefit of treating food with a combination of irradiation and heat, low temperature storage, modified-atmosphere packaging, or conventional preservatives is recognized. Rational combinations of ionizing radiation and other antimicrobial factors may allow the use of lower radiation doses. The overall effectiveness of combination treatments will depend on the target organisms present in the product and the specific sequence of the individual treatments. A combination of mild heat treatment (immersion in hot water), low-dose irradiation, and proper packaging may be successfully applied to those fruits that are sensitive to higher radiation doses. Particularly, irradiation can be involved in apparently synergistic combinations with other preservative factors to further increase antimicrobial treatment efficiency, as such improving microbiological safety and keeping quality of foods.

Radiation treatment offers a great potential for improving microbiological safety of minimally processed foods. 'Minimally processed' is an equivocal term that is applied to such different types of products as precut, prepackaged fresh produce, or fresh meat for short refrigerated shelf life, and mildly cooked or pasteurized foods (meals or meal components) that can be stored under refrigeration for more than a week. Many food processors also take advantage of using modified atmosphere packaging (MAP). It is frequently used, for example, for precooked chilled food for catering and retail sale. Such foods aimed for refrigerated storage may enhance its microbiological safety by using a quality-friendly, nonthermal microbicide treatment such as irradiation. Special versions of cook–chill items are the 'sous-vide' foods. These foods are cooked in vacuum packaging and usually receive only a mild heat treatment, and rely heavily on vacuum packaging and refrigeration for preservation. The combined effect of irradiation and sous-vide cooking was investigated with promising results with respect to improvement of the microbiological safety and keeping quality. Postprocess recontamination, for instance, of heat treated ready-to-eat (RTE) products before packaging, is a serious safety concern, especially for pathogens whose infective doses are low (e.g., *Salmonella* spp., *Shigella* spp., or *E. coli* O157) and where there is a potential for growth. Low-dose irradiation could play a role in elimination of such postprocessing contaminants.

Radiation Sterilization of Enzyme-Inactivated High-Moisture Foods

Radappertization is the term used to describe a specific combination process that involves application of ionizing radiation to prepackaged, enzyme-inactivated, deeply frozen foods at radiation doses sufficient to reduce the number and activity of viable microorganisms to such an extent that in the absence of postprocessing contamination no microbiological spoilage or toxicity of microbial origin occurs, no matter how long and under what conditions the food is stored (radiation sterilization). A mild heat treatment is sufficient for enzyme inactivation. The dose requirement for radappertization is determined by the most radiation-resistant microorganisms that may be associated with the enzyme-inactivated food. For nonacid, low-salt foods this is *Clostridium botulinum* type A spore. By analogy with thermal processing (canning), the microbiological safety of the radappertized foods is based on a 12-D reduction in the viability of botulinal spores. For such foods the required dose is approximately 45–50 kGy. The irradiation of the product is performed in deep-frozen condition to prevent sensory changes and significant vitamin losses when applying the high radiation dose. Radiation sterilization has been successfully applied, for instance, for meals for army personnel and astronauts as well as for meals prepared for hospitalized patients whose immune systems have been suppressed by disease or therapy. The safety, palatability, and nutritional quality of meals for such patients can be improved if radiation sterilization is used instead of thermal sterilization. It seems reasonable that this application may prove useful among other population groups, such as air travelers, and the young and old residents of nursing facilities.

Quality Matters

The Necessity of Good Manufacturing and Hygienic Practices

One should keep in mind that the quality of irradiated foods, as is the case for any other preserved food, is dependent on the quality of the original material. To obtain good results,

therefore, good hygienic practices and good manufacturing practices are essential. The longest shelf life may be obtained if the quality of the raw material is good and proper hygienic conditions are maintained. In certain cases, the use of more thorough inspection and sorting to remove substandard quality materials before treatment (or treatments such as washing or blanching) can be used to reduce the radiation dose required and to produce a high-quality product. The benefits of irradiation should never be considered as a substitute for product quality or as compensation for poor handling and storage conditions.

Dose Limitations, Unwanted Changes of Sensorial or Technological Quality

Depending on the absorbed dose level, irradiation can cause sensory changes (e.g., off-flavors, especially in foods of animal origin, such as dairy products). In fresh fruits and vegetables, radiation may cause softening and increase in the permeability of tissue. These effects may limit the permissible dose because they are often accompanied by accelerated spoilage if the product becomes contaminated by microorganisms after the irradiation treatment. However, because irradiation slows the rate of ripening of fresh fruits and vegetables, properly stored and packaged products remain in a usable condition considerably longer than they would be without radiation processing. The extent of radiation-induced organoleptic changes in foods is dose related and there seems to be a threshold dose below which these changes are not detectable. For this reason, the selection of dosage, and, often, the decision to employ supplementary processing to contribute to the intended result are critical factors. Environmental conditions prevailing during the growing phase may also strongly affect the dose–response of the product from both textural and organoleptic points of view.

Technical Aspects

Irradiation Facilities

Food irradiation is carried out in specific containment areas applying the necessary shielding to prevent the escape of ionizing radiation and to avoid exposure of personnel. In automated irradiation facilities, conveyors or other carrier systems move the products to be irradiated at a predetermined speed or rate to deliver the necessary dosage. With the exception of Florida and Hawaii, USA, there are no irradiation facilities devoted exclusively to the processing of food; in general, food irradiation is conducted in commercial irradiation facilities treating a wide variety of other nonfood products.

Gamma rays emitted by radioactive sources (^{60}Co or ^{137}Cs) are highly penetrative and their energy levels are constant. Electron beams produced by electron accelerators are much less penetrative. Photons of high-energy X-rays produced when an electron beam hits specific heavy metal targets (the X-ray converter) are also able to treat large packages of food (e.g., pallet boxes). However, unlike photons emitted by the above radionuclides, X-rays have a photon energy spectrum. Both electron beams and X-rays are produced by machine sources which can be switched off if they are not in use.

Irradiation facilities can be operated as a part of the food-processing operation such as a processing line or of handling lines in storehouses. They can, alternatively, be operated as service facilities on a fee basis. Examples of a high-capacity commercial cobalt-60 irradiation facility and an electron-beam machine facility are illustrated by Figures 2 and 3, respectively.

The International Atomic Energy Agency (IAEA) maintains a Food Irradiation Facilities Database, containing the facilities' location, contact details, type of irradiators, etc.

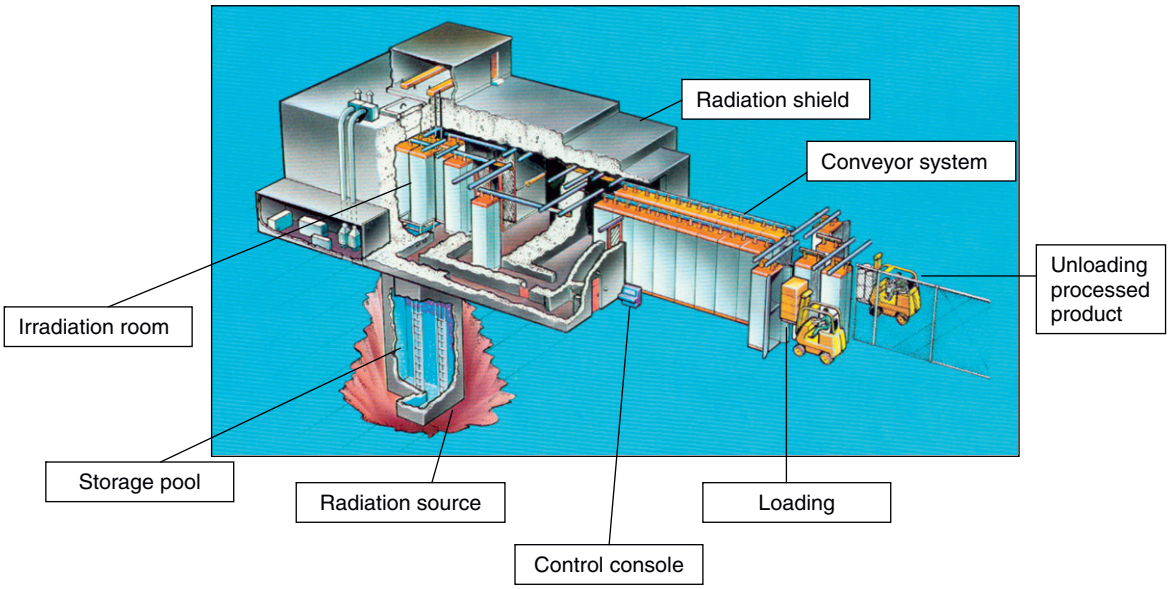

Figure 2 A commercial scale, high-capacity, and carrier-type cobalt-60 irradiation facility. Courtesy of Wageningen Academic Publishers, The Netherlands.

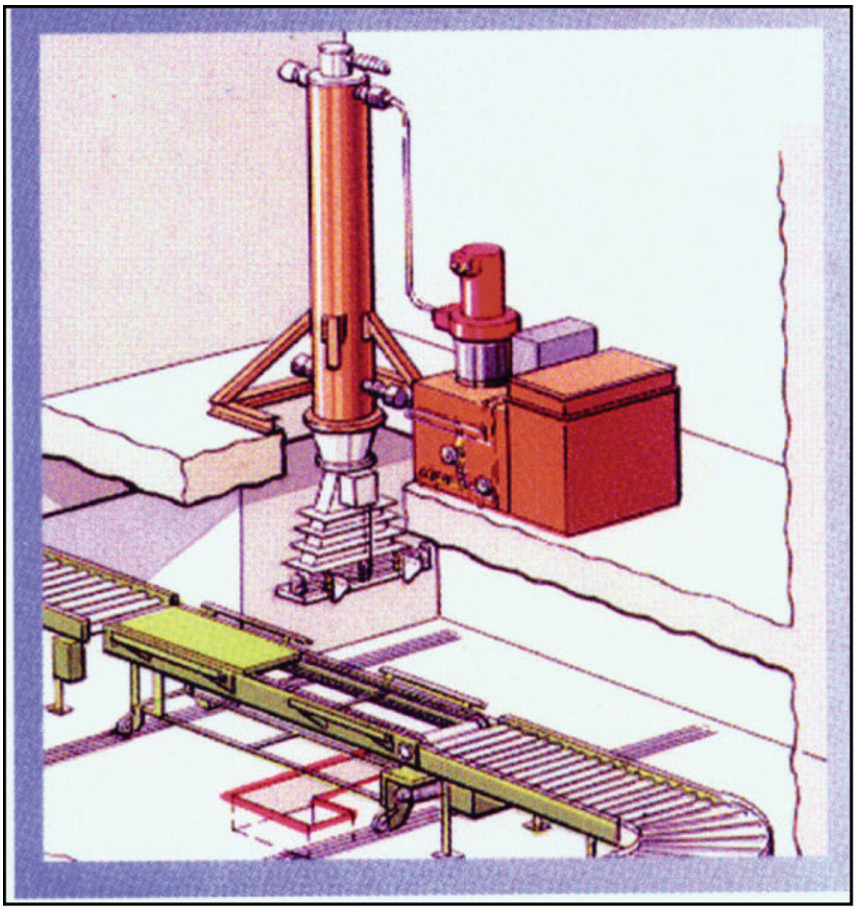

Figure 3 An electron-beam facility based on a linear accelerator which is placed beneath the product delivery conveyor. Courtesy of Wageningen Academic Publishers, The Netherlands.

Food Irradiation Process Control and Detection of Irradiated Foods

Process control of food irradiation involves monitoring absorbed radiation dose, radiation dose distribution, machine parameters such as voltage and beam current as well as speed of the conveyor or carrier system.

Chemical and physical changes in foods induced by irradiation are very small and mostly nonspecific to this treatment. Therefore, food irradiation results in a product that is virtually indistinguishable from unprocessed food by the consumer. However, a number of ingenious chemical, physical, and biological methods were developed and adopted, for example, by the European Committee for Standardization (CEN) to assist regulating authorities in determination whether a particular food sample has been irradiated or not. Several such methods have also been adopted by the Codex Alimentarius.

Detection of irradiated food products on the marketplace also serves the enforcement of labeling regulations; most countries require clear information to inform the consumer. Mostly, labeling phrases as 'irradiated' or 'treated by ionizing radiation' are required; France has chosen 'ionized.' Codex Alimentarius proposes the use of the Radura logo (Figure 4) some countries require different design and varied color, however, the EU directives exclude the use of the logo. The logo is considered to give the consumer an easily recognized symbol for the information about the treatment by ionizing radiation.

Figure 4 The Radura logo.

Irradiation Costs/Economic Feasibility

Economics of food irradiation are one of the key factors for utilization just like in other technologies, and the costs

involved in the process must be less than the benefits that can be derived from it. Economic feasibility is greatly affected by local circumstances. In general, however, one can state that the unit cost of irradiation would be in the range of a few percent of the value of food treated, and in many cases they are in the same order of magnitude as those of other preservation or decontamination methods. As with any food process, many items of costs make up the total costs and many variables exist that affect the actual cost for a practical application, for example, capital cost (building and radiation source), source efficiency, plant utilization, cost of labor, overheads, rate of depreciation, and interest. Irradiation has relatively low labor cost. The input requirement for conventional energy is low. The capital cost, however, is relatively high, especially when an economically effective scale of operation is involved. Evidently, there must be a product volume sufficient to support the capital cost, and the unit cost of irradiation decreases rapidly with increasing plant utilization.

There are several arguments about the costs of radiation processing. As it relates to microbiological safety and the protection against food-mediated infections, estimates of authorities see such extra costs much lower than the costs of illnesses and even death from the unirradiated product; however, such advantages in costs would mainly be counted in relation to public budgets for health issues. Practical experience in the marketplace proves that the irradiated product (e.g., hamburgers or exotic fruit) has an insignificant increase in the price.

Legislation of Food Irradiation

Wholesomeness (radiological safety, toxicological innocuity, nutritional adequacy, and microbiological safety) of irradiated food has been carefully evaluated by an unprecedented width of research and testing. Specific research programs and international projects by specialized agencies of the UN, such as the Food and Agriculture Organization (FAO), the IAEA and the World Health Organization (WHO) have been important partners in assisting progress. The scientifically acceptable evidence described in detail in a separate article in this Encyclopedia supports the safety of irradiated food for consumption. Considering the potential role of food irradiation in the safety of food supply, the WHO formed a positive attitude toward the utilization of food irradiation. For example, in collaboration with the FAO, the WHO published in 1988 a booklet entitled 'Food Irradiation – a technique for preserving and improving the safety of food'. In its 'WHO Golden Rules for Safe Food Preparation,' the rule No.1 ('Choose foods processed for safety') states "…always buy pasteurized milk as opposed to raw milk, and if you have the choice, select fresh or frozen poultry treated with ionizing radiation…." The WHO set up a Consultation on Food Irradiation in 1992, and published its detailed report in 1994, with the endorsement that "food irradiation is a thoroughly tested technique, that it has not been shown to have any deleterious effects when performed in accordance with good manufacturing practices, and that it can keep to ensure a safer and more plentiful food supply by extending shelf life, eradicating pests and inactivating pathogens". These efforts assisted a worldwide development of clearances on food irradiation.

Food irradiation is approved for use in an increasing number of countries worldwide for various application and purposes in a wide variety of foodstuffs. The IAEA is also updating and maintaining a Food Irradiation Clearance Database. At the EU level, there are two main pieces of legislation regulating the irradiation of food: Directive 1999/2/EC and Directive 1999/3/EC. The Joint FAO/WHO Codex Alimentarius Commission adopted a Codex General Standard for Irradiated Foods as well as a Recommended International Code of Practice for Radiation Processing of Food. These Codex documents were revised in 2003, and are publicly available on the websites indicated below.

Legislative authorities require that irradiated food products be labeled. In general, the international food irradiation symbol, the so-called Radura logo is required with a statement that the product has been intentionally subjected to ionizing radiation.

See also: Foodborne Diseases: Overview of Emerging Food Technologies. Safety of Food and Beverages: Safety of Irradiated Foods

Further Reading

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Relevant Websites

http://www.codexalimentarius.net/web/more_info.jsp?id_sta=16
Codex General Standard for Irradiated Foods (CODEX STAN 106–1983. Rev1-2003).

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Codex Recommended International Code of Practice for Radiation Processing of Food (CAC/RPC 19–1979, Rev2-2003).

http://nucleus.iaea.org/apps/FICDB/Browse.asp
IAEA Food Irradiation Clearance Database.

http://nucleus.iaea.org/apps/IFDB/Browse.asp
IAEA Food Irradiation Facilities Database.

FOOD TECHNOLOGIES

Freezing

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Glossary

Conduction, thermal Mechanism for heat transfer. The process of heat transfer through a solid material/medium in which kinetic energy is transmitted by the particles of the material from particle to particle without gross displacement of the particles.

Convection, thermal Mechanism for heat transfer. The process of heat transfer through a liquid or gas by means of circulating currents caused by changes in density.

Drip Exudative liquid from meats and fish containing water and proteins. Produced by protein denaturation/structural breakdown during chilling/freezing and storage, particularly freezing and thawing. Also referred to as exudate, purge fluid, press fluid, etc.

Heat transfer coefficient Coefficient used in thermodynamics to calculate heat transfer, typically by convection or phase change, between a fluid and a solid.

Latent heat of fusion The amount of thermal energy required to change the state of a substance without a change in temperature. The thermal energy absorbed or rejected by a substance during its conversion, respectively, from a solid to a liquid, as from ice to water, or from a liquid to a solid, as from water to ice.

Radiation, thermal Mechanism for heat transfer. Electromagnetic radiation generated by the thermal motion of charged particles in matter. All matter with a temperature greater than absolute zero emit thermal radiation.

Refrigeration May be defined as the process of removing heat from any substance to: (1) render colder – reduce temperature, (2) change its state – for example, water to ice, and (3) maintain its state – preserving foods, storing ice.

Water activity (a_w) A measure of the available water in a substance. 'High a_w' foods support bacterial growth, 'low a_w' do not. This is not the same as water content. Some foods with a high water content have a relatively low a_w because the water is bound up with dissolved salts or sugar, for example, jam.

Introduction

Freezing, i.e., holding a food in a refrigerated environment with the majority of the water in the food turned into ice, is a major preservation method for foods. In foods, containing free water, ice can form at any temperature below the initial freezing point of that food. In addition once ice has been formed it will remain present unless, or until, the temperature of the food is raised above the initial freezing point (more properly described in such case as the final melting point). Whereas water has a single freezing point, foodstuffs (as they are a mixture of different constituents, between 10% and 30% of substances other than water) have a freezing range. The 'freezing point' of a food is usually considered as the initial freezing point, the temperature at which ice formation starts following supercooling. In foods with low water contents and high sugar or salt contents the initial freezing point may be as low as -4 to $-10\,^{\circ}C$, or even lower. International Institute of refrigeration (IIR) Recommendations for the Processing and Handling of Frozen Foods state that "a food product is regarded as a frozen food product when the product temperature is $-10\,^{\circ}C$ or colder, or when a high proportion of the freezable water in the product is converted into ice (usually more than 80% of water content)."

The freezing of a food can be divided into five distinct periods (Figure 1):

1. An initial cooling period, where cooling takes place without a phase change.
2. A supercooling period, where the product temperature falls below the freezing point without there being a phase change, due to the requirement of high activation energy for starting nucleation (of the crystallization).
3. A phase change period, which starts when nucleation occurs and ice begins to form in the surface layer. Although the water is initially changing to ice there is little change in temperature due to the heat required for the change in state (the latent heat of fusion). The freezing front gradually advances into the product, until it reaches the thermal center and there is ice throughout the food.
4. A completion of freezing period, during which the required amount of heat is extracted to create the desired ice content throughout the food.
5. An equalization period, as the food temperature tends to the temperature of the heat transfer medium.

In general, the term freezing refers to the initial process in which the temperature of the food is reduced to a temperature below its freezing point, whereas the term frozen is used to

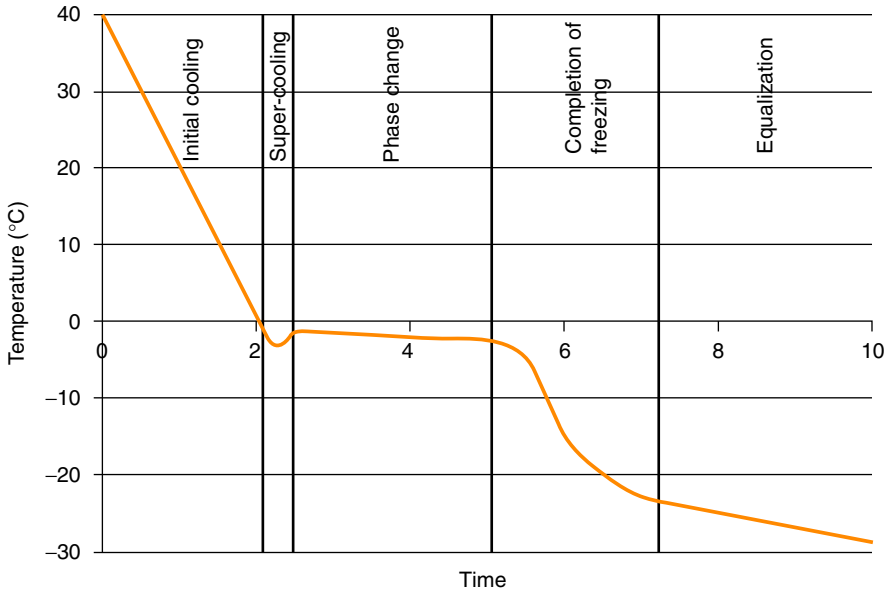

Figure 1 Typical representative freezing curve showing periods of freezing.

describe the subsequent state the food is kept in, i.e., the maintenance of the food below that temperature during the rest of the cold-chain.

A frozen food has a 'safe' storage and distribution life that can be measured in years when compared to the days or months of a chilled product; the storage life being controlled by chemical changes that affect the taste of the food not microbiological safety. A reliable frozen cold-chain initially reduces the temperature of the food below a temperature (−12 °C) that prevents the growth of any pathogenic or spoilage microorganisms and stops it rising during the rest of the cold-chain, i.e., storage, distribution, retail display, and domestic handling.

With many foods, freezing is the best long-term food preservation method because it produces no significant changes in the texture, taste, smell, or appearance of the food at the point of consumption. Changes to the structure and loss of water-holding capacity are noticeable after thawing and are important if the food, i.e., soft fruit, is to be consumed uncooked. In addition nutrients such as vitamins are better preserved in freezing than traditional preservation methods, including chilling. It is therefore preferable to traditional alternatives such as smoking, drying, salting, or canning.

The removal of heat from a material diminishes the kinetic energy of that material, thus decreasing the temperature (temperature being a measure of the rate of motion of molecules, or atoms, within a body). The rate at which molecules and atoms move within a material determines the rate at which they react with one another. In food products many important reactions and processes are, among others, controlled by temperature:

- the growth of microorganisms;
- chemical and enzymatic reactions, such as browning reactions, lipid oxidation, cold-shortening, vitamin degradation, and pigment degradation; and
- physical processes, such as moisture loss.

In almost all cases, reducing the temperature reduces the rate of these changes in foods and thus extends their preservation. Provided the food is of a safe quality before freezing, as long as the temperature remains below −12 °C during storage there will be no growth of microorganisms so the food will remain safe. Frozen storage life will ultimately be limited by enzymic reactions, which will in time affect the taste of the frozen product. The rates of these reactions are a function of temperature, so the storage life will generally be longer at lower temperatures. Once thawed, however, any microbes present can again become active, and under the right conditions will multiply to levels that can lead to foodborne illness.

Freezing Process

Freezing is a process of removing heat and can only be achieved by four basic mechanisms: radiation, conduction, convection, or evaporation. Conduction requires a good physical contact between the food to be frozen and the cooling medium, and this is generally achieved only with foods that can be shaped into regular shapes, such as blocks of meat and fish or in the freezing liquids, i.e., ice cream, fruit juices, etc. Radiation does not require any physical contact but a large temperature difference is required between the surface of the food being cooled and that of surrounding surfaces to achieve significant heat flow. In primary freezing, radiation is only important in the initial stages of the process in a system where the food is not surrounded by other products. Again, in the initial stages of the freezing of cooked food products (e.g., pies, pastries, joints), radiant heat loss can be substantial if the products are surrounded by cold surfaces. Evaporation from a food surface reduces yield and is not desirable in most food refrigeration operations but can be useful again in the initial cooling of cooked food products. However, as soon as the surface of the food is frozen then any heat loss due to

evaporation is minimal. Convection is by far the most important heat transfer mechanism employed in the majority of food freezing systems. In most cases, refrigerated air is the transfer medium; however, in some cases brine or a cryogenic gas can be used. The rate of heat removal depends on the following:

1. Surface area available for heat flow.
2. Temperature difference between the surface and the medium.
3. Surface heat transfer coefficient.

Each combination of product and cooling system can be characterized by a specific surface heat transfer coefficient whose value depends principally on the thermophysical properties and velocity of the medium. Typical values range from 5 $W\,m^{-2}\,K^{-1}$ for slow moving air to 500 $W\,m^{-2}\,K^{-1}$ for immersion in an agitated refrigerant. Heat must also be conducted from within the food to its surface before it can be removed. Most foodstuffs are poor conductors of heat and this imposes a severe limitation on attainable freezing times for either large individual items or small items cooled in bulk. However, because the thermal conductivity of ice is approximately three times that of water, as freezing proceeds it becomes easier to extract heat from the deeper regions.

Effect of Freezing on Food Safety

Microorganisms vary in their ability to tolerate freezing and frozen storage. Survival is affected by the type and age of microorganism. In general, Gram-negative bacteria (which include pathogens such as *Escherichia* and *Salmonella* spp.) are more susceptible to freezing and frozen storage than Gram-positives, with bacilli being more susceptible than cocci. Yeasts and molds are more resistant than bacteria, in part due to their tolerance to reduced water activity (a_w). Psychrophilic and psychrotrophic microorganisms are generally more tolerant to freezing and frozen storage due to their ability to synthesize larger amounts of enzymes to compensate for reduced enzymic activity at low temperatures, and their reduced susceptibility to cold shock in comparison to thermopiles and mesophiles.

Spoilage microorganisms do not grow below *ca.* -10 to $-12\,^{\circ}C$ and pathogens below $-1\,^{\circ}C$, thus the growth of pathogenic microorganisms is only normally relevant to handling before freezing or during thawing. In these contexts, frozen foods behave like their unfrozen counterparts, if surface temperatures are reduced rapidly during freezing this allows less time for any microorganisms to grow, although growth rates may be faster after thawing due to increased drip. Also, thawing may take a long time and on large objects subjected to long uncontrolled thawing cycles, surface spoilage can occur before the center regions have fully thawed.

Although salmonella, staphylococci, and other potential pathogens can survive freezing and frozen storage, the saprophytic flora (spoilage bacteria) tend to inhibit their growth. During freezing and thawing of food, the temperature favors the growth of psychrophilic organisms, most of which are spoilage organisms. Hence, in nearly all cases, if a frozen product is mishandled, spoilage is apparent before the food becomes a health hazard.

Repeated freeze-thaw cycles have been shown to disrupt and destroy bacteria; however, the effects of cyclic freezing on most microbial pathogens are not well documented. As bacteria generally grow more rapidly than fungi, mold spoilage of foods is thought to develop only when competing bacteria are inhibited. Temperature is usually assumed to be the critical factor, mold spoilage being classically associated with frozen meat. It has been generally accepted that molds can develop on meat at temperatures as low as -10 or $-12\,^{\circ}C$. However, there is some evidence that this is an exaggeration and that for practical purposes the minimum temperature for mold growth on meat should be taken to be approximately $-5\,^{\circ}C$, and that surface desiccation rather than temperature is the factor that inhibits bacterial growth. In the past, carcass meats were imported at temperatures of -5 to $-10\,^{\circ}C$. At these temperatures there were problems with the growth of psychrotrophic molds such as strains of Cladosporium, Geotrichum, Mucor, Penicillium, Rhizopus, and Thamnidium, causing 'whiskers' or 'spots' of various colors depending on the species. Because little meat is now stored at these temperatures, mold spoilage is largely of historic importance and thus any reports of mold growth on frozen meats should be taken as indicative of particularly poor temperature control. Despite this, many meat microbiology textbooks continue to discuss this subject in great detail.

Freezing and crust-freezing has been suggested as a means to reduce numbers of campylobacter organisms on poultry carcasses, and is one of a number of measures taken to reduce the incidence of campylobacteriosis in Iceland, although the exact impact of this measure is unclear. This work in Iceland has been very influential and many risk assessment models have incorporated freezing as an import factor due to this work. Freezing to approximately $-20\,^{\circ}C$ has been reported by a number of studies to result in an initial fall in numbers of campylobacter organisms, followed by a slower decline during storage. Freezing has been recommended as a control measure for reducing campylobacter by the European Food Safety Authority. The mechanism of damage during freezing has been attributed to mechanical damage caused by ice crystals, desiccation due to the reduced water activity, and oxidative damage.

Freezing has little effect on viruses, or on histamine and microbially produced toxins. Inadequate freezing and thawing procedures have been identified as factors in cases of scombroid poisoning associated with the consumption of spoiled fish flesh that is high in histamine (from pelagic species such as mackerel, sardines, pilchards, and certain tuna species).

Nematode parasites are very susceptible to freezing and freezing is a control measure for inactivating trichinae in pork and nematode parasites in seafood (particularly for lightly processed seafoods that will receive no cooking before consumption). The United States Department of Agriculture recommended holding times for pork to inactivate *Trichinella spiralis* range from 106 h at $-18\,^{\circ}C$ to 0.5 h at $-37\,^{\circ}C$. Freezing is also used as a control measure for inactivating tape worms (*Taenia saginata*) in beef carcasses with localized infections in the EU by holding at $-10\,^{\circ}C$ or less for 14 days or more.

Many of the chemical changes that limit the storage life of frozen foods are enzymic. Although freezing inhibits some enzymes, a considerable number, such as invertases, lipases, lipoxidases, catalases, peroxidases, etc., remain active. Because frozen foods are stored for relatively long periods, these enzymic reactions although slow (reactions that take 45 min at 37 °C will take over a week at −29 °C) can cause significant problems. In time this can lead to off-flavor development and color and texture deterioration. It has been demonstrated that food deterioration due to enzymes of microbial origin can occur at temperatures too low for the activity of the microorganisms that generated them. For vegetables, and to a lesser degree fruits, where enzymic deterioration during frozen storage is a problem, blanching, a mild heat treatment, is required to deactivate the enzymes. Typically, blanching is done by treating the product with steam or hot water for 1–10 min at 75–95 °C, the time/temperature combination depending on the specific product. Such treatment times and temperatures are also capable of reducing, to varying extents, the numbers of viable microorganisms on the food.

Whether 'rapid' freezing offers any clear advantages to product safety will depend on what biological hazards (pathogens, etc.) are present, at what numbers they are present, and whether they are on, or in, the food in question, and how 'rapid' the rate is in comparison to other rates. There is no definition of 'rapid' and 'slow' rates. Size of product will also have a big effect on relative rates of freezing, because conduction through the product will be the rate limiting factor.

Effect of Refreezing on Food Safety

General advice to consumers is that once thawed previously frozen food should not be refrozen. The rational for such advice is that microbial growth may have occurred during thawing and that further microbial growth may occur during subsequent thawing of the refrozen product. However, commercially many foods, particularly fish and shellfish, are repeatedly frozen and thawed during production and supply. Provided conditions are controlled to prevent any growth of pathogens during the period the food is no longer frozen, foods can be repeatedly frozen and thawed safely. In general it is recommended that product temperatures should be kept below 10 °C during thawing. Ideally two-stage thawing procedures should be used, in which the thawing medium temperatures are initially high (15–20 °C) but are reduced to $\leq$5 °C as the product surface temperature rises. There is evidence that in some cases repeated freeze-thaw cycles can disrupt and destroy bacteria. Concerns have been expressed that thawed food may represent an increased safety hazard due to the release of nutrients caused by ice crystal damage, however, there is little evidence that thawed foods represent any more of a hazard than foods that have not been frozen, although exudate from thawed products such as meat may contain pathogens and be a source of contamination. The use of carbon dioxide has been suggested during thawing and post thawing to mitigate any increased hazard. There is some evidence that refreezing may cause additional quality damage to some products (such as gaping in fish, further drip in meat, etc.), although this is not always detectable in a final cooked product.

Freezing Operations

Freezing systems for foodstuffs will contain many, if not all, of the following unit operations:

- Preparatory treatment; conditioning, waxing, cooking, pasteurizing, blanching, etc.
- Freezing.
- Storage.
- Transportation.
- Retail display.
- Domestic storage.
- Thawing/tempering.

During the preparatory treatment there can be a range of temperature responses, from a large gain to a small decrease in the temperature of the product. During freezing there is a substantial decrease in the mean temperature of the product. During thawing/tempering there is a substantial increase in the mean temperature of the product. Within a correctly designed frozen chain there should be no significant change in mean product temperature during storage, transport, retail display, or domestic storage.

Freezing Equipment

From a hygiene/hazard analysis and critical control points (HACCP)-based approach, prepacking the food before freezing may lower the risk of contamination/cross-contamination during the freezing process, however, it will significantly affect the rate of freezing (particularly in systems with low surface heat transfer rates such as low-velocity air), and this in turn may allow the growth of any microorganisms present during the initial cooling stage. Provided the cooling media (air, refrigerant, etc.) and refrigeration equipment used is kept sufficiently clean, no one freezing method can be said to be intrinsically more hygienic than any other.

Blast Air Freezers

Air is by far the most widely used method of freezing food as it is economical, hygienic, and relatively noncorrosive to equipment. Systems range from the most basic in which a fan draws air through a refrigerated coil and blows the cooled air around an insulated room, to purpose-built conveyorized blast freezing tunnels or spirals. Relatively low rates of heat transfer are attained from product surfaces in air cooled systems. The big advantage of air systems are their versatility, especially when there is a requirement to freeze a variety of products particularly of irregular shapes.

Batch Air Freezers

Placing food in large refrigerated rooms is a common method of freezing. With few exceptions the product is always wrapped and packaged before freezing.

In simple batch air freezing systems air distribution is a major problem that is often overlooked by the system designer and the operator. The freezing time of the product is reduced as the air speed is increased. An optimum value exists between the decrease in freezing time and the increasing power required to drive the fans to produce higher air speeds. Up to 30% of the total energy consumed in a blast freezer may be consumed by the fans and the extra refrigeration required to extract the heat generated by the fans. This optimum air velocity can be as low as 1.0 m s^{-1} when freezing pallets or large cartons to greater than 15 m s^{-1} plus for thin products. In addition even when a system has been designed to distribute the air through the product, poor management and/or poor understanding of the requirement of the plant commonly leads to uneven cooling. Products stacked or racked irregularly will leave channels around the stacks that are larger in cross sectional area than those within the stacks and channels of differing area through the stacks. Air leaving and returning to the refrigeration coil will take the path of least resistance through the largest gaps, instead of passing evenly through or over the product.

Continuous Air Freezers

Conveying the product through the freezing system overcomes the problem of uneven air distribution because each item is subjected to the same velocity/time profile. In the simplest continuous air freezing systems the food is suspended from an overhead conveyer and moved through a refrigerated room.

In more sophisticated plants the product is conveyed through a freezing tunnel. The main advantage of this method is that the refrigeration capacity and air conditions can be varied throughout the length of the tunnel. Large capacity evaporators can be installed in the initial stage to cater for the high rates of heat release encountered at the start of the freezing process.

Some small cooked products are continuously frozen on racks of trays (8 to 16 high) that are pulled or pushed through a freezing tunnel using a simple mechanical system. In larger operations it is more satisfactory to convey the cooked products through a linear tunnel or spiral freezer. Linear tunnels are far simpler constructions than spirals but their use is often ruled out due to constraints on floor area. In spirals, advantages are claimed for either horizontal or vertical systems of air distribution. In horizontal systems a lower pressure drop and smaller temperature difference across the products and coil result in less weight loss from unwrapped products. In systems utilizing a vertical air distribution, there is increased turbulence and higher, and more uniform, heat transfer coefficients, which lead to reduced freezing times.

The use of impingement technology to increase the surface heat transfer in freezing systems is now common practice. Impingement is the process of directing a jet or jets of fluid at a solid surface to effect a change. The very high velocity (20–30 m s^{-1}) impingement gas jets 'breakup' the static surface boundary layer of gas that surrounds a food. The resulting medium around the product is more turbulent and the heat exchange through this zone becomes much more effective. Impingement freezing is best suited for products with high surface area to weight ratios, i.e., hamburger patties or products with one small dimension. Testing has shown that products with a thickness less than 20 mm freeze most effectively in an impingement heat transfer environment. When freezing products thicker than 20 mm, the benefits of impingement freezing can still be achieved, however, the surface heat transfer coefficients later in the freezing process should be reduced to balance the overall process efficiency. The process is also very attractive for products that require very rapid surface freezing.

Fluidized bed freezing is a modification of air-blast freezing. The principal behind fluidization is that fairly uniform particles are subjected to an upward air stream. At a certain velocity the particles will float in the air stream, each separated from the other, surrounded by air and free to move. In this state the particles act in a similar fashion to a fluid, thus the term 'fluidized.' Products are fed into the higher end of a sloping tunnel, where they are simultaneously conveyed and frozen by the same air. Fluidized bed freezers can also be combined with a conveyorized system. Additional agitation, in the form of a movable base, may be required for some irregular products such as French fries. Fluidized bed freezers are used to produce free-flowing products, most notably vegetables (such as peas, sliced carrots, green beans, etc.) and fruit, but can also be used for peeled cooked shrimps, diced meats, etc. Products frozen by this method, as well as in other in-line blast freezers, are commonly referred to as individually quick frozen (IQF). Fluidization achieves a very efficient air-to-product contact, thus giving much higher heat transfer rates than for conventional air-blast freezing tunnels or belt freezers and subsequently shorter freezing times.

Plate Freezers

Modern plate freezers differ little in principle from the first contact freezer patented in 1929 by Clarence Birdseye. Essentially product is pressed between hollow metal plates containing a circulating refrigerant. A hydraulic cylinder is used to bring the refrigerated plates into pressure contact with the product. These plates can be either horizontal or vertical. They tend to be expensive, especially if automatic loading and unloading is required, but have low running costs. Good heat transfer is dependent on product thickness, good contact, and the conductivity of the product. Plate freezers are often limited to a maximum thickness of 50–70 mm. Air spaces in packaging and fouling of the plates can have a significant effect on freezing time. With thin materials a plate freezing system has the potential to halve the time required in an air-blast system.

Belt Freezers

Belt freezers employ a contact method of freezing similar to plate freezers. Simple belt freezers consist of an endless steel belt (approximately 1 mm in thickness), the underside of which is cooled either directly with brine, glycol, or cryogenic sprays, or by sliding over a stationary cold surface. Because only one side of the product is in contact with the cooling surface relatively thin products are required, such as

hamburgers, fish fillets, or liquid and semiliquid products such as purées and sauces.

In double-band systems the product is frozen between two endless belts of which the top is flat and the lower belt corrugated. The product is spread into the corrugations, the top belt enclosing the exposed surface thus freezing the product as IQF pellets. Liquids and semiliquids are often frozen into pellets using this method.

Scraped Surface Freezers

Scraped surface, or cylindrical, freezers are designed for freezing liquid products either on the inner or the outer surface of a cooled cylinder. The layer of frozen product formed on the surface of the cylinder is continuously scraped from the cylinder surface, thus achieving high heat transfer and a rapid freezing rate. Scraped surface freezers are used for manufacturing ice creams and similar products.

Immersion/Spray Freezers

Immersion/spray freezing systems involve dipping product into a cold liquid, or spraying a cold liquid onto the food. Because heat transfer medium temperatures of $<0\,^{\circ}C$ are required this necessitates the use of nontoxic salt, sugar, or alcohol solutions in water, or the use of cryogens or other refrigerants. The type of salt depends on the required temperatures, calcium chloride solutions are capable of temperatures as low as $-55\,^{\circ}C$. This produces high rates of heat transfer due to the intimate contact between product and cooling medium. For example, bulk freezing times of less than 1 min can be obtained for peas, diced carrots, snow peas, and cut green beans using a 23% sodium chloride solution.

Clearly if the food is unwrapped the liquid has to be 'food safe.' Any uptake of the cooling medium, whether 'food safe' or not, by the product may present problems both in terms of flavor changes and the requirement for periodic replacement of the medium. This transfer can be minimized by packaging, although this will hinder heat transfer.

Cryogenic Freezers

The term cryogenic simply means very low temperature. Cryogenic cooling uses refrigerants, such as liquid nitrogen or solid carbon dioxide, directly. Cryogenic freezing is often treated as a specific type of freezing method on its own, however, it is essentially an immersion/spray system, depending on how the cryogen is utilized.

Although it is common in laboratory studies to freeze samples with liquid nitrogen by direct immersion, few commercial liquid nitrogen freezers employ this technique. One reason for this is that many foods will shatter and split if frozen in this way; due to rapid ice expansion, it is also inefficient. Cryogens are typically employed as sprays in tunnel, spiral, or batch cabinet systems.

Cryogenic freezing is often cited as the fastest method of freezing a food. Rapid freezing in comparison with other methods is principally due to very low operating temperatures. In general, commercial cryogenic freezers do not provide substantially higher surface heat transfer coefficients between the product and medium than other refrigeration systems, unless the cryogen comes in direct contact with the product. Cryogenic systems are best suited to freezing thin products with a high surface area to weight ratios in which heat conduction within the product is not rate limiting. Although running costs of cryogenic systems can be expensive, capital investment is low, with cryogenic suppliers often renting the equipment to users. Also installation and maintenance costs are lower than mechanical refrigeration systems.

Frozen Storage

Publications such as those in the IIR provide data on the storage life of many foods at different temperatures. Storage lives for food can be as short as 3–4 months for IQF, polybag-packed shrimps at $-18\,^{\circ}C$. However, lamb stored at $-25\,^{\circ}C$ can be kept for over 2.5 years.

Traditionally the frozen food industry was interested in two problems that were detrimental to the appearance of the frozen food; 'freezer burn' and 'in-package frosting,' both of which may occur during storage. Freezer burn is caused by water loss from the surface of the frozen food due to sublimation. The resulting desiccation produces a dry fibrous layer at the surface that has the appearance of a burn. It is irreversible. It only occurs in unwrapped, or poorly wrapped, foods and its development is fastest at high storage temperatures and high air movements. It occurs during storage and not during the freezing process (unless the freezing process is excessively long); it is not caused by fast freezing. In-package frosting results from a combination of water loss from the surface, lose packaging, and temperature fluctuations during storage. The water lost from the surface is deposited and frozen on the inner surface of the packaging. The use of suitable packaging and good temperature control should eliminate both problems. Neither has an effect on food safety.

Thawing and Tempering Systems

Frozen raw material as supplied to the industry ranges in size and shape, although much of it is in blocks packed in boxes. Thawing is usually regarded as complete when the center of the block has reached $0\,^{\circ}C$, the minimum temperature at which meat or fish can be filleted or cut by hand and fruits and vegetables hand sorted. Lower temperatures (e.g., -5 to $-2\,^{\circ}C$) are acceptable for produce that is destined for mechanical chopping, but such meat is 'tempered' rather than thawed. The two processes should not be confused because tempering only constitutes the initial phase of a complete thawing process.

Thawing is often considered as simply the reversal of the freezing process. However, inherent in thawing is a major problem that does not occur in the freezing operation. The majority of the microorganisms that cause spoilage or food poisoning are found on the surfaces of foods. During the freezing operation, surface temperatures are reduced rapidly

and bacterial multiplication is severely limited, with microorganisms becoming completely dormant below $-12\,^{\circ}C$. In the thawing operation these same surface areas are the first to rise in temperature and microbial multiplication can recommence. On large objects subjected to long uncontrolled thawing cycles, surface spoilage can occur before the center regions have fully thawed.

Most thawing systems supply heat to the surface and then rely on conduction to transfer that heat into the center of the food. A few use electromagnetic radiation to generate heat within the food. In selecting a thawing system for industrial use a balance must be struck between thawing time, appearance and bacteriological condition of product, processing problems such as effluent disposal, and the capital and operating costs of the respective systems.

Transportation

Frozen foods are transported around the world and locally via a range of transportation systems. All these transportation systems are expected to maintain the temperature of the food within close limits to ensure its optimum safety and high quality shelf-life. As with chilled food it is important that the food is at the correct temperature before loading because the refrigeration systems used in most transport containers are not designed to extract heat from the load but to maintain the temperature of the load.

Overland Transport

Overland transportation systems range from 12 m refrigerated containers for long distance road or rail movement of frozen products to small uninsulated vans supplying food to local retail outlets or even directly to the consumer. The rise in supermarket home delivery services where there are requirements for mixed loads of products that may each require different storage temperatures is introducing a new complexity to local overland delivery.

The majority of current road transport vehicles for frozen foods are refrigerated using either mechanical, eutectic plates, or liquid nitrogen cooling systems. Irrespective of the type of refrigeration equipment used the product will not be maintained at its desired temperature during transportation unless it is surrounded by air or surfaces at or below the maximum transportation temperature. This is usually achieved by a system that circulates moving air, either forced or by gravity, around the load. Inadequate air distribution is probably the principal cause of product deterioration and loss of shelf-life during transportation. Conventional forced air units usually discharge air over the stacked or suspended products either directly from the evaporator or through ducts toward the rear cargo doors.

Sea Transport

Most International Standard Organization (ISO) containers for food transport are either 6 or 12 m long, hold up to 26 t of product and can be 'insulated' or 'refrigerated.' The refrigerated containers incorporate insulation and have refrigeration units built into their structure. The units operate electrically, either from an external power supply on board the ship or dock or from a generator on a road vehicle. Insulated containers either utilize the plug-type refrigeration units already described or may be connected directly to an air-handling system in a ship's hold or at the docks. Close temperature control is most easily achieved in containers that are placed in insulated holds and connected to the ship's refrigeration system. However, suitable refrigeration facilities must be available for any overland sections of the journey. When the containers are fully loaded and the cooled air is forced uniformly through the spaces between cartons, the maximum difference between delivery and return air can be less than $0.8\,^{\circ}C$. The entire product in a container can be maintained to within $\pm 1.0\,^{\circ}C$ of the set point. Refrigerated containers are easier to transport overland than the insulated types, but have to be carried on deck when shipped because of problems in operating the refrigeration units within closed holds. On board ship they are therefore subjected to too much higher ambient temperatures and consequently larger heat gains, which make it far more difficult to control product temperatures.

Retail Display

No frozen food, with the possible exception of ice cream, should be unwrapped when in a retail display cabinet. Traditionally frozen food was displayed in a 'well-type' cabinet with only the top faces of food packs being exposed. In many cases the cabinets were fitted with a see-through insulated lid to further reduce heat infiltration.

Increasingly there is marketing pressure to display an increasing amount of frozen food in open multideck display cabinets. Maintaining the temperature of products below set limits while they are on open display in a heated store will always be a difficult task. Radiant heat gain on the surfaces of exposed packs can result in the food thawing in extreme cases. During display, temperature, temperature fluctuations, and packaging are the main display parameters that control quality.

Temperature fluctuations can increase the rate of weight loss from wrapped meat. Higher rates of dehydration have been measured in a retail cabinet operating at $-15\,^{\circ}C$ than another cabinet operating at $-8\,^{\circ}C$. Fluctuations in air temperature in the $-15\,^{\circ}C$ cabinet ranged from -5 to $-21\,^{\circ}C$ compared with $\pm 1.5\,^{\circ}C$ in the $-8\,^{\circ}C$ cabinet. Successive evaporation and condensation (as frost) caused by such a wide temperature differential resulted in exaggerated in-package dehydration.

The extent of temperature fluctuations will be dependent on the air temperature over the product, the product packaging, and the level of radiant heat. Retail display packs have a relatively small thermal mass and respond relatively quickly to external temperature changes. These can be from store and display lighting, defrost cycles, and heat infiltration from the store environment. In products where air gaps exist between the packaging and the meat, sublimation of ice within the product leads to condensation on the inside of the packaging,

resulting in a build-up of frost. This dehydration causes small fissures in the surface of the food, allowing the ingress of any packaging gases into the food. This can aid the acceleration of oxidative rancidity within the product. Minor product temperature fluctuations are generally considered to be unimportant, especially if the product is stored below $-18\,^{\circ}C$ and fluctuations do not exceed $2\,^{\circ}C$.

Domestic Handling

After a frozen product has reached the operating temperature of a domestic freezer it is very unlikely that its temperature will rise above $-12\,^{\circ}C$ during domestic storage, unless there is electricity cut. However, safety can be compromised during handling before and after the product reaches the storage temperature.

When removed from display cabinets the temperature of frozen foods can rise rapidly if exposed to ambient conditions. Surveys have shown that the majority of consumers do not use insulated bags or boxes to transport frozen food to their homes. Once the food has warmed during transportation it can take many hours in a domestic freezer for the food temperature to fall below a safe temperature of $-12\,^{\circ}C$.

It is common for consumers to purchase chilled products and freeze them at home. Studies have shown that it can take over 6 h for the temperature of a chicken portion to cool from 0 to $-5\,^{\circ}C$ in a domestic freezer.

There have been relatively few published surveys of temperatures in domestic freezers. In a survey of domestic freezers in New Zealand, mean temperatures ranged from -11.5 to $-23.3\,^{\circ}C$ with an overall mean value of $-16.6\,^{\circ}C$. Only 28% of freezers operated at $-18\,^{\circ}C$ or lower, with 68% operating between -13 and $-18\,^{\circ}C$. The type of freezer did not influence the mean air temperature values recorded. These ranged from -16.3 to $-16.6\,^{\circ}C$, irrespective of other considerations such as data logger location and freezer loading. The warmest freezer temperature encountered was in a top-loading fridge-freezer, whereas the coldest was in a vertical freezer. Temperature control in freezers does not appear to have improved over 20 years. Freezers ≤ 10 years old and freezers ≥ 21 years old had similar mean temperature values. Freezers of 11–15 and 16–20 years of age had respective mean air temperatures approximately $3\,^{\circ}C$ lower and $2\,^{\circ}C$ higher than the other freezer age groups but came from small sample sizes. The mean air temperature recorded in the top sections of surveyed freezers was on average 2–2.5 $^{\circ}C$ warmer than the middle and bottom sections, respectively, which suggests that freezing could be slightly slower in the upper areas of the freezer compartment.

It is usually recommended that large individual frozen items, i.e., meat joints, whole poultry carcasses, are thawed to above $0\,^{\circ}C$ before cooking. If carried out in a domestic refrigerator this process may take many days but the food will not reach a temperature that will support pathogenic growth. Thawing product in ambient air or water is far quicker but the process should be controlled so that the food is only above $5\,^{\circ}C$ for a minimum time before cooking.

Conclusions

Frozen foods generally have an excellent overall safety record. However, the production of safe frozen foods requires the same attention to good manufacturing practices and HACCP principles as the chilled or fresh counterpart.

Provided the food is of a 'safe' condition before freezing, freezing is a very safe method of preserving food. During the time the food remains below $-12\,^{\circ}C$ there will be no growth in food poisoning or food spoilage microorganisms. Freezing has little effect on viruses or on histamine but will inactivate nematode parasites in pork and seafood. As long as the temperature of the food is reduced to below $-12\,^{\circ}C$ in a reasonable time (hours rather than days) during the initial freezing operation and not allowed to rise above $7\,^{\circ}C$ during thawing, it will generally be as safe to consume after thawing as it was before freezing. Once thawed, however, any microorganisms present can again become active, and under the right conditions will multiply to levels that can lead to foodborne illness. Overall it is therefore very important from a food safety point of view that control measures are put in place to ensure that processing before freezing results in safe food when frozen and that the thawing process does not result in any growth of food poisoning organisms.

See also: Foodborne Diseases: Overview of Biological Hazards and Foodborne Diseases

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http://www.fao.org/
Official Site for the Food and Agriculture Organization of the United Nations.
http://www.iifiir.org/
Official Site for the International Institute of Refrigeration.
http://people.ucalgary.ca/~kmuldrew/cryo_course/course_outline.html
Short Course on Cryobiology from the University of Calgary.

High Pressure Processing

MF Patterson, Agri-Food and Biosciences Institute, Belfast, UK

Glossary

Adiabatic heating or adiabatic heat of compression The work of compression during the high-pressure treatment will increase the temperature of foods through a process known as adiabatic heating. The extent of the temperature increase varies with the compression fluid and composition of the food. In water-based systems, the temperature is approximately 2 °C/100 MPa.

Megapascals (MPa) The SI unit of pressure is the pascal (Pa). 1 Pa is equivalent to 1 N m^{-2}. This is a very small unit of pressure and most food applications require pressures in the megapascal range (MPa), where 1 MPa is equivalent to 1×10^6 Pa. Other units of pressure have been, and still are used and a conversion table is given below.

MPa	*kbar*	*kgf mm^{-2}*	*atmospheres*	*lbf in^{-2}*	*tonf in^{-2}*
100	1	10.20	986.9	14 504	6.475
600	6	61.20	5921.40	87 024	38.85

Introduction

High pressure processing (HPP) is being used around the world to treat a wide variety of foods. The rapid increase in commercial use of the technology in the last decade is largely due to the increase in availability of equipment that can routinely and reliably operate at up to 600 megapascals (MPa) at 5–35 °C (~90 000 pounds per square inch or 6000 atmospheres). This is sufficient to destroy many microorganisms in foods, leading to improved safety and shelf-life without adversely affecting sensory characteristics or nutritional quality. However, these conditions are not sufficient to inactivate bacterial spores, so the treatment has been referred to as a 'cold pasteurization' process. It cannot, currently, be used commercially to sterilize foods. The pressure treatment is normally carried out on prepackaged foods so as to minimize the risk of recontamination, post-treatment.

High pressure is generally a batch process for solid foods, although it can be a semicontinuous bulk process for liquid foods. A typical high-pressure system consists of a pressure vessel and a pressure generator (Figure 1). Food packages are loaded into the vessel and the top closed. The pressure transmission fluid, usually water, is pumped into the vessel from the bottom. Once the desired pressure is reached, the pumping is stopped, valves are closed, and the pressure is held without the need for further energy input. Processing costs are claimed to be £0.04–0.20 per liter or per kilogram depending on factors such as the pressure applied, process time, and throughput. Pressure vessels, up to 600 l capacity, capable of processing foods are now available and >150 commercial facilities are in operation around the world, treating a wide range of products with a relatively high throughput, compared to early machines. For example, a 350 l machine can process two tons of poultry products per hour.

A detailed description of how the technology works in practice and the type of equipment involved can be found in articles in the Further Reading section of this article.

Effects of High Pressure on Microbial Cells

There has been much research into the effects that pressure treatment has on microbial cells, which include alterations to the cell membrane, effects on proteins (including enzymes), and effects on the genetic mechanisms of microorganisms. Most authors agree that the lethal effect of high pressure on

Figure 1 Horizontal HPP facility, processing deli meats. Photograph with permission from Avure Technologies and Maple Lodge Farms.

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bacteria is due to a number of different processes taking place simultaneously. In particular, damage to the cell membrane and inactivation of key enzymes, including those involved in DNA replication and transcription, are thought to be primary sites of pressure damage in microorganisms. Stationary-phase cells are normally more pressure-resistant than exponential-phase cells and this may be due to differences in the composition of the cytoplasmic membranes. The complex tertiary and quaternary structure of proteins can be affected depending on the level of pressure applied and this protein denaturation can lead to physical damage to cell membranes. This causes alterations to membrane permeability and has been demonstrated as leakage of adenosine triphosphate (ATP) or ultraviolet (UV)-absorbing material from bacterial cells after pressure treatment or increased uptake of fluorescent dyes such as propidium iodide that do not normally penetrate the membranes of healthy cells. Changes in protein structure can lead to enzyme activity being enhanced or decreased by pressure, either due to changes in the structure of the enzyme itself or to changes in the substrate. In contrast, covalent bonds are generally not affected by pressure, so many compounds associated with the nutritional and sensory quality of foods, such as vitamins, flavor, and color components, are not destroyed.

Factors Affecting the Microbiological Safety of Pressure-Treated Foods

Many factors can affect the response of microorganisms to high pressure. Treatment conditions in terms of the level of pressure applied, the hold time, and process temperature are parameters that need to be optimized. There are significant variations in pressure resistance between different species and even between strains of the same species and the composition of the substrate can also influence the level of inactivation achieved. The response of a variety of foodborne pathogens to pressure treatments in different foods is given in Table 1.

In general terms, applying higher pressures normally results in greater levels of kill in vegetative cells. However, in many cases the inactivation curves do not follow first-order kinetics, and a plot of hold time versus $\log_{10}$ survivors does not give a straight line. Tailing effects are common, with plots showing an initial decrease in numbers, followed by a leveling of the curve, where there is little further inactivation as treatment time increases. Tailing effects have also been reported in thermal processing studies but seem to be more common and more pronounced with pressure treatment.

Table 1 Response of selected foodborne pathogens to high hydrostatic pressure

Pathogen	*Foodstuff*	*Processing conditions – pressure (MPa)/initial temperature (°C)/hold time*	*Level of inactivation*
Aeromonas hydrophila[a]	Ground pork	253/25/15 min	7 $\log_{10}$ inactivation
Campylobacter jejuni[b]	Whole milk	400/25/10 min	>8 $\log_{10}$ reduction
Campylobacter jejuni[b]	Chicken pureé	400/25/10 min	>8 $\log_{10}$ reduction
Campylobacter jejuni[c]	Pork slurry	300/25/10 min	5 $\log_{10}$ reduction
Chronobacter (Enterobacter) sakazakii[d]	Reconstituted infant formula	500/25/26.3 min or 500/40/7.9 min	90% probability of obtaining a 5-log reduction
C. botulinum type A spores[e]	Crab meat	827/75/15 min	3.2 $\log_{10}$ reduction
Clostridium sporogenes spores[f]	Meat emulsion	621/98/5 min	>5 $\log_{10}$ reduction
C. parvum[g]	Apple juice	530/20/1 min	>4 log reduction of oocysts
Eimeria acervulina[h]	DMEM containing 5.8 $\log_{10}$ oocysts	550/40/2 min	No pathogenicity detected in chickens and no fecal shedding of oocysts
Escherichia coli O157:H7 NCTC 12079[i]	UHT milk	600/20/15 min	<2 $\log_{10}$ reduction
E. coli O157:H7 NCTC 12079[j]	Raw poultry mince	600/20/15 min	3 $\log_{10}$ reduction
E. coli O157:H7 (cocktail of 3 strains)[j]	Grapefruit juice pH 3.2	615/15/2 min	>8 $\log_{10}$ reduction
E. coli O157:H7 (cocktail of 3 strains)[j]	Carrot juice pH 6.2	615/15/2 min	>6 $\log_{10}$ reduction
Hepatitis A virus[k]	Experimentally contaminated Pacific oyster (*Crassostrea gigas*)	400/9/1 min	Reduction of >3 $\log_{10}$ plaque forming units
Hepatitis A virus[l]	Green onions	375/21/5 min	Reduction of $\log_{10}$ 4.75 plaque forming unit g^{-1}
L. monocytogenes[m]	Cold-smoked salmon	450/12/5 min	~ 2 $\log_{10}$ reduction
L. monocytogenes[n]	Human milk	400/21/2 min	8 $\log_{10}$ reduction
Murine norovirus[o]	Experimentally contaminated oyster tissue	400/5/5 min	Reduction of $\log_{10}$ 4.05 plaque forming unit g^{-1}
Salmonella spp. (5 individual serovars)[j]	Grapefruit juice pH 3.2	615/15/2 min	>8 log 10 reduction

(*Continued*)

Table 1 Continued

Pathogen	*Foodstuff*	*Processing conditions – pressure (MPa)/initial temperature (°C)/hold time*	*Level of inactivation*
Salmonella spp. (5 individual serovars)[j]	Carrot juice pH 6.2	615/15/2 min	5.31–7.81 $\log_{10}$ reduction, depending on strain
Salmonella spp. (cocktail of 5 serovars)[p]	Orange juice (pH 4.3 and pH 3.7)	600/18/5 s	5 $\log_{10}$ predicted from mathematical modeling of inactivation data
Salmonella enteritidis[q]	Raw chicken breast fillets	400/8/20 min	>5 $\log_{10}$ reduction
S. aureus[n]	Human milk	400/21/30 min	8 $\log_{10}$ reduction
S. aureus[j]	Raw poultry mince	600/20/30 min	<4 $\log_{10}$ inactivation
Vibrio parahaemolyticus[r]	Oysters	300/10/3 min	5 $\log_{10}$ reduction
Yersinia pseudotuberculosis 197 (surrogate for *Y. pestis*)[s]	Orange juice	500/10/2 min	>5 $\log_{10}$ reduction

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These tailing phenomena cannot be ignored, for several reasons. First, the effectiveness of any high-pressure treatment must be known in order to produce pressure-treated foods that are safe to consume. Accurate information on inactivation kinetics is therefore essential and a number of models, including Weibull and log-logistic models, have been used successfully to describe nonlinear inactivation data. Second, from a commercial viewpoint, the optimum treatment time needs to be established. HPP is a batch process, so it is important not to have hold times that are longer than necessary, in order to maximize throughput.

Treatment temperature can be another important factor in the level of inactivation achieved. Currently, most commercial treatments are carried out when the initial temperature is in the range of 5–25 °C. There is normally an increase in temperature during processing, due to adiabatic heating. In water-based systems, this equates to approximately a 2 °C rise in temperature per 100 MPa, and the heat quickly dissipates once the pressure is released. Operating at these temperatures means that the fresh appearance and flavor of many foods can be maintained – which is an important advantage of HPP over other technologies. For many commercial applications, the exact initial temperature is not critical, provided it is in the 5–25 °C range. However, in some cases, treatment temperature can have a significant effect. For example, studies with murine norovirus in oyster tissue found that the inactivation achieved was significantly greater at 5 °C than at 20 or 30 °C.

Bacterial spores, including *Clostridium botulinum*, can withstand exposure to very high pressures (>1000 MPa) for long periods of time (>60 min) at room temperature. Therefore, currently, HPP cannot be used commercially to sterilize foodss. However, recent research as shown that the

combination of high pressure and high temperature can be effective in inactivating spores. The process, also known as pressure-assisted thermal sterilization (PATS), requires the food to be preheated to approximately 90 °C, followed by pressurization to approximately 600 MPa for a few minutes and then a rapid decompression. The temperature increase during pressurization, due to adiabatic heating, means that the final product temperature can reach 121 °C, whereas the product can cool rapidly once the pressure is released. Overall, the processing times are shorter and there should be less damage to the quality attributes than when foods are subjected to conventional heat sterilization.

The first PATS product was approved by the US Food and Drug Administration (FDA) in 2009. This was mashed potato, which had the quality expected of the traditional pasteurized product but with the ambient shelf-life capability of a sterilized product. This is a positive development but a number of technical challenges still need to be addressed before PATS will be suitable for widespread commercial use. From a food safety perspective, there is a need to demonstrate control of temperature variation within the processing vessel so as to ensure that products receive a consistent minimum process regardless of spatial position within the vessel.

Vegetative bacteria, including those associated with foodborne disease, are much more sensitive to pressure than spores. Cells in the stationary growth phase tend to be more resistant than cells in the exponential phase, and Gram-negative rods such as *Vibrio* spp. and *Campylobacter* spp. tend to be more sensitive than Gram-positive cocci such as *Staphylococcus aureus.* Significant variation between different strains of the same species has been noted in, for example, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *S. aureus*, and *Salmonella* spp. All of these factors need to be taken into account when preparing suitable cocktails of strains for use in inactivation studies.

There is relatively little information on the effects of pressure on parasites. However, *Cryptosporidium parvum* does appear to have a sensitivity similar to those of vegetative bacterial cells. Treatment of 530 MPa at 20 °C for 1 min gave >4 $\log_{10}$ inactivation of oocysts in apple juice, whereas all treatments between 305 and 550 MPa for 1–3 min gave a significant reduction in oocysts recovered from experimentally contaminated oysters.

The chemical composition and nature of the food can affect the response of microbial cells to pressure. Bacteria are usually most pressure-resistant at a neutral pH and become more sensitive as the pH reduces. This is useful in acid products, such as fruit juices, where the combined effect of pressure and low pH results in a greater level of inactivation than in more pH neutral products such as cooked meats. Water activity (a_w) also affects pressure resistance and a number of studies have demonstrated that reducing a_w from 0.98–1.00 (typical of foods such as fresh meat, fish, and fruits) down to 0.94–0.96 (typical of some cheeses as well as mayonnaise and other sauces) increases pressure resistance. However, these effects may vary, depending on the processing conditions. For example, increasing the processing temperature may counteract the protective effect of a reduced a_w. The baroprotective effect can also vary, depending on the type of solute even at the same a_w. For example, in a model gel system with an a_w of 0.95 and treated between 400 and 600 MPa, sodium chloride appeared to confer less protection than glycerol or fructose, whereas sorbitol had the most protective effect on the inactivation of *E. coli* K12.

The complex composition of foods can make it difficult to predict the response of pathogens to pressure. There are numerous reports that show that the inactivation obtained in buffer systems is generally greater than in real foods. For example, *E. coli* O157:H7 and *L. monocytogenes* appeared to be more resistant when treated in UHT milk than in poultry meat, which was, in turn, more protective than phosphate-buffered saline (PBS) buffer. Cations such as Ca^{2+} can be baroprotective and this may partly explain why many microorganisms appear more pressure resistant when treated in certain foods, such as milk.

As discussed earlier in this section, there are many interacting factors that must be taken into consideration when designing the optimum pressure-processing conditions for the production of safe, high quality foods. These conditions are likely to vary, depending on the product.

Commercial Applications of HPP

In 2008 there were over 150 commercial HPP facilities in use around the world, processing approximately 130 different products. This compares to just 9 commercial facilities worldwide in 1998. For an application to be successful, HPP has to give a unique or significant commercial advantage over existing technologies. These advantages include the fact that vegetative bacteria can be killed without affecting the fresh qualities of the product. This is important for some products such as fruit juices and sauces, where the fresh taste, color, and odor, along with retention of vitamins and antioxidants are unique selling points and can command a premium price. Another important application of HPP is the fact that it can be used to easily and cleanly remove (shuck) meat from shellfish, including oysters, lobster, and crab without affecting the raw, fresh characteristics of the product. The level of pressure required is lower than for many other applications, but will still be sufficient to give some reduction in microbial numbers, including viruses. In addition, this method of shucking significantly improves meat yield, and in the case of lobster, is the only method whereby raw meat can be cleanly removed from the shell and gives the possibility of new products such as lobster sashimi.

HPP can also reduce, or replace, the use of chemical preservatives in certain products such as cooked and cured meats, thus leading to 'clean labels,' which consumers perceive to be more natural and healthy. Other advantages of using HPP include a reduction in waste from spoiled food, as it can extend shelf-life and it is relatively energy efficient, compared to thermal processing, as normally heat is not required.

The main commercial applications are discussed in more detail in the following five sections.

HPP Applications for Cooked and Ready-to-Eat (RTE) Meats

This is one of the most common HPP food applications, in terms of commercial throughput. One of the main drivers

for this use of the technology has been the need to improve the safety of RTE meats. This is particularly true in North America, where there have been a number of product recalls due to contamination with pathogens, particularly *L. monocytogenes*. Within the USA, the USDA Food Safety and Inspection Service (FSIS) published its interim rule in 2003 for the control of *L. monocytogenes* in RTE meat and poultry products (9 CFR 430.). This required meat processors to implement and declare the food safety protocols they use to control *L. monocytogenes*, based on one of three risk-based alternatives (1–3). One obvious way of controlling the problem is to use a suitable postpackaging preservation treatment. An application was made to FSIS, and they have subsequently issued a letter-of-no-objection (LNO) for the use of HPP as an effective postpackaged intervention method in controlling *L. monocytogenes* in RTE meat and poultry products. Within Canada, Health Canada also issued a similar LNO for the control of *L. monocytogenes* in cured and uncured RTE pork products. HPP is now commonly used by many US and Canadian processors to meet the FSIS Alternative 1 category.

Typically pressures up to 600 MPa are applied for several minutes with an initial temperature of less than 10 °C. This is sufficient to give a 5 $\log_{10}$ reduction in *L. monocytogenes*. The treatment has the added advantage of extending shelf-life without affecting product quality.

HPP Applications for Fish and Seafood

One of the main reasons for pressure-treating shellfish is the fact that it allows meat to be easily shucked from the shell without adversely affecting eating quality. The pressure conditions required (typically 400 MPa or less for a few minutes) can also give useful reductions of typically approximately 4 $\log_{10}$ for pathogens such as Hepatitis A virus and *Vibrio* spp. Other viruses, such as murine norovirus-1 (MNV) and Feline Calicivirus, which have been used as surrogates for human noroviruses, are also relatively sensitive to high pressure. For example, with experimentally contaminated oysters, a 5 min treatment at 400 MPa at 5 °C was sufficient to give a 4.05 $\log_{10}$ reduction in plaque forming units.

Overall, these reductions in pathogen loads are particularly useful for oysters and other bivalve shellfish which are often eaten raw or lightly cooked.

Cold-smoked salmon is commonly prepared as an RTE food. The current manufacturing trend is have a less dry product which has lower levels of salt and smoke than in the past. Thus, the antimicrobial hurdles have been reduced, favoring the development of pathogens such as *L. monocytogenes*. HPP has been investigated as one way of improving the microbiological safety of the product. *L. monocytogenes* is relatively resistant to pressure and a treatment of >450 MPa for 5 min would be necessary to achieve a 2 $\log_{10}$ reduction in numbers. However, this treatment has adverse effects on the color and texture of the product. Therefore it is more likely that HPP will be useful in the development of other salmon products where consumers do not expect the specific traditional characteristics of cold-smoked salmon.

HPP Applications for Fruit and Vegetable Products

Fruit purees and sauces became the first HPP foods to be available commercially when they were launched on the Japanese market in the early 1990s. These products generally have a pH <4.5 and the primary reason for using high pressure was to extend shelf-life through the inactivation of yeasts and molds, rather than for reasons of microbial food safety. In the more recent years pressure-treated fruit juices and smoothies have become increasingly popular due to the fact that the treatment better retains quality attributes such as color, flavor, and vitamins, compared to conventional heat pasteurization. Typically pressures of 500 MPa are applied at room temperature or below for 1–2 min and the juices have a shelf-life of 3 weeks with a storage temperature of 4 °C. There have been a number of foodborne disease outbreaks attributed to the presence of pathogens such as *E. coli* O157:H7 and *Salmonella* spp. in high-acid foods, including nonpasteurized apple cider and orange juice. The pathogens generally cannot multiply at low pH, but some strains are acid tolerant and can survive storage in acidic fruit juices. High pressure combined with low pH results in increased inactivation and sublethally injured cells are less able to repair and so die more rapidly during subsequent storage of the juice. Thus, HPP can be incorporated into hazard analysis and critical control points (HACCP) programs to achieve the FDA requirement of 5 log reduction of pathogens in fresh juice.

Vegetable products generally have a higher pH and can potentially support the survival and growth of a wide range of pathogens. However, one of the most successful pressure-treated foods in the USA is guacamole. Its market share continues to grow and is reportedly based on the consumer preference for the 'fresher' taste of guacamole processed in this manner compared to heat-treated or frozen products. Treatment of approximately 500 MPa for 2 min is sufficient to extend shelf-life from 7 to 30 days at refrigeration temperatures. Challenge studies with a variety of pathogens have shown that this treatment is sufficient to give a 5-$\log_{10}$ reduction in numbers.

Other HPP vegetable products available commercially include wheatgrass, broccoli, and carrot juices. In these cases the products are acidified to pH <4.5 to ensure microbiological safety.

HPP Applications for Dairy Products

Currently there are relatively few HPP applications for milk and dairy products, probably because of the widespread acceptance and acceptability of heat treatments.

Patents have been filed for the use of HPP to treat extended shelf-life probiotic dairy-based foods such as yoghurt. Pressure-resistant and acid-resistant strains of lactic acid bacteria are used that can survive the processing conditions and subsequent storage. The use of HPP in the manufacture of cheese has also been the subject of much research. In some cases, the milk was treated with pressure instead of heat as a way of removing pathogens, leading to the possibility of microbiologically safe, high quality cheese with 'raw milk' characteristics. In other cases, mature cheese was treated with pressure in order to stop the ripening process.

There have been a few studies on the use of HPP as a method for inactivating *Cronobacter sakazakii* in liquid infant formula, before spray drying. The organism seems to be relatively pressure-resistant, compared to other pathogens. In one study, a modeling approach was used to determine treatment conditions required to achieve a 90% probability of achieving a 5-$\log_{10}$ reduction in numbers. The pressure-holding times required at 500 MPa were 26.3 and 7.9 min at 25 and 40 °C, respectively.

HPP Applications for Raw Meats, Fish, and Poultry

Research investigations have shown that HPP can be used successfully to inactivate vegetative pathogens in raw meat, fish, and poultry, thus giving safer products with a longer shelf-life. However, until recently, this has not been applied commercially as the pressures required (generally >500 MPa) generally cause changes in the appearance of the raw muscle, which leads to a cooked appearance. However, this problem can be overcome by covering the meat in a suitable sauce. This will also add value to the product as well as improving the microbiological quality. The products can be sold at a premium and there are now some of these in the marketplace.

Future Prospects for HPP

High-pressure-treated foods represent only a small share of the market, compared to foods processed by heat. However, the use of the technology is increasing, especially where it can give unique advantages over existing methods. To date, where pressure is used commercially to improve the safety of foods, this has been applied for the purpose of 'cold pasteurization' rather than sterilization. The next major development in the technology may be the ability to produce shelf-stable foods through the combined use of moderate heat and high pressure. Small experimental facilities are now available, which are capable of operating at temperatures of approximately 100 °C and pressures up to 600 MPa. These systems have been used to produce a limited range of shelf-stable products. Further research is required before this will be commercially feasible. This includes the need for sufficient experimental data to validate the microbiological safety of the foods produced in this way.

One major drawback of using HPP is the fact that it is a batch process. Modern commercial vessels can have a capacity of up to 600 l and such systems can produce 2 or more tons of product per hour per system, depending upon the vessel utilization and packaging configuration. This production rate can be increased further if multiple systems are used in parallel. However, this will still not give the same throughput that can be achieved with other methods, such as heat pasteurization, and this can add to the unit cost of pressure-treated foods. It is likely, therefore, that for the foreseeable future, HPP will mainly be used commercially for high value products, where it gives a unique advantage over other technologies, and where any additional processing cost can be justified.

See also: Foodborne Diseases: Overview of Emerging Food Technologies

Further Reading

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Microwave Heating

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Glossary

Athermal- or nonthermal effects Reactions that do not require the transfer of electromagnetic energy into thermal energy thus enhancing the temperature. Instead, the electromagnetic energy itself directly couples to energy to the molecule or lattice.

Electromagnetism Is one of the four fundamental interactions of nature and causes the interaction between electrically charged particles or magnetic dipoles; the areas in which this happens are called electromagnetic fields. Electromagnetism manifests as both electric and magnetic fields. Both fields are different aspects of electromagnetism and are intrinsically related. The classical interactions are governed by Maxwell's equations.

Emission The emission is the emitted (microwave) radiation power of an equipment.

Exposure The exposure is the quantity of absorbed (microwave) radiation energy by a human body or body part.

Pasteurization A heat-treatment process the aim of which is to inactivate or remove most microorganisms but definitely all pathogenic microorganisms from the product with a predefined probability.

Sterilization A heat-treatment process the aim of which is to inactivate or remove all viable forms of microorganisms from the product with a predefined probability.

Introduction

Starting in the 1960s–70s thermal continuous short-time processing operations of food (often combined with high temperatures) became more and more necessary. This is due to the increasing trend to produce foods industrially with high throughputs as well as the trend to produce food with minimum loss of sensorial and nutritional valuable ingredients or even increased nutritional quality, keeping its safety untouched (minimum processed food). The pasteurization of milk in the high-temperature short-time process (ultra-high temperature (UHT) or extremely high temperature (EHT) process) at 140 °C ensuring a safe product needs only a few seconds, during which the product suffers only slight quality deterioration is just one well known example.

For such short-time thermal processes, the heating rate period cannot be neglected compared with the holding times. Short heating times depend on rapid heat transfer mechanisms, which cannot be achieved by conventional heat conduction especially for solid foods, giving a technological push to alternative heating techniques as microwave heating. Although the heating capacity of microwaves was already found in the 1950s, its use in food processing is still relatively limited to niche applications – apart from the domestic use.

Physical Principles

Microwave heating is based, as most other alternative heating techniques, on electromagnetism. The main difference between ohmic heating, microwave heating and infrared heating is in the frequency of the electromagnetic field used. The coupling of the electromagnetic field (mainly the electric component) with the matter is responsible for the energy conversion into heat. Waves of frequencies between 300 MHz and up to 300 GHz are called microwaves (the corresponding wavelength in vacuum or air range from 1 m down to 1 mm).

Electromagnetic waves with frequencies smaller than ultraviolet rays and thus also microwaves can be summarized as nonionizing radiation, which means that the wave photon does not have enough energy to directly produce ions from neutral atoms or molecules. The latter is important for the chemical/physical impact of the radiation.

Generally, electromagnetism waves may be basically described using Maxwell's equations. The interaction of electromagnetism with matter is included in constitutive relations (1–3), where the permittivity or dielectric constant ε, interaction of nonconducting matter with an electric field, the electrical conductivity σ and permeability μ, interaction with a magnetic field, describe their behavior. The zero indexed values belong to vacuum values:

$$\vec{D} = \varepsilon_0 \varepsilon \cdot \vec{E} \quad [1]$$

$$\vec{B} = \mu_0 \mu \cdot \vec{H} \quad [2]$$

$$\vec{j} = \sigma \cdot \vec{E} \quad [3]$$

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Equation [3] is equivalent to Ohm's law, when the definitions of the current density j and of the electric field (within a homogeneously filled plate capacitor with plate distance l and plate area A) are taken into account.

In general, the material parameters do show a directional-dependent behavior. For most food substances, for practical use this directional dependency is neglected and the relative permeability can be set to $\mu = 1$. Thus, the permittivity tensor can be reduced to a complex constant with real (ε') and imaginary part (ε''), which may include the conductivity σ when an isotropic material is assumed.

Starting from Maxwell's equations the corresponding wave equations (for the electric eqn [4] or magnetic field) or the more general Telegrapher's eqn [5] can be easily derived:

$$\Delta\vec{E} - \mu_0\mu\varepsilon_0\varepsilon\frac{\partial^2\vec{E}}{\partial t^2} = 0 \qquad [4]$$

$$\Delta\vec{E} - \mu_0\mu\sigma\frac{\partial\vec{E}}{\partial t} - \mu_0\mu\varepsilon_0\varepsilon\frac{\partial^2\vec{E}}{\partial t^2} = 0 \qquad [5]$$

showing the wave velocity as defined by eqn [6]:

$$c = \frac{1}{\sqrt{\mu_0\varepsilon_0\mu\varepsilon}} = \frac{c_0}{\sqrt{\mu\varepsilon}} \qquad [6]$$

Furthermore, the conductivity is an additional term to the imaginary part of the dielectric constant (eqn [7]):

$$\varepsilon''_{\text{total}} = \varepsilon'' + \frac{\sigma}{\varepsilon_0\omega} \qquad [7]$$

as the exponentially damped waves are a solution of the above differential equations.

The corresponding electric field penetration depth, the distance in which the electric field is reduced to the fraction $1/e$ can be calculated by eqn [8]

$$\delta_E = \frac{1}{\omega}\cdot\sqrt{\frac{2}{\mu_0\mu\varepsilon_0\varepsilon'\cdot\left(\sqrt{\sqrt{1+\frac{\varepsilon''^2}{\varepsilon'^2}}-1}\right)}} \qquad [8]$$

An important consequence of the frequency dependency of δ_e is that microwaves of 915 MHz do have an approximately 2.5 times larger penetration depth than waves of 2450 MHz, when similar permittivities at both the frequencies are assumed. This larger penetration depth helps to heat larger (industrial) pieces more homogeneously.

With the assumption of the excitation and the propagation of a plane wave, first estimations of the field configurations within applicators and food are possible. For example, the laws of the geometric optics can be inferred, which are also valid for microwaves when the object size is much larger than the wavelength.

So the particular center heating of objects of cm-dimensions with convex surfaces (like eggs) can be easily understood, as at the convex surface the microwave 'rays' are refracted and focused to the center.

For objects that are of the same size as the wavelength or smaller, as well as complex food and applicator geometries direct field modeling by numerical solutions of Maxwell's equations becomes more and more important.

To calculate temperature changes within an object by microwave heating, the power density starting from the electromagnetic field configuration is necessary. Mostly, the electric field is essential to calculate the power dissipation into heat. This power dissipation (per unit volume) p_V is determined by ohmic losses which are calculable by

$$p_V = \frac{1}{2}\sigma_{\text{total}}\cdot\left|\vec{E}\right|^2 = \frac{1}{2}\omega\varepsilon_0\varepsilon_{\text{total}}\cdot\left|\vec{E}\right|^2 \qquad [9]$$

The increase of the power dissipation with increased microwave frequency is obvious (when the permittivity is constant). The power dissipation penetration depth δ_p is reduced by a factor of 2 compared with the electric field penetration depth δ_E (eqn [10]):

$$\delta_p = \frac{1}{\omega}\cdot\sqrt{\frac{1}{2\cdot\mu_0\mu\varepsilon_0\varepsilon'\cdot\left(\sqrt{\sqrt{1+\frac{\varepsilon''^2}{\varepsilon'^2}}-1}\right)}} \qquad [10]$$

Typical values for the penetration depth are listed in Table 1.

Table 1 Penetration depth of microwaves in food materials

Material	*Temperature* $\theta/°C$	*Penetration depth* δ_p*/mm at 915 MHz*	*Penetration depth* δ_p*/mm at 2450 MHz*
Water			
Distilled/deionized	20	122.4	16.8
0.5% Salt	23	22.2	10.9
Ice	−12	–	11 615
Corn oil	25	467	220
Fresh fruits and vegetables			
Apples (red delicious)	22	42.6	12.3
Potato	25	21.3	9.0
Asparagus	21	21.5	10.3
Dehydrated fruits			
Apples (red delicious) %Moisture (wet basis)			
87.5	22	48.9	12.9
30.3		33.7	11.9
9.2		387	289
68.7	60	33.1	14.5
34.6		36.8	13.2
11.0		71.5	29.9
Animal products			
Yogurt (premixed)	22	21.2	9.0
Whey protein gel	22	22.2	9.6
Cooked ham	25	5.1	3.8
	50	3.7	2.8
Cooked beef	25	13.0	9.9
	50	9.5	8.9

Source: Data from Tang J (2005) Dielectric properties of foods. In: Schubert H and Regier M (eds.) *The Microwave Processing of Foods*, pp. 22–40. Cambridge: Woodhead Publishing.

Microwave Heating Equipment

Safety Regulations

The frequency range of microwaves is used for telecommunication purposes like mobile phones, radar, or in modern times bluetooth connections, too. To prevent interference problems special frequency bands have been assigned for industrial, scientific, and medical (the so-called ISM) applications, where a certain radiation level has to be tolerated by other applications like bluetooth communication devices. The microwave ISM bands are located at 433, 915, and 2450 MHz whereby the first frequency is not often used, the second is not generally permitted all over the world.

For microwave ovens at home the only used frequency is 2450 MHz, whereas 915 MHz (as well as combinations) has some considerable advantages for industrial applications. Apart from the interference regulations, two types of safety regulations exist:

1. the regulation concerning the maximum exposure or absorption of a human, working in a microwave environment, and
2. the regulation concerning the maximum emission or leakage of the microwave equipment.

Concerning the food safety for microwave equipments no special regulations exist, but microwave-specific safety concerns are addressed in the next article.

1. *Exposure*: The exposure limits for humans is based on the estimation of thermal effects that microwaves can cause in the human body. Thus, the limit for human exposure that is generally considered to be safe is at a level of 1 mW cm^{-2} body surface in most countries. Furthermore as common in the case of ionizing radiation, there is also a trend to express the exposure or absorption by humans by the value of the specific absorption rate (SAR), which is defined as the quotient between the incident power and the body weight. For microwaves the International Commission on Non-ionizing Radiation Protection (INIRC, 1998 as confirmed in a statement of 2009) recommends a value for the SAR to be set to 0.4 W kg^{-1}.
2. *Emission*: The maximum emission of a microwave equipment is limited to a value of 5 mW cm^{-2} measured at a distance of 5 cm from the point where the leakage has the maximum level. Thus, the permissible leakage level is higher than the maximum exposure limit. However, the power density of nonfocused radiation, what is normally the case for leakage, decreases proportional to the inversed square of the distance from the source. So a leakage which just manages to stay in the limits of 5 mW cm^{-2} at 5-cm distance is already below the maximum exposure limit of 1 mW cm^{-2} at a distance of 11.2 cm.

Setup

Three basic parts are generally necessary in a microwave heating device: a microwave source, a waveguide, and an applicator.

The generally used microwave sources for industrial and domestic applications are magnetron tubes with a maximum power of 1.5 and 25 kW for air- or water-cooled systems, respectively, and efficiencies of approximately 70%.

Transmission lines (e.g., coaxial lines) and waveguides are used for guiding the microwaves with minimum losses to the applicator.

Already the waveguide itself can be used as applicator for microwave heating when the material to be heated is introduced by wall slots and the waveguide is terminated by a matched load (the so-called traveling wave device). More common in the food industrial and domestic field are standing wave devices where the microwaves irradiate by slot arrays or horn antennas.

Tuners are waveguide components used to match the load impedance to the impedance of the waveguide, so that tuners minimize the amount of reflected power, which results in the most efficient coupling of power to the load. Furthermore, circulators – directional-dependent microwave traveling devices – are used that let the incident wave pass and guide the reflected wave into an additional load.

Common applicators can be classified into three types based on the type of field configurations:

1. *Near-field applicators*: In the case of near-field applicators the incident microwaves are completely absorbed by the product to be heated.
2. *Single-mode applicators*: To heat substances with low dielectric losses efficiently by microwaves, applicators with resonant modes that enhance the electric field at certain positions (where the material to be heated is located) are used. The applicator has a relatively small dimension to avoid standing wave modes that differ from the desired mode.
3. *Multimode applicators*: By increasing the dimensions of the cavity a fast transition from the single mode to the multimode applicator occurs due to the strongly increasing mode density with applicator size. In industrial as well as in domestic applications the multimode applicators play by far the most important role although a nonhomogeneous field distribution is often dominant. In opposition to the case of the single-mode application, usually this inhomogeneous field distribution which would result in an inhomogeneous heating pattern is not desired as it is hard to control. An undesired inhomogeneous heating pattern can be prevented by changing the field configuration by varying cavity geometries (e.g., mode stirrer) or by moving of the product (conveyor belt and turn table), which also influences the field distribution.

Regulations require the avoidance of leakage radiation through the product flow cavity through the inlet and the outlet. For fluids or granular products with small dimensions (cm range), this can be guaranteed by the small in- and outlet sizes together with the absorption of the entering product sometimes coupled with additional dielectric loads just in front of the openings.

Especially in the case of larger product pieces, inlet and outlet gates that completely close the microwave application device have to be used.

Microwave-Specific Safety and Quality Aspects for Foods

Effect on Microorganisms and Chemical Compounds

Some literature in the past as well as today report on the experimental results that cannot be explained solely due to the thermal effect of microwaves on chemical structures and microorganisms. Thus, frequently assumptions have been made if and how a possible athermal effect of microwaves on cells, spores or chemical compounds could be explained.

However, it is generally believed that inactivation due to dielectric heating in the range of microwave frequency is solely caused by thermal effects. This is also supported by the ICINRP statement on the electric, magnetic and electromagnetic fields (EMF) guidelines from 2009, where only thermal effects are taken into account.

The most impressive theoretical reason for the no-existence of athermal effects is that electromagnetic energy can only be absorbed in quantums. For any athermal effect of microwaves their quantum energy has to exceed the bond energy of chemical bonds but in reality lay 4–5 orders of magnitude below.

Elevated but also reduced inactivation levels that are sometimes observed in microwave applications could be explained in the way that before a temperature equilibration can take place temperatures in hot or cold spots falsify integrated measured values. An elevation of the temperature of a few degrees in the critical temperature range can potentiate the success of inactivation, whereas lower temperatures may serve as 'life-boats' for microorganisms.

Whereas improbable athermal inactivation effects would even support the microbial food safety, a reduced inactivation by microwave heat compared with 'conventional' heat would be much more problematic but in the literature there is no evidence for an elevated heat resistance of pathogenic bacteria exposed to microwaves.

Thus, the United States Food and Drug Administration recommends to act on the assumption that solely thermal effects exist, the more so as all proposed athermal effects increase the inactivation rate. So the ignorance of athermal effects in pasteurization and sterilization processes improves food safety.

n general, it is to be stated that – as opposed to the cell phone and communication sector – in the food sector possible athermal effects do not endanger the consumer. If athermal effects had an influence on food safety, it would increase. This is due to that no chemical changes that can be induced by microwaves that do not occur in conventionally heated foodstuffs.

IActually, the possible risk for food safety in microwave processes stems from another microwave specificity: The temperature distribution in microwave-treated foodstuffs develops completely different compared with conventional processes. So unexpected changes in quality – positive or negative – can occur due to uncommon heating patterns. In principle, it can be stated that where microwaves increase heating uniformity an enhancement in quality can be expected; meanwhile, in processes where extreme uneven heating patterns occur, quality levels fall below the ones of conventional processes.

Inhomogeneous Heating

Depending on the compounds, the geometry of a food sample as well as the used microwave input uneven heating can occur in microwave processing. Several effects that even interact with, amplify and cancel each other, may contribute to inhomogeneous temperature distributions.

On one hand the electric field independent of the load does often not develop homogeneously in the applicator. Especially in geometrically simple structures as, for example, cuboids used in household standing wave patterns will cause the load to be heated more intensely in some parts of the oven than in others.

On the other hand, the dielectric properties of the heated body are temperature dependent and vary in space caused by an inhomogeneous composition of the sample. Sometimes, 'runaway heating'-effects can occur if the absorbed energy by far exceeds the energy that can be transported by heat conduction and when the dielectric loss factor increases with temperature. This happens, for example, if small areas of food melt, meanwhile, most of the sample stays frozen.

Furthermore, the electric field is influenced by the load due to the change in dielectric constant and dielectric loss. In a simple approximation, the electric field is damped exponentially by traveling in a lossy load. The higher the frequency, the more will be the electric field strength already damped in the outer regions of the load.

In addition to that, the geometry of the heated sample plays an important role. Edges and corners often overheat faster than round surfaces. Small bodies with a diameter below some millimeters barely heat, bodies with a diameter below some centimeters tend to overheat in the center due to microwave refraction, superposition of different waves, and focusing effects.

Different from conventional heating processes, on the surface of microwave-heated foodstuffs the effect of evaporative cooling may be pronounced even in drier products, when surface moisture is driven to the surface due to internal vapor pressure buildup.

Nutritional Value of Microwave-Heated Food

A broad literature overview on the nutritional value of microwave-processed food is given by Cross 1982 stating that no significant nutritional differences exist between foods prepared by conventional and microwave methods and any differences reported in the literature are minimal. Again it should be emphasized that differences stem from thermal effects (different temperature distributions) supported by following heat and mass transfers. Nevertheless, for consumer products the worst case scenario for temperature distributions in a household microwave oven has to be taken into account, thus the minimum time and power to reach the necessary temperature has to be labeled on the packaging as well as additional explanations to ensure a homogeneous heating pattern (if wanted). For example, stirring or equilibration times should be stated.

Use of Microwaves in the Industry

Microwave processes often compete with well known and well established standard processes. Of course, only when strong

quality or financial reasons support the use of microwaves these standard processes may be overcome, as the implementation of 'new' techniques also expose some risk.

A possible problem is the right choice of the packaging material. As of high-temperature peaks that can occur in microwave applicators it must have a sufficiently high melting point. Generally, it should be transparent to microwaves, but some metal may on one hand be used as shielding to avoid the overheating of edges and corners. On the other hand, the so-called susceptors allow for additional heat dissipation at selected parts of the product. Thus, for example, browning of surfaces, what cannot be achieved by ordinary microwave processes, is possible. Nevertheless, due to extreme temperatures that can develop in these processes it cannot be generally excluded that chemical compounds migrate from the susceptor material to the food.

Pasteurization and Sterilization

As mentioned in Section Introduction, a shift toward elevated temperatures and shorter process times for microbial inactivation while avoiding intense loss of quality is necessary. This effect is caused by the stronger dependency of inactivation kinetics of microorganisms from temperature than the corresponding degradation kinetics of quality attributes as vitamins and texture. Owing to the limited heat conductivity this advantage cannot be used for the processing of solid and highly viscous foods, where surface regions degrade much faster than central ones. Here lies one chance for the use of microwaves as they overcome the slow heat conduction by heat generation within the product.

A process for microwave based-microbial inactivation consists of four phases: heating, temperature equilibration, holding, and cooling. The special relevance of the second phase due to inhomogeneous heating patterns in microwave fields may exist.

Thus, the most critical point in microwave pasteurization and sterilization processes is to assure that any point in the treated body has reached the working temperature for a selected time. As temperature distributions cannot be easily measured three dimensionally alternative approaches are necessary for the validation of the sterile criteria. One approach is the use of time-temperature integrators.

Often, the effect of evaporative cooling on the surface gives rise to smallest temperatures at the surface in microwave heating of moist foods. This cannot be overcome by simply prolonging the times for equilibration and holding, but by humid atmospheres, waterproof packaging or by using external heating sources.

Tempering and Thawing

Possible benefits of microwave thawing are shorter thawing time, smaller processing space, reduced drip loss, less microbial problems, and chemical deterioration (both due to shorter times). However, the difference of the dielectric loss between frozen water and liquid water of three orders of magnitude is the cause for the so-called runaway heating as soon as a part of the product is completely thawed.

To overcome this problem, instead of fully thawing the food, it is tempered by microwaves to just some degrees below the melting point. Furthermore, surface cooling at temperatures just below the freezing point bewares the surface from melting and minimizes drip loss and quality deterioration.

To achieve a more uniform heating especially for larger products usually a frequency of 915 MHz is used due to its larger penetration depth.

Drying

Microwave-assisted drying has possible advantages: The correlation between dielectric loss and moisture content, the so-called leveling effect, makes the electromagnetic energy rather dissipate in wet regions than in regions of the product that have already been dried. Furthermore, a preferred volumetric heating (even of the product center) that cannot be as easily reached by heat conduction from the surface is possible. Besides, microwave drying processes show often a high efficient drying in the falling rate period that is even delayed observing an extended constant rate period. So, as soon as the evaporation temperature has been reached, the drying rate is proportional to the applied microwave power that makes controlling relatively easy. The volumetric heating and evaporation also reduces the migration of water-soluble constituents to the surface and may increase the internal porosity, which facilitates rehydration.

Nevertheless, some problems may also occur, which also influence the food quality and safety. If at the end of drying, regions of high water content are still existent (due to inhomogenities in heating and evaporation) they may give rise to microbial spoilage. Another critical point may be the occurrence of plasma due to a dielectric breakdown of the atmosphere. Even when the process starts at an uncritical power level where all the power is delivered directly to the load the fall of lossy (moist) material may give rise to an increasing maximal field strength in the applicator (especially at metallic corners). Thus, unadjusted microwave power can allow for a glow discharge (or plasma) that may scorch or burn the product when it appears close to it. Of course, the quality of the product is reduced or even toxic combustion products may develop.

See also: Food Technologies: Aseptic Packaging; Food Irradiation; Pasteurization; Sterilization. Risk Analysis: Risk Communication: Novel Foods and Novel Technologies

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Relevant Websites

www.ampereeurope.org
Association for Microwave Power in Europe for Research and Education (AMPERE).

www.icnirp.de
International Commission on Non-Ionizing Radiation Protection (ICNIRP).

www.impi.org
International Microwave Power Institute (IMPI).

Nanotechnology and Food Safety

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Glossary

Material A single or closely bound ensemble of substances at least one of which is in a condensed phase, where the constituents of substances are considered to be atoms or molecules.
Nanoparticles At present generally accepted as particles with one or more external dimensions in the range 1–100 nm.
Nanoscience The study of materials at the atomic or molecular level.
Nanotechnology The manipulation or materials at the atomic or molecular level.
Particle A minute piece of matter with defined physical boundaries.
Particulate nanomaterial It is currently considered to be a material in the form of particles with one or more external dimensions in the size range 1–100 nm.

Introduction

There are several aspects of nanotechnology that relate to food safety. These include the use of the products of nanotechnology in protecting the authenticity and ensuring the traceability of foods, and the use of nanotechnology in the design of coatings, surfaces, and other food contact materials that will inhibit microbial contamination and detect microbial spoilage. On the other hand, there is considerable debate over the issues and processes of the regulation of nanotechnology, because of concerns about the safety issues related to the ingestion of certain particulate nanomaterials that may persist and accumulate in the body.

Authenticity and Traceability of Foods

Perhaps the most successful use of nanotechnology is in the miniaturization of electronic circuits that has led to vast improvements in computing, communication, and data accumulation and processing. These aspects of nanotechnology offer considerable promise for enhancing food safety. The use of labels or packaging materials, capable of monitoring and reporting on changes in environmental conditions, in combination with radio frequency identification and satellite tracking, provides a basis for tracking food products and their quality throughout distribution and storage. There is considerable interest in the development of a range of sensors that can be incorporated into labels or packaging materials. The particulate nanomaterial components of these sensors, surfaces, labels or coatings will be enclosed within larger solid matrices and are unlikely to lead to migration of nanostructures into food: Migration of particulate nanomaterials into food structure on contact will be an important aspect of the evaluation, regulation, and approval of these products. The use of hand held electronic devices during shopping and to interrogate labels, electronic remote shopping, and the data bases collected by major retailers on consumer purchasing are all benefits of nanotechnology that are regarded as safe and acceptable.

Food Contact Materials

In Europe and in many countries worldwide there is currently debate on the definitions and regulatory procedures needed to evaluate the use of nanotechnology in the development of food contact materials. Recent guidelines from bodies such as World Health Organisation/Food and Agricultural Organisation have stressed the need for universally accepted definitions and regulatory procedures. While this debate continues a perusal of the internet reveals the advertisement and availability of a growing number of food related products claimed to be 'nanoproducts'. A large proportion of these products are food contact materials, and most of these products are claimed to reduce microbial contamination and improve the shelf life of food produce. Although individual manufacturers may have evidence to support their health claims and the safety aspects of the use of these products, there is generally no independent verification of these claims or approval for use in the countries in which these products can be purchased through the internet. The labeling of these products as nanoproducts is used as a branding tool and does not necessarily confirm that they contain nanostructures, or are really produced through the use of nanotechnology. Examples of such products include packaging materials, containers, kitchen utensils, cutting boards, eating utensils, pots, pans, cloths, kettles, and larger objects such as refrigerators. Many of these products use silver

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nanoparticles as antimicrobial agents (although nanoparticles of other materials such as magnesium oxide and zinc oxide have also been reported to have antimicrobial activity). For these applications the (silver) nanoparticles are enclosed in a solid matrix at the surface of the products. Given that manufacturers of food and food contact materials have a requirement to ensure that their products are safe, and that any approval for use will require studies to establish lack of migration of the silver nanoparticles into foods, it is likely that these products will be safe for use. If they reduce microbial contamination and improve shelf like then they will be of benefit to the food industry. However, there may be ecological or social arguments for restricting the use of such products, particularly if such nanoparticles were incorporated into biodegradable or edible coatings.

An alternative to the use of nanoparticles as antimicrobial agents is the use of nanotechnology to coat surfaces to inhibit the adsorption of microorganisms and the growth of biofilms. This may include the deposition of single layers or multilayers to reduce the roughness of the surface, or to change the physical properties, in order to repel bacteria and ease cleaning and reuse of the materials.

There are suggestions that sensors could be designed to detect the presence of microorganisms and then trigger release of antimicrobial agents. There is also research on the development of sensors that can detect products of microbial spoilage that could be used to signal that a food product is no longer safe to eat – the sensor could for example trigger a color change on a label.

There are already commercial examples of the incorporation of nanoparticles into packing to enhance shelf life on storage: Incorporation of clay nanoparticles into plastics has been used to control the flow of gases into and out of plastic bottles by introducing lengthened diffusion paths for the gases within the plastic composites.

Ingestion of Nanoparticles

There is considerable interest in the use of nanoencapsulation as a tool for the delivery of a variety of components such as vitamins, lycopene, beta-carotene, lutein, phytosterols, coenzymes such as Q10, natural colors, and unsaturated fatty acids. The components themselves can be fabricated as nanoparticles that can be amorphous or crystalline, which often require surface modification to enhance dispersion and prevent irreversible aggregation on dispersion. Examples of nanocrystals include starch, lycopene, and certain fatty acids. Delivery vehicles include nanomicelles, nanoemulsions, molecular cages that can be organic or inorganic and certain biopolymer latex nanoparticles. There are benefits common to nanoencapsulation and microencapsulation. Both techniques can be used to reduce transport and storage costs through the production of powders, to protect components from deterioration on storage, to partially protect or target delivery during digestion, to disguise unpleasant tastes and odors, and to improve the dispersion of fat soluble materials in aqueous medium and enhance their bioavailability. Nanoencapsulation is considered to enhance availability through improved uptake at cellular surfaces. The smaller size of nanoparticles means that they will remain in suspension longer, and scatter less light than larger colloidal particles, allowing use in a wider range of transparent aqueous food and drinks.

The nanoparticles used in nanoencapsulation will be novel food additives or ingredients and the foods they are incorporated into will be novel foods. As such they will require approval for use. At present there is debate as to whether the current regulatory systems are capable of adequately assessing these new types of products. Where the encapsulated components and the capsules themselves are completely broken down and metabolized during digestion the products are probably covered by current regulatory procedures. However, if the components themselves, or the carriers used in nanoencapsulation, are not metabolized or excreted then there is need for more detailed evaluation procedures. Because of their size these materials may enter and accumulate in areas of the body that larger colloidal particles cannot enter: In this case there is a lack of information and methods for assessing the consequences of this persistence and bioaccumulation.

Definitions, Regulation, and Labeling

It is widely accepted that there is a need for worldwide acceptance of definitions of engineered nanomaterials and a procedure for the evaluation and approval of uses of nanoscience and nanotechnology in foods, food additives and ingredients, and in food contact materials. Such definitions need to be enforceable and for this reason it is suggested that they are based on size alone. There is a wide range of definitions of nanostructures and most are summarized in the EU Joint Research Center Reference report EUR 24403 EN. This report suggested particulate nanomaterials as a class of materials that could be identified and that represented those nanostructures that raised concern over their ingestion. The growing consensus is that the evaluation procedure should involve a tiered approach that looks at whether a particular 'nano' product is covered by current regulatory procedures and, if not, identifies classes of products requiring additional information, or new types of methodology for their evaluation. In this context the definitions of nanostructures adopted for regulatory purposes may be important. The term nanomaterials does by definition exclude natural food macromolecules, such as polysaccharides, proteins, and certain lipids, which by most definitions would be called nanoparticles: Materials are by definition structures composed of atoms and molecules. The term particulate nanomaterials would exclude most naturally occurring self-assembled nanostructures present in plant and animal food products, and those nanostructures introduced during processing to generate food gels, foams, and emulsions. Finally, there is growing awareness of the fact that there are essentially two classes of particulate nanomaterials: nonpersistent and persistent particulate nanomaterials. The former, which are completely broken down during digestion are probably covered by current regulatory procedures but will require additional information related to how size affects the site and level of adsorption. For the latter class new information and methodologies will in general be required to evaluate the safety of these products as, on ingestion they can penetrate into regions of the body where larger colloidal particles cannot reach.

Any definitions of nanostructures used for regulatory purposes need to be enforceable. Hence it is likely that they will be based on size alone. The size range chosen is usually between 1–100 nm, although the upper limit is open to debate. This size range is currently chosen such that it will include all particulate nanomaterials where reduction in size leads to significant changes in physical and chemical properties. Given that most of the concern is with the ingestion of persistent particulate nanomaterials that accumulate in the body, and which because of their reduced size penetrate into regions that colloidal particles cannot reach, it may be that this aspect offers an alternative basis for defining the upper size limit.

Environmental Concerns

The incorporation of nanoparticles into solid matrices that limit or prevent migration of the nanoparticles into food or drinks will result in nanoproducts that are safe for use as food contact materials. The products may provide benefits to the food industry, primarily through preventing or detecting spoilage or microbial contamination, thus enhancing shelf life, reducing food wastes and improving food safety. However, there must remain some concern, and some need to consider as part of any approval process, the 'whole life' aspects of these new food contact materials, particularly if persistent particulate nanomaterials are incorporated into biodegradable food contact materials. Approval for food use should consider whether the disposal of these products may ultimately lead to release and accumulation of nanoparticles in the environment and entry into the food chain: There is growing evidence that particulate nanomaterials can be passed up the food chain once they are released into the environment. Thus there may be a need for specific recycling of these products to prevent contamination and/or labeling to enable consumers' choice in the purchase, use, and disposal of these products. The nanoparticles when incorporated into these food contact materials have antimicrobial properties this will need special consideration in terms of the environmental effects. The widespread use of particulate nanomaterials as antimicrobial agents in food contact materials or as food supplements raises concerns that their ingestion or release into the environment may lead to low-level exposure and acquired bacterial resistance to these materials. This may preclude their future medical use as antimicrobials. Where there are ecological concerns this may warrant selective labeling in order to enable consumer choice and to identify any need for special recycling of the products.

See also: Disciplines Associated with Food Safety: Food Safety Toxicology. Food Safety Assurance Systems: Labeling and Information for Consumers. Foodborne Diseases: Overview of Emerging Food Technologies. Hazards of Food Contact Material: Nanotechnologies and Nanomaterials. Institutions Involved in Food Safety: FAO/WHO Codex Alimentarius Commission (CAC). Other Significant Hazards: Physical Hazards in Foods. Public Health Measures: Assessment of Novel Foods and Ingredients.

Risk Analysis: Risk Communication: Novel Foods and Novel Technologies

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Packaging

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Glossary

Good manufacturing practices All practices regarding the conditions and measures necessary to ensure the safety and suitability of food at all stages of the food chain.
Hazard A biological, chemical, or physical agent in food or the condition of food itself with the potential to cause an adverse health effect.
Package A metal can, glass bottle, plastic bag, or pouch suitable for its function of containing the food product, besides providing protection, convenience, and communication for the product.
Permeability The transport of a gas, vapor, or liquid through a barrier without affecting it physically or chemically.
Shelf life Consumer acceptability of product under specified conditions of storage.

Food Packaging

Food packaging is of paramount significance to preserve the quality of fresh and processed foods. It would be practically impossible for the food processors to distribute food without packaging. The several packaging functions include prevention of spoilage and contamination, preservation of food quality, physical protection and product information, bring convenience, and facilitate transportation as well as distribution. Therefore, food packaging may be defined as, 'the enclosure of food products in a wrapped pouch, bag, box, tray, can, bottle or any other packaging material with the functions of containment, protection, preservation, communication, utility and performance.' Packaging may be performed before processing (canning, retort pouch, dairy fermentations, etc.) or can be followed after major processing steps (pasteurization, ultra high temperature (UHT) processing, baking, frying, etc.).

Packaging Functions

The primary function of packaging is to protect the food quality and to prevent the spoilage and pathogenicity during its entire period from production to consumption (Figure 1). The basic packaging functions are elaborated as follows:

Protection

The deterioration of food is either caused by external factors like oxygen, moisture, off flavors, toxins, microorganisms, mass transfer, physical and mechanical damage, or by internal factors like inherent microorganisms. Therefore, the primary function of the package is to protect the food from adverse effects of environment, retain the beneficial effects of processing, extend the shelf life and maintain the quality and safety of fresh or processed foods. Moreover, the diversity in composition, structure, and physiology of fresh and processed foods demands varying levels of protection throughout the supply chain. Therefore, the packaging should be aimed to protect the food from physical, chemical, and biological hazards.

Physical protection shields the food from mechanical damage due to shock and vibration during transportation and distribution. Packaging materials like paperboard, corrugated materials, etc. resist impact, abrasion, and crushing damage caused to fruits, eggs, cakes, etc., during transportation. The replacement of glass bottles with plastic packaging material is another example for increasing the physical protection levels to liquid foods. Chemical protection is aimed to reduce the compositional changes caused due to, for example, oxidation, light, and the resulting degradation of color, vitamins, and nutrients. Glass packaging materials, for example, are inert with absolute barrier to substances, but lack protection against light, whereas metal packaging materials act as an absolute barrier but with some migration issues. Plastic packaging materials as commonly used in food packaging show a wide range of different levels of barrier properties. The third role is to protect the food from biological deterioration caused by spoilage and pathogenic microorganisms (bacteria, yeasts, fungi, and viruses), and animals like insects or rodents.

Containment

The other basic packaging function is to contain food products in specific containers (packages) to facilitate transportation and distribution throughout the supply chain. Without packaging, there would be a huge product loss due to several environmental factors. Moreover, it would be impossible to move liquid products without packages; furthermore, mixing

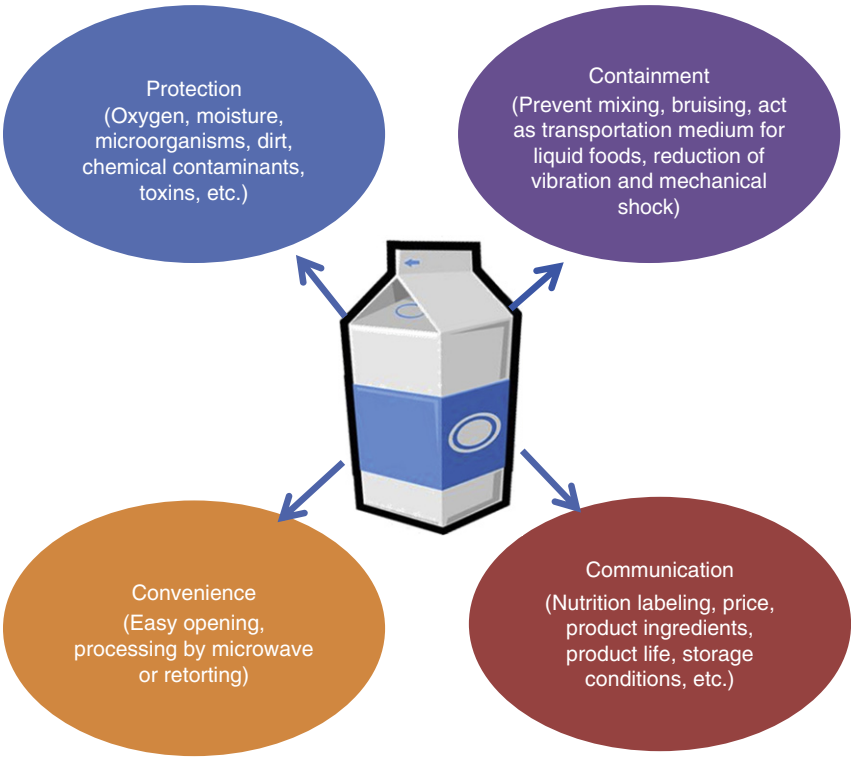

Figure 1 Major functions of food packaging.

of different solid foods is possible due to shock and vibration during transportation. The other associated problems include mixing of graded food stuffs, bruising of soft fruits, physical damage due to friction of loose materials, contamination with air suspended particles, etc. Therefore, packaging reduces the huge loss of food products in the entire product chain.

Convenience

Industrialization and busy work schedules demand the food and packaging industry to bring convenience to food through innovative packaging solutions. The packaging industry has brought convenience by incorporating features such as easy opening, reclosability and processing in the package via, for example, microwavable packaging, oven safe trays or boil in bags. This has enabled the consumers to prepare food in shorter time, which in turn has increased the global demand for packed food.

Communication

Each package is labeled to inform the consumers about the product contents, brand, shelf life, and storage conditions. This communication is important to consumers in order to know the product quality, characteristics, and handling, besides assistance in marketing strategies. Several packaging designs are used to accommodate the product information (nutrients, weight, brand labels, certification, ingredient labeling, barcodes, etc.) in order to satisfy the legal requirements and to promote the product branding. Moreover, the package conveys important information on product storage, cooking instructions, price, and life cycle.

Developments in Packaging

The developments in packaging have moved beyond these basic packaging functions. The introduction of modified atmosphere packaging, active packaging (oxygen scavenging, antimicrobial activity), and intelligent technologies have performed beyond basic functions and increased the product quality, safety, and shelf life beyond consumer expectations. Such innovative technologies need further experimentation to optimize them for cost effectiveness.

Packaging Materials

A number of packaging materials are currently in use for food applications, which are accepted by regulatory bodies like the United States Food and Drug Administration (FDA), European Union (EU), etc. The packaging design and material properties determine the package end use and shelf life of packaged foods. Glass, paper, metal, and plastics are the most important groups of materials used for food packaging.

Glass

It is an inert packaging material with absolute barrier to gases and moisture making it versatile packaging material to retain the flavor and freshness of delicate food products like beer and wine. The other advantages of using glass are that it can withstand high thermal processing conditions, provides good insulation, can be formed into different shapes, and can be supplied in different colors with an optical transmission

between nearly opaque and nearly transparent. Additional oxide coatings help to improve the mechanical properties and give a barrier against chemical attack. Heavy weight, and fragility to internal pressure, impact and thermal shock are some of the limitations for their extensive use in food industry.

Paper

Paper is another commonly used material in the packaging of several food products. Plain paper is hardly ever used for food packaging alone. It has to be modified with several additives (lacquers, waxes, resins, etc.) or extrusion coated with other polymers to improve the barrier properties. Paper and paper boards are used in several forms such as corrugated boxes, cartons, bags, sacks, and wrapping paper. Selected forms of paper used for packaging are given in the following:

1. Kraft paper – Available as natural brown, unbleached, and bleached white. It is the strongest type of paper and is used for bags and wrappings.
2. Sulfite paper – It is usually glazed to improve the appearance, wet strength, and oil resistance. Sulfite paper is relatively lighter and weaker than kraft paper, but has high print quality. It is often used in plastic or foil laminates.
3. Densified and greaseproof papers of different types, e.g. glassine or parchment. They offer fat resistance but give a high permeability to moisture and are used for packaging of biscuits, confectionery and fats.
4. Paperboard – Available in several forms (white board, solid board, chip board, fiber board, and paper laminates), mainly used as secondary packages to improve the handling and distribution of food products.

Metal

The basic metals used for food packaging are steel and aluminum. Steel has to be plated against corrosion by tin, thus giving tinplate or chromium. They offer excellent barrier properties, physical protection, printability, consumer acceptance, and recyclability. Tinplate is produced from low carbon steel, which is coated on both sides with a thin layer of tin. In most applications, they are further lacquered with epoxy or polyester resins to provide an additional barrier to the food materials against corrosion. Containers from tinplate or aluminum are commonly used in retort processing of fruit and vegetables, meat, fish, pulses, cans for drinks, containers for baby foods or powders, confectionery, etc.

Aluminum is further used to produce flexible packaging materials like foil, laminated paper/plastic films, and metalized films. It has several advantages over other metals such as light weight, corrosion resistance and good recycling properties.

Plastics

Plastics can be classified into thermosets and thermoplastic polymers, the latter constituting the major packaging material in form of films, bottles, cups, jugs, etc. for food industries. Although polymers from petrochemical sources are mainly used for food packaging (see Table 1 for details), a tendency has emerged to replace them with bioplastics for reasons of sustainability and environmental issues. The other specific aspect of plastics in food packaging includes the impact of the migration of monomers, oligomers and additives into food, thereby affecting the food quality and, in the worst case, human health. These concerns shall be discussed in more detail in the latter section of the article. However, the use of plastics in food packaging has continuously increased due to the low cost and other functional advantages such as optical properties, thermoselability, microwavability, optical properties, etc. The details of the most important plastics in food use are listed in Table 1.

Special Packaging Technologies

Vacuum Packaging

This is based on the principle of excluding oxygen to prevent the oxidation in oxygen sensitive foods, for example, nuts, beer, chocolates, fats and oils, fried products, etc.

Modified Atmosphere Packaging

This technology makes use of an internal gas composition in the headspace of the package that differs from the composition of the surrounding atmosphere to preserve the food quality for both fresh (fruits and vegetables, meat, etc.) and processed foods (cakes, pastries, etc.) for a longer time. Whereas fresh foods often create a different internal atmosphere due to their own respiration, processed foods need the admission of specific gas mixtures into the headspace of the package.

Active and Intelligent Packaging

Active packaging uses active principles that are generated by or originate from the package and exert a specific effect on the packed food product like antimicrobial agents, oxygen absorbers, ethylene scavengers, etc. The purpose of its use is to preserve and maintain the food quality throughout the supply chain and the storage period at the consumer. Intelligent packaging uses different principles to monitor the status of the packed product and to communicate it.

Aseptic Packaging

This technology creates a package that is virtually free from fertile microorganisms via separate treatment of the product and the package. Thus, a sterile product and a sterile package are combined in the filling step and directly closed afterwards to avoid recontamination. Aseptic packaging gives less thermal loads to the filled product than conventional retorting and allows for a shelf life in the range of several months.

Product-Specific Packaging Materials and Technologies – Shelf Life and Food Safety Aspects

Owing to an array of responsible factors, food quality tends to deteriorate following the produce harvest, processing steps, or

Table 1 Some properties of the commonly used plastic packaging materials for food products

Type	*Physical properties*	*Mechanical, chemical, and miscellaneous properties*	*Barrier properties*	*Food use*
Polyolefins				
PE-LD	Density (910–925 kg m^{-3}); transparency (poor–fair); low crystallinity, temperature range (– 50 to 80 °C)	Tough, flexible, resistant to grease and chemicals, and good sealing properties	High moisture barrier, very low gas barrier	Bread and frozen food bags, flexible lids, squeezable food bottles, etc.
PE-LLD	Density (910–940 kg m^{-3}); transparency (poor–fair); high crystallinity, temperature range (– 30 to 100 °C)	Tough, extensible, good resistance to grease, and good sealing properties	High moisture barrier, very low gas barrier	Stretch/cling wrap, heat sealant coating, etc.
PE-HD	Density (945–967 kg m^{-3}); transparency (poor); high crystallinity, temperature range (– 40 to 120 °C)	Tough, stiff, strong, resistant to grease and chemicals, good sealing properties, and easy to process and form	Very high moisture barrier, very low gas barrier	Used for bottles of milk, juice and water, cereal box liners, margarine tubs, trash and retail bags
PP	Density (900–915 kg m^{-3}); transparency (fair); low crystallinity, temperature range (– 40 to 120 °C), high meting point of 160 °C	Moderately stiff, strong, and good resistance to grease and chemicals	High moisture and low gas barrier	Used for bottles of milk, juice and water, cereal box liners, margarine tubs, hot filled and microwavable packaging, trash and retail bags
PET	Density (1380–1410 kg m^{-3}); High transparency (good); low crystallinity, temperature range (– 60 to 200 °C)	Stiff, strong, and good resistance to grease and chemicals	Good barrier to gases and moisture, very good barrier to flavors and contaminants	As containers (bottles, jars, and tubs), semirigid sheets as (trays and blisters), and thin oriented films (bags and snack food wrappers)
PEN	Density (1.36 g cm^{-3}); transparency (good); applicable at both high and low temperatures	Stiff, chemical and hydrolytic resistance, and thermal & thermo-oxidative resistance	Good gas and moisture barrier properties, UV light barrier	Suitable for hot refills, rewashing, and recyclable Suitable for beer and wine bottles to preserve the flavor
PC				
PVC-U	Density (1350–1450 kg m^{-3}); transparency (good); temperature range (– 2 to 80 °C)	Strong, stiff ductile, resistant to chemicals, and stable electrical properties	Good moisture barrier, moderate oxygen barrier, good resistance to grease and oil	Used in bottles and packaging films Limited use in food applications
PVdC	Density (1600–1700 kg m^{-3}); transparency (good); temperature range (– 20 to 130 °C)	Strong, stiff ductile, resistant to chemicals, and stable electrical properties	Excellent oxygen and moisture barrier properties, very good grease and oil resistance	Suitable for poultry, cured meats, cheese, tea coffee, snack foods, and confectionary. May be used in hot filling, low temperature storage, and modified atmosphere storage conditions
Polystyrene	Density (1030–1100 kg m^{-3}); transparency (very good); temperature range (– 20 to 90 °C)	Hard and brittle with low meting point	Low moisture and gas barrier, fair to good resistance to oil and grease	Used as protective packaging for eggs, disposable plastic ware, cups, plates, bottles, and trays. Expanded form may be used as cushioning material
EVOH	Density (1140–1210 kg m^{-3}); transparency (good); applicable temperatures (– 20 to 150 °C)	Stiff, strong, and very strong oil & grease resistance	Excellent gas barrier, high moisture barrier, very good resistance to grease and oil	Used in coextruded films to avoid its contact with water
PA 6	Density (1130–1160 kg m^{-3}); transparency (good); applicable temperatures (– 2 to 120 °C)	Stiff, strong, and good resistance to grease and chemicals	High gas barrier, low moisture barrier, good resistance to grease and oil	Used for boil-in-bag packaging

Abbreviations: EVOH, ethyl vinyl alcohol copolymer; PA 6, polyamide 6 UV, ultraviolet; PE-HD, high-density polyethylene; PE-LD, low-density polyethylene; PE-LLD, linear low-density polyethylene; PEN, polyethylene naphthalate; PET, polyethylene glycol terephthalate; PP, polypropylene; PVC-U (not plasticized), polyvinyl chloride; PVdC, polyvinylidene chloride.

Source: Adapted from Marsh K and Bugusu B (2007) Food packaging – Roles, materials, and environmental issues. *Journal of Food Science* 72(3): R39–R55; Lee DS, Kit LY, and Piergiovanni L (2008) *Food Packaging Science & Technology.* USA: CRC Press.

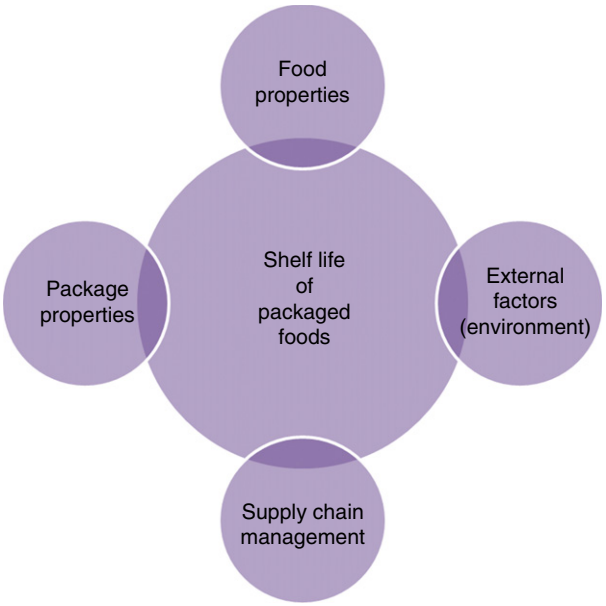

Figure 2 Responsible factors for shelf life of packaged foods.

during storage and handling (Figure 2). The influencing factors may be intrinsic (pH, water activity, enzymes, microorganisms, sensitive food ingredients like poly unsaturated fatty acids, etc.) or extrinsic (temperature, relative humidity, light, storage, and handling). The latter can be controlled to preserve the food quality. The expected shelf life of food products can also be used to group them into product categories from the shelf life perspective, for example, highly perishable, perishable and nonperishable foods. The other aspects of food deterioration have been discussed in detail in other sections of this encyclopedia. Good manufacturing practices in the processing & packaging play a significant role in preventing the food safety hazards. The product-specific packaging considerations may be classified as follows:

Meat, Fish, and Poultry Products

Meat and meat products are one of the most demanded food categories throughout the world. The consumer-related quality indicators for fresh red meat are color, juiciness, and microbial safety. Once the animal is slaughtered, the red meat color pigment (myoglobin) tends to oxidize to either to an unwanted grey-brownish color (metmyoglobin) upon exposure to low levels of oxygen or to the red oxymyoglobin at high oxygen levels. The color change towards grey in meat is an undesirable quality, which is determined by consumers during the meat purchase. Other changes after slaughter include the exudation of fluid from meat muscles that besides resulting in shrinkage of myofibrils, allows a pool of spoilage and pathogenic microorganisms to grow on a nutrient-rich substrate. To preserve the meat quality, packaging plays a vital role in maintaining the shelf life and safety of meat and meat products. Several packaging methods are used by the meat industry for marketing the fresh meat. Vacuum packaging has been widely used to pack meat either for retail or bulk storage. Vacuum packaging may be performed either as shrink packaging, nonshrink packaging, or as thermoformed trays. This method is effective for the prevention of oxidative greying, oxidative rancidity and aerobic microbial spoilage. Some of the limitations to vacuum packaging include proliferation of anaerobic pathogenic bacteria, release of muscle water due to pressure on muscle filaments, and residual oxygen resulting in surface color changes due to oxidation of surface meat pigments. Vacuum packaging is suitable for refrigerated bulk storage with an estimated shelf life of 10–12 weeks at 0 °C. Oxygen permeable trays for meat are in use for a limited shelf life of 2–3 days. The advantage of these packaging systems is the rapid oxidation of the myoglobin to oxymyoglobin, giving the bright red meat color, generally preferred by consumers. However, discoloration proceeds rapidly due to aerobic microbial growth. In the recent years, modified atmosphere packaging (MAP) has emerged as an innovative approach to pack fresh red meats. Modified atmosphere packaging has several advantages over vacuum packaging as it prevents the drip loss, restricts the growth of microorganisms, and retains the meat color. Several gas combinations are in practice and use depending on the type of the meat, for example, high-oxygen MAP, low-oxygen MAP, and ultra low-oxygen MAP. Further details on the MAP of fresh meat and the food safety aspects have been discussed in detail by Robertson (2006) and McMillin (2008). For red meats, a gas composition of 60–80% N_2 and 20–40% CO_2 is reported to be optimal for both the preservation of meat color, avoidance of oxidative processes and prevention of microbial hazards. Industrial practice, however, is to use gas compositions with excess oxygen up to 80%, thus allowing for a very fast formation of oxymyoglobin, but also at the risk of faster oxidative degradation of fats and proteins. Meat is also stored under frozen conditions for long-term storage or during long distance transportation for retail marketing. This is mainly done to arrest the microbial growth. Generally, shrink vacuum packaging is used for frozen meats and restructured meat products. Fresh meat is often processed into different meat products such as cured meats, smoked meat, sausages, minced meat, etc. They require attention against high spoilage risks due to restructuring of meat and exposure to contaminants during processing and handling. Extrinsic factors like proper temperature control is one of the main factors to restrict microbial spoilage and pathogenicity. For processed meat, the packaging requirements include oxygen and light barrier films to prevent especially light-mediated oxidation.

Poultry meat is also prone to spoilage due to favorable pH that supports the growth of a number of microorganisms during storage and distribution. Although the color degradation is not a major concern for poultry, spoilage and pathogenic microorganisms have been the limiting factor. Vacuum packaging of poultry meat is limited to strict refrigerated storage conditions. However, commercially MAP may be suitable at 25–50% CO_2 and 50–75% N_2 levels. The microbial safety of MAP packed poultry has often raised safety concerns. However, MAP with strict temperature management has been successful in preventing spoilage and foodborne outbreaks. Fish is another source of white meat that is highly prone to microbial and enzymatic spoilage. In the natural habitat, the microorganisms are generally attached to the fins

and skin of the fish besides their intestines. Often, these microorganisms are adapted to lower temperatures. Once the fish is caught, postmortem changes follow a series of enzymatic reactions, leading to putrification and spoilage. The spoilage is further aggravated by rapid bacterial growth due to suitable muscle pH. Traditionally, fresh fish is packed in plastic containers directly on ice. Vacuum packaging of fish is limited due to the possible growth of anaerobes. However, the appropriate MAP conditions combined with super chilling have proved efficient for the packaging of seafood. The gas combinations for nonfatty fish and shellfish vary from 25% to 35% O_2, 35% to 45% CO_2, and 25% to 35% N_2, whereas for fatty and smoked fish, the gas combinations vary from 35% to 45% CO_2 and 55% to 65% N_2. However, high CO_2 levels may increase drip loss, pack collapse, clouding of eyes, and acid flavor, all being the negative attributes of fish quality. MAP has been successful in extending the shelf life from a few days to a week's time depending on the fish type, temperature abuse, and other intrinsic and extrinsic factors.

Dairy and Dairy Products

Milk and milk products are highly prone to spoilage and need special packaging requirements to protect them from adverse conditions. A discrimination has to be made between shorter shelf life products like pasteurized milk or yoghurt and longer shelf life products like UHT milk treated at very high temperature. For the latter group, the package should be impermeable to light, oxygen, and moisture. Owing to high water activity, suitable pH and sufficient nutrients, bacteria rapidly grow in milk, thereby spoiling milk and milk products. Fresh pasteurized milk is generally packaged in opaque materials of glass, plastic (PE-HD or PET) or liquid packaging board laminates. If glass containers are used, the headspace is sometimes flushed with inert gas to avoid light-induced flavor changes. In developing countries, pasteurized milk is still packed in PE-LD pouches without a light barrier, which results in loss of Vitamin C and light mediated oxidation. On the other hand, UHT milk is often packed under aseptic conditions in sterile packaging materials to avoid any further contamination. UHT milk is generally packaged in sterile paper-based laminates with an anticipated shelf life of 90–150 days at ambient temperature. Butter is packaged in greaseproof paper laminated with aluminum foil for retail marketing to avoid the contact of any moisture, light, and oxygen. For bulk marketing, butter is also packaged in plastic tubs that are fabricated from PVC. Being sensitive to oxygen and moisture, dry milk products require packaging materials with specifically selected barrier properties. To prevent oxidation, they are often packed in tin cans and flushed with nitrogen gas or vacuum packaged. Aluminum foil/plastic laminates with gas flushing are also used as flexible packaging material for the packaging of milk powders. The class of fermented milk products constitutes cheese and products like yoghurt, kefir, etc. The packaging requirements depend on the product type. Moisture and light barrier properties are required for most types of fermented milk products. However, some cheeses like mold ripened cheese (Camembert) require oxygen for mold growth, and oxygen-permeable films are used to facilitate the fermentation. Modified atmosphere packaging is quite often used nowadays for retail marketing (CO_2 and N_2 in the ratio of 80:20 or 70:30) to avoid undesirable quality changes. Sliced MAP packaged cheese in reclosable tray packages has acquired high consumer acceptance as a convenience product.

Fruits and Vegetables

Packaging requirements largely vary for fresh, fresh cut, and processed fruits and vegetables. They also depend on the respiratory pattern (climacteric and nonclimacteric) of the fresh produce. Temperature, gas conditions in storage, relative humidity, released ethylene, and fruit type are the major factors affecting the postharvest life of fresh fruits. Therefore, packaging materials with shock and vibration resistance as well as different permeation properties are the primary considerations for packaging materials for fruits and vegetables. One has to differentiate between consumer packages used for storage under normal atmosphere and those intended for storage in larger rooms equipped with controlled atmosphere. The latter type has to be highly permeable like corrugated paper board boxes with cushion pad or fruit trays with larger openings to allow a fast exchange of gases with the surrounding. Sometimes, the fresh produce itself is coated with wax or edible emulsions to prevent moisture loss during storage or transportation. The use of MAP for fresh produce and fresh cut fruits and vegetables has gained paramount significance due to retention of flavors, texture and color during marketing. Due to the respiration of the products, the oxygen level in the package is maintained at lower values in relation to the surrounding atmosphere whereas carbon dioxide is enriched. Thus, the fresh produce creates its own modified atmosphere with a composition specific to respiration rate and permeability of the package. Therefore, oxygen barrier films should not be used as it would result in off flavor development during storage. The safety for MAP packed fresh cut fruits has been a challenge in developing countries, especially due to the temperature abuse during marketing of these products. Several microbes of concern include *Clostridium botulinum*, *Pseudomonas* spp., *Erwinia herbicola*, *Flavobacterium*, *Xanthomonas*, *Enterobacter agglomerans*, lactic acid bacteria such as *Leuconostic mesenteroides* and *Lactobacillus* spp., molds, and yeasts. Moreover, the growth of pathogenic microorganisms (*Aeromonas hydrophila*, *L. monocytogenes*, *Yersinia enterocolitica*, etc.) under refrigerated conditions has raised safety concerns. Multiple hurdles can be incorporated in the processing steps and supply chain to inhibit survival, growth, and toxin formation of microorganisms during the shelf life of fresh produce. Laurile and Ahvenainen have suggested the below mentioned hurdles for the minimal preservation in order to extend the shelf life of fresh cut fruits and vegetables:

- Short-time heat treatment
- Lowering the pH (≤ 5)
- Reduction in aw (≤ 0.97)
- Addition of NaCl (3.5%)

Packaging materials used in MAP are usually highly permeable, such as PE-LD, PE-LLD, often coextruded with EVA copolymer, or BOPP, often microperforated to adapt the gas

exchange rate to the requirements of the produce. For cut products with their lower respiration rates and higher sensitivity, an initial gas atmosphere has to be provided. Common gas mixtures for cut fresh salads are 1–5% O_2 and 5–20% CO_2 with N_2 as balance. Sometimes, Argon is added in levels up to 17%. Fruits and vegetables are also thermally processed in metal cans for long-term storage. Defects in cans may be observed when the food leads to corrosion of the metal via pinholes in the interior lacquer coating. In some cases, the cans may bulge on both ends, mostly due to inadequate heat processing, which allows the survival of spoilage or pathogenic microorganisms. *Clostridium botulinum* is of serious concern in the bulged cans as it produces heat stable neurotoxin, which can prove fatal. Glass jars also find their way in the packaging of high moisture fruit products, pickles, chutneys, jams, jellies and soups. Some dehydrated fruits and vegetables are also marketed and they need protection against moisture and oxygen. They can be easily packaged either under vacuum or inert gas atmospheres in extruded flexible plastic pouches, with aluminum lining in order to prevent the oxidation of pigments or color loss.

Cereal and Cereal-Based Products

This group constitutes a diverse range of food products from cereal grains, breakfast foods, to bakery products. Therefore, the packaging requirements considerably vary with the product type. After drying, cereal grains are quite stable due to low moisture content, which does not support any microbial growth. An increase in moisture during storage may be detrimental for e.g. the quality of flour due to the action of amylases. To prevent the permeation of moisture cereal grains are generally packed in kraft paper bags, solid board boxes or even jute bags, all with polyethylene lining. Breakfast cereals are commonly packaged in flexible pouches, sometimes from simple PE-HD, for hot and humid environment also coextruded films of PVdC–PP or PVdC–LDPE in order to prevent permeation. Often, the pouches are further packed in paper cartons to prevent them from light mediated oxidation and mechanical damage. Except for dried baked products like biscuits, crackers and cookies, standard bakery products like bread, buns, pizza, cakes, and doughnuts are prone to microbial spoilage. Antimicrobial agents in use have raised health concerns and research for alternate preservation methods is needed. MAP has attained attention and is currently used in the packaging of several cereal-based baked food products.

Beverages

One of the largest and vital groups of food products (water, tea, coffee, juices, carbonated soft drinks, beer, and wine) requires special packaging requirements to preserve their essence and quality. The consumption of packaged bottled water has increased significantly in the past decade due to the concerns over the safety of municipal water supplies. Bottled water is processed through a series of steps and finally packaged into glass bottles now largely replaced by PET, PP, PVC, or HDPE. The major concern in the packaged bottled water is to avoid microbial growth and the formation of off-flavour. Microbial growth may either be caused by inappropriate UV or ozone treatment or due to microbial contamination from the packaging materials. An associated problem is the development of off-flavors in ozone treated water packed in PE-HD containers. This is due to the migration of butylated hydroxytoluene (BHT) in water. For fruit juice and carbonated soft drinks, packages should have strong barrier for microorganisms, oxygen, and light. Fruit juices are usually packed in glass bottles, metal cans, paper–aluminum–plastic laminates or plastic packaging materials. Glass is mainly used for high quality fruit/vegetable juices, beer, and wine. It has the advantage of being an absolute barrier, which shifts the weakest point of the package to the closure. It allows both for hot filling as well as for thermal treatment of the filled and closed container. to avoid further contamination risks. The cleaning of glass bottles for reuse needs proper attention as they may act as a source of contamination. Metal cans give the same options for filling as glass containers. As many beverages have a low pH value, stable lacqer formulations for interior coating have to be selected to avoid corrosion. Board laminates with plastic and aluminum foil are used for aseptic packaging of juices, whereas plastic film laminates with aluminum foil allow for an easier thermal treatment after filling. The major consideration for the use of plastic packaging materials for beverages is to achieve low oxygen permeability in order to prevent the oxidation of valuable ingredients, like vitamins, and of other constituents, to avoid phenomena like color changes or the formation of off-flavors. For carbonated soft drinks with their added flavors, the barrier against permeation of carbon dioxide and flavor substances becomes another important issue. Although glass and metal containers are frequently used as packaging medium for premium beverages, the gas barrier properties of plastic packaging materials have improved considerably over the last decades with the introduction of new multi-layer materials, including oxygen-absorbing layers, and new barrier coating techniques. This has created a large market segment of plastic containers, mainly on the base of PET for all types of beverages.

Snack Foods and Confectionary

Snack foods are valued for their crisp and crunchy texture, which is lost with moisture admission. The other major cause of deterioration is off flavor development due to the oxidation of fatty constituents like vegetable oil. This group primarily constitutes fried snack foods (potato chips, banana chips, French fries, etc.), extruded puffed snacks, and fruit-based snacks. Their common property is the porous structure which allows the fast access of moisture and oxygen. The second group, confectionary, has a closer structure and is, therefore, much less sensitive. Examples are, sugar boiled confectionary (hard boiled candies, toffees, chewing gum, etc.), and chocolates. The packaging materials for snack foods, have to meet high requirements for oxygen and moisture barrier properties in order to protect them from the above deteriorations. Vacuum packing or inert gas flushing is often used. For confectionary, the influence of elevated temperature is of highest importance. In such instances, strict temperature control as well as optimal

product formulation and design should be taken into account while marketing such products, and the package should be labeled with a temperature storage warning.

Migration Aspects of Packaging Materials

Migration, i.e. the transport of potentially harmful substances from the different packaging materials into the packed food products has been a serious concern for the consumers. Many factors influence this transfer: The composition of the packaging material, the conditions of material conversion and processing and the properties of the food itself determine the amount of substance being transferred into the food. Most of these phenomena are known for plastics packaging which is the reason that this packaging sector has been regulated worldwide to the largest extent. Examples of the migration of monomers or additives from polymeric packaging materials are vinyl chloride, acrylonitrile, styrene (as monomers), diethyl hexyl phthalate, acetyltributyl citrate (as plasticizers), among many other additives. For the single substances, maximum admissible values have been recommended by the relevant regulatory bodies on the basis of toxicological considerations, for example in the form of specific migration limits (SML) in the EU.

Package Opening

The package opening has been a serious issue in the past and has undergone many innovations, leading to the development of easy-to-open packages. True consumer risk with package opening mainly existed with glass bottles and metal cans. Today, opening of a package has to be consumer friendly, which depends on the gender and target group. Females generally exert 75% of the forces that males exert while opening a specific package. Fraunhofer IVV has laid force guidelines for designing easy-to-open packages for different age groups (children, adults, and seniors). The package intended for children (3–4 years old), children (8–10 years old), adults (20–60 years old), and older adults (61–80 years) should open with a force capacity of 4, 10, 17, and 11 N respectively. The opening process, opening principle, removal of the products, consumer safety and behavior, and resealing wherever applicable are major considerations while designing the package opening system.

Conclusion

In summary, the critical factors for food quality and safety considerations of packaging materials are:

Mechanical stability – The material should have sufficient mechanical stability strength to maintain the package integrity during storage and distribution of its entire life cycle.
Permeability – The material should have adapted and sufficiently low permeability for gases, moisture, and flavors as per the product requirements to preserve the quality of fresh and preserved food. Moreover, the package design should prevent changes in water activity during transportation mainly due to incorrect storage temperature.
Light-barrier properties – The packaging medium should protect the food from adverse effects as light-mediated oxidations and color changes.
Microbial contamination – The packaging medium should prevent microbial contamination of the packed product from the environment and the package itself. The microbial growth may also take place inside the package due to inappropriate water activity. This needs to be taken care of while designing the product and the package.
Sealing and lid closure – Proper procedures for sealing and lid closure and, if needed, online leak detection tests shall be adopted to ensure safe product and to avoid recontamination.
Migration aspects – All relevant components of the package should meet the migration limits as per the standards and regulations, thereby taking into account the properties of the specific product and the conditions of filling, distribution, storage and preparation.

See also: Food Technologies: Aseptic Packaging. Foodborne Diseases: Overview of Chemical, Physical, and Other Significant Hazards; Overview of Emerging Food Technologies. Hazards of Food Contact Material: Bisphenol A and Endocrine Disruption; Food Packaging Contaminants; Nanotechnologies and Nanomaterials; Phthalates

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Pasteurization

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Glossary

***D* value** Decimal reduction time: the time ins minutes at a given temperature to inactivate 90% (one log cycle) of a microorganism leaving one tenth of survivors.

HTST High temperature short time heat treatment, bulk pasteurization at 70–80 °C for 15–20 s.

Pasteurization units (PU) The cumulative lethal equivalent expressed in the reference temperature, generally 80 °C.

UHT Ultrahigh temperature treatment at 130–150 °C for a few seconds.

***z* value** The temperature change required to change the *D* value by a factor of 10.

Introduction

Pasteurization treatment should be sufficient to kill all non-sporeforming pathogenic bacteria. The more general objective of pasteurization is to extend product shelf-life and maintain keeping quality by inhibiting microbial or enzymatic spoilage. Pasteurization typically applies temperatures 65–70 °C for 15–30 min for products filled in bottles, jars, or cans. Nowadays, however, most liquid foods, such as beverages, fruit and vegetable juices, milk, and some dairy products are pasteurized in bulk by the high-temperature short-time (HTST) method, also called flash pasteurization. In the HTST process, liquids are streamed through heat exchangers (metal plates or pipes) and heated to 70–73 °C for 15–20 s.

Pasteurization is commonly associated with milk for which it is used all over the world. Pasteurization is carried out at temperatures approximately 80 °C for some meat products such as sausages, pickles and sauerkraut, tomatoes, fruit products, juices, and preserves, which have generally low pH below 4.5. To be effective in inhibiting microbial or enzymatic spoilage, pasteurization is frequently combined with other means of preservation such as acidification, concentration, and chemical preservatives.

Scope

Heat treatment with high temperatures is the most important method of preservation that is used widely in the food industry. Pasteurization means a comparatively low order of heat treatment, generally at a temperature below the boiling point of water. In the recent times, however, thermal treatment applying temperatures far over 100 °C only for seconds is also called pasteurization. The process is named in honor of Louis Pasteur, who found that microbes were responsible for the putrefaction of meat and milk, as well for 'disease' of wine, and developed a heat process (later called pasteurization) to preserve wine.

The aim of pasteurization is manifold. Firstly, the heat treatment should destroy most foodborne pathogenic microorganisms except those forming heat-resistant spores. Secondly, the heat destruction should decrease the number of surviving microorganisms to a degree to slow down, delay, or stop the spoilage of food, assuring the product an acceptable shelf-life. Further, heating should inactivate enzymes within the food, which otherwise would cause unwanted organoleptic change. Finally, the moderate heat treatment is able to retain the food quality at a higher degree compared to the more severe sterilization process.

Thermal Death of Microorganisms

The method of pasteurization rests upon the principles of thermal death of microorganisms, according to which the death of a cell population follows the kinetic of a first-order reaction. When the logarithm of surviving cell number is plotted against time, a straight (linear) line is obtained, the slope of which is related to the death rate coefficient. The decimal reduction time *D* is the time in which the number of survivors decreases to one tenth. The value of *D* (in minutes) is independent of the size of population but depends on the degree of temperature. Thus, the *D* value is also a measure of the heat resistance of a given kind (species or strain) of microorganism. The dependence of *D* on temperature is expressed by the value of *z* (in °C or °F) defined as the degree of temperature causing a decimal change of *D*.

The extent of microbial destruction during the process of heat treatment depends on the combined action of temperature and time. Analogously to the calculation of the sterilization requirement and efficacy in F_o value, in the case of

pasteurization the D and z parameters are to be related to fixed reference values, and the cumulative thermal destruction equivalent of changing temperatures and times is expressed in pasteurizing units (PU) or pasteurizing equivalent (P). The reference temperature should be marked; for example, at 80 °C the value is P_{80}:

$$P_T = \int_{T_w}^{T_c} 10^{(T-T_r)/z}\,dt$$

where P_T is the pasteurization equivalent at T, the temperature of heating, integrated between the cooling temperature T_c and the warming temperature T_w, related to the reference temperature T_r and t is the time of heating.

Heat Resistance of Microorganisms

The heat resistance of microorganisms is primarily a genetically determined specific characteristic that can be modified by the environmental conditions. In general, heat resistance is in proportion to the growth temperature (Table 1). Psychrophilic vegetative bacteria become inactivated already at approximately 40 °C, whereas mesophiles have a decimal reduction time of approximately 1 min at 55–60 °C. Certain thermoduric bacteria (e.g., *Enterococcus*, *Microbacterium* species) may survive 30 min heating at 60 °C, with a fairly large z value of 15–20 °C. Heat resistance of most vegetative pathogenic bacteria occurring in foods is similar to those of mesophiles, and they can be inactivated with the conventional pasteurizing treatments at temperatures below 100 °C. Unusually high heat resistance approaching that of thermoduric species is shown by the serotype *Salmonella* Senftenberg.

The majority of yeasts and molds possess heat resistance similar to mesophilic vegetative bacteria. Heat resistance of sexual spores or asexual conidia does not surpass that of vegetative cells. However, ascospores of certain molds, such as species of *Byssochlamys*, *Neosartorya*, and *Talaromyces*, have rather high heat resistance with 7–22 min D value at 88 °C, and these can survive 30 min heat treatment at 90 °C, causing spoilage of pasteurized fruit juices and canned fruits.

Although the vegetative cells of sporeforming bacteria are equally sensitive to heat as other bacteria, their endospores possess high heat resistance (Table 2). The thermophilic sporeforming species are remarkably more heat resistant than mesophiles. Heat resistance of mesophilic spores is characterized with $D_{121\,°C}$ of 0.01–0.1 min, whereas that of thermophiles may reach 2–5 min decimal reduction time at this temperature. From the point of food safety, *Clostridium botulinum* is the most important of the pathogenic sporeformers, having 0.1–0.2 min $D_{121\,°C}$. Among the sporeformers causing spoilage in canned foods spores of *Geobacillus stearothermophilus* and *Clostridium thermosaccharolyticum* have D_{121} values of 3–5 min, and these can survive heat treatments calculated for the destruction of *C. botulinum* (see commercial sterility). Heat resistance of spores is also characterized with z values two or three times higher than that of vegetative cells, in the order of 8–12 °C, and some spores may reach 20–30 °C.

Table 1 Average heat resistance of vegetative microorganisms

Physiological group	D *value (min)*		
	40 °C	*50 °C*	*60 °C*
Psychrophilic bacteria	0.3	–	–
Psychrotrophic bacteria	–	1–5	–
Mesophilic bacteria	–	5–40	0.2–1
Thermoduric bacteria	–	–	1–30
Thermophilic bacteria	–	–	100
Yeasts and molds	–	1–5	0.02–0.4

Factors Influencing Thermal Resistance

The thermal resistance and thermal death of microorganisms and their spores are influenced by several environmental factors. Moreover, although the heat resistance is a specific characteristic, it may differ between strains of a species, and may change according to the physiological state of cells. Cells in the exponential phase of growth are usually more sensitive to heat than those in stationary phase. Spores developed at elevated temperature are somewhat more heat resistant. For the practice of heat processing, the most important factor influencing heat resistance is the composition of product, in particular its water activity and pH.

Decrease of water activity significantly increases thermal resistance (Table 3). This is often the case in foods with high sugar concentration or containing many proteins or fats (Table 4). Spores embedded in drops of fat become subjected to the effect of dry heat, against the effect of which they are more resistant than to moist heat (steam). Acidic environment and low pH also decrease heat resistance (Table 5). Product pH is of outstanding importance for heat processing. pH 4.5 signifies a dividing line; products with pH lower than 4.5 can be pasteurized at 100 °C or below, whereas foods of higher pH than 4.5 must be sterilized over 100 °C. The fundamental safety reason for this is that the most important pathogenic endosporic microorganisms, *C. botulinum*, cannot grow or

Table 2 Thermal resistance of microorganisms

Mirobe	D *value (min)*	z *value (°C)*
Pasteurization at 65 °C		
Salmonella spp.	0.02–0.25	4.4–5.5
Salmonella Seftenberg	0.80–1.00	4.4–6.7
Staphylococcus aureus	0.20–2.00	4.4–6.7
Yeasts, molds	0.50–3.00	4.4–6.7
Pasteurization at 100 °C		
Alicyclobacillus acidoterrestris	3.0–8.0	6.0–8.0
Bacillus cereus	5–10	7.0–10.0
Clostridium botulinum E	15–50	5.0–8.9
Clostridium sporogenes	60–190	9.0–13.0
Sterilization at 121.1 °C		
Clostridium botulinum A, B	0.10–0.20	7.8–10.0
Geobacillus stearothermophilus	4.00–5.00	7.8–12.2

Source: Adapted from Stumbo CR (1973) *Thermobacteriology in Food Processing.* London: Academic Press.

produce toxin at pH <4.5, and the spores that may survive heat treatment cannot germinate either.

Factors affecting heat resistance are in force before, during, and after heat processing. Cells surviving heat treatment become damaged, which could be repaired only under optimum conditions but not in a product that may contain certain chemicals, such as preservatives and nitrite or stored at low temperature. These products, although they may contain living bacteria, however they are not able to start growing, and such products remain in a state of 'commercial sterility' without spoilage.

Methods and Equipment

There are two basic methods, batch or continuous, and these can be applied for bulk products or filled in containers. The batch method operates with a jacketed vat heated by circulating hot water or steam, or by heating coils. The vat is filled with bulk liquid food (e.g., milk), heated for the holding period, during which it is agitated. Thereafter the product is cooled in the vat and filled into containers. Similar batch process can be used for products distributed in containers before heating. Some liquid foods (beers and fruit juices) are pasteurized after filling into cans or bottles. Batch pasteurizer for bottles is a simple water bath in which containers in crates are immersed and the water heated to approximately 20 °C higher than the temperature at filling to avoid thermal shock. After the appropriate holding time containers are cooled to 40 °C. Hot water bath can be operated continuously when containers carried by a conveyor are immersed into heating than cooling parts. Another continuous method applies hot water spray on containers conveyed through the heating zone of a tunnel, followed by cold spray in the cooling zone.

A simple way of pasteurization is the hot-fill method by which the product is heated to pasteurization temperatures (above 60 °C) and filled directly into containers, destroying the microorganisms by the heat of the product. After sealing, the closures are decontaminated by turning upside down the container. This method can be applied for high acid products (sauces, purees, and dressings), whose pasteurization can be achieved by the residual heat of the filled product.

Continuous pasteurization saves time and energy over the vat method. For most cases of HTST (see Modes of Pasteurization) pasteurization heat exchangers are used. These equipment offer uniform heat treatment, greater energy efficiency, flexibility for product types, high throughput rates, and control and automation of pasteurization conditions. Continuous heating can be done directly and indirectly. For direct method the heating media (steam) is directly introduced into the product (steam injection), or the product is injected into steam (infusion); whereas by the indirect method the heat is transferred through a surface between the product and the heating media (Figure 1).

There is a large variety of heat exchangers falling into three main types: plate and tube systems and the scraped-surface models. A plate heat exchanger consists a stack of stainless steel plates bounded in a frame. Through the plates flows the liquid, which is heated outside by hot water in a counter current providing $>90\%$ energy regeneration. A tubular system may consists of strait tube surrounded by an outer tube, and can be of spiral or coiled double and triple tubular set. These work under high pressure to assure high flow rates, and bends and wall corrugations effect turbulence to maintain uniform heating and avoid overheating. Scraped-surface heat exchangers are more suitable for viscous foods with particles (e.g., stew). The product flows through a jacketed tube with the heating medium is scraped from the sides with a rotating knife. Modern heat exchangers operate fully automatically and

Table 3 Effect of water activity on the heat resistance of some bacteria

Sucrose	a_w *(wt%)*	$D_{57\,^\circ C}$ *(min) at pH 6.9*		
		E. coli	S. *Typhimurium*	S. *Senftenberg*
15.4	0.99	1.2	0.8	1.2
39.6	0.96	10.1	5.3	35.5
51.3	0.93	33.9	14.3	68.7
58.6	0.90	46.5	21.1	80.0
63.7	0.87	43.7	26.6	95.0

Table 4 Increase of heat resistance in the presence of proteins

Milk (%)	$D_{58\,^\circ C}$ *(min)*		*z* (°C)	
	E. coli	S. *Typhimurium*	E. coli	S. *Typhimurium*
10	1.4	1.4	4.6	4.0
30	2.4	4.9	4.9	4.6
42	7.3	9.8	6.3	6.0
51	13.5	26.6	7.9	6.8

Table 5 Effect of pH on the heat resistance of some microorganisms (D_T values, min)

pH	Clostridium botulinum D_{121}	Clostridium subtilis D_{110}	Bacillus faecalis D_{100}	Enterococcus sporogenes D_{65}
5.0	1.1	10.6	3.6	0.5
5.5	1.6	13.5	7.0	1.0
6.0	2.0	15.0	9.2	3.3
6.5	1.8	17.8	10.5	7.2
7.0	2.6	15.9	11.0	13.0
7.5	–	10.5	11.0	3.8
8.0	–	–	10.2	1.8

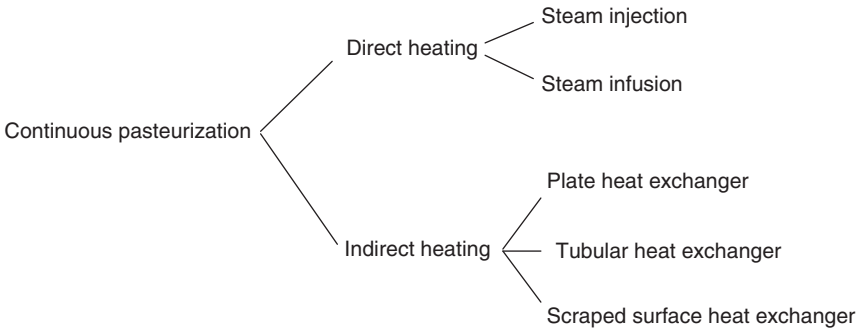

Figure 1 Modes of continuous pasteurization.

continuously, and are cleaned in place. With heat exchangers temperatures can be achieved up to 135 °C, and can be used for HTST and ultrahigh temperature (UHT) treatment as well. Heat exchangers are discussed in more detail by Emond in 2001.

Containers for thermally processed canned products can be constructed of metal, glass, plastic laminates or composites of plastic, cardboard, and metal laminates. The most frequently used form of packaging for canned products is tinplate, which is fabricated into two and three piece cans of a wide variety of shapes and sizes. Glass can be equally used for processing canned products. Jars, bottles, and flasks of glass are widely used for pasteurized products (tomato juice, fruit juices, etc.). Plastic or plastic and aluminum foil pouches are now available to withstand pasteurization. Containers used for aseptic filling after heat sterilization are not exposed to heat and pressure, hence a great variety of materials and size are suitable, including laminated cartons, plastics, pouches, cups, and other packs. The first aseptic filling plant for milk was established in Switzerland in 1961. The Tetra Pak Company later became one of the leading suppliers of processing and packaging equipment.

Modes of Pasteurization

There are two main types of pasteurization according to the temperature and time applied. One is a slow process using low temperatures for long time, the other is a flash process using high temperatures for short time (HTST). Important are also the modes of pasteurization being either batch or continuous, and whether the heating process is applied for unpackaged bulk product or after packaging in containers. Pasteurization treatment should suffice to kill all nonsporeforming pathogenic bacteria. The more general objective of pasteurization is to extend product shelf-life and maintain quality. To be effective in inhibiting microbial or enzymatic spoilage, pasteurization is frequently combined with another means of preservation such as acidification, concentration, and chemical preservatives.

Traditional slow pasteurization typically applies temperatures 65–70 °C for 15–30 min for products filled in bottles, jars, or cans. Jacketed vats or tanks can be used for batch process, whereas tunnel pasteurizers are used for continuous heat treatment. However, nowadays most liquid foods, such as beverages, fruit and vegetable juices, milk, and some dairy products are pasteurized in bulk by the HTST method. In the HTST process, liquids are streamed through heat exchangers (metal plates or pipes) and heated to 70–73 °C for 15–20 s. This thermal treatment should achieve 5 log reductions in the number of viable microorganisms, destroying almost all spoilage bacteria, yeasts, and molds, and nonsporeforming pathogens, with the exception of heat-resistant spore formers. Flash pasteurized bulk liquids are filled into containers after heat treatment under rigidly maintained sterile conditions, similar to aseptic processing.

Table 6 Comparison of parameters of various methods of pasteurization

Method	*Temperature (°C)*	*Time (min s)*
Batch (vat)	65	30 min
HTST	72	15 s
Ultrapasteurization	89–100	1 s
UHT	138	2 s

Applications

Pasteurization is commonly associated with milk, for which it is used all over the world. Milk pasteurization was first applied by Franz von Soxhlet in 1886, and since then it has remained the most important operation to extend its shelf-life. Pasteurization is also widely applied to various liquid and certain viscous and particulated foods such as juices, soft drinks, beer, cider, wine, cream and processed cheese, liquid eggs, syrups, sauces, soups, and some ready meals.

For the pasteurization of milk temperatures below boiling temperature are typically used because at very high temperatures casein micelles will irreversibly aggregate (or 'curdle'). There are two main types of milk pasteurization used today: the conventional batch method, by which the milk is heated in a vat at 63 °C for not less than 30 min, and the HTST method, by which the milk is pasteurized at 72 °C for 15 s using a continuous heat exchanger. In the recent times, UHT is also used for milk treatment. It is in fact a sterilization process at 135 °C for 2–5 s only before packaging of milk, which is then filled into containers aseptically (Table 6).

Batch pasteurization is usually made for milk already bottled and has a shelf-life of several days when refrigerated. HTST pasteurized milk typically has a refrigerated shelf-life of 2–3 weeks, whereas UHT milk can last much longer even when unrefrigerated, sometimes 6–9 months.

The HTST pasteurization should achieve a 5-log reduction of the number of viable microorganisms in milk, killing almost all yeasts, mold, and common spoilage and pathogenic bacteria. Exceptionally, some heat-resistant vegetative pathogens, such as *Mycobacterium tuberculosis*, and *Coxiella burnetii*, may survive pasteurization. UHT treatment is expected to destroy bacterial spores as well.

Ultrapasteurization (UP) is a process similar to HTST pasteurization, but using slightly different equipment and higher temperatures. UP pasteurization results in a product with longer shelf-life but still requiring refrigeration. Another method, UHT sterilization, raises the temperature of milk to at least 280 °F for 2 s, followed by rapid cooling. UHT-pasteurized milk that is packaged aseptically results in a 'shelf stable' product that does not requires refrigeration until opened.

Pasteurization regimes for certain dairy products differ depending on the fat content of the product. Ice cream, dairy dessert mix, cream, or processed cheese require more robust treatment, for example, 70 °C for 25–30 min or 80 °C for 25 s.

Most meat products, cured or uncured, are often subjected to heat treatment during or at the end of processing or both times. Pasteurization is carried out at temperatures of approximately 80 °C for sausages filled in natural and artificial casings, resulting in a limited shelf-life and the need for refrigeration. Although cooking would destroy vegetative pathogens and most spoilage bacteria, heat-resistant lactobacilli and streptococci may survive, and psychrotrophic species (e.g., *Lactobacillus viridescens*) may cause spoilage. If stored above 10 °C spoilage can involve mesophilic sporeformers, *Bacillus licheniformis*, *Bacillus cereus*, and *Clostridium putrefaciens*, whereas if heating is lower than 45 °C inside the sausage batter, *Listeria monocytogenes* and *Escherichia coli* O157:H7 would survive.

Most vegetables are low acid products with pH >4.6 and have to be sterilized with the exception of pickles and fermented vegetables, which represent high acid products. Acidified pickled products in salt brine with 0.6–1.0% vinegar and also containing sugar are pasteurized at 80–85 °C. Examples of such acidified vegetables are cucumbers, onions, peppers, and various mixed vegetables.

Tomatoes are fairly acidic with a pH value of approximately 4.6 or less, hence they can be preserved by mild heat treatment generally with pasteurization (e.g., 45 min at 85 °C). Tomato paste is a main product obtained by removal of peel and seeds from tomatoes, followed by concentration of juice by evaporation under vacuum. From the evaporators the paste is passed continuously through a tubular heat exchanger, from which it emerges at a temperature of 90–92 °C. Hot-filling can be applied without further pasteurization. Other tomato products (juice, sauce, soup, and ketchup) are usually pasteurized, assuring the microbiological stability. Depending on the concentration and ingredients (vinegar, sugar, and spices) in addition to pasteurization, further measures can be necessary (e.g., cold storage or salt addition).

Fruit products, juices, and preserves have generally low pH of 3.2–3.8, and can be preserved by pasteurization. Hot filling or tunnel pasteurization can be used for bottled juices; modern thermal processes use the HTST technology and aseptic packaging. Pasteurization of beverages should assure a 5 log cycle reduction of vegetative form of pathogens (*E. coli* O157:H7, *Salmonella enterica*, *L. monocytogenes*). These bacteria, at the usual pH of fruit beverages lower than 4.0, have D values of a few seconds at 71 °C, and z value of 5 °C; however, the heat resistance of spoilage yeasts far surpasses these values, in particular in ascosporic forms. Hence, yeasts are the primary spoilage agents in fruit-based beverages and soft drinks. Heat-resistant molds and alicyclobacilli may also survive pasteurization; however, being aerobic organisms, their spoilage potential in carbonated beverages is limited.

Most canned fruits have a pH lower than 4.5, and these are packed in whole, halved, diced, or sliced cans or jars, and are usually pasteurized at 70–75 °C. Resistant spores of the acid tolerant *Bacillus coagulans* are possible organisms surviving heat treatment and causing flat sour spoilage. Frequently, heat-resistant ascospores of certain fungal species may survive the heat treatment and cause spoilage. Among these are *Byssochlamys fulva*, *Byssochlamys nivea*, *Neosartorya fischeri*, *Talaromyces flavus*, *T. bacillisporus*, and *Eupenicillium baarnense* and some other *Eupenicillium* species. Fruit preserves (jams, jellies, and marmalades) with a sugar concentration of at least 60% and pH between 3.2 and 3.8 can also be pasteurized for 1–5 min at 80 °C.

Among other foods and beverages, pasteurization of beer is widely used. Normally, draft and keg beer is not pasteurized, only the beer filled and sealed in cans and bottles is subjected to mild heat treatment. The process involves running the package in a tunnel through a hot water spray (approximately 140 °F or 55 °C) for 2–3 min. Pasteurization definitely extends the shelf-life of beer; however, its impact on the taste and flavor is debated.

Wine is traditionally stabilized by racking and filtration and its high alcoholic content. However, if the alcoholic content is less than 12 vol%, chemical stabilization may be necessary. Heat treatment of wine is less frequent than that of beer; it is sometimes practiced for religious reasons.

Eggs are important ingredients for several foods. Shell eggs are nearly always contaminated by pathogenic bacteria like salmonellae. Instead of shell eggs, industry prefers to use processed egg products in the form of whole eggs, egg yolk, egg white in liquid (pasteurized), and dried (powered) preparations. Recently, pasteurization of shell egg without cooking has been developed and patented in the US by applying controlled temperature regime.

See also: Bacteria: *Clostridium botulinum*. Disciplines Associated with Food Safety: Food Microbiology. Food Technologies: Aseptic Packaging; Food Irradiation. Organisms of Concern but not Foodborne or Confirmed Foodborne: Spoilage Microorganisms

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Pulsed Ultraviolet Radiation Processing

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Glossary

Ablation The ability of high-intensity light to remove materials from a surface or a liquid due to the absorption of pulsed light energy causing sudden ionization (charging) and electrical repulsion that prompts the particle's removal from the surface.

Advanced oxidation processes In general, it refers to a set of chemical reactions designed to remove organic and sometimes inorganic materials in water and wastewater by oxidation through reactions with hydroxyl radicals ($^{\bullet}$OH). UV radiation generates hydroxyl radicals in any moist environment including the surface of fruits and thus advanced oxidation processes is a mechanism for disinfection induced with UV radiation.

Cavitation An enhancement of range of penetration in materials due to pressure differentials, hydrodynamic factors, or to high intensity, high-power light sources.

Diffuse reflectance Diffuse reflectance is the reflection of a single incident UV light photon from a surface in multiple angles and depends somewhat on the properties of the surface.

Electromagnetic radiation spectrum The complete range of the wavelengths of electromagnetic radiation (waves), beginning with radio waves, radiofrequency, microwaves, infrared, visible, ultraviolet, X-rays, and gamma rays.

Fluorescence Part of a group of luminescence processes in which susceptible molecules or atoms emit light photons from electronically excited states created by physical, mechanical, or chemical mechanisms.

Specular reflectance The mirror-like reflection of UV (or other kinds of waves) from a surface in which UV from a single incident direction is reflected into a single outgoing (reflected) direction.

UV fluence It refers to the amount of UV energy (mJ, J) per unit area (cm^2) and per unit time (s) emitted by a UV source. For pulsed UV sources, UV fluence may be expressed as per pulse instead of per unit time.

Introduction

Ultraviolet radiation (UV) is a form of wave energy (photon) generally recognized as a form of light belonging to a specific portion of the electromagnetic radiation spectrum (ERS). Photon energies in the ERS are directly associated with specific wave properties such as frequency (v) in Hz or cycles per second [cycles per s] and wavelength (λ) in meters (m). Wave properties are related according to Einstein's classic expression

$$E = hv = hc/\lambda \qquad [1]$$

where E is the photon energy (Joules or electron volts [eV]; 1 J = 1.609×10^{-19} eV); h is the Planck's constant (6.626×10^{-34} J s; 4.136×10–15 eV s); v is the frequency (Hz or cycles s^{-1}); c is the speed of light (2.998×10^8 m s^{-1}); and λ is the wavelength (m).

In the ERS, UV photons belong to a wavelength range between 10 and 400 nm (1 nm = 10^{-9} m) and therefore possess wavelengths shorter than visible light (400–800 nm) but longer than X-rays (< 10 nm). Photon energy and wavelength are also related by eqn [1], but the relationship can be simplified and expressed as

$$E\,(\text{eV}) = \frac{1240}{\lambda}\,(\text{nm}) \qquad [2]$$

International standards (ISO-21348 – Process for Determining Solar Irradiances. (http://www.spacewx.com/ISO_solar_standard.html) identify UV bands as given in **Table 1**.

UV light is present in solar radiation (sunlight) and is therefore a natural source of radiation energy invisible to the human eye that propagates at the speed of light and in straight paths. As the earth's atmosphere absorbs most of the space (solar) UV radiation, any UV application is based on man-made UV sources.

In the solar spectrum, the high-energy UV photons (10–120 nm; 124–10 eV) are capable of ionizing molecules by a single-step energy transfer process to electrons which, when lost, leave molecules (or atoms) with positive charges in a process called ionization. Molecular oxygen (O_2) and other gases (i.e., ozone, sulfur, and nitrogen oxides) in the earth's atmosphere are effective in absorbing (blocking) high-energy solar UV photons. Therefore, only low-energy non-ionizing UV photons reach the earth's surface. However, the absorption of low-energy UV photons leads to an excitation process in which the absorbing molecule (or atom) is excited to higher vibrational and rotational levels increasing the reactivity and onset of a photochemical reaction.

Today's ultraviolet C (UVC) applications ranges from industrial to medical areas and are summarized in **Table 2**. For food safety applications, the germicidal UVC radiation has

Table 1 Ultraviolet (UV) bands, boundaries, and general properties

UV band	*Notation*	*Wavelength (nm)*	*UV photon energy (eV)*	*Chemical effects*
Before UV	Visible	>400	<3.10	None
Long wave UV	UVA	400–315	3.10–3.94	Excitation
Near UV	NUV	400–300	3.10–3.94	Excitation
Medium wave UV	UVB	315–280	3.94–4.83	Excitation
Short wave UV (germicidal)	UVC	300–100	4.1–12.4	Excitation Ionization
Far UV	FUV	200–122	6.20–10.2	Ionization
Vacuum UV	VUV	200–100	6.20–12.4	Ionization
Low UV	LUV	100–88	12.4–14.1	Ionization
Super UV	SUV	150–10	8.28–124	Ionization
Extreme UV	EUV	121–10	10.2–124	Ionization
Beyond UV	X-rays	<10	>124	Ionization

Table 2 Applications of ultraviolet (UV) radiation in different energy ranges

UV energy (eV)	*Wavelength (nm)*	*Applications*
6.2–3.1	200–400	Forensic analyses, drug detection
5.6–4.4	220–300	UV disinfection (germicidal band)
5.4–3.4	230–365	UV identification; barcodes; label tracking
5.4–3.1	230–400	Optical sensors; UV spectrophotometry
4.6–3.4	270–360	Chemical analyses (proteins; DNA)
3.3–3.1	280–400	Medical imaging (cellular structures)
4.1–3.1	300–400	Solid-state lightning
4.1–3.9	300–320	Medical therapy
4.1–3.4	300–365	Polymer and printer ink curing
3.5–3.4	350–370	Insect controls
92	13.5	Lithography

special affinity for some proteins and enzymes and in particular, for the nucleic acids. Therefore, UVC is considered as a desirable technology to disinfect foods.

Its effectiveness in controlling the growth of single-cell organisms (i.e., fungi, bacteria) and viruses is well established. However, a lesser known fact is UVC's effectiveness with complex, multicellular organisms like nematodes and arthropod (insects and mites) pests. The inactivation occurs as a result of exposing a pest with UV energy intensity (I) over a certain time of exposure (t). This $I \cdot t$ factor varies between organisms but once a threshold value is reached, the UVC radiation is an effective sanitation technique.

However, UVC radiation has limited depth (range) of penetration in most materials and especially in biological (plant) tissue. In air, UV penetrates thousands of meters and several meters in optically clear water. However, its penetration is severely limited (few microns) in biological tissues due to the presence of absorbing chemicals. Therefore, UVC inactivation is restricted to the surface of solids or to a narrow depth in liquids.

Historical Background

Since the early part of the twentieth century, UV radiation from mercury (Hg) lamps was used successfully for the sterilization of drinking water. In 1903, Niels Finsen was awarded a Nobel Prize for the work on using UV radiation to treat tuberculosis.

Since its inception, UVC disinfection competed with chemical disinfectants, particularly with chlorine alternatives (established in the nineteenth century). Since the 1970s, in most developing nations, UV techniques were chosen to sterilize potable (drinking) water. With improved designs, higher power, and reliability, the electronics industry was involved in the commercialization of the Hg lamp technology, a trend enhanced by health apprehensions related to the use of chlorination, in particular for wastewater disinfection. Concerns were due to the formation and carcinogenic activity of disinfection by-products (DBPs) by UV-induced photolytic effects on the organic-rich wastewater streams. New regulations were adopted for the enhancement of worker, general public, and environmental health protection. (In 1974, the US Congress passed the Safe Drinking Water Act. In 1979, the US EPA developed drinking water rules of tetra-halo-methane (THMs) DBPs allowed in drinking water.)

Since the late 1970s, the pharmaceutical, cosmetic, liquid food (beverages), and food sectors successfully incorporated Hg lamp UVC systems to provide higher quality disinfected water. In addition, air- and insect-control techniques were incorporated (UVC is effectively blocked by the Earth's atmosphere. Therefore, applications require man-made UVC radiation sources.).

Today, UVC radiation has a small portion of the potable water disinfection market although food disinfection is considered as an emerging opportunity due to the increased consumer awareness regarding microbial contaminants in foods as well as by concerns with pesticide residues. (In 2012, the Center for Disease Control in the US reported that one out of six consumers (~48 million) are affected by foodborne diseases; ~128 000 need medical assistance resulting in ~3000 deaths. (www.cdc.gov/foodborneburden.)) UVC technology, as a physical energy process, is viewed as a non-invasive (residue free) technical alternative.

UVC Source Technologies

The food safety applications are based on two man-made sources of UVC radiation: (1) Hg germicidal lamps (black lights) to deliver continuous UV power; and (2) arc or flash lamps for pulsed UV (PUV) applications. New potential applications for food safety rely at present on the readily-available Hg germicidal lamps, on arc lamps with continuous emission, and on flash lamps operating with PUV emissions. Limited power and facility (space) cost are the major concerns with Hg lamps but a major factor for their use is the past record of operation in water disinfection. The presence of toxic Hg in food processing facilities is a lesser concern.

Hg Germicidal Lamps

Mercury UV lamps emit long wavelength UV with a small fraction of visible light. Quartz tubes containing a set of electrodes and liquid Hg have become a technology of choice for low- to medium-intensity fluorescence sources of continuous UVC radiation. Fluorescence is an energy absorption process of certain atoms or molecules exposed to high-energy photons (lower wavelength) or particles which excite their electronic energy levels to higher levels, followed by the emission of lower energy photons (longer wavelength) through a subsequent de-excitation process. Remaining energy in the absorbing molecule (or atom) is converted into higher vibrational and rotational energy levels (www.bing.com/Fluorescence). The quartz tube could be coated and the interior surface modified with a deep bluish-purple glass (a nickel oxide doped glass) to enhance UV emission while stopping visible photons (i.e., black light lamp).

Fluorescent lamps without any phosphor coating (i.e., clear quartz Hg lamps) emit 253.7 nm (85–90%) and 185 nm (5–10%) photons and few other emissions. The 185 nm photons are absorbed by a special coating, thus the formation of ozone (O_3; a powerful oxidizer) from photoactivation of oxygen (O_2) is prevented. The 253.7 nm emission is very near the most absorbent UV wavelength for deoxyribonucleic acid (DNA) and provides an efficient process to damage DNA and prevent its replication. This is the rationale for using this UV source technology to provide germicidal effects.

Germicidal lamps use liquid Hg that vaporizes when a steady voltage is applied causing a low-pressure Hg vapor to form and normally operate at approximately 30 °C with 30–35% overall efficiency in converting electric to UV power. The applied current excites the Hg electronic levels which return to the ground (standard) states by emitting noncoherent (all directions) characteristic UV rays.

UV emission can be enhanced with Hg amalgams that increase the operating temperature to near 100 °C doubling or tripling the UVC output. Total emission power is dependent on the arc lamp length allowing a range of germicidal lamps to operate in the 100–400 W UV power range.

Germicidal lamps are readily available from many manufacturers/distributors worldwide (www.globalsources.com/manufacturers/Mercury-Lamps.html). As a result, design and UV power range are broadly based on intended use and throughput needs. Support and services for the Hg-based UVC sources are also well developed and available for most disinfection and air- and insect-control applications.

A schematic of a low-pressure Hg lamp is shown in Figure 1.

Arc or Flash Lamps

Arc or flash lamps are a specialized group of gas discharge lamps mostly filled with xenon (Xe) in which an electric

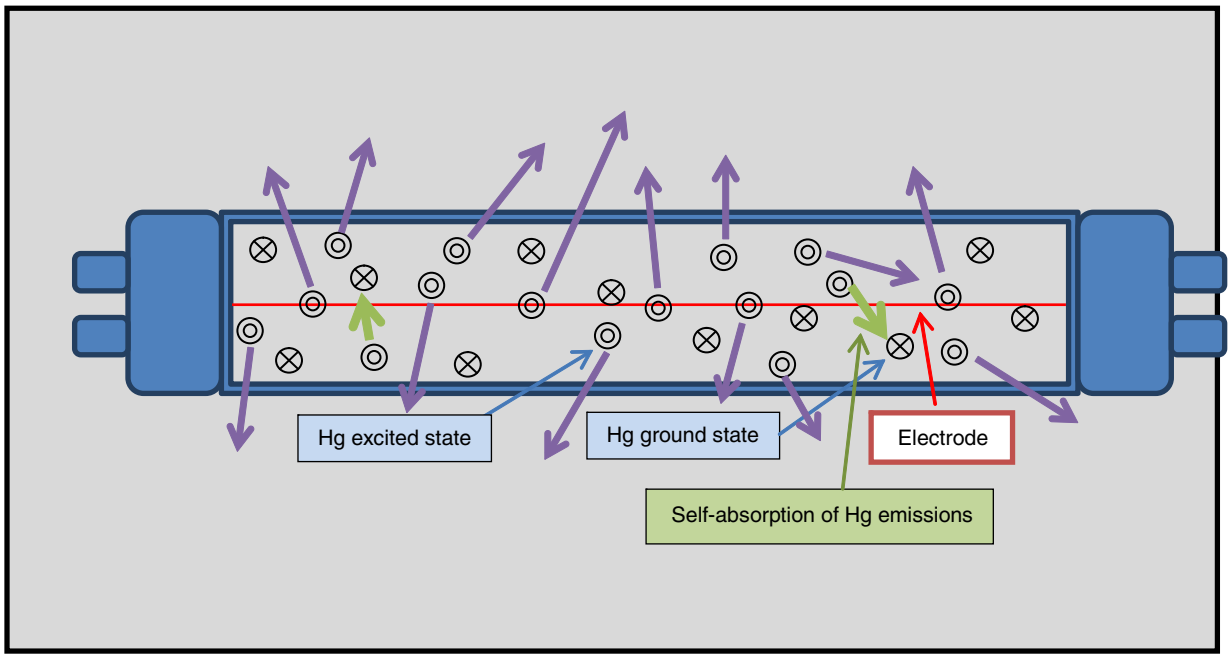

Figure 1 Schematic of an Hg-based germicidal lamp. Emission of UV photons is noncoherent (all directions) and self-absorption occurs by ground-state Hg, a limiting factor in pressurizing Hg lamps for higher output.

current is applied to a pressurized gas (up to 30 atm). (Because of the high gas pressure, the operation of these arc lamps presents worker safety issues requiring special handling to prevent accidents due to explosion.) The gas ionizes allowing the passing of electricity and producing a bright white broadband radiation emission (light). A typical Xe-filled arc lamp with continuous emission of UV and visible light is shown in Figure 2.

The emission spectrum from arc or flash Xe lamps contains not only a significant band of UV radiation but also visible light and some infrared radiation (see Figure 3). Other gases may be used to generate different emission spectra shifted to other UV bands (i.e., nitrogen gas for vacuum UV). The emission spectrum is broad and similar to the solar radiation spectrum (natural sunlight) and is used in producing natural lightning conditions for illumination and projection applications. These lamps are made with glass or fused quartz tubes to prevent absorption of UV photons and with tungsten metal electrodes at both ends. Some arc lamps with high power (> 10 kW) ratings require water cooling as a significant fraction of the generated power is converted to thermal (heat) power.

Xenon-filled arc lamps were developed in Germany in the 1940s and introduced commercially in the early 1950s. Today, Xe arc lamps with continuous emissions for use in illumination, projection, and research applications, operating in the 10 W to ~20 kW power range are commercially available.

However, recent developments have incorporated a pulsed power Xe 'flash' lamp option using ultra-short pulses (milliseconds) for similar applications but using high peak UV power (several megawatts per pulse). Overall, Xe arc and flash lamps generate a substantial UV radiation that reaches up to 18–20% of the total emission spectrum per pulse.

A comparison of the emission spectra from Hg-based germicidal lamps, several excimer lamp systems, and the broadband high-power Xe-filled flash lamp, is also shown in Figure 3.

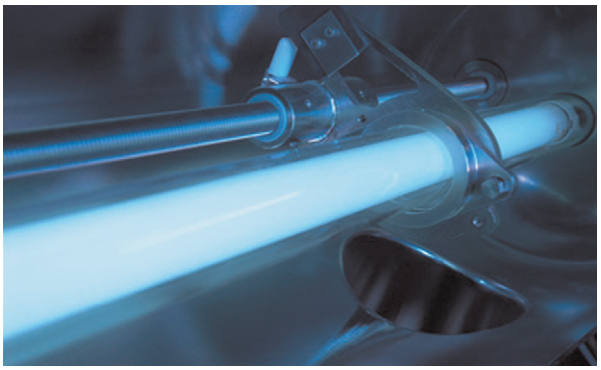

Figure 2 Typical Xe-filled excimer lamp UV with external water cooling. http://halmapr.com/news/aquionics/files2007/03

Mechanisms of UV Disinfection

Photochemical Reactions

UV radiation is effective in altering the chemical structure of biological molecules including the nucleic acids. DNA represents the most likely target as its nucleotide bases (purine [adenine; guanine] and pyrimidine [thymine and cytosine] derivatives) are highly UV-absorbent chromophores (i.e., sites). Therefore, UV-induced DNA changes are critical and

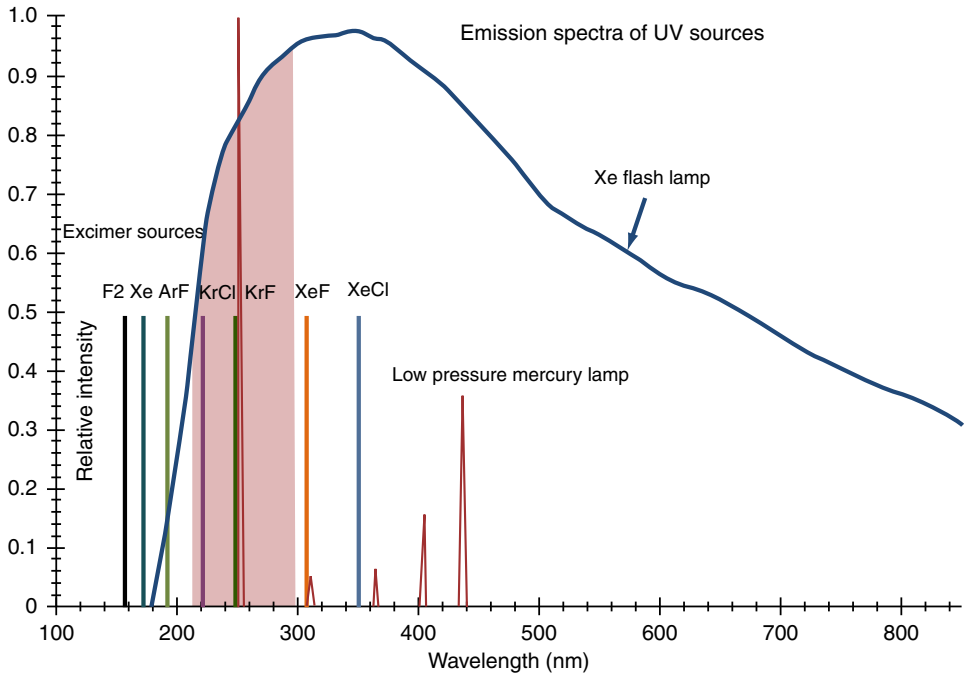

Figure 3 Comparison of emission spectra for different UV radiation sources. The shaded area indicates the germicidal UV photons emitted from the broadband high-peak power PUV Xe flash lamps.

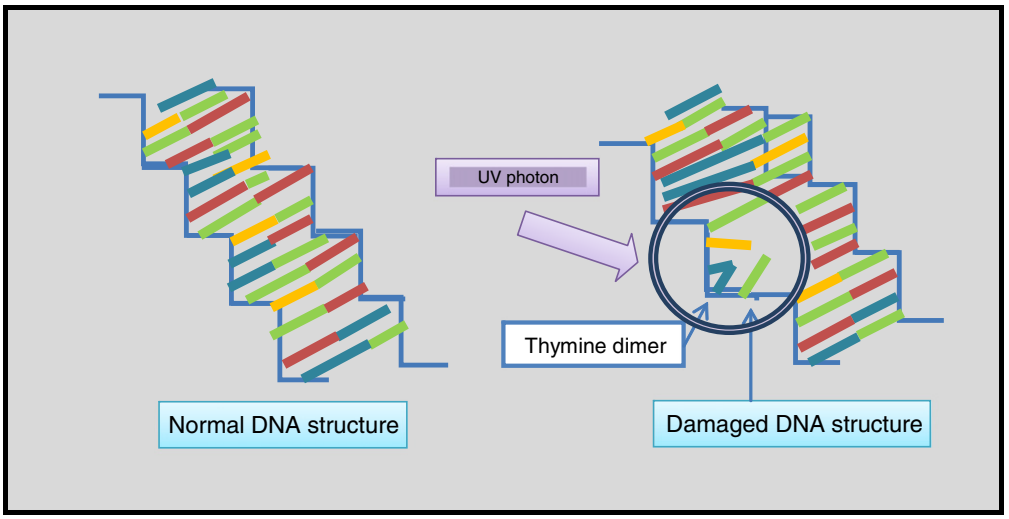

Figure 4 Thymine-dimer formation by photochemical-induced lesions in DNA with UVC radiation.

responsible for the disinfection action of UV photons. DNA has a maximum UV absorption band at 265 nm and the photobiology effects results in DNA mutagenic and cytotoxic lesions that damages microbes and humans.

By comparison, proteins absorb mostly at ~280 nm and at equal concentrations are lower absorbents than the nucleic acids. Other lesser chromophores include porphyrines, carotenoids, steroids, and quinones.

The structural alteration of UVC radiation in DNA is illustrated in Figure 4, and is based on the formation of cyclobutane pyrimidine dimers (CPD), known as thymine dimers. This lesion occurs when neighboring thymine bases holding DNA's helix structure are excited by the absorption of UV energy, breaking bonds and prompting the formation of new bonds between thymine base pairs as opposed to across the individual single strands as in the normal DNA structure. This results in the deformation (distortion) of DNA's helix structure restricting its effective coupling with catalytic enzymes, a critical initial step in the cell's transcription and replication processes.

UVC-Induced Cellular Lesions

Two major UV-induced lesion mechanisms in cells form CPDs and/or 6–4 photoproducts (6–4 PPs) and their valence isomers. These are the principal lesions leading to mutagenesis. The formation of 6–4 PPs also may result in changes in cell metabolism as they can inhibit or induce other combined cytotoxic effects.

Other lesions are usually initiated by: (a) alkylating agents that play an essential role in many biosynthetic reactions but that can modify the structure of nucleotides into a mutagenic compound, a miscoding alteration, or even a lethal noncoding damage; (b) by hydrolytic processes that deaminate nucleotide nature; and (c) by formation of short lived but highly reactive free radicals including reactive oxygen species induced by UV (i.e., photolysis reactions).

Any UVC source is effective in inducing critical lesions that inactivates vital cell processes. Therefore, all UVC radiation sources are potentially useful for food safety applications as will be discussed below in Section Applications of Pulsed UVC (PUVC) Radiation in Food Sanitation.

Repair Mechanisms

The initial controlling action of absorbed UVC radiation may be totally or partially counteracted by cell mechanisms to repair the initial photochemical lesions. These repair mechanisms (dark and/or enzymatic) allow for cell survival and may lead to mutagenesis. The cell's capability to repair also leads to different resistance (tolerance) levels to UVC absorbed radiation doses.

The ability to repair lesions is governed by second-order kinetics with the limiting reactant being the specific chemical initiating the repair process. Therefore, to overcome repair and mutagenesis, the choice of UV source technology is critical. High peak of UV power is preferred as it leads to the short timed formation of excited DNA at concentrations above the capability of repairing molecules. This is further discussed below in Section Potential Uses of High-Power PUVC Radiation Sources.

The different consequences following UVC exposure in generating DNA lesions are further illustrated in Figure 5.

Applications of Pulsed UVC (PUVC) Radiation in Food Sanitation

By nature, the UV process is a physical method of photon energy transfer that offers nonthermal and nonchemical (residue free) sanitation alternatives apt for temperature-sensitive commodities (i.e., fresh produce); for processed foods (i.e., fruit juices and milk) and for other food and agriculture commodities. UV disinfection of air and water is already practised and extension of its use to irrigation, processing (washing), recycled, and waste water is being developed.

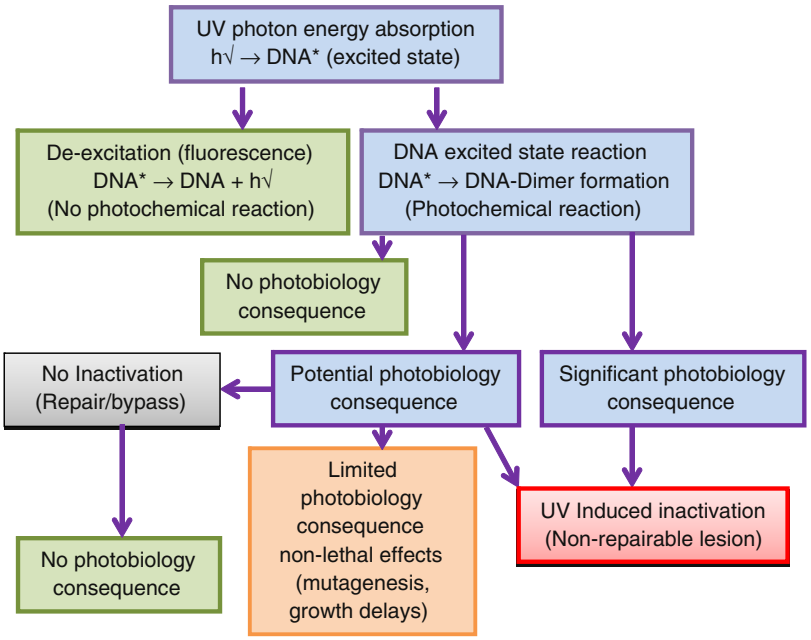

Figure 5 Photochemical, repair, and cell mechanisms following absorption of UVC radiation.

Continuous and PUVC Sources

Today, commercial scale Hg lamp technologies for water and air disinfection are successful and credible processes are providing a strong basis to consider new UVC applications. However, the choice of UVC technology is again critical. For example, for surface disinfection of fresh fruits, Hg lamps have limited power with limited throughput that imposes substantial changes in infrastructure and practices. By contrast, new and reliable PUV systems are now available commercially and operate with high repetition rates at competitive costs. More importantly, their compact modular designs and small physical profile allows implementation within the existing infrastructure.

Potential Uses of High-Power PUVC Radiation Sources

Studies at the University of California, Davis, since 1992, have firmly established the potential uses of PUVC sources for many applications in food and agriculture. Results and rationales for potential commercial uses with liquid and solid commodities are discussed in Sections Liquid Foods (Volumetric Disinfection) and UVC disinfection of fruit juices

Liquid Foods (Volumetric Disinfection)

The UVC disinfection action with liquid commodities is volumetric as pathogens are dispersed throughout the entire volume of a commodity. However, for process uniformity, special mixing techniques are required. For fruit juices, several prototype UVC reactors have been considered. For larger volumes (i.e., irrigation water), reactors are simply combined to achieve the needed flow throughputs.

UVC disinfection of fruit juices

Today, the leading commercial method for the sanitation of fruit juices is pasteurization, using a mild thermal process ($\sim$72 °C [162 °F] for 15 s) efficient to inactivate vegetative microorganisms including *Escherichia coli* spp., *Listeria* spp., and *Salmonella* spp., but not heat-resistant spores (e.g., *Bacillus* spp.). However, pasteurization reduces the nutritional value of fruit juices by destroying some essential vitamins (i.e., C, B1, B2, and natural enzymes) and noticeably changing flavor. Nevertheless, the lack of alternatives has forced the industry to adopt pasteurization although in full recognition of its shortcomings. However, pasteurization in the US is not mandatory and therefore the development of alternative methods has been encouraged including the use of nonthermal UVC irradiation (US Food and Drug Administration-approved 21 US Code of Federal Regulations 179) to achieve >5 $\log_{10}$ reductions. UVC radiation processing of liquid milk has already been investigated and it is a nonthermal potential alternative to heat pasteurization.

Nonthermal UVC disinfection of fruit juices is not new but is conducted with Hg germicidal lamps limiting the flow rate capabilities. High processing rates are required, thus several hundred Hg lamps are used adding complexity in operation and maintenance. In addition, special handling methods (i.e., annular, concentric, and rotating tubes) and reflecting devices are needed to process the juices effectively and uniformly.

Current turbulent-flow UVC reactors include $\sim$90 m ($\sim$300 ft) of UV transparent tubing surrounded by hundreds of Hg lamps. By comparison, if laminar flow conditions are used, very thin layers of juices may be exposed but this simpler approach is restricted to optically clear, low viscosity fruit juices, thereby limiting throughput.

A PUVC reactor system Figure 6, was designed based on tests with a single PUV Xe flash lamp at the University of California, Davis. It combines six Xe-filled flash lamps for a total peak UV power of $\sim$2.4 kW pulse^{-1} at 10 pulse per sec(pps) repetition rates. It uses Spectralon^{TM} reflectors, a commercial fluorinated polymer with $>$99% diffuse

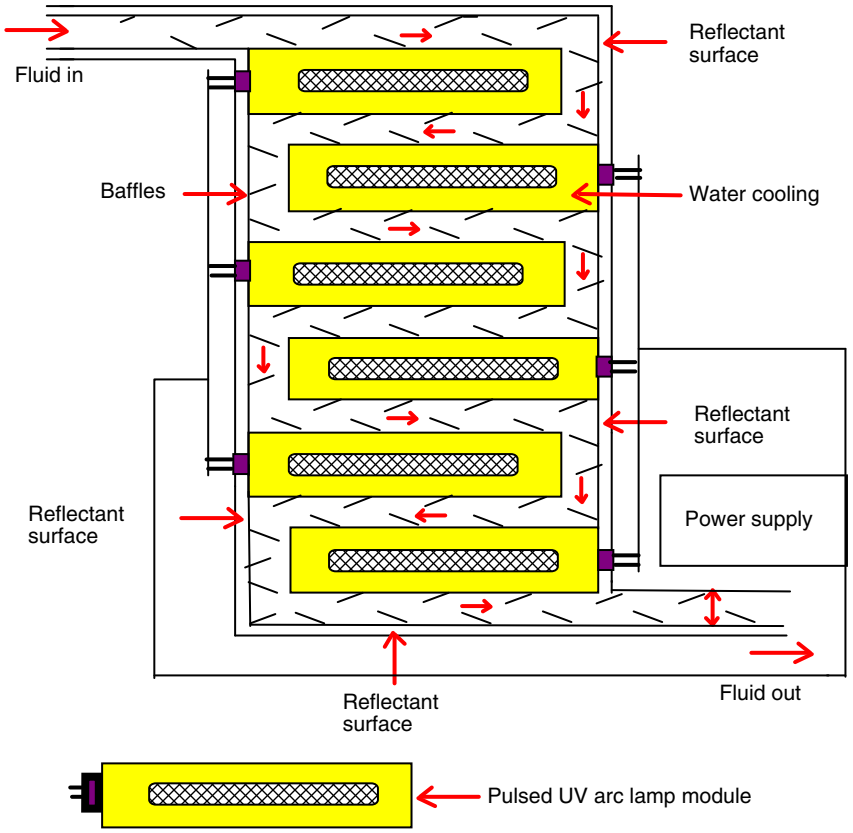

Figure 6 Multiple Xe arc (flash) lamp UVC reactor for the disinfection of liquid products.

Figure 7 PUV Xe arc (flash) lamp system used in experimentation at the University of California, Davis. Power supply and pulsing control system (left) and Xe-filled water-cooled quartz (right). Picture courtesy of UC Davis.

reflectance (www.en.wikipedia.org/wiki/Spectralon). The liquid channels also contain baffles to force a turbulent-flow process.

The single, Xe-flash lamp prototype system Figure 7 used in evaluating process efficacy and throughput for disinfecting food surfaces and liquid products operates reliably for >5 million pulses/lamp and its throughput equivalence is several hundred Hg germicidal lamps. This UVC reactor operates at highly competitive costs as compared with other emerging techniques. Cost estimates with alternative disinfection techniques range from ~USD$0.15 gal^{-1} (hydrostatic pressure) to ~US $0.05 gal^{-1} for carbon dioxide and pasteurization

Table 3 Pulsed ultraviolet (PUV) disinfection of fruit juices

Fruit Juice	*Type of inoculum*	*Initial inoculum (cfu ml^{-1})*	*Threshold ultraviolet (UV) energy (mJ cm^{-2})*	*Observations (Up to 5 J cm^{-2} UV doses)*
Apple	*Escherichia coli* O157:H7	3.9×10^7	600–800 >5 $\log_{10}$ reduction	High UV tolerance, no color or flavor changes
Orange	*Salmonella typhimurium*	1×10^6	800–1000 >5 $\log_{10}$ reduction	High UV tolerance, no color or flavor changes
Raspberry	Natural flora	$\sim 5 \times 10^3$	400–600 >3 $\log_{10}$ reduction	High UV tolerance, no color or flavor changes

treatments; to $\sim$USD\$0.002 gal^{-1} for conventional Hg germicidal lamps (In: 'Fresh today, Safe next week,' Food Engineering, Feb 2001). Current estimate for PUV Xe flash lamps is $<$USD\$0.001 gal^{-1}.

Results of several investigations at the University of California, Davis, using a PUV Xe-flash lamp UVC reactor, are summarized: Apple, orange, and raspberry juices: Freshly made, nonpasteurized apple juices (filtered and unfiltered) prepared from Granny Smith varieties (inoculated with *Escherichia coli* O157:H7); orange juice (no pulp; thermally pasteurized) obtained from commercial markets (inoculated with *Salmonella typhimurium*); and fresh raspberry (Heritage) juice containing a natural contamination flora were tested.

Microbial assays, before and after UVC processing, were conducted using standard methods. Above the threshold UVC energies, bioassays of the UV-treated samples indicated no pathogenic colony formation. Results of these experiments under optimized mixing conditions during PUV exposure are summarized in Table 3.

Results with fruit juices have indicated that UVC disinfection is effective and technically feasible. Besides, the tested fruit juices showed high UVC tolerance and maintained quality attributes with minimal effects on nutritional quality. Therefore, this new approach offers a new UVC alternative for the disinfection of fruit juices and offers increased and broad range capabilities; low profile compact systems technology development is underway at RF Biocidics Inc., Vacaville, CA (www.rfbiocidics.com).

Disinfection of Irrigation and Wash Water

Irrigation and wash (processing) water are critical resources in agriculture but they may be direct sources of plant pathogens (i.e., microbes and nematodes). Contaminated irrigation water affects productivity, forces the implementation of disease management strategies (usually pesticides), whereas the wash water used in postharvest sanitation needs disinfection to assure safety and quality of many fresh commodities. Furthermore, due to new regulatory and economic concerns, the nursery and greenhouse industries have adopted recycling as a water conservation strategy that prompted new risks by an increased reliance on prophylactic fungicides, filtration (slow and limited efficiency), chlorination (worker and public safety risks), ozonation (worker safety risks and limited efficacy), and Hg lamp UV irradiation (limited throughput and efficacy).

Plant pathogens like fungi (i.e., *Pythium* spp. and *Phytophthora* spp.) and plant parasitic nematodes (i.e., *Aphelenchoides* spp., *Meloidogyne* spp., and *Pratylenchus* spp.) are naturally present in many crops. Besides, sources of irrigation water have limited UV optical transparency even when filtered. Therefore, the potential for UVC disinfection of irrigation water has similar challenges as described before for fruit juices, and requires a large processing capacity.

Hg germicidal lamp systems are available for disinfecting irrigation and processed water for greenhouse and nursery operations. UVC processing of 2000–8000 l min^{-1} with 30–60% UV transmission at 30–50 mJ cm^{-2} UV doses is used, but increased dissolved organic matters reduces throughput as higher UVC doses (200–600 mJ cm^{-2}) are required.

Advanced oxidation processes are also considered for wash water disinfection by combining UVC radiation with added hydrogen peroxide which forms short-lived but powerful oxidizing (disinfecting) species like hydroxyl peroxide (HO_2) and hydroxy radicals ($^{\bullet}OH$) known to be powerful antiseptics.

As for disinfection of fruit juices, high-power, PUV sources are advantageous when combined with ozonation or with vortex techniques to remove suspended solids. A single PUV Xe-filled flash lamp (shown in Figure 7) delivers approximately 4 MW of germicidal UV ($\sim$50% UVC). This UVC power output is equivalent to several hundred $\sim$400 mW cm^{-2} Hg lamps. An exact equivalence is not given as other factors include reactor design, fluid optical quality, and flow. Finally, reduced chlorination levels may be added to provide residual disinfection effects.

Efficacy of PUV disinfection in irrigation water was demonstrated with mixtures of zoospores and cysts of *Phytophthora spp*. (i.e., *citrophthora*, *nicotianae*, and *capsici*) treated with a 248 nm PUVC beam from a KrF excimer laser. Results are given in Figure 8 and indicate full inactivation in the 10–30 mJ cm^{-2} UVC dose range.

A schematic of a potential UVC combined process for the disinfection of large volumes of irrigation and processing water is shown in Figure 9.

In summary, PUVC combined with available technologies may help provide alternative disinfection for water resources in the food and agriculture industry. The ability to safely recycle many water resources and reduce recontamination of soils, crops, and harvested commodities would greatly enhance food safety for many food commodities presently at risk.

Potential Uses of High-Power Pulsed UVC Sources in the Disinfection of Solids

Fresh produce is a preferred choice for consumers based on their attributes as being healthy, tasty, convenient, and fresh.

Over the past 20+ years, the fresh produce industry (conventional and organic) has experienced solid growth and is estimated to reach an USD$150 billion retail and service market in the USA with a similar growth scenario around the world. However, the industry also faces challenges as it needs to protect consumers against microbiological hazards as it operates with multiple scenarios related to regional, national, and international markets with many practices and opportunities to introduce microbial contamination.

Options to attempt safety include chemical pesticides (fungicides and bactericides), direct chlorination, ozonation, and/or a combination of disinfectants. Others are directed to produce disinfected or activated wash (processing) water to directly eliminate pests in the commodity's surface.

Combination of various disinfection approaches have also been attempted using chemical and physical methods. Furthermore, physical methods including brushing surfaces and ultrasound, hot water treatments for somewhat temperature-tolerant fresh commodities have also been considered.

Among these approaches, PUVC sources offer again many comparative advantages similar to those indicated for the disinfection of liquid commodities. Besides, PUV will again be capable of offering large-scale processing capabilities unequaled with Hg lamp technologies and with low-profile compact systems.

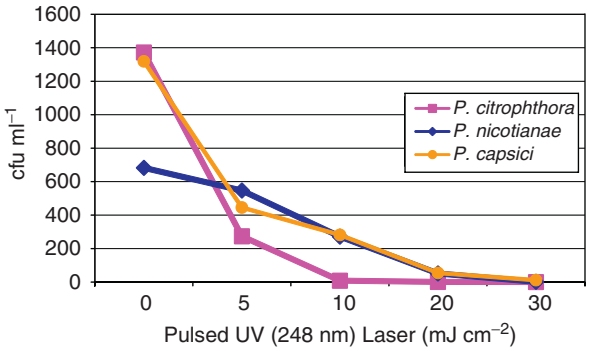

Figure 8 PUV (248 nm) disinfection effects on mixtures of zoospores and cysts of *Phytophthora spp.* using high-power UV radiation.

UVC Photon Interactions with Fresh Fruit Surfaces

UVC radiation can effectively disinfect the surface of many foods including temperature sensitive, high moisture fresh produce, and temperature tolerant, low-moisture nuts, grains, and seed products. However, the nature of the exposed surface determines the practical effectiveness of UVC radiation to reach exposure levels for adequate disinfection.

In fruits and in many vegetables, the presence of high UV-absorbent chemicals limits UVC depth of penetration of the surface (epidermis) to 10–40 μm. In addition, natural and added waxes (lipids) contribute to diffuse and specular UVC reflectance. Specular reflectance is the mirror-like reflection of UV (or other kinds of waves) from a surface in which UV from a single incident direction is reflected into a single outgoing (reflected) direction (www.en.wikipidia.org/wiki/Specular_reflection) and decreases UVC exposure levels. No measurements of reflectance in fruits and vegetables have been reported and therefore no quantitative estimation of this energy loss mechanism is known. A natural physical barrier is also present as trichomes combine with the natural waxy layers in some cultivars and with some physical roughness (i.e., holes and crevices). This combination results in effective shadows or shields for microbes to UVC photons and therefore establishing UVC exposure conditions is limited to experimental observations. Other limitations are due to the

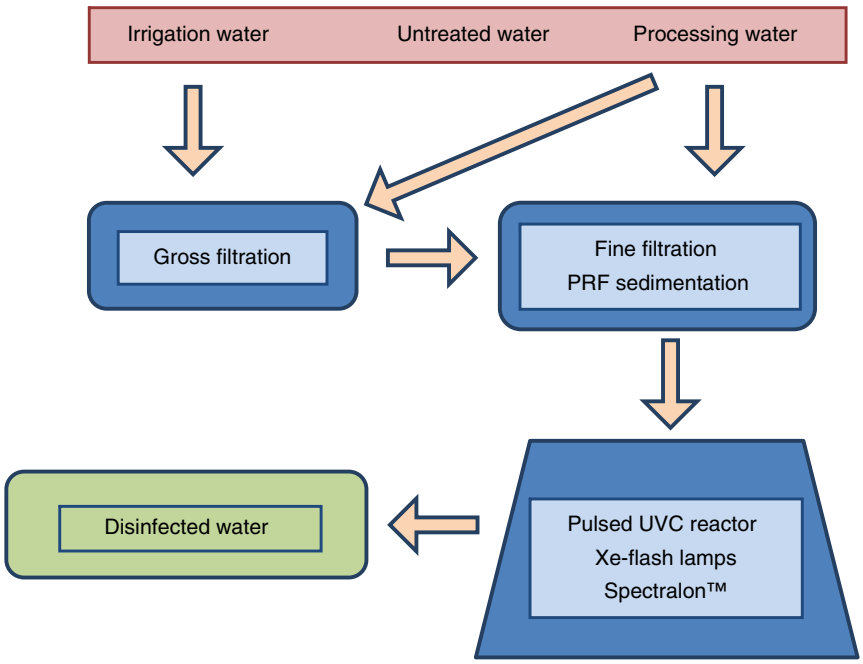

Figure 9 Combination technologies for a PUVC reactor system for disinfection of irrigation and/or wash (processing) water.

geometric characteristics of some fresh fruits and vegetables (e.g., bunches of fruit, folded leaves in vegetables, etc.) as again biological tissue is an effective barrier to UVC penetration. Finally, as UVC photons move in straight-path trajectories from their source, the round or oval geometry of many commodities provides effective shielding that force the need to expose a surface with different UV-path incident angles. The round surfaces, particularly when moving (rotating), enhance the natural shielding or shadowing effects and minimize the direct interaction of the straight-path incident UVC photons with the targeted pathogens. These natural and combined effects on fruit surfaces are illustrated schematically in Figure 10.

For bulk processing of nuts, grains, and seed products, other physical barriers are present due to additional UV shielding provided by the grouping of the commodities unless effective single layering and UV exposure uniformity techniques are applied. Furthermore, the choice of UVC source and the availability of techniques to assure homogeneous, surface-uniform UV exposure are critical.

Despite these limitations, the combination of high-peak power UVC sources from Xe-filled flash lamps with specialized UV reactors operating with diffuse reflectance allow for adequate uniformity of UVC exposure of fruit surfaces when combined with readily available surface randomization techniques. This approach offers an emerging alternative capable of large throughput as well as reliability. This rationale is explained in Section Effects of PUVC Radiation on Fruit Commodities with the support of experimental data including trials with several different fruit commodities.

As many fruits are climacteric and have physiological activity after harvesting and during storage (i.e., respiration and ripening) and many are colored with UV-sensitive pigments, studies to evaluate fruit tolerance and the overall sensory and physiological response to UVC exposure have been conducted with commercial-quality fruits. The results of these investigations have provided a strong rationale to place this approach as a new alternative process with practical, logistical advantages and competitive economics.

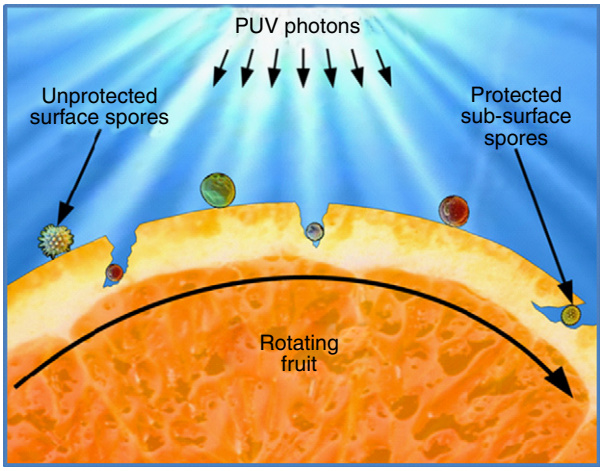

Figure 10 Schematic representation of UV shadowing effects on rounded rotating fruit surfaces. Surface, unshielded microbes should be easily inactivated by UVC radiation. Rotation during UV exposure allows changing the angle of incidence on the fruit surface and the orientation of a crevice or hole hosting microbes may provide an effective shield to UVC exposure.

Effects of PUVC Radiation on Fruit Commodities

Selected fruits of commercial quality were included in a study conducted at the University of California, Davis. The choice of fungal organisms was based on their relevance and impact on the spoilage of the selected fruits. The threshold PUV exposure energies (mJ cm^{-2}) used to completely inactivate the fungal organisms were also determined in appropriate culture media, on fruit surfaces, or in distilled/deionized water using a 248 nm KrF excimer laser. Results are given in Table 4.

Clearly, the presence of effective UV barriers in some fruit surfaces does increase the level of UVC exposure when compared with nonshielded surfaces (i.e., culture media) or in water (optimal UV transparency). Therefore these measurements provided an experimental determination of the practical levels needed to be used in achieving a desired level of disinfection. Factors ranging from 2 to 6.5 were determined except for *Aspergillus niger* which has melanin, a highly UV-resistant coloring pigment. Other limiting UV barriers include rotation effects and the presence of surface injuries. Therefore, under commercial (large scale) operations, even higher PUV exposure

Table 4 Threshold pulsed ultraviolet (PUV) (248 nm) energy doses for effective control of fungal organisms on fruit surfaces, in water, and on culture media[a]

Fungal organisms	*Host media*	*Threshold PUV energy (mJ cm^{-2})*
Alternaria alternata	Fruit surface (apple, pear)	500
	Solid culture medium	200
Aspergillus niger	Fruit surface (grape)	1900
	Solid culture medium	300
Botrytis cinerea	Fruit surface (apple, kiwi)	150
	Solid culture medium	30
Fusarium oxysporum	Fruit surface (pear, peach)	100
	Solid culture medium	35
Fusarium roseum	Fruit surface (pear, peach)	100
	Solid culture medium	35
Monilinia fructicola	Fruit surface (nectarine)	100
	Solid culture medium	50
Penicillium expansum	Fruit surface (orange)	150
	Solid culture medium	50
Penicillium digitatum	Fruit surface (orange)	150
Phytophthora citrophthora	Water	100
Rhizopus stonolifer	Fruit surface (nectarine)	130
	Water	20

[a]Experiments in duplicate with triplicate samples each ($n = 6$). The uncertainty in these measurements amounted to $\pm 21\%$ (root-mean-square (RMS); $\pm 1\sigma$).
Source: Reproduced from Lagunas-Solar MC, Piña C, MacDonald JD, and Bolkan L (2006) Development of pulsed ultraviolet light processes for surface fungal disinfection of fresh fruits. *Journal of Food Protection* 69(2): 376–384.

Table 5 Tolerance and effects of selected fruits exposed to pulsed ultraviolet (PUV) (248 nm)[a]

Fruit	*Variety*	*Max PUV (mJ cm^{-2})*	*Observations*
Apple	Red Delicious	<3000	High UV reflectance and tolerance Potential for external injuries with long-term storage
	Granny Smith	<3000	High UV reflectance and tolerance No effects on external quality
	Fuji	<3000	High UV reflectance and tolerance No effects on external quality
Kiwi	Hayward	>3000	High UV tolerance. No color changes No external injuries expected
Orange	Valencia	<3000	Medium UV reflectance and tolerance Potential for skin damage and skin color
Lemon	Eureka	<1500	Low UV tolerance Potential for skin damage and skin color
Nectarine	August Red	>2000	Medium UV tolerance No skin damage. No changes in external, ground color
Peach	Autumn Flame	<2000	Potential color changes at higher UV doses No external damages at <2000 mJ cm^{-2}
	O'Henry	<2000	Potential color changes at higher UV doses No external damages at <2000 mJ cm^{-2}
Pear	Bosc	>2000	Medium UV tolerance No skin damage. No changes in external, ground color
Raspberry	Heritage	<2000	Medium UV tolerance No skin damage. No changes in external, ground color
Table Grapes	Red Globe	<2000	Medium UV tolerance No skin damage or color changes
	Thompson Seedless	<1000	Medium UV tolerance No skin damage. Potential for lower color hue

[a]The total uncertainty of PUV exposure is $\pm 6\%$ (RMS; $\pm 1\sigma$).

Source: Reproduced from Lagunas-Solar MC, Piña C, MacDonald JD, and Bolkan L (2006) Development of pulsed ultraviolet light processes for surface fungal disinfection of fresh fruits. *Journal of Food Protection* 69(2): 376–384.

levels are anticipated and the commodity's UV tolerance needs consideration.

Tolerance and Quality Effects in Fruits Exposed to PUVC Radiation

The potential sensory and physiological effects in PUVC-treated fruits were studied with 11 different cultivars and a total of 14 varieties. Fruit quality was evaluated before treatment as well as at +15 and +30–40 days after exposure while at the refrigerated storage under simulated commercial conditions. Fruit quality evaluations including flesh firmness, skin color, soluble solids, pH, titratable acidity, respiration, ethylene production, decay, external/internal injury, and weight loss for both control and PUVC-treated samples were done in duplicate. The general conclusions of this study are summarized in Table 5.

PUVC Reactor for Fruit Disinfection

To reach the expected potential for PUVC radiation for surface disinfection of solid products, an efficient UVC reactor is needed. The UVC reactor needs to safely house single or multiple Xe flash lamps to allow for a broad range of processing capacity and to maximize the UV-energy use efficiency of the emitted (available) radiation energy. In a UVC reactor, diffuse reflectance is therefore a critical operational feature to allow for directing the noncoherent UV radiation toward the target and as a means of randomizing fruit surfaces to allow uniform exposure. The design and engineering features of such a PUVC reactor is illustrated Figure 11.

With the exception of table grapes, all fruits handled in conveyorized operations may be treated by PUV if all the needed operational features are introduced. Most importantly, the operating characteristics of PUV sources, particularly Xe flash lamps would have the potential for high disinfection rates as exposure can be varied to match product throughput and thus be able to disinfect large treatment areas in short times.

A yet to be documented potential effect of PUV is the ability of high-power energy deposition to cause the partial or total destruction of contact pesticide residues by photolytic effects including advanced oxidation processes. In principle, this potential can make the PUV technique able to simultaneously provide disinfection, disinfestation, and chemical decontamination effects in a single process.

Consumer response to the concept of PUV treated food was already studied and suggested a positive reaction by most consumers but indicated the need to further educate the conventional consumer.

Test Results of PUVC Exposed Fruit Surfaces

The disinfection effectiveness of high-power PUVC combined with diffuse all- direction exposure fields can be summarized in a few selected examples from laboratory experimentation.

The ability of an optimized PUVC (248 nm) radiation field on oranges inoculated with *Penicillium digitatum* spores and

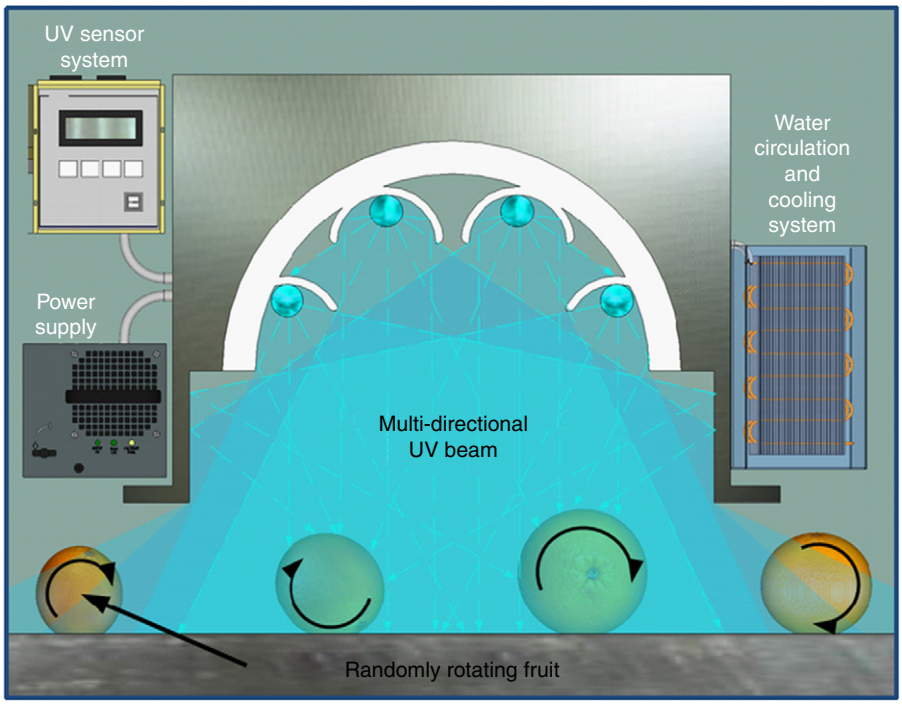

Figure 11 PUVC reactor for disinfection of fruit surfaces. All components are readily available from commercial sources.

Figure 12 Comparison of control (right) and PUVC (248 nm) excimer laser-treated (left) oranges inoculated with $\sim 2 \times 10^4$ cfu of *Penicillium digitatum* at ~ 5 mm under the epidermis. Photo courtesy of the University of California, Davis.

Figure 13 Instant cavitation and ablation effects of high-peak power PUVC (248 nm) sources on table grapes (Ribier). Observation was after 2 days of treatment. Photo courtesy of the University of California, Davis.

exposed to a well-tolerated dose of 2000 mJ cm^{-2} is shown in Figure 12. Inoculum was injected ~ 5 mm deep (in three sites) and represented a common practical problem as wounded, deep infection sites can normally develop into larger infections and are inaccessible to sprayed pesticides or fumigants.

PUVC sources have the potential for enhancing disinfection effects by cavitation, ablation and reflectance events. Cavitation refers to a physical process in which increased UV penetration is obtained due to the multiple scattering of photons from diffuse reflecting surfaces including waxy fruit surfaces. Ablation is an instantaneous physical removal process of debris on fruit surfaces due to the absorption and charging of the UV energy by the materials covering or adsorbed on the surface. This includes soil particles and pesticide residues. Diffuse reflectance occurs from waxy, round surfaces, but the ablation effects remove the debris (soil dust and pesticide residues). These effects are fully dependent on the UVC power density only available with PUVC power sources. These effects are shown in Figure 13.

Conclusions

UVC radiation is clearly an alternative disinfection method with a potential role in processes directed to the treatment of liquid and solid commodities. However, the choice of UVC source is important and determines the practical application conditions for the different commodities. Both the conventional use of standard, low-power continuous emission Hg-based germicidal lamps and the specialized, high-peak power, PUVC sources have a role depending on throughput and infrastructure (space availability) requirements. Significant larger demand for space is required for Hg lamps, whereas PUVC sources are compact and low-profile units.

Economics was not largely addressed in this work but it is estimated that both types of UVC sources offer competitive economics and reduced costs as compared with other alternatives. However, Hg lamps are readily available and some operating systems are already operating in the field, yet there is no known commercial operation that is supported by PUVC sources, although some semi-commercial tests have been conducted and plans for commercial development are underway.

The UVC systems appear to be adequately capable for covering a broad range of operations with small (greenhouse and nursery) to large (industrial level, packing houses, etc.) product throughputs. As for the choice of UVC sources, Hg lamps are expected to dominate the small markets whereas PUVC sources have the features to meet demands for larger processing needs. However, because of efficiency and added effects, PUVC technology shall become available in smaller market operations.

Current research information is clearly dominated by reports based on using Hg lamps although many investigations, with similar results and limitations, are available and will continue to be generated using the more advanced (Hg free) PUVC technologies.

For liquid foods (fruit juices), whereas Hg lamps can render effective but limited throughput systems, the PUVC technologies offer better cavitation (increased range) and throughput capabilities. For irrigation water, the scenario is similar and both types of UVC sources appear to have a role and cover the needs of focused markets.

For solid foods (fresh produce), Hg lamps are limited to small throughputs than PUVC with the latter offering unique advantages associated with their capabilities for diffuse reflectance and reaching the shielded areas, to use cavitation, and to clean surfaces by ablation while providing much larger processing capabilities with compact systems.

For other solid commodities such as nuts, grains, and seeds, UVC radiation offers the potential for effective disinfection if process uniformity techniques are successful. Material handling techniques to assure single layering and randomization for these products are available but the design and engineering effort to integrate it with any UVC source has not been reported.

Finally, the advancement of UVC disinfection technologies is expected to continue and even expand as the entire fresh produce industry needs to rely on nonthermal, nonchemical processes to improve wholesomeness and safety of fresh produce.

Acknowledgments

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Pulsed Electric Field Technology

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Glossary

Electroporation Pore formation in the membrane of biological cells caused by the application of an external electric field.

Hurdle concept The concept of applying combined methods in order to have a series of preservation methods resulting in multitarget effects for microbial inactivation.

Numerical simulation Computational modeling used in pulsed electric field (PEF) process analysis and optimization, for example, for calculating electric field strength or temperature distribution in the treatment chamber. This requires experimental information as well as validation.

Sublethal injury Structural or functional damage of microbial cells that is repairable under optimum growing conditions resulting in a recovery of physiological fitness.

Treatment intensity The term 'treatment intensity' of a PEF treatment may encompass electric field strength level, number of pulses, frequency, treatment time, pulse energy, total specific energy input, and treatment temperature.

Introduction

Pulsed electric field (PEF) treatment is a nonthermal alternative to heat pasteurization of liquid products. Other applications are the disintegration of plant and animal raw materials and also the induction of stress responses in biological cells. However, because of considerably higher treatment intensities applied for PEF food preservation, the following outline on food safety aspects related to PEF will focus on this application.

According to several studies, the quality of PEF pasteurized food product is closer to that of the fresh product than the heat-pasteurized product and the safety of the fresh product is enhanced by the inactivation of vegetative pathogenic microorganisms. Because PEF is a novel technology, special legal requirements have to be fulfilled (e.g., for the European countries the regulation of the European Commission No. 258/97 on novel foods and novel food ingredients) and safety concerns have to be addressed. The first commercial PEF application was installed in 2005 in the US for fruit juice preservation. Clearance by the Food and Drug Administration was already available since 1996, indicating the techniques potential for safe and gentle preservation. However, to make further use of this promising technology and to advance its industrial implementation, several aspects need to be taken into account in order to guarantee a satisfying process performance.

To conduct a PEF safety assessment, two main starting points are suggested: (1) a product-based approach (substantial equivalence study) answering the question of alterations occurring in the composition of a PEF-treated product or (2) a process-based approach that identifies critical control points using available knowledge on mechanisms and the description of the process.

This article aims to give an overview of important hazards regarding food safety that may occur during a PEF treatment. Underlying phenomena will be discussed and possible control actions to improve the process performance will be pointed out.

PEF Basic Principles

PEF technology can be used to induce nonthermal permeabilization of cell membranes. Depending on the treatment intensity (external electric field strength, number, and duration of the electric pulses) and cell properties (size, shape, orientation, and conductivity), the pore formation may be permanent or temporary.

Treatment consists of the application of very short electric pulses (1–100 μs) at electric field intensities in the range of 0.1–1 kV cm^{-1} (reversible permeabilization for stress induction in plant cells), 0.5–3 kV cm^{-1} (irreversible permeabilization of plant and animal tissues), and 15–40 kV cm^{-1} for the irreversible permeabilization of microbial cells. These field intensities lead to the formation of a critical transmembrane potential (TMP), which is regarded to be the precondition for cell membrane breakdown and electroporation.

The irreversible electroporation results in a loss of turgor, the leakage of cytoplasmic content and lysis. Reversible permeabilization leads to the formation of conductive channels

across the cell membrane but electrically insulating properties will recover within seconds. The inactivation of microorganisms in liquid products or changes in the microstructure and texture of treated solid raw materials can be expected as a consequence of the irreversible permeabilization of cell membranes.

High-intensity electric pulses can be generated by the switched discharge of a suitable capacitor bank. The characteristics of the discharge circuit determine the shape of the time-dependent potential in the treatment chamber. Exponential decay pulses represent a complete discharge of the capacity. A rectangular shape of the pulse can be produced by using special switches, capable of interrupting the current at high potentials, or the implementation of a pulse-forming network. If an additional capacitor is used together with a parallel switch, bipolar pulses can be obtained. The geometry of the treatment chamber has a considerable effect on the electric field distribution and on the total resistance and therewith on the discharge circuit. The treatment chamber design needs to be optimized also taking into account a homogenous distribution of the electric field strength as well as characteristics of the fluid flow, viscosity, and particle sizes.

Microbial Inactivation by PEF

Effect of PEF on Microorganisms

Till date there has been no clear evidence on underlying mechanisms at a cellular level but the main effects have been described to be triggered by the electric field, ionic punch-through, and dielectric breakdown of the membrane.

The exposure of the biological cell to an intense external electric field leads to an increase of the TMP, which imposes an electrocompression of the membrane. When a critical level of the TMP – which depends on the compressibility, permittivity and initial thickness of the membrane – is exceeded, an electrical breakdown of the membrane occurs. The TMP induced by an external electric field depends on the intensity of the external electric field and cell size, shape, and composition of the membrane. The minimum field strength level of the external electric field that is required to increase the TMP and cause pore formation is defined as critical electric field strength E_{crit}, which depends on the before-mentioned cell and membrane characteristics. Hence, electric field conditions in the treatment chamber, especially consideration of the nonuniform distribution in most cases, needs to take into account the existence of such threshold values.

Effective inactivation for most of the spoilage and pathogenic microorganisms has been shown and colony count reductions depending on treatment intensity, product properties and type of microorganism in the range of 4–6 log cycles are comparable to traditional thermal pasteurization.

Most studies conducted in the past showed that PEF is only effective against vegetative microorganisms, yeasts, and molds as well as mold ascospores but ineffective against bacterial endospores and viruses. Recent investigation indicated increased inactivation of *B. subtilis* endospores when thermal treatment (75–115 °C) was combined with PEF application (15 kV cm^{-1}, 60–120 kJ kg^{-1}).

Relevant Impact Factors

The factors that affect microbial inactivation during PEF treatment are process related such as electric field intensity; pulse width and shape; treatment time; and temperature; as well as microbial factors such as type, shape, size, concentration and growth stage of microorganism, and media factors such as pH; antimicrobials and ionic compounds; electrical conductivity; and medium ionic strength.

The microbial inactivation by PEF depends on characteristics such as type, shape, growth phase, and inoculation amount of microorganisms.

The cells in the logarithmic growth phase are more sensitive to inactivation by PEF. Inactivation by PEF is therefore higher in the logarithmic growth phase than in the stationary phase. Principally, the effect of the electric pulses on the cell membrane is given by the membrane potential theory. This theory assumes that the electrical potential across the membrane (TMP) depends on the cell size. The cell size has an inverse relationship to the external electric field strength necessary to induce a specific TMP. It has been demonstrated by several authors that for smaller particles, higher electric field strength is necessary to reach the critical membrane potential.

Microbial inactivation by PEF also depends on the characteristics of the microorganism-containing medium or food. Factors such as the electrical resistance, pH, water activity, viscosity, presence of solid particles, bubbles, or oil droplets have an impact on the PEF effectiveness.

The conductivity of a medium, defined as the ability to conduct electric current, is an important variable in PEF technology. Treatment chambers with high conductivity foods have poor resistance and it is necessary to apply higher voltage to achieve similar microbiological inactivation that is achieved when processing low-conductivity foods. To obtain the same degree of microbiological inactivation in foods with very different conductivity, treatment conditions, such as the inter-electrode gap in the treatment chamber, pulse width, and voltage must be adapted.

The influence of pH on the microbial inactivation during PEF treatment plays an important role. Both acidic and alkaline pH values induce additional stress to cells, and consequently increases their susceptibility to physical and chemical treatments. The pH can be modified and alters the inactivation kinetics by PEF. Depending on the type of microorganism and strain how pH affects the microbial resistance against PEF has also been shown.

Sublethal Injury and Hurdle Concept

To obtain maximum food safety, a direct transfer of cells from the vital to the lethal fraction during microbial inactivation is favorable. However, because the impact on food quality characteristics limits the applicable treatment intensities, a limited number of dead cells may result. Membrane damage and inactivation of microorganisms due to PEF was first considered as an all-or-nothing event, but a differentiated approach is required even if the critical parameters for the electrical breakdown of cell membranes are exceeded. Membrane damage and sublethal injury was found to be repairable

under certain conditions and the extent to which cells repair their injuries was reported to depend on the treatment intensity, microorganism, and treatment medium pH. On the one hand, sublethally injured cell fractions are a risk from a food quality and safety point of view because these cells may recover and regain their initial vitality. On the other hand, sublethally injured fractions have a potential for subsequent complete inactivation by the application of additional hurdles such as suboptimal storage conditions or the other inactivation methods such as the application of antimicrobials or other food preservation technologies.

The microbial inactivation rates differ considerably between simple media and a complex matrix. This was partly attributed to the protective effect of some food compounds such as xanthan, proteins, or fat. Other studies did neither reveal differences in the microbial inactivation conducted in either buffer or complex media, nor detect the occurrence of sublethally injured cells after PEF treatment of complex food systems. However, inactivation kinetics obtained from PEF treatment in buffer systems have only limited comparability with real food products. In addition, model microorganisms used in most studies and the method of sample preparation differ significantly from the native state of microbial population present in the real food system. The diverse and heterogenous microbial flora present in real foods is not comparable to inoculated microorganisms in most cases due to the strong variability of microbial species and physiological state of microorganisms. Consideration of the microbial growth state, adaptation to treatment media, and existence of inhomogenous microbial populations with less sensitive subpopulations seem to be the most challenging aspects when transferring inactivation results to real products and industrial implementation. Assured food safety and stability, along with a desired level of microbial inactivation requires an accurately defined treatment intensity followed by a predictable microbial inactivation. The transfer of inactivation results from model systems to real foods and the determination of appropriate PEF treatment parameters require the consideration of existing particularities.

Protective Effects of Food Constituents

The impact of food constituents on PEF effectiveness is not fully elucidated. Some authors report a protective effect of xanthan, proteins, or fat. Other studies did not reveal differences in the microbial inactivation conducted in buffer or complex media. Investigation of *Escherichia coli* inactivation in an ovalbumin solution, fish egg suspension, dairy cream, and in phosphate buffer did not show any protective effect of emulsified lipids, soluble proteins, or conductive food particulate. However, inactivation of *Enterobacter sakazakii* in peptone water and infant formula milk by PEF at 40 kV cm^{-1} for 300 μs was found to cause 2,7 log-cycle and 1,2 log-cycle reductions, respectively; thus, showing an impact of the complex composition of infant formula milk in comparison to peptone water. When considering PEF effectiveness as a function of the treatment media, the critical field strength and treatment time required was found to be dependent not only on cell geometry but also on the properties of the media.

Inactivation in complex media like an orange juice–milk-based beverage was also under investigation. The authors concluded the need for further investigation on the effectiveness and mechanism of action of the complex food composition during PEF treatment, as the inactivation results obtained in the complex orange juice–milk based beverage were lower than in simpler substrates.

In addition to the protective effect of food constituents, food matrix properties such as electrical conductivity, heat capacity, and viscosity have to be considered. These parameters affect the power requirements necessary for the generation of a desired electric field level as well as the power consumption during operation. Viscosity will determine the flow behavior and residence time distribution in the treatment chamber resulting in treatment time variations and subsequently also in a nonuniform temperature increase with the occurrence of hot spots.

Shelf Life Studies

An effective microbial inactivation (5 $\log_{10}$) must not only be accompanied by a minimal impact on food nutrients and vitamins but also by a shelf stable quality. The shelf life of a product is defined as the period in which the product is still acceptable for human consumption. Products are spoiled by microbial, chemical, and physical processes. The shelf life is determined by the raw material quality, product formulation, processing, packaging, and storage conditions. Numerous researchers have studied the shelf life of a food product in terms of microbial spoilage.

Combinations of kinetic models which determine and predict the shelf life of a product after microbial or enzyme inactivation are described. The basis is an inactivation of up to 5 $\log_{10}$ units of vegetative pathogenic microorganisms. The pathogenic *Salmonella*, *E. coli*, and *Listeria monocytogenes* species can be considered as most important targets for inactivation due to its higher resistance to PEF treatments. In products with low pH values, spore-forming bacteria (*Bacillus* species) are not able to grow. This means that a pasteurization treatment is sufficient to achieve a microbiologically shelf stable product. In case the product is recontaminated, for example, during packaging after treatment, mainly yeasts and lactic acid bacteria will be introduced in the product. Because lactic acid bacteria are not able to grow in acidic pH foods, yeasts are considered to be the target microorganisms during storage. Therefore, a combination of the highest PEF-resistant microorganisms and the microorganism that is most likely to grow under the storage conditions are suggested for consideration.

Validation and Availability of Indicators for PEF Performance

Strategies for process validation may include a record of parameters to prove the appropriate performance of a treatment. The temperature increase as a result of the dissipation of the total specific electrical energy input can be used as another measure in order to evaluate the treatment intensity in terms of energy input. However, it only reflects an average dose

parameter. Measurement possibilities for the process parameters inside the treatment chamber would be necessary in order to detect nonuniformities but they are limited owing to the small dimension of the treatment chamber and the physical conditions. However, numerical simulation provides a capable tool in order to obtain relevant data on inhomogeneities of electric field strength, treatment time, and temperature. Other methods have been proposed to use enzymes or chemical indicators to detect thermal or chemical side effects occurring during PEF treatment. It has to be stated that sensitivity of microorganisms to PEF is different from their sensitivity to thermal treatment. Hence, matrix-specific inactivation kinetics have to be established in order to define product relevant treatment parameters for different target microorganisms.

Technical Aspects Relevant for Safety Considerations

Treatment Chamber Design

The treatment chamber is the key component of the PEF system for direct application of the electric field in contact with the treatment media. Its design should consider the uniformity of the electric field distribution in combination with flow characteristics in continuous applications as well as the extent of temperature increase.

Various treatment chamber designs such as parallel plates, coaxial cylinders, or colinear configurations have been used for PEF processing and some modifications of these basic designs have been proposed. A comprehensive overview of different treatment chamber configurations can be found in the further reading list.

Adequate shape of electrodes and the insulator is a prerequisite for an optimal electric field distribution and will reduce dielectric breakdown effects of the food, because local high electric field strength levels can be avoided, for example, by providing a round-edged insulator geometry. Dielectric breakdowns of food are undesired as they cause arcing, which leads to the destruction of the food, damage on the electrode and insulator surface, as well as explosion of the treatment chamber due to pressure increase. Electric field homogeneity and the avoidance of low field intensities are not only desirable from the microbial inactivation point of view but also from the fact that energy dissipation and power consumption will take place in low field regions without contributing to the microbial inactivation.

In addition to distribution of the electric field strength and flow velocity considerations, hygienic aspects need to be taken into account for the design and operation of a PEF unit.

General hygienic guidelines for the food industry (e.g. guidelines of the European Hygienic Engineering & Design Group, www.ehedg.org) apply and particular attention should be paid to treatment chamber design and the possibility of fouling and accumulation of deposits during processing and their removal during cleaning. Apart from construction elements, process aspects and in particular process start up with the incoming product after sanitation requires particular attention. PEF treatment needs to be started before a contaminated product passes the treatment chamber, to avoid recontamination of the presanitized processing line. However, the electric field can only be switched on when the treatment chamber is filled. Therefore, to avoid insufficiently treated product passing the treatment chamber and recontaminating the line, the installation must be filled with water of similar electrical conductivity to start the system at targeted process parameters.

Electric Field Strength Distribution

Microbial and media properties have been widely investigated. However, the treatment chamber design and its influence on processing parameters such as the electric field strength and its distribution have hardly been discussed. One of the main aspects related to chamber design is the insulator shape and its impact on the flow and electric field distribution. The insulator and its specific design have a considerable impact on the process performance as they affect the electric field strength distribution and therefore the microbial inactivation. The velocity profile in the treatment chamber (laminar, transitional, or turbulent) determines the residence time distribution and therefore the total treatment time of the product. Because the fluid velocity differs depending on the location in the treatment chamber, longer treatment times will occur near the wall owing to lower flow velocity and shorter treatment times occur in the center of the treatment chamber where there is higher flow of velocity. Examples for modifications as insulator gap/diameter for colinear treatment chambers can be found in literature, where it is stated that a gap increase will provide smaller electric field strength, but will also provide a more homogenous distribution.

The alteration of electric field distribution by modifying the insulator geometry can be used to improve the microbial inactivation results. However, this depends strongly on other processing conditions, such as number of pulses, media conductivity, and velocity profile, that have to be considered during the microbial inactivation process. Owing to high inhomogeneities presented in a continuous treatment system, PEF processing should be characterized in terms of the electric field strength, treatment time, and specific energy input. An average value from the previously mentioned parameters with their respective standard deviation as well as occurring maximum and minimum values should be described in detail for the process. The lowest value of electric field strength, treatment time, and energy input will be the limiting factors for food safety reasons and the maximum value for the resulting product temperature should be considered with regard to product quality. The modification of a colinear treatment chamber by inserting static mixing devices provides a possibility to improve electric field strength distribution, with the aim of increasing the average value and reducing standard deviation. This will result in an increased microbial inactivation. The insertion of static mixing devices also provides a more homogenous velocity profile (thus a more homogenous treatment time as well) and a more homogenous temperature distribution, which aims at higher retention of heat sensitive compounds.

Thermal Effects and Impact on Safety and Quality

Although the PEF treatment is a nonthermal food processing technology, there is a significant temperature increase during high intensity PEF treatment, when applied for pasteurization purposes due to Joule heating. Many authors have described the temperature distribution in a PEF treatment chamber and reported the occurrence of high local temperatures owing to inhomogenous field distribution of the electrical field, limited flow velocity, and recirculation of the liquid. For this purpose, numerical simulations using computational fluid dynamics have gained growing interest because experimental measurement of the related parameters is not possible in most cases due to small dimensions of the treatment chamber as well as the interference of the measuring device with the product flow and electric field. Apart from the overall liquid temperature measured at the outlet of the treatment chamber, treatment inhomogeneity and the occurrence of temperature peaks within the treatment chamber have to be considered as thermal impact factors. This is of particular importance when discussing PEF effects on functionality of heat sensitive compounds, such as proteins or when conducting inactivation kinetic studies. Additional thermal effects may occur in case of application of high total energy inputs, insufficient temperature control, or unfavorable treatment chamber design.

However, the application of PEF in combination with mild heat seems to be a promising technique for a gentle, multihurdle preservation process. The synergism between temperature and PEF membrane electroporation can be used to improve the inactivation efficiency. The phospholipid bilayer structure of the cell membrane changes from a gel-like to liquid crystalline state when increasing the temperature and increased membrane fluidity leads to reduced membrane stability and facilitates the electroporation of the cell membrane. The synergetic effect of temperature during PEF inactivation of microorganisms can be used to improve the inactivation results and/or to reduce the electrical energy costs.

PEF Impact on the Food Matrix

Food Constituents Affected by PEF

When PEF treatment of liquid or semisolid raw materials is used for microbial inactivation, the desired effect is primarily nonthermal pasteurization of the food product considering the microbial cell as target of the treatment. The modification of other food constituents as well as functional food properties may be considered as an unwanted side effect in most cases. However, potential applications to use the electric field effect to modify functional properties of food constituents will gain increasing attention.

The evaluation of the effect of PEF on food compounds is complex. Available reports are limited and different experimental setups and processing parameters make them difficult to compare. The consideration of electric field side effects such as temperature increase and temperature hot spots due to Joule heating effects within a nonuniform electric field and the occurrence of electrochemical reactions and pH shifts is the most challenging aspect within this context. The nonthermal inactivation of microorganisms by PEF is based on the electroporation of membrane structures. High-intensity electric fields are unlikely to affect covalent chemical bonds but the electric field application and related side effects may show an impact on food compounds and process modifications toward the inactivation of microorganisms and enzyme structures are also possible. Owing to the application of PEF, changes in the conformational state of proteins might cause changes in protein structure and enzyme activity.

In general, the mechanisms involved in the inactivation of enzymes and the modification of proteins by PEF are not fully understood. Possible mechanisms could entail polarization of the protein molecule; dissociation of noncovalently linked protein subunits involved in quaternary structures; changes in protein conformation so that hydrophobic amino acid or sulfhydryl groups are exposed; attraction of polarized structures by electrostatic forces; and hydrophobic interactions or covalent bonds forming aggregates. The effect of PEF on milk proteins may also be explained as a result of the modification of the apparent charge after exposure to intensive electric fields and subsequent modification of ionic interactions of the proteins. A comprehensive overview of PEF effect on food material properties can be found in the literature given below.

Electrochemical and Toxicological Aspects

In addition to the microbial safety and control of process parameters relevant for a high level of microbial inactivation, possible chemical, and toxicological safety issues related to PEF need to be considered.

In PEF systems working at electric field intensities are suitable for pasteurization applications, electrochemical reactions can occur at the electrode–solution interface. Related effects could be a partial electrolysis of the solution or the corrosion of the electrode material and an emission of metals from the electrodes in the liquid.

However, these unwanted phenomena can be limited or avoided by suitable selection of electrode materials and adaptation of the electrical pulse shape and duration.

The modification of the electrode material such as the use of titanium, which is oxidized to water insoluble titanium dioxide at the electrode surface or the application of innovative materials such as carbon loaded polymers have the potential to decrease the amount of metal release into the product.

However, to control the electrochemical reaction it is preferable to eliminate causes rather than eliminating results such as the corrosion of electrode material. Therefore, it is required to modify the electrical characteristics of the PEF systems in two ways: (1) avoiding direct leak currents by improving the electrical switching components and (2) reducing low frequency alternating voltages and currents by improving the pulse conditions.

In properly designed PEF systems working at adequate processing conditions, the metal release can be reduced far below the existing standards for maximum concentrations in drinking water or fruit juices.

Comprehensive studies on the impact of PEFs on food constituents are limited. Most studies focus on single compounds such as enzymes or vitamins. However, to conduct a product specific substantial equivalence study, a complex analytical approach has to be undertaken which also includes the formation of process induced contaminants. A statement

of the Senate Commission on Food Safety of the German Research Foundation on the treatment of food using a PEF is available since 2008 indicating the need for a case-by-case evaluation of PEF-treated products and for the availability of standardized process parameters and assessment criteria including indicators.

Summary

PEF technology was shown to effectively inactivate microorganisms in liquid foods resulting in shelf stable safe products. Because the PEF resistance of microorganisms is different compared to thermal treatment and strongly depends on matrix properties, inactivation levels between microorganisms and products differ significantly. In addition, technical PEF treatment parameters with impact on microbial inactivation are multifaceted and provide various adjustment options. Nonuniform distribution of these parameters in continuous industrial scale PEF units and occurring thermal effects result in complex process–product interactions. Hence, the design of a PEF process requires the consideration of process and product related characteristics to guarantee food safety. To have maximum process performance, process optimization needs to consider particularities regarding the microbial inactivation such as the occurrence of sublethally injured cells as well as the protective effect of a complex food matrix. In addition, process analytical tools such as numerical simulation are required to exactly define process conditions. Technological aspects such as pulse characteristics, electrode material, and treatment chamber design can be considered as key aspects to improve process performance.

See also: Characteristics of Foodborne Hazard and Diseases: Sublethally Injured and Viable but Nonculturable Cells. Foodborne Diseases: Overview of Emerging Food Technologies. Risk Analysis: Risk Communication: Novel Foods and Novel Technologies

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Sterilization

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Glossary

Botulinum cook Heat treatment to achieve 12 decimal reduction in the number of *C. botulinum* spores. If D_{121} of *C. botulinum* is 0.21 min, then a botulinum cook will have an F_0 value of $12 \times 0.21 = 2.52$ min.

Commercial sterility Commercial sterile food is not necessarily free from extremely heat-resistant viable spores, however, these are not capable of growing in the product under normal storage conditions.

***D*-value** Decimal reduction time, the time in minutes at a given temperature to inactivate 90% (one log cycle) of a microorganism, leaving one-tenth of survivors.

***F*-value** Time equivalent of different inactivation temperatures in relative fractions of 121.1 °C.

F_0-value Sum of the lethality of a heat process expressed in that of the reference temperature of 121.1 °C.

***z*-Value** The temperature change required to change the *D*-value by a factor of 10.

Scope

Sterilization means the near complete destruction of microorganisms usually by temperatures greater than 100 °C using equipment with pressure (autoclaves or retorts). Because the resistances of bacterial spores to heat are different, sterilization frequently means a treatment of at least 121 °C (250 °F) of wet heat for 15 min or its equivalent to inactivate to a large extent of spores of the pathogenic *Clostridium botulinum* and most of the spore-forming spoilage microorganisms. Sterilization also means that every particle of the food must receive the adequate heat treatment. Hence, the slowness of heat transfer through the food should also be considered in determining the overall heat destruction effect of the sterilizing treatment.

In practice, however, a product subjected to sterilization may not be sterile. From the principle of exponential death of microbial population it follows that absolute sterility cannot be achieved and there will not be zero survivor; only the probability of survival can be minimized to an acceptable degree. This has been set to be a 10^{-12} part survival of *C. botulinum* spores, called 12D concept. Even in this case, some more heat resistant spore formers, for example, *Clostridium thermosaccharolyticum* or *Geobacillus stearothermophilus*, may survive this and more intensive heat treatment. Being of thermophilic nature, these surviving microorganisms cannot grow under the normal condition of storage (at ambient temperature without refrigeration), and this condition is termed commercial sterility.

Food industry applies several environmental (extrinsic) factors for the inhibition and/or inactivation of microorganisms in order to manufacture safe product with long keeping quality and shelf-life. Among others, heating, freezing, drying, irradiation, high pressure, and other methods alone or in combination can be applied. Heat treatment at high temperatures is the most important method of preservation that is used widely in canning industry. The practice of canning started at the end of the eighteenth century, much earlier than when the scientific fundamentals of the heat sterilization process became understood. In 1795, motivated by a tender of Napoleon Bonaparte offering 12 000 francs for developing a practical way to preserve food, a confectioner named Nicholas Appert successfully preserved meat placed and boiled in glass bottles. He patented the process and this was the beginning of food preservation by canning. It passed 50 years until another Frenchmen, Louis Pasteur, demonstrated the role of microbes in food spoilage. He found that microbes were responsible for the putrefaction of meat and milk, as well for 'disease' of wine, and developed a heat process (later called pasteurization) to preserve wine. The sound scientific foundation for the process of sterilization was laid down in the 1920s by the Americans Ball, Bigelow, and others.

Fundamentals of Thermal Death of Microorganisms

In practice, we never deal with a single microbial cell but rather with the population of microbes consisting of numerous (often millions of) cells. The death of an individual cell is instantaneous; however, the destruction of microbial population is a continuous process in time. Also, for methodical reason, it is only the living cells that can be studied and enumerated; hence the death of cell population in time can be followed by counting of the cells *surviving* the destructive treatment. Thus, the death kinetics can be described on the analogy of a first-order reaction by the change of surviving cell count in time:

$$dN/dt = -kN$$

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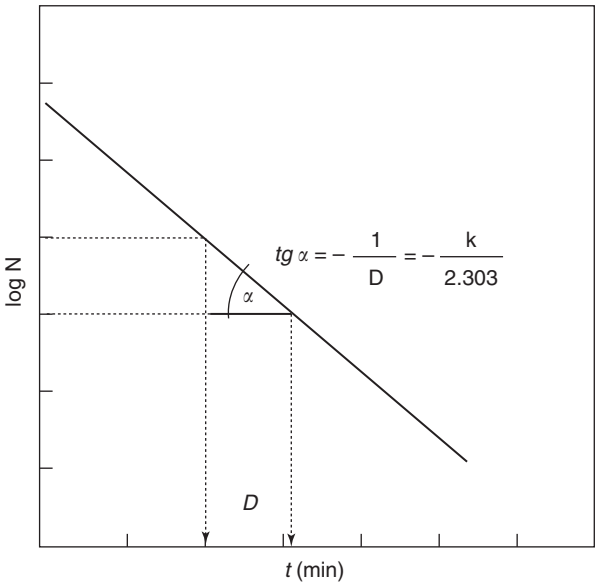

Figure 1 Survivor curve and the *D*-value. Reproduced from Deák T (2006) *Élelmiszer Mikrobiologia*. Budapest: Mezögazda.

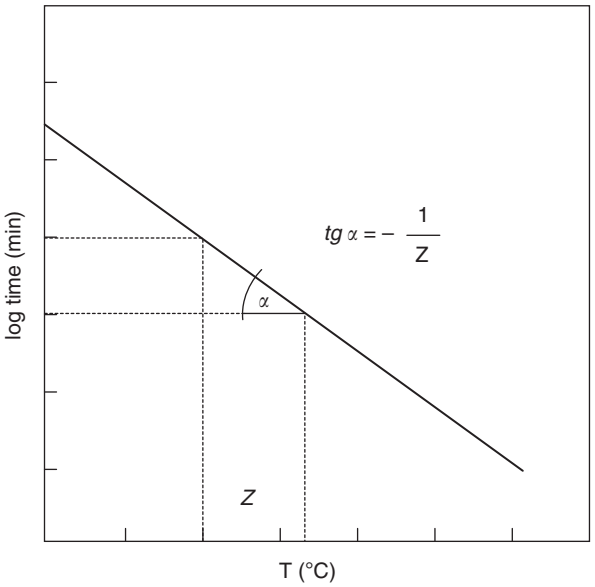

Figure 2 Thermal death curve and the *z*-value. Reproduced from Deák T (2006) *Élelmiszer Mikrobiologia*. Budapest: Mezögazda.

that is, the change in number of survivors (dN) in a given time (dt) is proportional to the actual number of living cells (N), where the k factor is called the death rate coefficient (with a negative sign as the cell number is decreasing). Integrating this equation between the limits of initial cell count (N_0) and surviving cell count (N_t) after t time, we arrive at the fundamental equation describing the death of microbial populations:

$$N_t = N_0\, e^{-kt}$$

often rewritten in logarithmic form, which is called the equation of survival curve:

$$\log N_t/N_0 = -kt$$

from which the death rate coefficient can be expressed as:

$$k = 2.303/t \log (N_0/N_t)$$

Accordingly, when the logarithm of surviving cell number is plotted against time, a straight (linear) line is obtained, the slope of which is related to the death rate coefficient (Figure 1).

The time through which the number of survivors decreases to one-tenth is the decimal reduction time, D:

$$D = t/(\log N_0 - \log N_t)$$

The decimal reduction time is also a measure for the degree of resistance of a microbe population to destruction; the longer the D-value, the more resistant the population against a destructive factor (heat or other).

When the log D-values are plotted against temperature, the thermal resistance curve is obtained (Figure 2). The slope of the line marks the change of resistance in function of temperature. This is represented by with the z-value, which represents the degree of increase in temperature through which the decimal reduction time (D) will decrease to one-tenth or one log order.

D and z are the two basic parameters defining completely the heat resistance characteristics of microorganisms.

Heat Resistance of Microorganisms

The heat resistance of microorganisms is primarily a genetically determined specific characteristic that can be modified by the environmental conditions. In general, heat resistance is in proportion to the growth temperature. Psychrophilic vegetative bacteria become inactivated even at approximately 40 °C, whereas mesophiles have a decimal reduction time of approximately 1 min at 55–60 °C. Certain thermoduric bacteria (e.g., *Enterococcus* and *Microbacterium* species) may survive 30 min heating at 60 °C, with a fairly large z-value of 15–20 °C. Heat resistance of most vegetative pathogenic bacteria occurring in foods is similar to that of mesophiles, and they can be inactivated with the conventional pasteurizing treatments at temperatures below 100 °C. Unusually high heat resistance approaching that of thermoduric species is shown by the serotype *Salmonella* Senftenberg. The majority of yeasts and molds possess heat resistance similar to those of mesophilic vegetative bacteria.

Although the vegetative cells of spore-forming bacteria are equally sensitive to heat as other bacteria, their endospores possess high heat resistance (Table 1). The thermophilic spore forming species are remarkably more heat resistant than mesophiles. Heat resistance of mesophilic spores is characterized with D_{121} °C of 0.01–0.1 min, whereas that of thermophiles may reach 2–5 min decimal reduction time at this temperature. From the point of food safety, *C. botulinum* is the most important of the pathogenic spore formers, having 0.1–0.2 min D_{121} °C; in particular the strains belonging to serotypes A and B, whereas the psychrotrophic E serotype

Table 1 Heat resistance of some spore-forming bacteria

Species	D_{121} *(min)*	z *(°C)*
Bacillus polymyxa	0.05	8
C. botulinum	0.21	10
B. subtilis	0.7	8
Clostridium sporogenes	1.5	10
Desulfotomaculum nigrificans	2–3	9–12
Thermoanaerobacterium thermosaccharolyticum	3–4	12–18
G. stearothermophilus	4	10

Source: Data from Stumbo CR (1973) *Thermobacteriology in Food Processing.* London: Academic Press, and Deák T, Farkas J, and Incze K (1980) *Microbiology of Canning-, Meat- and Freezing Industries (in Hungarian).* Budapest: Mezőgazdasági Kiadó.

strains are less resistant, characterized by D_{80} value of 0.3–3 min. Among the spore formers causing spoilage in canned foods, spores of *G. stearothermophilus* and *C. thermosaccharolyticum* have D_{121} values of 3–5 min, and these can survive heat treatments calculated for the destruction of *C. botulinum* (see commercial sterility). Heat resistance of spores is also characterized with *z*-values two or three times higher than those of vegetative cells, in the order of 8–12 °C, and some spores may reach 20–30 °C.

Factors Influencing Thermal Resistance

The thermal resistance and thermal death of microorganisms and their spores are influenced by several environmental factors. Moreover, although the heat resistance is a specific characteristic, it may differ between strains of a species. Decrease of water activity significantly increases thermal resistance. This is often the case in foods with high sugar concentration or containing many proteins or fats. However, acidic environment and low pH markedly decrease heat resistance. Product pH is of outstanding importance for heat processing. The pH of 4.5 signifies a dividing line; products with pH lower than 4.5 can be pasteurized at 100 °C or below, whereas foods of pH higher than 4.5 must be sterilized at temperature greater than 100 °C. The fundamental safety reason for this is that the most important pathogenic endosporic microorganisms, *C. botulinum*, cannot grow or produce toxin at pH <4.5 and the spores that may survive heat treatment cannot germinate either. In the canning practice, the interaction of heat with other preservative factors can be utilized favorably.

Determination of Heat Process Requirement

The most important field for application of the thermal death curve is the heat treatment in canning. In the practice of heat treatment, various degrees of temperature are applied. The values of *D* (or its multiple, the thermal death time, TDT) at any given temperature can be obtained using a reference value, *F*, at a reference temperature (Figure 3). For the latter, 121.1 °C was chosen, a temperature important in the sterilization practice (this value corresponds to a

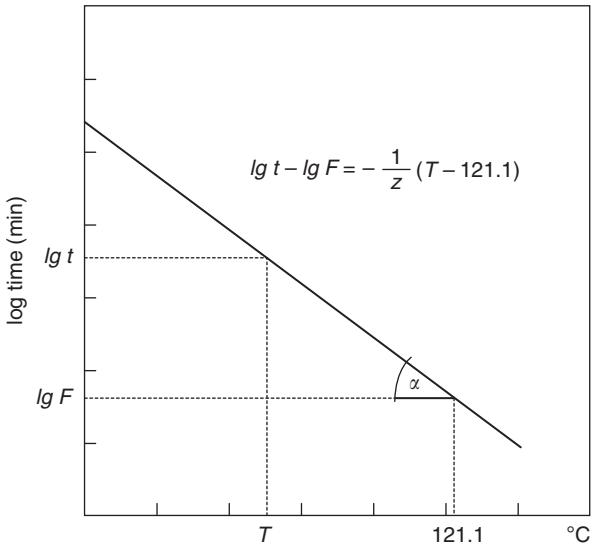

Figure 3 Reference thermal death curve. Reproduced from Deák T (2006) *Élelmiszer Mikrobiologia.* Budapest: Mezögazda.

round figure, 250 °F). Also, a *z*-value of 10 °C was selected as the slope of this particular thermal death curve. With these determined points, the equation of the thermal death curve is:

$$\log (t - F) = -(T - 121.1)/z$$

This equation is used for the calculation of the thermal processing requirement and the lethality of the sterilization process. For these calculations, the lethal effect of any other temperature should be compared to that at the reference temperature, 121.1 °C. From the above equation, the relative rate of heat destruction at various temperatures compared to that of 121.1 °C can be obtained as

$$F/t = \text{antilog}\,(T - 121.1)/z$$

In the practice of heat treatment (sterilization and pasteurization) the temperature is not constant but changes, increasing during warming up and decreasing during cooling. In calculating the heat process requirement, the task is to sum up the lethal effects of changing temperatures for changing time. This can be done if the TDT (or rate) at various temperatures is expressed in a similar manner to be integrated. As shown above, the death time of any temperature related to the reference temperature 121.1 °C is expressed as F/t, and the integrated time equivalent, F_i, of different temperatures in relative fractions of 121.1 °C can be obtained as

$$F_i = \int_0^t F/t\,\mathrm{d}t$$

For example, if the time equivalent of a thermal process is $F_i = 3$ min, it means that the sum of lethality of all corresponding temperature–time combinations during heat treatment will be equal to the effect of 3 min instantaneous treatment at 121.1 °C. In this interpretation, the F_i value does not relate to a given kind of microorganism but to a given heat process; hence it can be used to compare the efficacy of different thermal processes.

The heat resistances of microorganisms are, however, different, and also change with temperature (as expressed by the D and z values). For safety reason, the minimal degree of thermal process chosen should be adequate to kill the most resistant pathogenic microbe that may occur in the practice of canning. According to common experience, it is the toxigenic *C. botulinum* that constitutes the greatest health hazard and whose endospores have high heat resistance. The D-value of the most resistant spores of *C. botulinum* at 121.1 °C is 0.21 min, and its thermal dependence, the z-value is 10 °C. This has been chosen universally for the calculation of thermal process requirements, and the summarized lethality value of temperatures related to 121.1 °C is distinctively marked as F_0-value, and called equivalent sterilization treatment. In contrast to the F_i thermal time equivalent, which may refer to any TDT curve no matter which z-value, the F_0-value relates to a thermal process characterized with a thermal death curve of $z = 10$ °C value.

The F/t values can be graphically integrated by taking the relative death rates corresponding to different temperatures of the heat penetration curve, and plotting with time to obtain the so-called sterilization curve. The area below the sterilization curve will be equivalent to the sterilization treatment in minutes of F_0 (Figure 4). For practical reason the summing up of F/t values usually starts when the internal temperature reaches 100 °C and includes also the cooling part until 100 °C. The F/t values associated with temperatures below 100 °C are very small and hence do not contribute significantly to the overall amount of heat treatment. However, omitting the effect of high temperatures during cooling would result in oversterilizing of the product, possibly resulting in quality losses. In the recent times, with the development of computing technology, programs are available to determine thermal process requirements, and also online monitoring and controlling of the thermal process.

Based on the sterilization equivalent F_0-values, not only the efficacy of various thermal processes can be compared but also the minimal degree of heat treatment required for safety can be determined. It is a universally accepted practice to apply a heat treatment that should destroy the number of *C. botulinum* spores to 10^{-12} proportion. This is the 12D concept, which is equivalent to $12 \times 0.21 = 2.52$ min heat treatment at 121.1 °C, which is the F_0 for *C. botulinum* (also called 'botulinum cook'). This provides high safety for heat sterilization. Since it has been introduced for commercial canning from the end of the 1920s, only a few cases of *C. botulinum* intoxication occurred (unfortunately much more in home canning).

The 12D principle of sterilization should be applied for low acid products with pH >4.5 in which *C. botulinum* can grow. In these products, however, spore-forming bacteria may occur whose heat resistance is higher than that of *C. botulinum* (Table 1). Although these do not present health hazards, they can survive the minimal requirement of safe heat treatment (i.e., $F_0 = 2.52$ min), and can cause spoilage. For economic reason, the spoilage ratio should be kept lower than 0.1%. When thermophilic spore formers are to be accounted for as contaminants having D_{121} values of 3–5 min, the equivalent sterilization treatment should be much higher, sometimes reaching $F_0 = 15$–20 values.

However, for the sterilization of product whose pH is lower than 4.5 (the so-called acid and high-acid foods), not even the minimal requirement for botulinum cook ($F_0 = 2.52$ min) should be applied. Partly, *C. botulinum* cannot grow at or below pH 4.5 and the acidic environment will decrease the heat resistance of microbes. These products can be pasteurized by heat treatment lower than 100 °C.

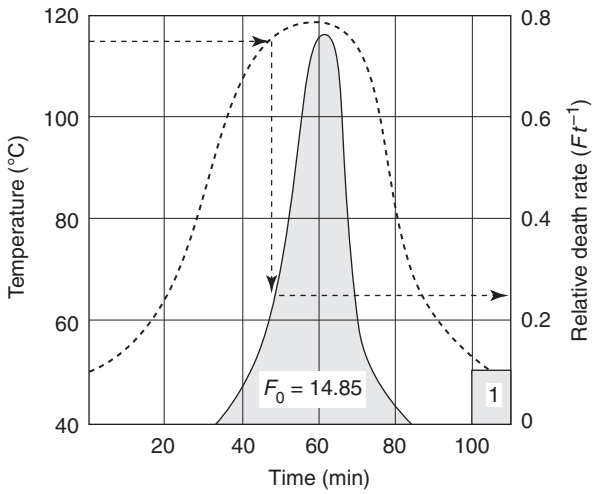

Figure 4 Sterilization curve and the sterilization equivalent value F_0. Reproduced from Deák T (2006) *Élelmiszer Mikrobiologia*. Budapest: Mezögazda.

Conventional Thermal Sterilization

Thermal sterilization is an established technology for canned foods. In the majority of cases, sterilization is applied for foods filled and sealed into containers. However, heat preservation can be applied in bulk food subsequently packaged aseptically. This technology is more frequently used for pasteurized products. Pasteurization temperature and time will vary according to the nature of product. Often high temperature (130–150 °C) is applied for very short time, less than 1 min. These modes of heat preservation are called ultra-high temperature (UHT) and high-temperature short-time (HTST) treatments.

The conventional process of thermal sterilization is made with foods filled in containers. Four main types of containers are used for sterilization: metal cans, glass jars and bottles, flexible pouches, and rigid trays. The length of time required to sterilize a food in container depends on:

- heat resistance and number of microorganisms,
- size and shape of container and penetration of heat into it,
- physical state of the food,
- pH of the food,
- means and conditions of heating.

In addition to the heat resistance of microorganisms, the other factor significantly influencing the efficacy of sterilizing process is the rate of heat penetration to the food container.

To effectively and safely preserve foods using heat treatment, two factors should be considered: (1) the required time–temperature combination to inactivate the most heat resistant pathogens and spoilage organisms in a particular food, and (2) the heat penetration characteristics in a particular food, including the type and size of container used. For the heat sterilization to be efficient, the lethal temperatures are to be achieved at the cold point of containers (Figure 5).

The course of temperature during thermal processing depends on several factors related to: (1) heating conditions (retort type, loading, and time–temperature formulae), (2) heating mode (still or agitated), (3) heating medium (water, steam, with/without overpressure), (4) product type (solid, liquid), and (5) container type, shape, and size. The thermophysical properties of the product, in particular its consistency, will influence the mode of heat transfer, and are of utmost importance for the speed of heat penetration. Basically, in solids it is by conduction and in liquids by convection; however, in real foodstuffs it is usually between the two extremes, and may change during the heating process.

Heat penetration is extremely important, because it is the determining factor for the success of the whole operation. The most suitable and practical method to speed up thermopenetration is the movement of containers during the thermal process. Rotation of containers around their axis is an efficient means to accelerate heat transfer, because this will rapidly mix the contents, enabling a more uniform heating of products, and reducing heating time and organoleptic degradation. Heat penetration is slow in glass containers compared to metal cans.

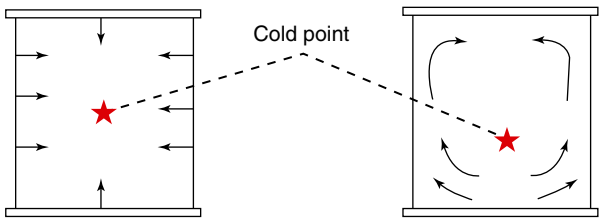

Figure 5 The slowest warming up cold point in containers. Heat transfer is (a) by conduction and (b) by convection. Reproduced by the authorization of the Food and Agriculture Organization of the United Nations from Heinz G and Hautzinger P (2007) *Meat Processing Technology*. Bangkok: RAP Publication.

In addition to the composition and moisture of the food, the acidity and pH value have tremendous impact on the efficacy of heat preservation. Foods can be divided into groups to receive different heat treatments according to their acidity. Acid foods have pH below 4.5 and low acid foods are those with pH above 4.5. Acid food includes most fruits, and pasteurization would suffice for preservation, and low acid foods are those like meat and most vegetables, which require sterilization treatment. Table 2 lists various types of foods and their pH value, together with the heat processing requirements.

Equipment for Heat Sterilization

Canned products are generally processed in a container that may be metal can, glass, or flexible pouch. Sterilization process at temperature greater than 100 °C is made under pressure in retorts or autoclaves. The heat transfer medium is usually saturated steam, hot water, or mixture of steam and air. As the pressure increases, the boiling point of water also increases, allowing superheating of water without boiling. Autoclaves are made in different sizes and shapes and function in different modes. Common types are vertical and horizontal, processing in batch and nonagitating mode; others are rotating the product during heating, and still others operate continuously. In rotary autoclaves the basket containing the cans rotate during sterilization. Cans move end-over-end while rotated. This technique is useful for cans with liquid or semiliquid content as it achieves a mixing effect of the content, resulting in accelerated heat penetration. Continuous retorts also include horizontal rotary models in which the containers conveyed through a spiral fashion within a vessel are heated. The cans rotate axially (side-over-side). Continuous retorts increase the rate of heat transfer in fluid products as well as increase the production rate.

A particularly efficient continuous and agitating sterilization arrangement is the hydrostatic sterilizer. It operates on the hydrostatic principle with the pressure of saturated steam maintained and balanced by the weight of water columns. Hence, a hydrostatic sterilizer consists of four or more towers with a height of up to 20 m (Figure 6). The first tower serves as a preheating section and the second one is the sterilizing section in which the temperature is directly related to the pressure of the saturated steam; by varying the pressure and water column height, the temperature can be controlled. After the sterilizing tower, the cans pass through the cooling tower,

Table 2 Heat processing requirements – dependence on product acidity

Acidity class	*pH value*	*Food commodity*	*Heat processing mode*
Low acid	5.3–6.0	Vegetables	High temperature sterilization
		Uncured meat, poultry, fish, soups	115–121 °C, 240–250 °F
	5.0	Tomato products	105–115 °C, 221–240 °F
Medium acid	4.5–5.3	Fruits, fruit juices	Pasteurization
			100 °C, 212 °F
Acid	3.7–4.5	Fruits	80 °C, 176 °F
High acid	3.0–3.7	Pickles, sauerkraut	80 °C, 176 °F

Source: Reproduced from Desrosier NW and Desrosier JN (1977) *Technology of Food Preservation*, 4th edn. Westport: AVI Publishing Co.

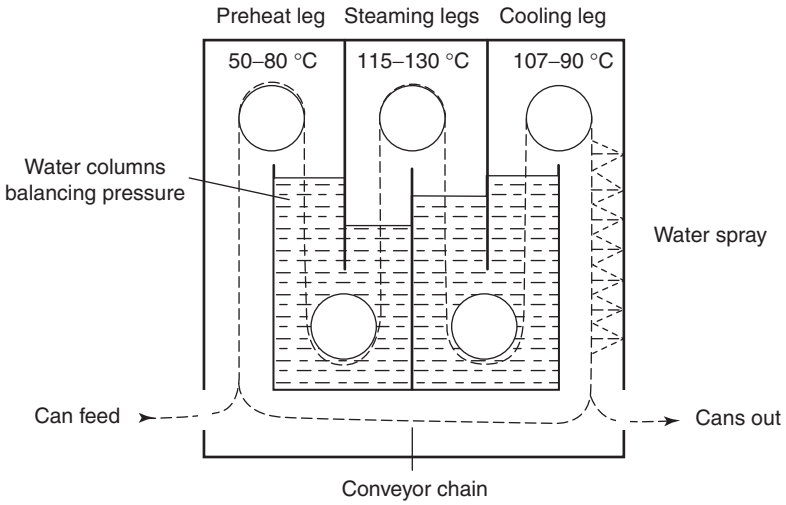

Figure 6 Scheme of a hydrostatic sterilizer.

where the pressure gradually decreases and the cooling is completed at atmospheric pressure. The cans rotate while being conveyed through the machine by endless stainless steel chains.

Aseptic system is a combination of processing and packaging whereby presterilized and cooled product is filled into sterilized container and sealed with a sterile cover under sterile (aseptic) condition. Products are processed outside the package and then filled under aseptic (sterile) conditions into a sterile package. This requires sterilizable equipment, sterile product, sterile packages, sterile environment, monitoring and recording equipments, and proper handling of finished products. Processing temperatures are generally high, 130–150 °C, applied for a short time (few seconds); hence it is called the UHT process. The process avoids the slow heat penetration inherent in the traditional in-container heating process as the food is heated in thin layers passed through a heat exchanger. The UHT process can be applied for liquid foods, including milk, fruit juices, and salad dressing; with liquids this aseptic technology has now almost completely replaced conventional sterilization in containers. It can also be adapted for foods that contain small particles (e.g., baby foods, tomato products, and soups). Containers used for aseptic filling after heat sterilization are not exposed to heat and pressure; hence a great variety of materials and size are suitable, including laminated cartons, plastics, pouches, cups, and other packs. They can be presterilized with UV or ionizing radiation, hydrogen peroxide, or steam.

The most frequently used form of packaging for canned products is tinplate, which is fabricated into two and three piece cans of a wide variety of shapes and sizes. The hermetic closing of cans by double seams is crucial to protecting the product sealed inside. Errors in double seaming can lead to loss of the hermetic seal and the possibility of postprocess contamination, giving rise to spoilage of canned food. Glass can be equally used for processing canned products although the thermal properties of glass would not tolerate rapid changes in temperature. Glass is also susceptible to breakage due to internal pressure and mechanical impact. Special lids and sealing are used for closing of glass jars and bottles.

Accurate filling is required for declared weight and filling also has an impact for subsequent heat processing. Hermetically sealed glass or metal cans are not filled completely; a headspace is left to form a partial vacuum after heating and cooling. Exhausting is made after filling and before closing cans or jars to remove air from the contents but leave a headspace for vacuum to be formed when the container is cooled.

Even if the thermal process schedule is strictly controlled to avoid underprocessing spoilage, product shelf-life will be compromised if there is postprocess leaker spoilage. Several factors may contribute to postprocess leaker spoilage; including contaminated cooling water, poor postprocess hygiene and sanitation, and container damage during handling and storage. Table 3 lists the possible causes of canned foods and the sign by which the cause of spoilage can be identified. Often the outer appearance of the containers indicates the possible cause of spoilage, in particular if gas development deforms the shape of the can with various degrees of swelling. Evancho et al. (2009) recently provided an overview on the microbiological spoilage of canned foods.

Canning

Food preservation by heat treatment is a common practice of the food industry called canning.

Although, from the microbial point of view, it would be ideal to employ such intensive heat treatment that would eliminate the risk of any surviving microorganisms, most canned food products cannot be submitted to such degree of heating because it would degrade the sensory quality and result in loss of nutritional value. Hence in practice, a compromise is looked for in order to provide a heat treatment intensive enough for the microbiological safety of the products and in the same time moderate enough for preserving the

Table 3 Spore-forming bacteria causing the spoilage of canned products

Type of spoilage	*pH*	*Products*	*Spoilage bacteria*	*Heat resistance* D_r *(min)*
Flat sour	>4.5	Vegetables, meat dishes	Thermophiles	
			G. stearothermophilus	4–5
Gaseous souring			*C. thermosaccharolyticum*	3–4
Sulfide stinker			*D. nigrificans*	2–3
Flat sour	>4.5	Vegetables, canned meat	Mesophiles	
			B. cereus, *B. subtilis*, *B. brevis*	0.001–0.004
Gaseous putrefaction			*C. sporogenes*, *C. botulinum*	0.1–0.2
			C. putrefaciens	0.001–0.01
Flat sour	<4.5	Vegetables, tomato products	Thermophiles	
			B. coagulans	0.01–0.07
Gaseous souring	<4.5	Pickles	Mesophiles	
		Tomato products	*B. polymyxa*, *B. macerans*	0.01–0.05
Gaseous putrefaction		Canned tomato	*C. pasteurianum*	
Butyric fermentation		Canned fruits	*C. butyricum*	0.004–0.01

Source: Reproduced from Deák T, Farkas J, and Incze K (1980) *Microbiology of Canning-, Meat- and Freezing Industries (in Hungarian).* Budapest: Mezőgazdasági Kiadó.

Table 4 Sterilizing time equivalent for some canned products

Product type	*pH*	F_0 *(min)*
Pickles	3.4–4.1	0.0002–0.004
High acid fruits	3.2–3.8	0.002–0.007
Tomatoes	4.2–4.5	0.01–0.07
Medium acid fruits	3.7–4.5	0.1–0.4
Medium acid vegetables	4.0–4.5	0.1–2.0
Cooked meats	5.0–6.5	2.5–5.0
Low acid vegetables	5.0–6.5	4.0–14.0
Ready-to-eat foods	4.5–6.5	5.0–30.0

product quality. Commercial sterility is a generally accepted practice of canning.

As discussed in the section Determination of Heat Process Requirement, *C. botulinum* is used as a reference organism for manufacturing of safe and stable products by a heat treatment with a minimum F_0-value of 2.52 min. Based on microbiological considerations and including a sufficient safety margin, most sterilized canned products should be produced with F_0-values of 4.0–5.5. The retort temperatures to be used may vary between 117 and 130 °C (depending on the heat sensitivity of the individual products). It is known, however, that certain thermophilic sorganisms such as xG. *stearothermophilus* or *C. thermosaccharolyticum* are extremely heat resistant and may survive F_0-values of 4–5.5. In the case of survival they will not grow under normal storage conditions of up to 25 °C and do not pose a risk in countries with moderate temperatures. However, they may grow under tropical conditions in particular with storage temperatures of 25 °C and above. Hence, F_0-values of 12–15 have to be employed in cases containing this risk (Table 4).

Table 5 Some commercial F_0 values of thermal processing

Product	F_0
Meat products	
Ham in brine	0.3–0.5[a]
Sterile ham	3–4
Luncheon meat in brine	0.5–1.5
Meat in gravy	12–15
Frankfurter sausage	3–4
Poultry	
Chicken boned	6–8
Chicken breast in jelly	6–10
Poultry whole in brine	15–18
Fish	
Mackerel in brine	3–4
Herrings in tomato sauce	6–8
Vegetables	
Beans in tomato sauce	3–4
Corn in brine	9.15
Green beans in brine	4–6
Peas in brine	7–10
Mushrooms in brine	8–10
Dairy products	
Cream	3–6
Evaporated milk	5
Milk pudding	4–10
Formulated products	
Baby foods	3–5
Meat soups	10
Tomato soups	3
Cream soups	4–10

[a]Range covers different container sizes.

Source: Data selected from Richardson P (ed.) (2004) *Improving the Thermal Processing of Foods*. Cambridge: Woodhead Publishing.

Traditionally, canned foods are understood as sterile or commercially sterile, shelf-stable food products packed in and sealed hermetically in containers. The name refers to the rigid metal canisters (cans) although the containers can be glass jars and bottles and also semirigid, flexible plastic materials (e.g., pouches). Today a large variety of canned foods are available and processed in cans or other containers. Many of them are fully sterilized products, which can be stored under ambient temperatures; others are only pasteurized and have limited shelf-life even under refrigerated storage condition. However, there are many kind of heat preserved foods manufactured beyond the traditional processes of canning. For example UHT milk in laminated cardbox, or pasteurized beer filled in metal can with pull tab. However, a cooked, vacuum packed, and chilled food (sous-vide product) of limited shelf-life hardly suits the notion of canned product.

Most canned meat and vegetable products fall in the category of low acid foods, and these must be submitted to heat treatment by the combination of temperature and time to be sterile or commercially sterile by applying a minimum botulinum cook ($F_0 = 2.5$ min) or higher. There are other foods of medium or high acidity (pH below 4.5) for which the application of botulinum cook is not necessary and can be sterilized at lower temperature and/or shorter time (e.g., tomato and most fruit products; see **Table 5**). These are also considered canned foods when packed and heated in hermetically sealed containers.

The fully canned foods are expected to have a shelf-life of at least two years from the date of processing. However, canned foods could retain safety and nutritional value well beyond two years, but they may show some change in quality, such as color and texture. Practically, a canned food has an almost indefinite shelf-life at moderate temperatures (20–25 °C, 68–77 °F). In fact, canned food as old as 100 years has been found in sunken ships and was still microbiologically safe for human consumption. Canning has remained a reliable, well proven technology, and the popularity and consumption of heat processed foods is only increasing. Acceptability of canned product can be attributed to the excellent safety record although the range of heat processed products has tremendously widened.

See also: Bacteria: *Clostridium botulinum*. Disciplines Associated with Food Safety: Food Microbiology. Food Technologies: Aseptic Packaging; Food Irradiation. Organisms of Concern but not Foodborne or Confirmed Foodborne: Spoilage Microorganisms

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SAFETY OF FOOD AND BEVERAGES

Contents

Fruits and Vegetables

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Glossary

Control measure Action used to prevent, eliminate, or reduce a food safety hazard.
Cross contamination Transfer of microorganisms from one surface or food to others.
Human pathogen An organism able to cause diseases in humans.
Postharvest Processes following harvest of the crops.
Preharvest Processes on the field or farm before harvest of the crops.

Introduction

Fresh fruits, vegetables, and herbs play an important role in human nutrition and contribute essentially to public health. This is reflected in many health promoting programs of health agencies in many countries in order to protect against many diseases, for example, cancer, cardiovascular diseases, diabetes, and chronic diseases, which in turn increased the demand

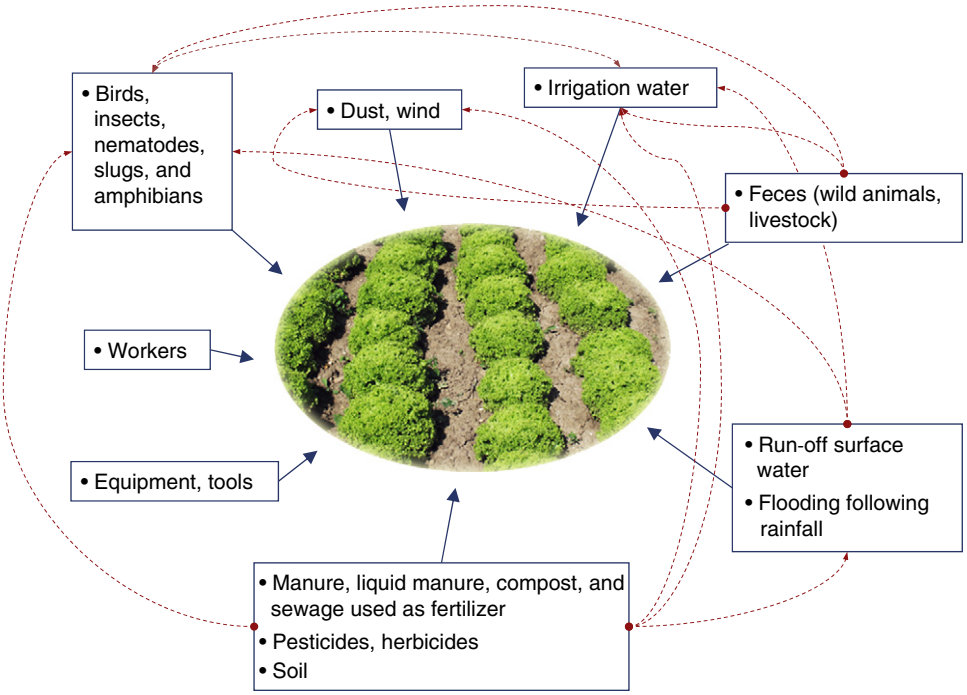

Figure 1 Possible sources of human pathogenic contamination in preharvest produce production.

for fruits and vegetables enormously. Therefore, human consumption of fruits and vegetables increased by 4.5% per year between 1990 and 2004. Global distribution and trading chains as well as the product varieties and minimally processed produce, for example, fresh-cut lettuce and fruit salads, have been widened. However, fresh fruits and vegetables, when consumed raw, caused several disease outbreaks in recent years including multicountry outbreaks – the number and frequency of foodborne outbreaks and the number of people affected because of such outbreaks revealed by epidemiological data increased in parallel to the increased production and consumption. Fresh produce eaten raw is therefore considered as high-risk food. Microbial contaminations may occur during pre- and postharvest stages. Severe outbreaks have been found to be caused after consumption of a wide variety of contaminated produce, for example, sprouts, spinach, lettuce, pepper, tomato, cantaloupe, and berries. Such outbreaks not only affect health but also economic aspects (growers and processors) due to recalls and loss of consumers' trust in fresh produce. There is a need to improve knowledge of the ecology of human pathogens and to adapt farm-to-fork strategies in order to enhance microbial safety of fresh produce. Over and above microbial agents there is a risk with chemicals, for example, pesticide residues or mycotoxins, which may be a major issue particularly in developing regions. Thus, this article focuses on the microbial food safety risks.

Preharvest Contamination

On the field, pathogens may find their way onto fruits and vegetables from contaminated water (run-off, flooding, and irrigation), soil, insufficiently composted manure used as fertilizer, feces from livestock and wildlife, through animals (e.g., insects and amphibians) having been in contact with contaminated water and intruding fields, workers, equipment, dust, and water used to apply herbicides and pesticides (Figure 1). It is known that human pathogens are able to survive and grow in herbicide-, insecticide-, and fungicide solutions. The contamination risk in production of fresh produce is dependent on the crop plant. Plants grown close to the ground, such as lettuce or cantaloupes, bear a higher risk than aerial fruits. Pathogens can grow on leaves or fruits of the crops or can enter the plants via root lesions. Obviously, the risk of internalization of pathogens via soil is lower than via leaves.

As is the case of outbreaks of foodborne pathogens associated with meat, milk, and other food, animals (cattle, swine, sheep, poultry, and wildlife species) play a key role as carriers of pathogens like *Escherichia coli*, *Salmonella enterica*, and *Campylobacter jejuni*. Transmission of pathogens occurs through the fecal–oral pathway. Colonization of animals by pathogens depends on the uptake pathway via feed, grass, and drinking water, as well as on pathogen factors (survival in the environment, soil, water, and intestinal tract) and animal factors (immune system, moving, defecation, and population density). Climatical conditions may increase or decrease the incidence of pathogens in environmental niches. Livestock in close proximity to agricultural land, where produce is grown, poses a threat for potential contamination and wild animals that may be present at feedlots might contribute to the spread of pathogens. Birds' feces were shown to contain a range of pathogens and insects; nematodes and slugs were shown to uptake and excrete pathogens.

Recent studies reported incidences of *E. coli* O157:H7 in 0.2–28% of cattle feces samples depending on the sampling

site (feedlot, pasture land, and slaughter) and kind of feeding. Surveys of pigs revealed 1.2–8.9% of samples being positive for *E. coli* O157:H7. *S. enterica* could be identified in 1.4–9% of cattle feces samples, in 23.4% of pig feces samples and in up to 13% of poultry samples. *Listeria monocytogenes* has been shown to appear in up to 50% of fecal samples from animals like cattle, sheep, goats, pigs, and poultry. Incidence of *C. jejuni* in animals mentioned above is similar to that of *E. coli* O157 and *Salmonella*. However, incidence in produce is absent or much lower. This is reflected by only a few outbreaks of *C. jejuni* associated with produce (lettuce, sweet potatoes, cantaloupe, strawberries, and cucumber). This might be explained by lower fitness of *C. jejuni* in environments linked to produce production. Compared to total foodborne infections, *Campylobacter*-related outbreaks associated with fresh produce are rather rare.

Persistence of *E. coli* O157, *E. coli* O157:H7 or *Salmonella* spp. in soil, water, or manure has been reported in several studies. Accordingly, these pathogens survived in some samples between more than 30 days and several months; an outbreak strain of *Salmonella* Enteritidis was detectable for more than 4 years in soil from an almond orchard. It has been shown that survival of *Salmonella* Typhimurium and *E. coli* O157:H7 in manure of cattle fed with high-fiber and low-energy diet was significantly lower than in manure of cattle fed with low-fiber and high-energy diet. Survival of theses pathogens is greater in dairy slurry than in solid dairy manure.

Quality of irrigation water plays an essential role in produce production as water is a key vector for transmission of pathogens. Quality of well- or surface water used is affected by geography, hydrology (salinity, nutrients, fecal dispersion, and concentration of pathogens), ecology (vegetation), and meteorology (temperature, rain fall, and floods). Flooding of fields following strong rainfall intensity is supposed to increase contamination through spread of feces, contaminated water, or soil. This is particularly critical after periods of drought.

Harvest and Postharvest Contamination

During harvesting, vegetables, herbs, and fruits may get contaminated by the field environment (e.g., soil and dust), by contact with harvesting equipment, and in case fruits and vegetables are harvested by hand, there is also an increased risk of transmission and spread of pathogens through workers. In particular, leafy vegetables and herbs are susceptible to mechanical injury leading to opening of the plant tissue, which in turn favors microbial growth and internalization. During subsequent processing, populations of pathogens having emerged on vegetables and fruits during preharvest stages or harvesting are able to proliferate. Postharvest processes in the packing house (grading, washing, cutting, and storage) play important roles as possible sources of contamination. Contamination may also occur through ill workers during processing or through rodents and contaminated containers during storage and transport. Fecal contamination, that is heterogeneously distributed in fields, orchards, on fruits, and vegetables, may lead to the spread of pathogens during postharvest handling. The postharvest microbial community on the surface of fruits and vegetables and storage conditions must be considered as critical factors because growth of human pathogens is affected by postharvest plant pathogens, for example, *Botrytis cinerea* or *Glomerella cingulata*. The latter was shown to promote growth of *L. monocytogenes* inoculated in decayed apple tissue in part due to an increase in pH from 4.7 to 7.0 in the infected tissue.

Cross-contamination during transport, at processing plants, for example, regarding fresh-cut produce, at markets or in restaurants needs to be taken into account. Packing technology and material must be carefully selected to avoid growth of pathogens. Leafy vegetables and herbs often exhibit high respiration. When packed under modified atmosphere for extending shelf life, levels of O_2 and CO_2 may change in a way favoring growth of, for example, *Clostridium botulinum* or *L. monocytogenes*. Proper processing of fruits and vegetables is a prerequisite to obtain safe food products. There have been outbreaks of spore-forming bacteria associated with faulty processing of fresh produce such as outbreaks of botulism associated with improper preservation of garlic-in-oil (pH >4.5, prepared without heat treatment or chemical or acid additives) or associated with canned bamboo shoots.

Outbreaks: Sources, Transmission, and Examples

Reasons which contribute to the observed increase in outbreaks may not simply be due to increased consumption of fresh vegetables, herbs, fruits, and ready-to-eat products. Several factors are discussed. An increase in contamination may contribute to higher numbers of outbreaks and illnesses. Production, processing, kind of produce, cultivar, and physiological state of plant and pathogens are critical factors and probably multiple events have to take place to cause an outbreak. In case an outbreak occurs, lack of information on consumed produce often hinders rapid identification of the product causing it. This is especially true as many salads including sprouts are consumed as mixtures. In addition, the number of consumers eating food prepared outside their home is increasing, thereby contributing to a greater risk of foodborne diseases. Global production, trade, and distribution of a wide variety of produce and its perishability pose major challenges for tracing the source of contamination and its mechanism. Longer food supply chains, enhanced pathogenicity of microorganisms due to genetic adaptation (greater virulence or drug resistance) or the more vulnerable older human population due to weakened immune systems may also lead to the increase in produce-associated diseases. More sensitive and specific identification and typing methods for elucidation of outbreaks and their sources are required (e.g., PulseNet in the US, national surveillance network).

The spectrum of microorganisms linked to produce-associated outbreaks comprises viruses, bacteria, protozoa, and helminths (Table 1). Originally, viruses have been reported to account for 20% of outbreaks. However, today, the number of norovirus-associated outbreaks has increased significantly, also due to improved surveillance and novel diagnostic and identification methods used. Most norovirus and hepatitis A infections caused by contaminated food are due to insufficient hygiene of infected workers on farms, in postharvest processes, or in gastronomy. Norovirus- and hepatitis A-related gastroenteritis has been reported to be

Table 1 Summary of some of the major outbreaks associated with produce

Date	*Place*	*Pathogen*	*Type of produce*	*Illnesses*	*Deaths*
Dec 2011–Mar 2012	USA	*E. coli* O26	Clover sprouts	29	
May–Jul 2011	15 countries in Europe, USA	*E. coli* O104:H4	Sprouts of fenugreek seeds	4075	50
Apr 2010	USA	*E. coli* O145	Lettuce – Romaine	26	
Sep–Oct 2007	Netherlands, Iceland	*E. coli* O157:H7	Lettuce, pre-packed	50	
Aug–Sept 2006	USA, Canada	*E. coli* O157:H7	Spinach bagged	199	3
Aug–Sept 2005	Sweden	*E. coli* O157:H7	Lettuce – Iceberg	135	
Oct–Nov 1996	USA, Canada	*E. coli* O157:H7	Apple juice, unpasteurized	70	
May–Dec 1996	Japan	*E. coli* O157:H7	Radish sprouts	9451	12
Jan–Aug 2011	USA	*Salmonella* Agona	Papayas	106	
Nov–Dec 2005	USA	*Salmonella* Braenderup	Tomatoes	82	
Jun–Jul 2004	USA	*Salmonella* Braenderup	Tomatoes	125	
Dec 2005–Aug 2006	Sweden	*Salmonella* Enteritids	Almonds	15	
Sept 2003–Apr 2004	USA, Canada	*Salmonella* Enteritids	Almonds	29	
Oct 2003–Jul 2001	USA, Canada	*Salmonella* Enteritids	Almonds	168	
Nov–Dec 2000	Netherlands	*Salmonella* Enteritids	Bean sprouts	27	
Jul–Sept 2007	Sweden	*Salmonella* Java	Baby spinach	172	
Jul 2004	Canada	*Salmonella* Javiana	Tomatoes	7	
Jun–Jul 2002	USA	*Salmonella* Javiana	Tomatoes	141	
Feb–Apr 2001	USA	*Salmonella* Kottbus	Alfalfa sprouts	32	
Jan–Apr 2008	USA, Canada	*Salmonella* Litchfield	Cantaloupe	60	
May–Jul 2007	USA	*Salmonella* Litchfield	Honeydew melon	30	0
Jun 1999	USA, Canada	*Salmonella* Muenchen	Orange juice, unpasteurized	207	
Jul–Nov 2006	USA	*Salmonella* Newport	Tomatoes	115	
Jul–Nov 2005	USA	*Salmonella* Newport	Tomatoes	72	
Aug–Sept 2004	UK	*Salmonella* Newport	Lettuce	368	
Sept–Oct 2002	USA	*Salmonella* Newport	Tomatoes	510	
Jun 2001	UK	*Salmonella* Newport	Ready-to-eat salad	9	
Nov–Dec 1999	USA	*Salmonella* Newport	Mangoes	78	2
Jun–Jul 2006	USA, Canada	*Salmonella* Oranienburg	Fruit salad	41	
Mar–May 2002	USA, Canada	*Salmonella* Poona	Cantaloupe	58	
Apr–May 2001	USA	*Salmonella* Poona	Cantaloupe	50	2
Apr–Jun 2000	USA	*Salmonella* Poona	Cantaloupe	47	
Jan–Jun 2007	UK	*Salmonella* Senftenberg	Basil	55	
Feb–Apr 2009	USA	*Salmonella* St.Paul	Alfalfa sprouts	235	
Apr–Aug 2008	USA	*Salmonella* St.Paul	Peppers	1442	2
Jul–Aug 2007	Sweden	*Salmonella* Stanely	Alfalfa sprouts	51	
Oct–Dec 2004	Norway	*Salmonella* Thompson	Rucola	21	
Sept–Oct 2006	USA, Norway	*Salmonella* Typhimurium	Tomatoes	190	
Jan–Feb 2005	UK	*Salmonella* Typhimurium	Lettuce – Iceberg	96	
May 2005	Finland	*Salmonella* Typhimurium	Lettuce	60	
Jul–Oct 2007	Norway, Denmark, Finland	*Salmonella* Welteverden	Alfalfa sprouts	45	
May–Jun 2009	Sweden	*Shigella dysenteriae*	Snow peas	35	
May 2001	USA	*Shigella flexneri*	Tomatoes	886	
Aug 1998	UK	*Shigella flexneri*	Fruit salad	36	
Apr–May 2009	Denmark	*Shigella sonnei*	Snow peas	16	
May 2009	Norway	*Shigella sonnei*	Snow peas	20	
Aug 2007	Denmark	*Shigella sonnei*	Baby corn	201	
Aug 2004	USA, Japan, Austrila, American Samoa	*Shigella sonnei*	Carrots	163	
Jul–Aug 1998	USA, Canada	*Shigella sonnei*	Parsley	479	
May–Jun 1994	Norway	*Shigella sonnei*	Lettuce – Iceberg	110	
Jul–Oct 2011	USA	*Listeria monocytogenes*	Cantaloupe	146	30
Aug–Sep 2008	Canada	*Campylobacter jejuni*	Raw peas	63	
Aug–Sep 2006	Finland	*Yersinia pseudotuberculosis*	Carrots	>400	
Oct–Nov 1998	Finland	*Yersinia pseudotuberculosis*	Lettuce – Iceberg	47	1
Jun–Jul 2004	USA	*Cyclospora cayetanensis*	Snow peas	40	
May 2001	Canada	*Cyclospora cayetanensis*	Basil	17	
May–Aug 1996	USA, Canada	*Cyclospora cayetanensis*	Raspberries	1465	
Sept 2008	Sweden	*Cryptosporidium* sp.	Parsely	21	
Aug 2005	Denmark	*Cryptosporidium hominis*	Carrots	78	
Oct–Nov 2003	USA	Hepatitis A	Green onions	>500	3
Nov 2000–Jun 2001	Sweden	Hepatitis A	Rucola	54	
Feb–Mar 1997	USA	Hepatitis A	Strawberries (frozen)	242	

(*Continued*)

Table 1 Continued

Date	*Place*	*Pathogen*	*Type of produce*	*Illnesses*	*Deaths*
Sept–Oct 2012	Germany	Norovirus	Strawberries (frozen)	>10 000	
Jan 2010	Denmark	Norovirus	Lettuce	260	
Dec–Feb 2009	Germany	Norovirus	Salad	101	
Jun–Aug 2006	Sweden	Norovirus	Raspberries	43	
May–Sept 2005	Denmark	Norovirus	Raspberries	>1000	
Mar 2005	France	Norovirus	Raspberries	75	

associated with raspberries, strawberries, cantaloupe, onions, or lettuce, respectively.

Protozoan parasites have been reported to account for 16% of the outbreaks related to produce in the US, mainly due to *Cyclospora cayetanensis*, *Giardia lamblia*, and *Cryptosporidium parvum*. *Toxoplasma gondii*, a ubiquitous occurring parasite with a wide host range among warm-blooded vertebrates causing toxoplasmosis poses a particular risk to pregnant women and their fetuses due to vertical transmission if infection occurs during pregnancy. Infections with *T. gondii* have been associated with consumption of unwashed produce that may have become contaminated with oocysts through cat feces in home gardening. Among eukaryotic parasites it is worth to note the possible risk with *Echinococcus multilocularis* causing alveolar echinococcosis, a severe liver disease. Raw fruits and vegetables from fields and gardens contaminated with eggs of *E. multilocularis* are likely to act as infection routes in humans. Carnivorous species like the fox are definitive hosts that excrete eggs with their feces which can contaminate the produce. It is therefore recommended not to consume unwashed produce from field or garden and not to pick low-hanging wild berries.

Pathogenic bacteria transmitted traditionally via food of animal origin have been linked to 60% of the outbreaks related to fresh produce between 1973 and 1997 in the US. Bacterial variety comprises pathogenic *S. enterica*, *E. coli*, *Campylobacter* spp., *L. monocytogenes*, *Shigella* spp., *Yersinia* spp., *Bacillus cereus*, and *Staphylococcus aureus*. *Salmonella* has been the major cause for outbreaks associated with fruits and vegetables – responsible for half of the bacterial outbreaks. Regarding the produce range reported to be linked with outbreaks of different *Salmonella* serovars, the following are documented most frequently: tomato, cantaloupes, peppers, rocket leaves, basil, and sprouts (e.g., alfalfa, mung bean, and radish).

Outbreaks caused by preharvest contamination have been reported from the US, Mexico, and Europe and comprise *E. coli* O157:H7, enteroaggregative *E. coli* (EAEC) and *Salmonella* associated with leafy greens, tomato, cantaloupes, sprouts, carrots, peppers, and almonds. But also outbreaks of *Shigella* spp. and *Yersinia pseudotuberculosis* associated with lettuce, parsley, tomato, or carrots have been linked with preharvest contaminations. An outbreak of *Shigella sonnei* infections associated with eating parsley has been linked with on-farm postharvest cooling processes. Postharvest contamination of lettuce through contaminated shredding equipment caused an outbreak of salmonellosis.

Several outbreaks of *Salmonella* and *E. coli* including Shiga toxin-producing *E. coli* worldwide have been associated with sprouts produced from contaminated seeds, which in turn have been produced in an agricultural environments not being sufficiently controlled for microbial safety. In subsequent steps of sprout production, conditions are also optimal for growth and multiplication of pathogens present on or in seeds, even after long storage periods. In Germany 2011, EAEC O104:H4 caused the largest outbreak of hemolytic uremic syndrome (HUS) and bloody diarrhea ever described worldwide, which has been linked with sprouts. This outbreak affected 3842 people (855 cases of HUS and 2987 cases of acute gastroenteritis). Of the HUS patients 35 died, and of the patients with acute gastroenteritis 18 died.

In many cases, preharvest contamination could be verified by isolating and identifying the same strain both in clinical specimens and environmental samples. This has been demonstrated particularly for several outbreaks linked to vegetables and the irrigation water used.

L. monocytogenes is considered as a major agent causing foodborne diseases as it differs from other foodborne pathogens by its ability to grow under diverse conditions (low pH, high osmolarity, low temperature, and low oxygen). A multistate outbreak of listeriosis in the US 2011, affected 146 persons. Thirty people died and a woman pregnant at the time of illness had a miscarriage. Cantaloupes from a farm in Colorado were identified as the source of the outbreak.

The situation with regard to developing countries is of particular importance, as standards of sanitation and Good Agricultural and Manufactory Practices (GAP and GMP) are often lacking or are less well established. Therefore, several agents are prone to be spread with fresh fruits, vegetables, and herbs. Infections with *Salmonella* Typhi and Paratyphi A occurring only in humans and causing typhoid fever are still common in developing countries. Fruits and vegetables can cause typhoid fever if they have been improperly handled by infected persons shedding the bacteria or if plants have been irrigated with water contaminated by sewage containing *S.* Typhi and Paratyphi A. Outbreaks of *Shigella* and *Vibrio* spp. related to fresh produce have been reported. Besides norovirus and hepatitis A, rotavirus is a major cause for gastroenteritis, particularly among children. It is likely that raw fruits and vegetables handled by infected persons cause illnesses. Foodborne parasitic infections due to several helminths often occur in developing countries through consumption of raw produce that has been contaminated with feces, soil, irrigation water, sewage, improper handling, or processing. Up to 10% of people in developing countries are infected with intestinal worms. Helminths as eukaryotic parasitic worms comprise cestodes, trematodes, and nematodes. Symptoms range from nonspecific symptoms, diarrhea, muscle pain, cough, skin lesions, malnutrition, weight loss, and neurological and other symptoms.

Data on Incidence of Pathogenic Contamination of Produce

The incidence of foodborne pathogens in fresh produce surveys has been reported in several studies ranging from 0% to 10%, occasionally up to more than 20%. *Salmonella* was found in 0.3% (range from 0.1% to 2.3%) of produce samples tested in 2007 within the European Union, of which mainly the precut ready-to-eat products revealed highest proportions of contamination. In total, 1.5% and 2.2% of sprouts samples were contaminated in the Netherlands and in Germany, respectively. *Salmonella* was found in 0.4% to 0.5% of herbs and spices from Hungary, UK, and Netherlands. A survey on minimally processed vegetables in Brazil showed 2.2% of the samples being contaminated with *Salmonella*.

A study by the US Department of Agriculture (USDA) (2002–2007), monitoring microbial safety of fresh produce (>59 000 samples) showed that the incidence of *E. coli* with virulence traits including Shiga toxin 1 and 2 ranged from 0.1% to 0.4%. A survey for generic *E. coli* on produce items (1183 samples) from Canada (2004) demonstrated that 0%, 1.3%, 6.5%, 11.6%, 4.9%, and 13.4% of tomato, cantaloupe, conventional lettuce, organic lettuce, cilantro, and parsley, respectively, were contaminated. Highest concentrations were found in cilantro (up to 7600 colony forming units (CFU)/g) and parsley (16 000 CFU/g). In another study, bagged precut spinach and lettuce mixtures from conventional and organic culture in the US were analyzed – 12.1% and 16.6% as well as 23.1% and 6.3% of the respective samples were tested positively for *E. coli*. Another survey from South Africa showed 2.2% of fresh vegetables to be contaminated with *E. coli* O157:H7. In general, results of these studies show a high variability of produce quality with respect to region, year and probably also to the methods used for identification and quantification. Significant data on correlations between incidence of generic *E. coli* and enteric human pathogens on produce are largely lacking. However, incidence of generic *E. coli* can be used as a marker for potential pre- and postharvest contamination. *C. jejuni* is rarely detected in produce grown in the Western world, but frequently in fruits and vegetables in developing countries. *L. monocytogenes* and *Listeria* species were found in cabbage, cucumber, potato, and radish. In addition, *L. monocytogenes* was isolated from ready-to-eat vegetables including beans, tomato, artichoke, peas, and cauliflower.

Microbial Attachment, Colonization, Survival, and Transmission

After attachment of microorganisms to the surface of fruits and vegetables, they are able to colonize further parts of the plant and to proliferate, and thus being transferred to the host. Proliferation is supported by high temperatures, humidity, and ideal nutrient status. Bacteria are not only able to attach to the surface of plants, but several studies showed that they are also able to internalize into inner tissue of plants via leaves, roots, or flowers. Studies showed that *E. coli* and *Salmonella* can persist for months after application on leaves. Serovars of *S. enterica* are able to colonize seeds, sprouts, leaves, and fruits. It has been reported that serovar-specific behavior in attachment and several modes of action during attachment of *Salmonella* exist. Pilus curli, the O-antigen capsule and cellulose synthesis are essential factors for adhesion. Curli and cellulose also promote the formation of biofilms, which was shown to strongly support adhesion and persistence on leaves. Obviously, flagellae also promote adhesion of the bacteria to leaves as shown for *Salmonella* Senftenberg. *E. coli* O157, in contrast to nonpathogenic strains, has been shown to attach to tomato fruit surface, spinach leaves, and sprouts via curli. Additionally, filamentous type III secretion system and flagella have been shown to be involved in adhesion to leaves. Human pathogenic bacteria, after having attached to the plant surface, are able to integrate into the phyllosphere microbial community. Several studies used *E. coli* and *Salmonella*, which have been sprayed or applied on leaves, inoculated into seeds and roots or applied directly in the soil. Furthermore, the rhizosphere might serve as another plant environment, where human pathogens are able to establish.

Risk and Control Measures

The application of GAP and GMP, Good Hygiene Practices, and implementation of Hazard Analysis and Critical Control Point systems are essential measures to assure safety of fresh produce from the farm to the consumer. As farming procedures exhibit diverse characteristics due to the kind of vegetable and fruit as well as production conditions (soil, irrigation, harvesting, sorting, packing, transportation, and storage), a well-designed food safety plan pointing to hazards and risks is required. The US Food and Drug Administration has published guides to minimize microbial food safety hazards of fresh fruits and vegetables, tomatoes, melons, and leafy greens comprising GAPs to reduce hazards along the entire food chain. It is worth mentioning that current technologies and practices are able to reduce microbial risks, but all hazards associated with fresh produce cannot be eliminated completely. Therefore, prevention of contamination from the field onwards is the most important issue to assure food safety.

Seeds used in produce production should be considered as an early critical point in hazard analysis as pathogens are able to survive on or in dried seeds. Appropriate storage conditions and exclusion of rodents from storage facilities to avoid fecal contamination are vital. It is worth mentioning that postharvest decontamination processes such as treatments with solutions containing chlorine do not fully prevent from bacterial regrowth. This is due to inaccessible sites of seeds and produce where pathogens are protected. It has been shown that chlorine in combination with ozone or ionizing radiation is efficient to reduce microbial load and to increase shelf life of leafy green vegetables. However, restrictions on using disinfectants with produce in some countries need to be considered.

To maintain and regularly control quality of water used in irrigation, crop protection, washing, and packing are prerequisites for safe production and processing of fruits and vegetables. The WHO standard for irrigation water in production of crops eaten raw is $<10^3$ coliform bacteria per 100 ml. Studies implicate that overhead irrigation increases the risk of contamination of vegetables as compared to drip irrigation.

Sufficient treatment of animal wastes by composting and heat treatment according to regulatory requirements decreases the risk of contamination when manure is applied to fields as soil amendment. Recommendations from national programs for time intervals between application of manure and harvest, for example, 90 or 120 days for fruits and vegetables whose edible parts have no or have contact to the soil, respectively, should be followed.

Access of livestock and wildlife species to produce and sprout seed production fields as well as irrigation water should be restricted to avoid fecal contamination of produce. Feces of birds was shown to contain pathogens. Avoidance of flooding of fields supports safety in the preharvest stage.

At harvest, sorting, and packing, the hygiene and health of workers is critical to prevent contamination of produce. Proper use of gloves, disinfectants, and toilets as well as efficient hand-washing significantly reduces the risk of contamination.

During processing of harvested produce, the equipment used in washing, sorting, cutting, and packing as well as the surface of facilities being in contact with produce need to be made of appropriate material allowing easy cleaning and regular disinfection. Washing water should be of drinking water quality. Formation of biofilms by nonspoilage, spoilage, and pathogenic microorganisms on equipment surfaces must be avoided as biofilms are difficult to eliminate, even when sanitized water is used. Regular surveillance, sampling, and analysis of surface samples help to reduce the risk of contamination and biofilm formation. Because temperature is the key factor for bacterial growth, control, and maintenance of appropriate temperature within the cold chain including precooling by icing, hydrocooling, or vacuum cooling, and during processing and distribution are essential measures to guarantee safety. Clear instructions and operating procedures for the process steps mentioned above need to be implemented, documented, and controlled.

Personnel at all levels of the chain must be educated and regularly trained, appropriate supervision must be arranged and management of the farms and processing plants must actively support a commitment of food safety and be aware of microbial hazards. Independent audits on farms and processing plants help to ensure that GAPs are implemented. Besides agricultural workers and food handlers, consumers are expected to follow guidelines that help to maintain food safety during transport, storage, and preparation at home. Selection at retailers should exclude damaged or slimy produce and subsequent transportation to home should be as rapid as possible using appropriate insulated transport bags. Leafy vegetables and herbs are recommended to be stored in a clean refrigerator at temperatures below 5 °C. Outer leaves should be removed and produce placed in plastic bags within the refrigerator. Good hygiene during handling of fresh produce includes application of proper hand washing techniques using soap and clean water for 20 s before and after preparing produce, use of hygienic utensils, cutting boards, and sinks in the kitchen protecting produce from cross-contamination, and finally potable running tap water should be used for washing just before eating. However, washing will never remove all microorganisms – it has limited effects on attached microorganisms. It should be a goal of consumers' education to increase their awareness of microbial risks related to fresh produce and to improve knowledge in hygienic handling of fresh and fresh-cut fruits, vegetables, and herbs. Stakeholders (government, industry, media, and consumer organizations) should communicate relevant information consistently and in an easily understandable manner.

There are promising results giving rise to the development of alternative practices on the field using natural antagonistic bacteria inhibiting growth of human pathogens in the phyllosphere. This has been shown for species of *Bacillus*, *Pseudomonas*, *Aeromonas*, *Pantoea*, *Klebsiella*, *Burkholderia*, and *Enterobacter*. Traceability systems of products as well as state-of-the art methods for sensitive, rapid, and robust identification and typing of human pathogens on fresh produce are prerequisites for preventing and elucidating outbreaks.

Currently, knowledge on contamination sources and spread of pathogens in the fresh produce chain as well as on colonization, internalization, and proliferation is still limited. These processes are less well-understood in fruit production than in vegetable production. Better insights into plant–microbe interactions, environmental conditions, and food processing and distribution are necessary to further reduce the risk of microbial contamination and to understand the occurrence of pathogens previously not associated with raw fruits and vegetables. This is particularly important when introducing new processes and when launching new products on the global market.

See also: Foodborne Diseases: Overview of Biological Hazards and Foodborne Diseases; Overview of Chemical, Physical, and Other Significant Hazards; Overview of Emerging Food Technologies

Further Reading

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SAFETY OF FOOD AND BEVERAGES

Seafood

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Glossary

Bivalve mollusks Filter-feeding lamellibranch mollusks.
Candling A method to detect worms embedded deep in the flesh of fish, which are not immediately obvious. It usually involves shining a bright light through the fillet. In commercial practice, candling is effective in detecting Phocanema in thin skinless fillets of white fish, particularly cod; the method does not work well on thick fillets with the skin on. Candling is less effective in detecting *Anisakis*. The simplest kind of candling table is a box about 50 cm square with a ground glass or Perspex top about 6 mm thick. The inside of the box is white, and is lit by two fluorescent tubes giving a white (not colored) light.
Decomposition The deterioration of fish, shellfish, and their products, including texture breakdown. It causes a persistent and distinct objectionable odor or flavor.
Harmful algal blooms (HABs) Rapid proliferation of single-cell algae causing environmental harm such as anoxia, objectionable odors, lethality to aquatic fauna, or accumulation of marine toxins in seafood species.
The Codex Alimentarius Commission The Commission was created in 1963 by the Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) to develop food standards, guidelines, and related texts such as codes of practice under the Joint FAO/WHO Food Standards Program. The main purposes of this Program are to protect the health of consumers, ensure fair trade practices in the food trade, and promote coordination of all food standards work undertaken by international governmental and nongovernmental organizations.

Introduction

Seafood is the only major food commodity that is extensively harvested from the wild. Additionally, aquaculture is the fastest growing sector of the food supply. The marine and aquaculture environment is a rich source of seafood hazards including bacterial and viral pathogens, parasites, marine toxins, heavy metals, and other chemical contaminants from anthropogenic sources, such as pesticides and pharmaceuticals. Almost every category of known biological, chemical, and physical hazard can occur in seafood and is also a common cause of food allergies. Some hazards that have been introduced preharvest can increase following postharvest handling, whereas others may be effectively mitigated. Additional hazards may be introduced postharvest, during production, or during processing, and vary considerably among seafood commodities. Of all available seafood commodities, live and raw bivalve mollusks are among the most risky products consumed by humans. Bivalve mollusks filter large volumes of seawater and bioconcentrate suspended particles including pathogens and harmful algal species when present in the water column. The risk is further exacerbated by certain cultural and social practices such as consumption of the entire bivalve mollusk, in either the live, raw, or lightly cooked state. Hazards associated with fish, crustacea, and other seafood commodities are largely related to species, trophic status, production region, habitat, harvest practices, and consumption patterns.

Preharvest controls are the most effective means to address microbial and chemical hazards that are introduced via the seafood production environment. Monitoring programs vary according to hazard and the circumstances of its introduction. Monitoring approaches include measurements either from the water column directly or in the seafood species affected. For instance, in the case of shellfish toxins, harmful algal bloom species are monitored by state health agencies and/or those responsible for fish and wildlife on a regular basis (e.g., weekly or biweekly) and harvests and growing areas are placed under temporary closure based on these cell density thresholds until toxin testing in shellfish/fish can be performed. Likewise, levels of indicator organisms such as fecal coliforms in bivalve mollusks or their overlying waters are used to manage risks associated with enteric pathogens. In some cases direct measurement of the hazard is not effective because analytical procedures are too slow or resource intensive and measurement of indicators are more appropriate. In an effort to provide early warning, predictive models incorporating climate and ocean conditions such as rainfall, water temperature, and salinity are under development for control of hazards including harmful algal blooms and naturally occurring pathogens such as *Vibrio* spp. Permanent closures are necessary for hazards that may be continuously introduced or persist in

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edible flesh of the seafood species, for example, industrial chemicals and heavy metals.

Commodity definitions are described in the Codex Code of Practice for Fish and Fishery Products (CAC/RCP 52-2003) and the associated hazard controls are published in this code and similar codes adopted by the Codex Committee for Food Hygiene. In the US, European Union and other developed countries, the safety of harvested seafood is controlled by a hazard analysis critical control points (HACCP) system and is also applied to developing countries that export seafood to developed countries. HACCP is a science-based systematic approach to the identification of significant food safety hazards and measures for their control or prevention to ensure the safety of food. In the seafood industry, business operators use the HACCP system and its prerequisite programs such as good hygienic practices to control hazards. One key element for successful HACCP development, implementation, and ongoing verification is to provide appropriate training for seafood processing plant employees.

This article presents an overview of seafood hazards and provides sources of information for assessing risk, control guidance, regulations, and advice on approaches for testing seafood for prominent hazards.

Pathogens

Pathogenic bacteria indigenous to the aquatic environment and naturally present in animals consumed as seafood, include *Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificus, Plesiomonas shigelloides, Aeromonas* spp., and *Clostridium botulinum*.

Vibrio spp. are ubiquitous in coastal environments and are a leading cause of illness and death associated with seafood consumption worldwide. Vibrio illnesses range from relatively mild diarrhea from ingestion of *V. parahaemolyticus* to life threatening diarrhea or septicemia from *V. cholerae* or *V. vulnificus*, respectively. Other than choleragenic *V. cholerae* that is typically transmitted via the fecal-oral route, nearly all human vibrio illnesses worldwide are associated with seafood consumption. Bivalve mollusks are typically the dominant vehicle of vibrio infections in the US and other western countries. In some Asian countries such as Japan, where fish (sashimi) and seafood other than bivalve mollusks are commonly consumed raw such as sea urchin and octopus, these products are a more significant cause of *V. parahaemolyticus* gastroenteritis than bivalve mollusks. The first *V. parahaemolyticus* outbreak ever reported was linked to a small salted fish product, shirasu, in Japan in the early 1950s and led to more careful oversight to assure appropriate salt content. Other articles of this volume are devoted to each of the above species. The Food and Agricultural Organization (FAO) and the World Health Organization (WHO) have published several risk assessments addressing vibrios in seafood.

Vibrios are most abundant in warm and moderately saline areas and outbreaks in temperate climates occur primarily during summer months when seawater temperatures are $>15\,^{\circ}C$. Monitoring of vibrios in the environment or the food supply is resource intensive and provides little public health value because products are generally consumed before analytical results are available. Vibrio abundance varies greatly but can be estimated based on water parameters such as temperature, salinity, and turbidity, which can be conveniently measured *in situ* or by remote sensing using satellite imagery. However, vibrio levels are not a reliable indicator of risk as the proportion of the vibrio population that is pathogenic is typically small and varies between the *Vibrio* spp. and regions.

Virulence in *Vibrio* spp. is multifaceted and much research remains to fully elucidate the mechanisms of pathogenicity. There are some well-established virulence determinants or markers in *V. parahaemolyticus* and *V. cholerae*. Greater than 90% of clinical *V. parahaemolyticus* isolates produce a thermostable direct hemolysin and/or a related toxin coded by the *tdh* and *trh* genes, respectively, but usually $<1\%$ of isolates from the environment or seafood contain either of these genes. Certain populations such as pathogenic *V. parahaemolyticus* (*tdh* and *trh*-positive) in the US Pacific Northwest or members of the pandemic clone of *V. parahaemolyticus* (*tdh*-positive, *trh*-negative) appear to have an attack rate that is more than 1000-fold greater than other pathogenic strains.

Cholera epidemics are caused by choleragenic *V. cholerae* strains possessing the cholera toxin gene, *ctx*, and belong to either serogroup O1 or O139. Contaminated drinking water and poor hygienic practices fuel epidemics in developing countries but epidemic strains also have reservoirs such as copepods in the aquatic environment. Shedding of *V. cholerae* in fecal material from infected humans is considered a major source of seafood contamination and detection of toxigenic *V. cholerae* is rare in nonendemic areas. Non O1/O139 serogroups of *V. cholerae* are frequently found in brackish water and cause mild to moderate gastroenteritis and are among the most common vibrio infections in developed countries. With the exception of a few serotypes such as O141 and O75, clinical strains of non O1/O139 *V. cholerae* rarely possess the *ctx* gene and their virulence mechanisms are poorly understood.

V. vulnificus primary septicemia has most often been associated with consumption of oysters harvested from coastal areas of the US Gulf Coast when water temperatures exceed 20 °C. They possess numerous putative virulence factors but their mechanism of virulence is poorly understood. The organism invades through the intestinal mucosa into the circulatory system causing primary septicemia. The disease progresses extremely rapidly and has the highest death rate of any foodborne organism. Foodborne cases occur almost exclusively in individuals who are immunocompromised including those with liver disease. Infection is relatively rare even with exposure rates of more than a million organisms.

Other than a zero tolerance (nondetectable in 25-g portion) for toxigenic *V. cholerae* for all foods including seafood, there are no internationally recognized standards for *Vibrio* spp. in seafood. In 2001 the EU Scientific Committee on Veterinary Measures relating to Public Health (SCVPH) concluded in their report that current scientific data did not support setting specific criteria for pathogenic *V. vulnificus* and *V. parahaemolyticus* in seafood. Few countries have official standards for *V. vulnificus* and *V. parahaemolyticus* but certain northern European countries reject lots of imported seafood with any detectable *Vibrio* spp. (25-g portion).

Seafood is a highly perishable commodity and typically spoils at storage temperatures permissive for pathogen proliferation before pathogens can reach risky levels. Unlike other seafood species that are slaughtered at harvest, bivalve mollusks survive for extended periods out of the water. After harvest, vibrios multiply readily in mollusks under improper temperature storage. Risk assessments indicate that these improper storage practices can greatly increase risk of illness. Control plans have recently been implemented in the US to require refrigeration within 1 h after harvest to control *V. vulnificus* growth in oysters harvested from the Gulf of Mexico during summer months. However, in most countries much longer duration of refrigeration after harvest of bivalves is allowed and time–temperature controls do not exist in many countries. A number of postharvest processing approaches including mild heat, freezing, ultra high hydrostatic pressure and irradiation have been used to mitigate vibrio levels in oysters. These processes have been shown to maintain raw sensory characteristics and greatly reduce vibrio levels and the risk for oyster consumers. Vibrios are highly susceptible to heat but illnesses are occasionally reported from consumption of lightly steamed or fried breaded oysters.

Aeromonas spp. are in the family Vibrionaceae and favor freshwater over marine and estuarine environments. *A. hydrophila* is commonly found in fish and fish products, meat, milk, poultry, and vegetables. Several studies have implicated *Aeromonas* species as spoilage organisms of raw meat, raw packed salmon, fish from warm tropical waters, and milk. Although aeromonads are mesophiles, they grow well in refrigerated seafood often reaching levels of 10^7–10^9 cfu g^{-1}. Growth of aeromonads is well controlled in foods with pH <6.5 and $>3\%$ NaCl. They are occasionally isolated from individuals with gastrointestinal symptoms and some strains possess putative virulence factors. However, their role in human pathogenicity is not clear and there are few established surveillance systems for these organisms.

The genus *Plesiomonas* also belongs to the Vibrionaceae family and consists of a single species, *P. shigelloides*. It is a natural inhabitant of both freshwater and marine waters and is commonly isolated from seafood. There appears to be seasonal variation, with the peak occurring during the warm summer months and does not grow $<8\,^{\circ}C$. *Plesiomonas shigelloides* can cause wound infections and septicemia. It has been isolated in those with gastroenteritis but failed to cause diarrhea in human volunteers after ingestion of 10^9 organisms.

C. botulinum is an anaerobic, Gram-positive, spore-forming bacterium that produces botulism toxin, one of the most potent toxins in nature. The spores of nonproteolytic types B, E, F *C. botulinum* are common in aquatic environments and have been found in the gills and viscera of finfish, crabs, and shellfish and can enter the processing plant on raw materials. *C. botulinum* type E is the most common form found in freshwater and marine environments. Illnesses associated with seafood have been reported from Egypt in 1991 (91 hospitalized and 18 fatal cases), Finland in 2006 (one case associated with vacuum-packed smoked whitefish), Germany in 1997 (two cases, hot-smoked vacuum-packed whitefish), Japan from 1951 to 1987 (479 cases), two outbreaks in Russia in 2004 and 2005 and two outbreaks in the USA in 1985 and 1987. Types A and B are generally found on land, but may also be occasionally found in water. Although the toxin is heat-sensitive, the destruction of spores requires commercial sterilization. The botulism risk usually appears after inadequate heat processing or loss of container integrity. This hazard in canned products can be controlled by limiting proliferation and contamination during processing and ensuring adequate heat processing, container integrity, sanitary postprocess cooling water and clean wet conveying equipment. These measures have been extremely effective in controlling *C. botulinum* in canned products and most illnesses occur in Native American and Asian cultures from traditional foods prepared with whole fish preserved by salting, smoking, or fermentation. Evisceration of fish before preservation by these processes greatly reduces this hazard. *C. botulinum* cannot grow and produce toxin at or below $3\,^{\circ}C$ or below a water activity of 0.94. If artificial smoked flavor blends are used as ingredients, then 5% aqueous phase salt would provide complete protection at temperatures between 3 and $10\,^{\circ}C$ and 10% aqueous phase salt would be required at any temperature over $10\,^{\circ}C$. Smoke-dried fish with a water activity of 0.75 or below (moisture content $<10\%$) inhibits the growth of all foodborne pathogens including *C. botulinum* and refrigeration is not required.

Listeria monocytogenes is considered indigenous to most nonaquatic environments, and is abundant on decaying plant material. It is seldom found in offshore waters or from fish caught or cultured in such waters but occurs at low levels in fish produced in waters impacted by agricultural runoff. The risk of infections from raw seafood products intended for cooking is negligible. *L. monocytogenes* grows at refrigeration temperatures in cooked or processed products like picked crab meat and smoked fish. *L. monocytogenes* prevalence in processed ready-to-eat products is 3–40%. Smoked fish is considered to be the riskiest seafood product; up to 80% of the samples at some processors are positive. In the US there is a zero tolerance (25-g portion) for *L. monocytogenes* in foods that support its growth and this is one of the most frequent causes of regulatory action against seafood businesses. *L. monocytogenes* most often affects the unborn fetus causing still birth but can also infect the elderly and immuocompromised. Although risk assessments consider ready-to-eat seafood products to be at high risk, there have been only a few instances in which seafood products have been implicated in *L. monocytogenes* illnesses and most have been with smoked products with long refrigeration shelf-lives. Product controls begin with reducing potential contamination, rinsing, and disinfecting fish surfaces and package instructions for consumers to freeze or to discard or thoroughly cook after a date when this pathogen could reach risky levels under typical refrigeration conditions. The processing environment is a common source of *L. monocytogenes* contamination. Frequent cleaning and disinfection of equipment, drains, and floor mats are recommended to control this hazard. Codex Alimentarius has established microbiological criteria including and recommendations for sampling plans in the Guidelines on the Application of General Principles of Food Hygiene to the Control of *L. monocytogenes* in food.

Clostridium perfringens is an anaerobic, Gram-positive, mesophilic spore-former widely distributed in the environment with highest levels in soil. It can also be isolated from water,

sediments and from feces of healthy individuals. Vegetative cells surviving gut passage can sporulate in the small intestine and produce an enterotoxin that causes nausea, abdominal pain, diarrhea, and sometimes vomiting 8–24 h after ingestion. In the US, approximately seven annual cases of *C. perfringens* are reported with links to seafood and it is estimated that approximately 200 seafood-caused cases occur every year. *Clostridium perfringens* growth can be controlled by chilling, pH <5, NaCl $>6\%$, and water activities <0.95. Observing proper time–temperature conditions and avoiding cross-contamination to heated foods are essential for the control of this hazard.

Bacillus cereus is an aerobic, Gram-positive spore-forming bacteria widely distributed in the environment. The spores are resistant to drying and are easily spread with dust. *Bacillus cereus* is found in a variety of foods but typically occurs only in low numbers, especially in raw foods. *Bacillus cereus* can cause either diarrhea in 8–16 h by producing a toxin in the intestine similar to *C. perfringens* or can cause nausea and vomiting by a toxin preformed in the food 0.5–5 h after ingestion. Seafood subjected to a mild heat-treatment combined with subsequent cold-temperature storage such as in the production of *sous-vide* products, may increase risk as heat processing induces spore formation. Except for the few psychrotrophic strains, *B. cereus* growth is well controlled by chilling.

Animals and humans are the major reservoirs of pathogens such as *Salmonella* spp., *Shigella* spp., *Escherichia coli*, *Campylobacter jejuni* and other mesophilic *Campylobacter*, and *Staphylococcus aureus.* Humans are the only known source of enteric viruses such as norovirus (NoV) and hepatitis A virus (HAV).

Contamination of fish products is almost always due to poor hygiene (poor personal hygiene, poor processing hygiene or poor water quality). Feces is the main source of most of these pathogens and seafood contamination usually occurs in production areas via direct discharge or after inadequate sewage treatment. Bivalve mollusks present the greatest risk to consumers for bacterial and viral pathogens of fecal origin as they concentrate these organisms through filter feeding and they are often consumed raw. Bacterial pathogens have been recognized as an important cause of illness due to consumption of raw bivalve mollusks for almost a century. Most developed countries have well-established control systems for bivalve mollusks and/or their growing areas based on analysis of certain populations of the coliform bacteria group. Early in the twentieth century, outbreaks of typhoid fever linked to consumption of raw oysters and clams were reported often in the US. Improved wastewater treatment, incorporation of better hygienic practices and a system that included sanitary surveys and continual testing of the overlying waters for coliform bacteria virtually eliminated shellfish related typhoid fever outbreaks in the US in less than a decade. Similar systems are used in the European Union, Canada, Mexico, and New Zealand based on testing of shellfish meats alone or combination of testing shellfish meats and overlying waters and this approach has been equally successful for controlling enteric bacterial pathogens. These systems rely on levels of indicator bacteria including total coliforms, fecal coliforms, and *E. coli*.

Sanitary controls have been much less effective in the control of viral outbreaks, especially those caused by NoV. Apparently, many enteric viruses including NoV and HAV survive various sewage treatments, persist much longer in the tissues of bivalve mollusks than indicator bacteria and have a lower infectious dose than the bacterial pathogens. In countries with cooler climates such as those in Europe outbreaks from NoV are much more common than those caused by all other pathogens and are primarily associated with consumption of raw oysters. HAV outbreaks are rarely reported in developed countries with modern sewage treatment facilities and well-established shellfish sanitation programs. However, an HAV outbreak associated with consumption of raw clams in China during the 1990s may have infected as many as a half of million individuals. NoV infections are characterized by mild to moderate diarrhea and vomiting, whereas HAV affects the liver and causes a more severe illness characterized by jaundice. There are currently no culture methods available for NoV and culture methods are not reliable for wild HAV strains and thus there are no means to reliably determine survival or infectivity of these viruses. These are RNA viruses and recently developed reverse transcription real time PCR assays are being increasingly applied to the detection of viral RNA in bivalve mollusks. NoV detection rates range from $<5\%$ to $>50\%$ in bivalve mollusk and higher levels are usually found during colder periods of the year and in shellfish implicated in outbreaks. There are no established tolerances for either NoV or HAV. The US and Canada are currently conducting a joint NoV risk assessment in bivalve mollusks and Codex guidance documents are under development. Bacteriophages specific to *E. coli* also referred to as coliphage appear to behave more like enteric viruses. RNA male-specific bacteriophages are of similar size and shape as the most common enteric viruses such as NoV and HAV and are most often used to model survival, uptake, and elimination of enteric viruses but have had limited application in official control programs. The most effective means for controlling enteric viruses is to prevent their introduction into shellfish growing areas and to restrict harvest after events that may lead to contamination of the growing waters. Enteric viruses are rarely associated with seafood products other than bivalve mollusks.

Salmonella is occasionally detected in finfish and shrimp reared in warm water, especially in fresh or brackish water species but has rarely been linked to human illnesses. The extent to which hygienic practices contributes to Salmonella contamination is uncertain as there are many natural reservoirs such as birds, reptiles, and amphibians that are in frequent contact with both wild and aquacultured species. The presence of Salmonella or other bacterial pathogens in finfish or shrimp is of much less concern than with bivalve mollusks as only muscle tissue is typically consumed and usually after cooking that inactivates most pathogens. The frequency of Salmonella detection in bivalve mollusks produced in countries with well-established shellfish sanitation programs generally ranges from 1 to 2% but has been reported to be as high as 30% in developing countries such as India. Considering the relatively high frequency of Salmonella occurrence in bivalve mollusks, these products are infrequently associated with outbreaks but there may be a significant risk for sporadic cases. It is not known whether Salmonella grows in live bivalve mollusks after harvest but the lack of outbreak reports suggest that significant postharvest growth is unlikely.

Although Salmonella testing is required in some countries, sampling plans are likely to provide only negligible additional protection unless numerous samples are analyzed and are not generally recommended.

Four species of *Shigella* are known and all are human pathogens. The primary route of infection is the fecal-oral route with person-to-person being the most common route of transmission. Unlike *Salmonella*, *Shigella* is not associated with particular food raw materials but its presence is exclusively a question of poor hygienic handling and humans are its natural reservoir. Outbreaks have been caused by a multitude of food products, including shrimp and clams. Although *Shigella* is not naturally present in water, but may survive for up to 6 months if water is contaminated and persists for long periods in clams and oysters. Similarly, the presence of pathogenic *E. coli* and *Campylobacter* spp. in seafood or production waters is indicative of human and animal pollution but these pathogens are seldom associated with illnesses from seafood consumption.

Staphylococcus aureus is Gram-positive bacterium that inhabits the skin and mucous membranes of animal and man. This organism is not a significant hazard associated with seafood production or processing of raw products. The main hazard is with crab meat that is usually handpicked after cooking. The presence of *S. aureus* on crab is an indication of postharvest contamination due to poor personal hygiene and its presence indicates a potential for food poisoning. Time–temperature abuse will allow rapid proliferation of *S. aureus* and formation of heat stable toxins in cooked foods such as crab meat. There is also the potential risk during canning of postprocess contamination with *S. aureus* if container integrity is compromised and containers are handled in an unsanitary manner.

Parasites

Although we are not aware of documented transmission of parasites of intestinal origin from consumption of bivalve mollusks, some studies have shown that oysters can accumulate Giardia and Cryptosporidium and these parasites can remain viable in shellfish tissues for significant periods. Few of the outbreaks of Giardia and Cryptosporidium can be attributed to a specific food commodity and further study is needed to better understand the significance of these findings to human health.

Some species of fish can serve as relatively specific intermediate hosts to a variety of parasites including trematodes, nematodes, and cestodes and can transmit these parasites to humans if fish are consumed raw or are inadequately cooked. Anisakiasis is a significant cause of acute gastrointestinal diseases from consumption of salt-water fish infected with nematodes of either *Anisakis* species or *Psudoterranova decipiens*. Human infection typically occurs by ingesting raw or undercooked fish such as tuna, salmon, herring, or mackerel for *Anisakis* species and cod or squid for *P. decipiens*. Inadequate fish treatment can allow *Anisakis* to attach to the human stomach and intestines where it can cause lesions and may require surgical removal. It is estimated that there are more than 2000 cases of Anisakiasis per year in Japan. Cases have also been described in coastal areas of the Netherlands, Germany, France, Spain, and US, but are much less prevalent than in Japan. Consumption of raw, lightly cooked, or pickled freshwater fish may cause an estimated 70–100 million infections globally with trematodes consisting of *Clonorchis* species. China, Japan, Korea, Taiwan, and Vietnam are the leading countries for trematode infections. Trematodes of other genera can infect humans including *Opisthorchis* spp. (prevalent in Poland, Eastern Germany, and in parts of the old Soviet Union), *Heterophyes heterophyes* and *Metagonimus yokogawai* (commonly occur in Japan, Laos, Thailand, Korea, Hawaii, Balkans, Philippines, China, Taiwan, Turkey, and Siberia) and, *Echinostoma* spp. including *E. ilocanum*, *E. revolutum*, *E. malayanum*, *E. echinatum* and *E. hortens* (commonly occur in Korea, the Philippines, Indonesia, Malaysia, and Thailand). *Capillaria philippinensis* causes infections in the Philippines and Thailand from consumption of raw or undercooked freshwater fish and appear to be spreading to other regions. The cestode, *Diphyllobpthrum latum* has caused infections in northern regions of Europe and North America, mainly from consumption of raw or undercooked lake trout. Similarly, *Diphyllobpthrum nihonkaiense* has been associated with infections in Japan, linked to consumption of salmon and trout.

Most parasites are very susceptible to heat inactivation and freezing with brief storage times. Cooking is generally sufficient to inactivate most parasites. Freezing of fish intended for raw consumption at $-20\,^{\circ}C$ or below for 7 days or at $-35\,^{\circ}C$ for 15 h also kills parasites. Other procedures such as brining, pickling, trimming away the belly flaps or candling, and physically removing parasites may reduce the hazard in fish but will not eliminate all parasites or reduce the hazard to an acceptable level.

Decomposition

Seafood commodities are among the most perishable of all foods and rapidly produce numerous compounds associated with objectionable odors such as ammonia and trimethylamine when improperly refrigerated. Decomposition is primarily a quality issue with most seafood commodities and readily detected by smell. However, scombroid (e.g., tuna and mackerel) and some nonscombroid (e.g., sardine and herring) fish contain relatively high levels of free histidine in their tissues for osmoregulation. Certain histamine forming bacteria (e.g., Enterobacteriaceae, *Clostridium*, *Lactobacillus*, *Vibrio*, and *Photobacterium*) can decarboxylate histidine into histamine if postharvest temperatures permit their growth. Although histamine can be produced in fish captured by drift nets or long lines if they die and soak in warm waters for extended periods before harvest, the most significant problems occur by delayed or inadequate cooling after harvest. Histamine is heat stable and is not inactivated by cooking or canning. Therefore, good hygienic practices for the preservation and handling from capture to consumption are essential to prevent histamine production. Human reactions to histamine vary from rashes and breathing difficulties to severe shock. The Codex Alimentarius Commission adopted a standard for some fish species for maximum histamine levels of 20 mg/100 g fish flesh.

Seafood producers or processors sometimes attempt to hide visual evidence of decomposition. For example, antioxidants such as sulfites can be used to disguise evidence of

spoilage such as black spots in shrimp; these compounds can cause allergic reactions in sensitive consumers. Carbon monoxide treatment is often used to produce a bright red color in tuna that can mask decomposition colors. The origin, postharvest history and supplier assurance certifications provide important information on whether one of these particular hazards is reasonably likely to occur.

Marine Toxins

Diverse and widespread marine microalgal species produce a variety of biotoxins that can be accumulated in bivalve mollusks. These can cause human illness syndromes including diarrhetic shellfish poisoning (DSP, caused by okadaic acid), paralytic shellfish poisoning (PSP, caused by saxitoxin), neurotoxic shellfish poisoning (NSP, caused by brevetoxin), amnesic shellfish poisoning (ASP, caused by domoic acid), or azaspiracid shellfish poisoning (AZP). The phytoplankton associated with these syndromes are typically bloom forming dinoflagellates and diatoms, known collectively as harmful algal blooms (HABs). These organisms are bioaccumulated by shellfish during filter feeding and then release their toxins into the shellfish tissues. In most cases a complex suite of toxins can be produced by a given phytoplankton species and these can be further metabolized by the shellfish, producing in some cases more potent derivatives of the precursor molecules. The existence of multiple toxin classes and multiple congeners within a given class complicates monitoring and analysis. For this reason, the mouse bioassay continues to be the basis of most national safety standards for these toxins due to the ability to detect total composite toxicity. The majority of shellfish toxins are heat stable and are not inactivated or degraded by cooking. For the PSP toxins, cooking can in fact increase toxicity by converting the less toxic derivatives into more toxic forms. Thus, it is crucial to monitor and control this hazard in the shellfish growing areas. In most cases, harmful algal blooms are visible in the water before shellfish become toxic but identification and enumeration of the toxic phytoplankton requires special skills and costly equipment so routine monitoring using the mouse bioassay is usually recommended in areas where HABs frequently occur. With the increasing availability of advanced instrumentation, such as mass spectrometry, paired with observations by electron microscopy, there have been numerous discoveries of new toxic algal species and novel toxins and derivatives. Additionally, well-studied toxins such as PSP appear to be expanding in range and some suggest that ballast discharge from international shipping and climate change are at least partially responsible for expansion in the geographical ranges of some toxic algal species. The control of this hazard in bivalve mollusks depends largely on knowledge of shellfish origin, environmental status of the harvest area, and monitoring efforts and controls in that area. When biotoxins are detected above safe thresholds in the tissues of bivalves, the growing area must be temporarily closed for harvesting, and shellfish tested until toxin levels return to safe levels. The Codex Alimentarius Commission adopted the following standards maximum tolerable levels (per kg) in bivalve mollusk tissues: saxitoxin group $\leq$0.8 mg; okadaic acid group $\leq$0.16 mg; domoic acid group $\leq$20 mg; brevetoxin group $\leq$200 mouse units or equivalent; azaspiracid group $\leq$0.16 mg.

Predatory reef fish such as barracuda, grouper, snapper, and amberjacks from tropical areas can bioaccumulate ciguatera toxin. Ciguatera toxin precursors are produced by dinoflagellates of the genus *Gambierdiscus* that colonize sessile macroalgae, corals, and seagrasses which are grazed by herbivorous fish. These toxins are metabolized and biomagnified by piscivorous fish and transferred to higher trophic levels of the coral reef food web. Toxin structure and associated toxicity varies substantially by region with structural variants identified in the Pacific, Caribbean, and Indian Oceans. More than 400 species of fish are known to be vectors of ciguatoxins. Likewise the fish species in which the toxin accumulates is important. For instance, Nassau grouper have rarely been implicated with ciguatera fish poisoning in the Caribbean, but the closely related yellowfin grouper is almost always toxic in this region. Ciguatoxins are among the most toxic compounds in nature and can cause serious and often long-term health effects. Ciguatoxins causes disease at levels below 1 ppb in the flesh of the fish. Clinical symptoms vary widely but are characterized by gastrointestinal, neurological, and cardiovascular disturbances not only within 10 min but also up to 24 h after ingestion of toxic fish, but death is rare. Controls are mostly limited to identification of the fish species and harvest restrictions in known 'hot spots.' Ciguatoxins are heat stable and cooking provides no protection and there are no effective treatments. The mouse bioassay may be used to detect ciguatoxins from fish but few laboratories have the analytical capabilities for their detection.

Tetrodotoxin (TTX) is a potent marine neurotoxin with a similar activity to the paralytic shellfish toxins. This toxin has been primarily associated with puffer fish in the marine environment but can also be found in the blue ringed octopus, newts, frogs, and a variety of freshwater fish. Consumption of these fish can cause severe foodborne intoxication. TTX is heat stable and is not affected by cooking or freezing. Certain puffer fish species are considered a delicacy and a special culinary license is required in order to prepare the fish flesh safely for consumption. Avoidance of known TTX-containing species and/or known toxic parts is the primary control measure for this toxin class as it is not associated with phytoplankton blooms or other known microorganisms.

PSP, DSP, ASP, AZP, TTX, and palytoxin may be found in the viscera of a variety of crab species in certain geographical regions; PSP can also occur in the hepato-pancreas of lobsters.

Chemical Contaminants

There are numerous classes of chemical contaminants present in seafood production environments and these vary greatly in their route of introduction, geographical distribution, and toxicity to humans. Some heavy metals and petroleum hydrocarbons have both natural and anthropogenic sources, others such as pesticides and industrial chemicals are always associated with human activity and some such antimicrobials are introduced intentionally as therapeutic or growth promotion agents.

Table 1 Tolerances and critical limits of environmental chemical contaminants in fish and fish products for US, EC and Codex

Substance	*Maximum levels*			*Food commodity*
	US (ppm)	*EU (mg per kg wet weight)*	*Codex*	
Arsenic	76 (crustaceans), –86 (mollusks)			Mollusks, crustaceans
Cadmium	3.0 (crustaceans), 4.0 (molluskan bivalves)	0.05–1.0 (bivalves, cephalopods)	2 (marine bivalve mollusks and cephalopods)	Fish, mollusks
Lead	1.5 (crustaceans), 1.7 (molluskan bivalves)	0.3–1.0	0.3 (fish)	Fish, mollusks
Methylmercury	1.0	1.0	0.5 (fish), 1.0 (predatory fish)	All fish
PCB	2.0			All fish
DDT, TDE	5.0			All fish
Diedrin	0.0			All fish
Dioxin		0.000004		

Methylmercury is the most widespread and serious risk among anthropogenic and/or natural (volcanoes) contaminants and can cause nervous system disorders in the developing fetus, infants, and young children. In 2003, the FAO/WHO Joint Expert Committee on Food Additives (JECFA) revised the provisional tolerable weekly intake (PTWI) for methylmercury from 3.3 to 1.6 μg per kg of body weight per week. Highest levels are found in larger, long-living predators (e.g., sharks, swordfish, and king mackerel) but greatest exposure occurs with consumption of tuna as it is one of the most heavily consumed seafoods globally. Some government agencies issue an advisory on consumption of fish for women and young children, focused on the benefits of eating fish while rducing their exposure to the harmful effects of mercury. Other heavy metals such as arsenic, chromium, and lead can cause adverse human health affects and various international tolerance for these are shown in Table 1. Exposure of aquatic animals to petroleum products can occur from tanker accidents, blow outs of offshore wells, discharges from vessel bilge water and natural seeps from seafloor vents. Polycyclic aromatic hydrocarbons (PAHs) are the most toxic components of petroleum products including crude oil and their proportions vary considerably depending on the source and weathering of the oil. After a petroleum spill or discharge areas with a visible sheen on the water surface are generally closed for both commercial and recreational fishing. After the sheen disappears, human sensory analysis is usually sufficient to detect odor indicative of elevated risk and is often used to screen suspected seafood samples. In high profile spills, sensory results are often confirmed using analytical methods such as gas chromatography-mass spectroscopy. Formation of PAH during smoking and drying of fish and other seafood may increase cancer risk. PAH formation can be minimized by following practices described in the Codex Alimentarius Code of Practice for the Reduction of Contamination of Food with Polycyclic Hydrocarbons (PAH) from Smoking and Direct Drying Processes (CAC/RCP 68-2009).

Exposure to agricultural chemicals such as pesticides and industrial chemicals such as dioxin is typically from direct discharge into rivers, leaching from contaminated soil or from storm water runoff. The main risk is elevated cancer rates from chronic exposures. Some of these chemicals persist for long periods in the environment and permanent closures or advisories are the only options to control these hazards once the environment has become contaminated as these chemicals are stored for extended periods in fatty tissues and are heat stable. Various international tolerances for these chemicals are shown in Table 1.

The use of veterinary drugs such as antimicrobial compounds and other chemical treatments are widely used in production of aquacultured finfish worldwide primarily to control disease. Unregulated/unapproved veterinary drugs administered to aquacultured fish pose a potential human health hazard. These substances may be carcinogenic, allergenic, and/or may cause antibiotic resistance. Low levels of antimicrobial residues are often detected and are the basis of regulatory action in countries importing finfish and other seafood products but have rarely been linked to adverse human health effects. There are antimicrobial agents that have been deemed safe by international organizations but the problem often arises due to off-label use or unapproved drugs. The following Codex standards were established for MRL of chlortetracycline/oxytetracycline/tetracycline at 200 $\mu g\,kg^{-1}$ in fish muscle in general and specific species. Deltamethrin (used also as pesticide) at 30 $\mu g\,kg^{-1}$ in salmon muscle, and flumequine at 500 $\mu g\,kg^{-1}$ in trout muscle including normal proportion of skin.

See also: Bacteria: *Aeromonas*; *Clostridium botulinum*; *Clostridium perfringens*; *Listeria monocytogenes*; Other Vibrios; *Plesiomonas shigelloides*; *Salmonella* Non-Typhi; *Staphylococcus aureus*; *Vibrio cholerae*; *Vibrio parahaemolyticus*; *Vibrio vulnificus*. Disciplines Associated with Food Safety: Food Virology. Environmental Contaminants: Dioxins, Furans and Dioxin-like Polychlorinated Biphenyls. Food Safety Assurance Systems: Good Practices in Fisheries and Aquaculture; Hazard Analysis and Critical Control Point System (HACCP): Principles and Practice. Foodborne Diseases: Overview of Biological Hazards and Foodborne Diseases. Natural Toxicants: Tetrodotoxin. Processing Contaminants: Biogenic Amines; Polycyclic Aromatic Hydrocarbons (PAHs). Toxic Metals: Arsenic; Cadmium; Mercury. Viruses: Hepatitis A Virus

Further Reading

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SAFETY OF FOOD AND BEVERAGES

Meat and Meat Products

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Glossary

Bacteriophage A virus that infects its specific bacterial cell host.
Bovine spongiform encephalopathy A neurodegenerative fatal disease in cattle that results in the formation of a spongy degeneration in the brain and spinal cord.
Campylobacteriosis The infection caused by the bacterium *Campylobacter*.
Competitive exclusion Competing bacterial species with one having an advantage over the other.
Cross contamination Transfer of bacterial cells from one food, and/or its environment, to another food.
Enteric Intestinal.
Enteritis Inflammation of the small intestine.
Enterohemorrhagic Intestinal bleeding detected in diarrheal stools.
Hemolytic uremic syndrome A rare, but potentially fatal, condition that can lead to kidney failure, mostly in children and immunocompromised adults, caused by shiga toxin-producing bacteria such as *Escherichia coli*.
Hemorrhagic colitis A self-limiting, but sometimes fatal, bacterial infection, often attributed to strains of *Escherichia coli*, that is characterized by abdominal cramps and bloody diarrhea.
Hurdle technology Application of a combination of individually sublethal antimicrobial hurdles for control of microorganisms and assurance of safety and stability in food products.
Listeriosis Invasive infection caused by *Listeria monocytogenes*.
Meningitis Bacterial infection of the meninges (membranes of the brain and spinal cord).
Meningoencephalitis A condition resembling meningitis and encephalitis or inflammation of the brain.
Mycotoxins Acutely toxic or carcinogenic (depending on level of exposure) secondary metabolites of certain fungi or molds that may form under certain conditions in some foods.
Nosocomial infection Infection acquired in a hospital.
Outbreaks At least two linked cases of foodborne disease of the same symptoms and associated with the same food.
Prebiotics Nondigestible ingredients that stimulate growth and/or activity of certain bacteria in the digestive system resulting in interference with pathogenic bacteria.
Prion Abnormal transmissible, pathogenic agents, inducing abnormal folding of specific normal cellular proteins called prion proteins in the brain and leading to brain damage and the characteristic signs and symptoms of prion diseases (i.e., transmissible spongiform encephalopathies (TSE), such as bovine spongiform encephalopathy).
Probiotics Live microbial cultures that may be beneficial by interfering with pathogens.
Septicemia Bacterial invasion of the blood (bacteremia).
Serotype Group of closely related microorganisms reacting to a characteristic set of antigens.
Shiga toxins, shiga-like toxins, verotoxins, or verocytotoxins Toxins, produced by the strains of enterohemorrhagic *Escherichia coli* (EHEC), causing hemolytic uremic syndrome.
Toxicoinfection Illness caused by toxins produced by noninvasive bacteria growing in human intestines.
Water activity The amount of chemically unbound water in a food that is related to relative humidity and is available for growth of microorganisms.

Introduction

The major challenges associated with meat safety and quality are microorganism-associated issues. Microbial pathogens cause mild, severe, brief or chronic human illness, or death. Loss of quality is caused by spoilage-causing microorganisms, which by shortening shelf life lead to reduced food supplies and economic losses. The microbiological status of meat products is influenced by system failures or abuses during animal food production, product processing and distribution, and preparation for consumption. Despite recent improvements in meat processing, documented disease episodes and concern about meat products acting as vehicles of meat-borne hazards are increasing rather than diminishing. Reasons for this may include increased international food trade, consumer preferences for minimally processed products, increased worldwide meat consumption, increasing numbers of consumers at risk for infection, emerging pathogens of increased virulence and resistance to control or clinical treatment, advances in microbial detection methodologies, and

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inadequate food handler and consumer education and training in proper food handling. In addition, there is increased interest, awareness, and scrutiny of foodborne illness episodes by news media, consumers, and consumer organizations. Major challenges associated with unsafe food include microbial foodborne illnesses and deaths, product recalls, and related issues of regulatory compliance.

The knowledge of sources of contamination and the properties of pathogens allows application of proper procedures for pathogen control and enhancement of food safety. As sensitive to contamination and microbial growth, meat products should be handled and preserved properly to maintain their quality and safety. Microbial control in meat products is accomplished through the implementation of interventions or procedures that: (1) prevent or minimize the access of microorganisms into the product; (2) reduce initial contamination by the removal or inactivation of microorganisms, which have gained access; (3) inactivate microorganisms on products; and (4) prevent, delay, or slow down growth of viable microorganisms, which have gained access and have not been inactivated. Proper implementation and management of such an approach ensures the safety of meat products or at least reduces the incidence of microbial meat-borne illness.

Contamination of Meat Products

The interior of muscle tissues in healthy animals is generally sterile before slaughter, whereas various organs (e.g., liver and lymph nodes) may sporadically contain low numbers of microbial cells. In contrast, animal surfaces exposed to the environment, such as hides, pelts, fleece, the mouth, and the gastrointestinal tract, are usually heavily contaminated. Sources contributing microbial contamination to animals and meat during animal slaughter and dressing of carcasses include feces, soil, water, air, feed, hides, intestines, lymph nodes, processing equipment, utensils, and humans. Identifying the sources and modes of meat product contamination is important in applying proper approaches and procedures for hazard control and enhancement of meat safety.

Overall, the cycle of contamination involves animal feces and manure, soil, decaying matter, water, air, pastures and other animal feeds, and animals and their products, as well as water and other foods or the environment. Additional vehicles of biological hazards within this continuum may include rodents, birds, insects, animal transportation crates and vehicles, and other equipment and utensils, which contribute to cross contamination. Animal manure may contaminate water used for drinking or to irrigate or wash plant crops resulting in cross contamination of other foods, as demonstrated by the increased occurrence of foodborne outbreaks with enteric pathogens present in vegetables. The extent of microbial transfer from the above sources to meat and other foods depends on sanitation and hygienic practices, product handling and processing procedures, and conditions of storage and distribution. In general, meat products become easily contaminated during animal slaughter, carcass dressing and cutting, meat processing, storage, merchandizing, preparation, and serving for consumption. Overall, animal production, and product processing and handling practices result in contamination, which may vary depending on animal type and age, geographic region, season of the year, and other variables; for example, *Escherichia coli* O157:H7 prevalence is usually higher during the warmer months.

As sources of contamination are diverse, and facilities and practices of slaughtering and processing operations are variable, there may be variation in the types of microorganisms introduced and, especially, in the extent (prevalence and density) of contamination of meat or other foods. Initial contamination of meat consists of Gram-negative and Gram-positive bacteria, yeasts, molds, parasites, and viruses. Presence of pathogens in animal products processed under sanitary and hygienic conditions should generally be infrequent and at low levels. As contamination is unpredictable, any raw, unprocessed, and uncooked meat product should be considered, treated, and handled as potentially contaminated with pathogens.

Food Safety Concerns in Meat Products

Chemical hazards that may be present in meat products include mycotoxins resulting from feeding animals with moldy feeds or due to uncontrolled mold growth in certain meat products aged improperly and for longer periods of time. Use of chemicals (e.g., antibiotics, hormones, and growth promoting agents) during meat animal production, for better growth, feed efficiency, and disease control, in some countries, is of concern to consumers, and causes trade conflicts among certain countries. Some consumers are also concerned with the use of additives (e.g., common salt, nitrate, nitrite, and other compounds) in processed meat products. In general, the contribution of additives and their residues to the overall food safety concerns in meat products is considered minor or negligible, particularly in industrialized countries where there is extensive legislation for their control. Presence of residues of certain toxic chemicals, such as dioxins, in meat products has caused major consumer scares from time-to-time. The debate over the positive and negative aspects of meat in the human diet also reemerges from time-to-time.

There is a long list of biological hazards, including bacteria, viruses, parasites, and prions that may potentially be present in animals and meat products, and sometimes transmitted to humans. Some hazards are transmitted to humans through consumption of meat products, whereas others have not been documented as being transmitted to humans through handling or consumption of meat products. Other hazards present in animals are associated with nonfood transmission routes such as aerosols and direct contact with animals or diseased tissues. Hazards of most concern in terms of severity of illness and deaths are bacterial, whereas viruses usually cause large numbers of mild gastrointestinal illness as a consequence of poor sanitation and unhygienic practices; parasites have minor involvement in meat-borne illness in developed countries (**Table 1**).

Bacterial pathogens that have been documented as transmitted through meat products include pathogenic *Bacillus* spp. (e.g., *Bacillus cereus*), *Campylobacter* spp. (thermophilic), *Clostridium perfringens*, *Clostridium botulinum*, *Escherichia coli*

Table 1 Biological hazards in meat products and their origin, products affected, type of illness, need for growth in food before consumption, and control approaches

Biological agent	*Product*	*Type of illness*	*Sources*	*Growth in food*	*Control approach*
Bacteria					
Bacillus cereus	Beef, pork, lamb, and poultry	Intoxication (emetic); toxicoinfection (diarrheal)	Soil and animals	Needed (some grow in the cold)	Inactivate spores (heat) or control growth (refrigeration)
Campylobacter spp. (thermophilic)	Beef, pork, lamb, and poultry	Invasive infection	Animals	Not needed	Inactivate (heat processing or cooking)
Clostridium botulinum	Beef, pork, lamb, and poultry	Intoxication (toxicoinfection, infants)	Soil, water, and animals	Needed	Inactivate spores (canning) or control growth (refrigeration)
Clostridium perfringens	Beef, pork, lamb, and poultry	Toxicoinfection	Soil, water, and animals	Needed	Inactivate spores (heat processing or cooking) or control growth (refrigeration)
Escherichia coli (STEC/EHEC)	Beef, pork, and lamb	Toxicoinfection	Animals	Not needed	Inactivate (pasteurization and cooking)
Listeria monocytogenes	Ready-to-eat beef, pork, and poultry	Invasive infection	Processing environment, soil, water, and animals	Needed (grows in the cold)	Inactivate (heat) or control growth (freezing, no long-term refrigerated storage)
Salmonella enterica	Poultry, beef, pork, and lamb	Invasive infection	Animals	Not needed	Control growth (refrigeration) and inactivate (pasteurization or cooking)
Staphylococcus aureus	Ready-to-eat beef, pork, lamb, and poultry	Intoxication (heat resistant toxin)	Humans, processing environment, and animals	Needed	Control growth (refrigeration)
Yersinia enterocolitica	Pork and poultry	Invasive infection	Animals, soil, and water	Needed (grows in the cold)	Control growth (freezing) and inactivate (pasteurization or cooking)
Parasites					
Cryptosporidium parvum	Beef and poultry	Invasive infection	Meat	Not needed	Freezing, salting, pasteurization, cooking, or irradiation[a]
Giardia duodenalis	Beef	Invasive infection	Meat	Not needed	Freezing, salting, pasteurization, cooking, or irradiation
Sarcocystis	Beef and pork	Invasive infection	Meat	Not needed	Freezing, salting, pasteurization, cooking, or irradiation
Taenia cysticercus	Beef and pork	Invasive infection	Meat	Not needed	Freezing, salting, pasteurization, cooking, or irradiation
Toxoplasma gondii	Beef, pork, and poultry	Invasive infection	Meat	Not needed	Freezing, salting, pasteurization, cooking, or irradiation
Trichinella spp.	Pork and game	Invasive infection	Meat	Not needed	Freezing, salting, pasteurization, cooking, or irradiation
Viruses					
Hepatitis E	Pork	Invasive infection	Animals and humans	Not needed	Sanitation[b] or cooking
Other foodborne viruses	Ready-to-eat meats and other foods	Infection	Humans	Not needed	Sanitation or cooking
Prions (encephalopathies)					
Bovine spongiform encephalopathy (BSE)	Beef	Invasive infection	Animals	Not needed	Special controls during animal growth and slaughter

[a]Irradiation is effective against all pathogens (exceptions: prions; bacterial spores and viruses need unacceptably high doses).
[b]Sanitation and hygienic conditions are crucial for control of all pathogens.

serotypes, especially shiga toxin-producing/verotoxigenic *E. coli* (STEC/VTEC) and enterohemorrhagic *E. coli* (EHEC) strains, *Listeria monocytogenes*, *Salmonella enterica* serotypes, *Staphylococcus aureus*, and *Yersinia enterocolitica*. Pathogenic bacteria considered as potentially transmitted through meat, include *Aeromonas*, *Arcobacter* (previously mesophilic *Campylobacter*), *Bacillus anthracis*, *Brucella*, *Clostridium difficile*, *Enterobacter*, *Helicobacter*, *Mycobacterium*, *Plesiomonas*, and *Shigella*.

The antimicrobial resistance exhibited by strains of some bacteria such as *Campylobacter* and *Salmonella* is also of concern. Strains may be more resistant to control procedures, survive better in their hosts, and be more virulent at lower doses. Adaptation and development of resistance by bacteria to antibiotics and potentially to traditional food preservation barriers such as low pH, heat, cold temperatures, dryness or low water activity, and chemical additives is an issue that needs further consideration and evaluation. A common sense approach for the control of antimicrobial resistance is not to overuse or abuse antibiotics in animals and humans; prudent use is recommended. Decisions on approaches for control should be based on risk analysis and examination of all issues associated with each specific type of antibiotic application and concern. Pathogen resistance issues and concerns become more important when considered in light of societal changes, including changes in consumer food preferences, lack of adequate consumer and food handler education in proper food handling, increases in number of populations at risk for microbial foodborne illness, complex meat distribution patterns that may lead to product abuses, increased international meat trade and associated risks, and better methods of testing for microbial detection.

Some microorganisms are of concern because they are responsible for spoilage and loss of eating quality in meat products. Spoilage causing microorganisms found on fresh meat include micrococci and Gram-negative rods. Common genera and groups include *Pseudomonas* spp., *Enterobacteriaceae*, *Acinetobacter* spp., *Alcaligenes* spp., *Moraxella* spp., *Flavobacterium* spp., *Aeromonas* spp., *Staphylococcus* spp., *Micrococcus* spp., *Brochothrix thermosphacta*, coryneforms, fecal streptococci, lactic acid bacteria, etc. Risks to consumers from consumption of stored meat products may also be associated with the presence of biogenic amines, such as histamine, putrescine, spermidine, and spermine, which are formed by the growth of spoilage and fermentative microorganisms.

Additional issues related to meat include the safety and quality of organic and natural products, the need for and development of improved and rapid testing and pathogen detection methodologies for laboratory and field use, regulatory inspection harmonization issues at the national and international level, establishment of risk assessment based food safety objectives, and routine implementation of the hazard analysis critical control points (HACCP) system at the production and processing level on the basis of food handler training and consumer education. Issues such as bovine spongiform encephalopathy (BSE) will continue to be of interest mostly as a target for eradication.

The issue of humane treatment and welfare of food animals deserves increased attention worldwide. Animal stressing should be avoided because it may damage meat quality and lead to higher pathogen shedding and increased contamination. In addition, ethics dictate the need for humane handling and treatment of animals.

Animal health pandemics, such as avian influenza and foot-and-mouth disease, irrespective of any impact on human health, may cause major economic losses to local, domestic, or international markets. In addition, they may develop into trade issues, and even technical, economic, political, or diplomatic challenges. National and international health authorities, through global cooperation for risk-based contingency planning, need to address such issues as effectively as possible based on early detection and diagnosis with the goal of prevention, containment, and eradication.

The ability to maintain verifiable custody of the identification of animals and their products, or traceability, from production to retail, is regarded as an important tool for the protection of animal and public health. It is increasingly accepted that traceability can play a major role in management of food safety risks and in product authentication. However, traceability of composite products, such as ground beef, is complicated and difficult, if not impossible, to apply in commerce.

Biological Hazards in Meat and Meat Products

Some pathogens may be of no major concern as meat-borne in developed world regions such as the US and the European Union. For example, *Bacillus anthracis* may cause endemic disease in Africa and Asia through direct contact with infected animals or carcasses. Extensive exposure through processing of hides and wool in enclosed facilities may result in pulmonary anthrax through inhalation of aerosolized spores. The cutaneous form of anthrax may be acquired through handling of contaminated hides or wool. Consumption of raw or undercooked meat from infected animals, showing clinical signs of the disease, may lead to gastrointestinal anthrax. *Bacillus cereus* causes intoxication (emetic form) or toxicoinfection (diarrheal form) after spore germination and multiplication in foods, including meat, but illness from consumption of other foods is more common (**Table 1**).

Spores of *Clostridium* are found in soil, dust, and water as well as in the intestines of animals. Thus, they may be present in many foods including meat products. Common species associated with food- including meat-borne transmission are *C. botulinum* and *C. perfringens*. They may become a problem when the food is temperature abused and allows growth of the pathogen. *Clostridium difficile* is an emerging animal pathogen with increased incidence and severity in nosocomial infections. Potential sources of the organism include farm (cows, pigs, and horses) and domestic (dogs and cats) animals. *Clostridium difficile* has been isolated from samples of meat and poultry products. The limited data available do not allow determination of the role, if any, of meat in the epidemiology of *C. difficile* human infections.

Bacterial hazards such as *Coxiella burnetii* are primarily transmitted to humans through aerosols, direct contact, or consumption of foods such as milk, but not meat. Similar modes of transmission exist for *Mycobacterium avium*, *M. avium* subspecies *paratuberculosis*, *Mycobacterium bovis*, and

Mycobacterium tuberculosis. Evidence for the presence of *M. bovis* in meat is limited and inconclusive, and transmission through meat consumption has not been verified. Confirmed modes of transmission include aerosols and consumption of unpasteurized milk. *Mycobacterium paratuberculosis* causes chronic enteritis in ruminants, such as cattle, named Johne's disease. Humans develop Crohn's disease, a similar chronic inflammatory condition of the intestine but any relationship of the pathogen with this disease has not been verified. The pathogen was recovered from raw and cooked meat samples containing mesenteric lymph nodes derived from carcasses of cows with advanced Johne's disease, indicating that human exposure to *M. paratuberculosis* should be avoided. *Shigella* becomes a problem only if introduced by humans in ready-to-eat products. *Staphylococcus aureus* may be present in raw meat but, because it is outcompeted by other bacteria, it may become a problem only in processed meat products where it is introduced usually by humans and, if the product is temperature abused, is able to produce heat resistant enterotoxins because competition by spoilage organisms is limited. *Yersinia enterocolitica* includes certain pathogenic serotypes, which may be transmitted with foods contaminated through water, as well as with pork products.

Most bacterial pathogens described above have been implicated in meat-borne illness. Currently, the bacterial pathogens receiving most attention by scientists, regulators, public health, and industry for their presence in fresh meat are *Campylobacter*, *E. coli* serotype O157:H7, and *Salmonella*, whereas *L. monocytogenes* is of concern in ready-to-eat meat products. Additional pathogens receiving attention presently are STEC/VTEC other than *E. coli* O157:H7, and antibiotic resistant strains such as those of *Campylobacter* and *Salmonella*.

Campylobacter

The pathogen is found in all food animals, but it is most common in poultry. Campylobacteriosis is one of the most frequently reported bacterial foodborne illnesses in the US and the European Union. Most illnesses are attributed to handling and consumption of broiler meat, whereas other meats and cross contamination are also important sources of *Campylobacter*. Its survival and growth at normal or cold conditions and in dry environments is limited, and it is sensitive to cooking temperatures.

Shiga Toxin-Producing or Verotoxigenic *E. coli*

Most serotypes of *E. coli* are harmless inhabitants of the gastrointestinal tract of humans and other warm-blooded animals. Strains of certain serotypes cause diarrheal type illness. Of the various disease causing *E. coli* groups, STEC/VTEC, including the EHEC strains are of most concern in undercooked meat products, especially nonintact meat products such as ground beef. From the gastrointestinal tract of ruminant animals, such strains contaminate animal exteriors, soil, and water, and consequently meat products and foods of plant origin. STEC/VTEC serotypes cause mild diarrhea, severe bloody diarrhea (i.e., hemorrhagic colitis) or in some cases hemolytic uremic syndrome (HUS), which is characterized by various complications including acute renal failure. Serotype O157 is involved in 80% of HUS cases in North America; approximately 6% of infected individuals develop HUS. Other foods implicated in infection are fermented meat products such as salami, unpasteurized milk and cheese, fruit juice, sprouts, lettuce, spinach, cantaloupe, and mushrooms. As the STEC/VTEC of most concern, *E. coli* O157:H7 has been declared as an adulterant for raw ground beef and other nonintact beef products in the US. Additional pathogenic STEC/VTEC serotypes (i.e., O26, O45, O103, O111, O121, and O145) are also considered for similar regulatory action in the US.

Listeria monocytogenes

This pathogen causes a severe invasive infection in sensitive individuals such as the elderly, immunocompromised, and the unborn, where it exhibits a case fatality rate of 20–30%. Invasive listeriosis is characterized by serious syndromes of the central nervous system such as meningitis and meningoencephalitis, whereas in its mild form the infection is a typical gastrointestinal foodborne illness. Although pregnant women may develop a flu-like illness, the infected fetus develops meningitis, neonatal septicemia, stillbirth, or spontaneous abortion. The incubation period of listeriosis may vary from a few days to 2–3 months. The infection is usually associated with ready-to-eat meat and poultry products and other foods, in which contamination occurs after processing and is followed by growth during prolonged storage even at refrigeration temperatures. The infectious dose should be greater than 100 cells g^{-1}; however, the possibility of lower infectious doses should not be ignored, especially for sensitive individuals. The organism is ubiquitous in the environment and may be harbored in many animals. It is of major concern because it is hardy and grows under conditions of high humidity, low temperatures, and limited nutrient levels. In meat processing environments it is found in floors, walls, drains, condensed and standing water, and food residues on equipment.

Salmonella

Strains of *S. enterica* subspecies *enterica* serotypes are causes of numerous foodborne illnesses throughout the world. Originating from animal feces, the organism is widely distributed in the environment. Thus, foods involved in human illness include animal products, as well as many other foods, including fruits, vegetables, and dry foods. Meat and poultry products are considered major sources because the main habitat of *Salmonella* is the gastrointestinal tract of food-producing animals. Salmonellosis is caused not only after ingestion of contaminated food or water but also through contact with animals or infected humans. Meat may be contaminated with *Salmonella* throughout the slaughtering, dressing, and boning processes. The United States Department of Agriculture Food Safety and Inspection Service (USDA/FSIS) has established microbiological criteria for *Salmonella* in animal carcasses and ground products as a measure of pathogen reduction since the implementation of HACCP programs. Procedures and interventions controlling STEC/VTEC should also be effective against *Salmonella* strains and similar enteric vegetative pathogens.

Other Biological Hazards and Concerns in Meat

Parasitic agents that may be transmitted with pork include *Trichinella spiralis*, *Sarcocystis* spp., and *Toxoplasma gondii*, resulting in trichinosis, sarcocystis, and toxoplasmosis, respectively (Table 1). Beef may be the source of tapeworms (*Taenia saginata*) and *Sarcocystis hominis*, and through fecally contaminated water may serve as an indirect vector for transmission of *Giardia duodenalis* and *Cryptosporidium parvum*. Poultry may transmit toxoplasmosis. Parasites, such as *Trichinella*, may also be transmitted through game meat. Parasitic hazards such as *Fasciola hepatica* and *Echinococcus granulosus* are primarily transmitted to humans through aerosols, direct contact, or consumption of foods such as milk, but not meat. Inactivation of parasites in foods is achieved through proper cooking, freezing, salting, chemical treatments, and ionizing radiation.

Common viral agents in meat products include norovirus, hepatitis A virus, and enteroviruses. Viruses are usually associated with the highest number of foodborne illness cases. Similar to parasites, viruses are unable to grow in foods and are generally sensitive to cooking. Their control in ready-to-eat foods should be through proper sanitation and hygienic practices of food service workers.

Prion diseases, and especially BSE, emerged as a major animal health problem in the 1990s. The concern was high because of the potential association with human transmissible spongiform encephalopathies such as the new variant Creutzfeldt–Jakob Disease. Preventive controls, including feed bans and control of 'specified risk materials' (brain, skull, eyes, spinal cord, small intestines, etc.) during slaughter of all cattle (small intestines) or those exceeding 30 months of age; increasing process controls for material obtained with 'advanced meat recovery' systems; banning the use of 'mechanically separated meat' in food products; and, banning the use of the above materials in dietary supplements and cosmetics, have resulted in control of BSE. Control programs should continue being implemented worldwide through international collaboration and coordination for potential eradication and prevention of reemergence.

Meat-Borne Illness Episodes

Meat and meat products are important vehicles in the transfer of foodborne hazards to humans. However, considering their high potential to be contaminated and the great quantities of products consumed on a daily basis in terms of servings, their safety record is considered, overall, as high. Nevertheless, any amount of products found potentially contaminated and recalled from the marketplace, as well as any illness, and especially death, through consumption of food, including meat, is unacceptable. Indicative data and reasons for product recalls in the US are presented in Table 2. Data in Table 3 show

Table 2 United States recalls of meat and poultry products in 2011 as reported by USDA/FSIS

Agent or product	*Number of recalls*	*Amount of product recalled (kg)*
Escherichia coli O157:H7	13	454 939
Salmonella	10	16 448 383
Listeria monocytogenes	11	238 589
Undeclared allergen	40	582 353
Other	29	284 405
Beef	35	609 199
Pork	14	337 650
Poultry	31	16 677 325
Mixed meats	23	384 495

Table 3 Food vehicles associated with outbreaks (%) in the European Union (2008 and 2009)

Food vehicle	*2008 (N = 890)*	*2009 (N = 977)*
Bovine meat and products	2.1	2.5
Pork and products	10.2	7.8
Broiler meat	3.7	3.6
Other or mixed meat products	2.6	3.4
Cheeses	1.8	3.4
Other dairy products	1.5	–
Shellfish	3.0	3.6
Fish and fish products	5.5	5.4
Bakery products	9.0	4.3
Eggs and egg products	23.1	17.3
Vegetables and juices	1.9	2.1
Fruits, berries, and juices	–	2.3
Mixed or buffet meals	9.2	8.1
Other foods	16.3	14.2
Unknown	9.9	22.1

Table 4 Outbreaks (%) of illness associated with pork in the European Union (2008 and 2009)

Biological agent	*2008 (N = 83)*	*2009 (N = 76)*
Bacillus	–	2.6
Clostridium	9.6	22.4
Escherichia coli (pathogenic)	–	2.6
Salmonella Enteritidis	3.6	2.6
Salmonella Typhimurium	26.5	6.6
Other *Salmonella*	6.0	6.6
Staphylococcus	9.6	6.6
Other bacteria	2.4	1.3
Viruses	–	2.6
Trichinella	42.2	39.5
Other agents	–	1.3
Unknown	–	5.3

Table 5 Outbreaks and cases (%) of illness associated with broiler meat in the European Union (2008)

Biological agent	*Outbreaks (N = 28)*	*Cases (N = 352)*
Bacillus	3.6	2.8
Campylobacter	21.4	13.9
Salmonella Enteritidis	46.4	57.7
Salmonella Typhimurium	7.1	15.3
Other *Salmonella*	10.7	4.5
Staphylococcus	7.1	1.7
Toxins	3.6	4.0

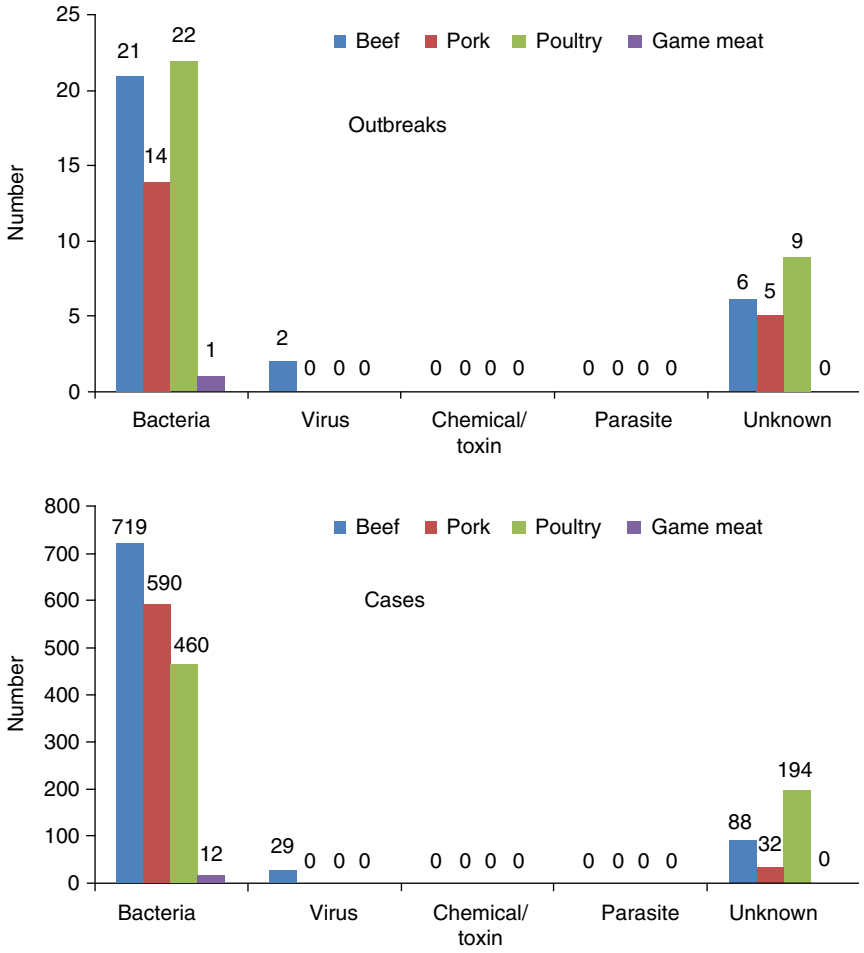

Figure 1 Numbers of confirmed outbreaks and cases due to various agents and meat products in the US (2008).

numbers of confirmed foodborne outbreaks in the European Union in 2008 and 2009, indicating that all meat products combined were responsible for 10–17% of the total outbreaks, compared to 19–25% and 8–9% for fruits and vegetables and fish and seafood, respectively. Data showing numbers of outbreaks and cases of illness associated with pork and broiler consumption in the European Union are shown in Tables 4 and 5, respectively. Figures 1 and 2 present numbers of outbreaks and cases of illness caused by various hazards and associated with consumption of meat products in the US. The data of Table 6 show the locations and settings associated with transmission of the pathogens for foodborne illness of confirmed outbreaks during 2008 and 2009 in the European Union. It is noteworthy that 21–23% and 36–38% of the outbreaks were associated with restaurant and household settings, respectively. The following are examples of outbreaks demonstrating the diversity of causes and reasons for their occurrence:

- In 1988, the first documented incidence of listeriosis due to a meat product was confirmed in the US. It afflicted a female cancer patient, who, on a daily basis, ate turkey frankfurters of the same brand, heated in a microwave oven. *Listeria monocytogenes* was isolated from an open package of product in the woman's home, from unopened product packages obtained from a local store, and from other opened foods in the woman's refrigerator suggesting the occurrence of cross contamination.
- Consumption of undercooked hamburgers from 73 Jack in the Box fast food restaurants in the Pacific Northwest of the US killed four children and sickened 700 others (171 hospitalizations) due to *E. coli* O157:H7 contamination in 1993. The extensive publicity associated with this unfortunate event resulted in political pressure that led to new USDA/FSIS meat inspection regulations.
- In 1994, one person was placed under mechanical ventilation after eating homemade beef stew. The stew was cooked and left on the stove for 3 days before eating without reheating. Botulism toxin type A was detected in the stew.
- An outbreak of *E. coli* O157:H7 occurred in Central Scotland in 1996. It involved a total of 496 cases and 21 deaths of elderly persons. The outbreak was traced to cross contamination between raw and cooked meat at a butcher shop operating under very poor hygiene.
- In 1998, a deadly listeriosis outbreak was linked to consumption of hot dogs and delicatessen meats in 24 states of the US, leading to at least 14 deaths and 4 miscarriages or

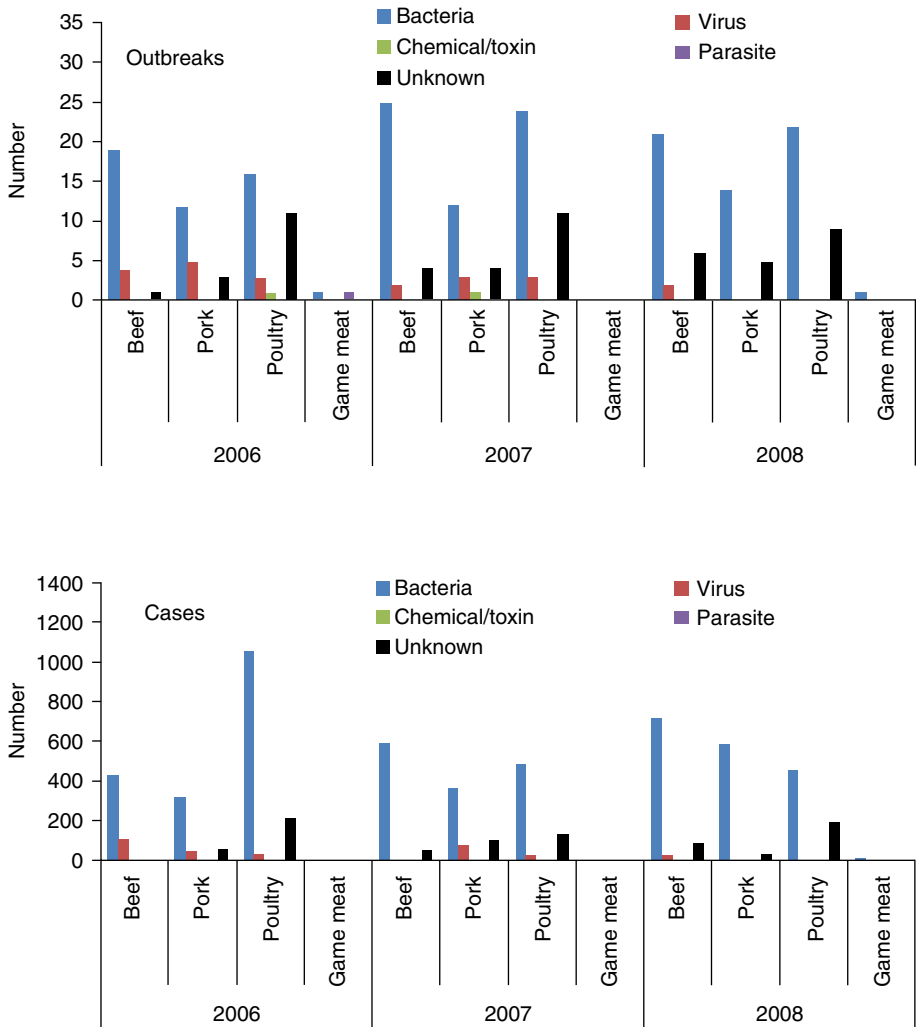

Figure 2 Numbers of confirmed outbreaks and cases associated with different hazards and various meat products in the US (2006–08).

Table 6 Settings involved in foodborne outbreaks (%) in the European Union (2008 and 2009)

Setting	*2008 (N=890)*	*2009 (N=977)*
Household	38.0	36.4
Restaurant, café, pub, bar, or hotel	23.1	20.6
Take away or fast food	2.7	–
Canteen or workplace catering	4.3	4.9
Fairs or festivals	–	2.8
School, kindergarten	5.3	5.5
Hospital or medical care facility	2.6	4.8
Other setting	10.8	9.9
Unknown	13.3	15.0

stillbirths. Illness onsets occurred from January 1998 to February 1999. Product contamination was attributed to demolition of a refrigeration unit, which increased environmental contamination in the production environment.

- *Staphylococcus aureus* intoxication occurred at a company picnic in an amusement park in GA, USA, in 2000. The illness was attributed to consumption of pork barbeque, which was kept in a cooler at high temperature.
- In 2002, processed chicken products contaminated with *L. monocytogenes* led to the death of seven and three miscarriages, whereas 12 250 000 kg of product were recalled.
- Trichinellosis, without deaths, occurred in Laos in 2005. It was associated with consumption of uncooked or fermented boar meat at funeral and wedding ceremonies.
- In 2006, two people became ill from *Campylobacter jejuni* and *Salmonella* Enteritidis after eating baked turkey in a private home in WI, USA.
- An outbreak of *Staphylococcus aureus* involved consumers of ham at a private home in NY, USA in 2008.
- An outbreak of *Bacillus cereus* and *Staphylococcus aureus* occurred in 2008 affecting people who ate prime rib beef at a banquet facility in AZ, USA.
- In 2009, an outbreak of Norovirus was attributed to eating chicken dishes, fruit, or vegetables at a restaurant in CO, USA.

- An outbreak due to *Bacillus cereus*, *Enterobacter*, and *Staphylococcus aureus* afflicted people who ate catered pork in Puerto Rico, in 2009.
- *E. coli* O157:H7 linked to a farm visit in Redhill, Surrey, England, in 2009, resulted in the hospitalization of 13 children.
- A total of 132 cases of salmonellosis occurred between August 5, 2010 and September 26, 2011 in 18 states of the US affecting people between 1 and 75 years of age. No deaths were reported and the source was attributed to handling turtles.
- In 2011, an outbreak of *E. coli* O157:H7 occurred among children visitors to an animal farm in WA, USA involving five laboratory confirmed, one probable case, and one hospitalization, but no deaths. Two calves were positive for the pathogen.
- *Clostridium perfringens* caused an outbreak, in 2011, at a business luncheon served by an unlicensed caterer, potentially due to improper reheating and hot holding of food.

Control of Biological Hazards in Meat Products

General

Meat products are rich in nutrients and provide a suitable environment for rapid microbial proliferation making them highly perishable foods. Therefore, microbial contamination of meat products needs to be adequately controlled in order for them to be preserved for maintenance of quality, delay of spoilage, and assurance of safety. A comprehensive and effective strategy for foodborne pathogen control should be based on an integrated approach from farm-to-table, and thus, it should be a shared responsibility of all involved in the meat production sector; producers, packers, processors, distributors, retailers, food service operators, and consumers. Control should be based on good production practices at the farm, slaughtering of animals that are disease free, processing of carcasses under sanitary and hygienic conditions, use of decontamination intervention strategies to reduce microbial levels, thermal or other (e.g., high pressure and irradiation) processing, drying, fermentation, acidification, prudent use of antimicrobials, maintenance of the cold chain during distribution, and proper storage and preparation procedures by food service and consumers.

The target of pathogen control at the preharvest level should be to minimize sources, levels, access, and transfer of contamination to the animal. Activities during slaughter and processing should be designed to minimize transfer and introduction of contamination on carcasses and meat, and to reduce existing contamination levels through implementation of decontamination or sanitization interventions, processing treatments for complete or partial destruction of contamination, or antimicrobial procedures for inhibition or retardation of microbial growth. The goal of pathogen control during storage and distribution, and at retail, food service and consumer level should be to prevent introduction of additional contamination, recontamination or cross contamination, and to inactivate or inhibit existing contamination. Discussion of microbial control in meat products is presented here in the context of control of contamination levels through sanitation, hygiene and decontamination; inactivation of contamination through sterilization (canning), pasteurization, and cooking; and, control of microbial growth through single or multiple antimicrobial hurdles. Successful implementation of pathogen control interventions should include adequate and effective education of food handlers and consumers, and should be managed based on the principles and spirit of the HACCP system.

Control of Microbial Contamination Levels

The first goal in microbial control is to maintain initial levels of contamination as low as possible because the amount of initial contamination influences the efficacy of all other antimicrobial interventions, and some agents are infectious at very low levels. Concentrations of contamination are reduced by procedures that restrict the sources, access, and transfer of microorganisms, including cleaning, sanitation, and hygienic practices, as well as packaging technologies such as aseptic packaging of thermally processed products. In addition, microbial levels on meat are reduced by decontamination technologies such as washing, application of hot water, steam, acids or other chemicals, and their combinations. Low levels of contamination ensure that acceptable intensities of subsequent antimicrobial interventions will be adequate to control contaminants and thus assure meat safety. In general, low contamination levels in meat assure that, even if the product is intentionally or unintentionally undercooked, the probability of illness will be lower.

Controlling animal contamination at the farm level (preharvest) may contribute to lower microbial loads reaching the abattoirs and the meat processing environment, and consequently contaminating carcasses and meat. Further, it reduces transmission of pathogens, through animal manure, to the soil and water, thus reducing the potential for contamination of foods of plant origin with enteric pathogens, as well as direct animal-to-human transmission of pathogens.

Antimicrobial interventions considered, or explored, for use to control contamination at the farm level include animal diet modifications, use of feed additives or supplements, antibiotic treatments, vaccination, bacteriophage therapy, competitive exclusion, prebiotics or probiotics, and good animal production management practices such as animal pen management, clean feed, water chlorination, clean and unstressful transportation of animals to slaughter, and clean animal holding environment before slaughter.

With the exception of good production management practices, and to some extent feeding of probiotics, the other approaches are still in the experimental stage or of limited use. Some countries (e.g., Scandinavian) have implemented some apparently successful preharvest pathogen control programs for certain animal types. Nevertheless, overall, preharvest pathogen control is still difficult due to limitations in existing relevant scientific knowledge related to sources and reservoirs for certain pathogens, existence of numerous and complicating variables, animal carriers being asymptomatic for

important pathogens such as *E. coli* O157:H7, pathogen shedding often being sporadic or of low prevalence and cell numbers, large levels of interfering total microbial contamination, lack of effective pathogen detection methodologies for use in the field, ubiquitous presence of some pathogens, lack of proven and approved pathogen control interventions, and economic considerations.

When at slaughter, animals are inspected antemortem and carcasses postmortem for animal health problems, and for the presence of parasitic agents and some animal pathogenic bacteria that also cause human illness (e.g., *M. tuberculosis*). These procedures, however, although necessary for verification of animal health and welfare, are not designed to determine the presence of enteric bacterial pathogens that contaminate meat and result in foodborne illness if not properly controlled.

In response to a major *E. coli* O157:H7 outbreak associated with consumption of undercooked ground beef in 1992–93, the USDA/FSIS established additional inspection requirements in 1996. These require meat and poultry processors to meet established performance criteria for biotype I *E. coli* contamination as a verification that HACCP-based process controls are adequate in preventing and removing fecal contamination. Another requirement is testing of carcasses or raw ground products in order to evaluate pathogen reduction performance standards for *Salmonella*. In addition to the US, microbiological performance criteria for meat have been established in the European Union, Australia, New Zealand, and other countries. In the US, processors and USDA/FSIS also test ground beef and raw materials destined for ground beef production for *E. coli* O157:H7, which is considered an adulterant in these products. To provide safer products and to meet regulatory requirements, as well as contractual specifications with major users of raw beef, the meat animal processing industry in the US and some other countries has employed extensive microbial pathogen reduction interventions as decontamination treatments for carcasses or meat cuts.

Decontamination interventions applied include animal washing, cleaning or partial hair clipping before slaughter, spot cleaning of visible soil on carcasses before evisceration by knife trimming or steam and vacuum, carcass water washing after visual inspection for absence ('zero tolerance') of physical contamination (e.g., feces, hair, etc.), and spraying, rinsing or deluging of carcasses before evisceration and/or before and after chilling with hot water or steam and with approved antimicrobial chemical solutions. Approved and used chemical agents include mostly organic acids (e.g., lactic-, acetic-, and citric acids), as well as acidified sodium chlorite, peroxyacetic acid-based preparations, etc. Frequently, these decontamination treatments are applied as multiple or combined, sequential or simultaneous interventions (multiple hurdles). Decontamination interventions reduce pathogen prevalence and contamination levels by 1–3 log units without eliminating it because they are mostly instantaneous or of short duration and of mild intensity. Their application should be optimized to avoid cell transfer, spreading, cross contamination, penetration, and biofilm formation in meat. In addition it is useful to examine whether they cause cell inactivation, injury, antimicrobial resistance, selection/adaptation/cross protection, or alteration of the natural microbiota of meat and the metabolic activity of survivors.

Pathogen Inactivation

Common processes that destroy microorganisms in foods include the thermal treatments of canning, pasteurization, and cooking. Additional inactivation processes include ionizing radiation and high pressure processing. Irradiation was approved in the US for use in fresh and frozen poultry meat in 1992 and fresh red meat in 1997, but it has found only limited application. Reasons for this may include consumer resistance and conflicting reports indicating potential negative effects of irradiation on meat color and undesirable odors. Ultra-high atmospheric pressure processing for pathogen control has found recent application in certain meat products, such as ready-to-eat ham, for the control of *L. monocytogenes* in the US. At the intensities applied, these processes are able to inactivate vegetative microorganisms, including pathogens, but not bacterial spores. Effectiveness may be increased if nonthermal and thermal technologies are used in milder combinations according to the multiple hurdle approach.

Inhibition of Microbial Growth

Microbial growth on meat products, as well as other foods, is affected, not only by the type and level of initial contamination but also by various factors associated with the product (intrinsic) or its environment (extrinsic). Approaches aiming to inhibit microbial growth are mostly based on manipulation or changes in these factors. These include storage at low temperatures, drying (evaporation) or reduction by binding of water levels (salting and sugaring) available for microbial growth (water activity), addition of acids (low pH), fermentation (low pH and production of antimicrobials), packaging under modified atmospheres such as vacuum, and use of chemical antimicrobials. Combinations of antimicrobial technologies, applied individually at sublethal levels (hurdle technology), are frequently used in many meat products as they result in microbiologically stable and safe products of desirable eating quality. It is important to properly select and apply sublethal hurdle combinations with the objective of pathogen control without stress adaptation or selection of resistant pathogens.

Management of Food Safety

Regulatory inspection authorities, in addition to microbiological performance criteria, have established requirements for the industry to implement sanitation standard operating procedures (SSOP) and HACCP programs for management of pathogen control interventions. Complete and routine implementation of validated and verified HACCP plans should be based on a solid foundation consisting of effective overall prerequisite programs, including proper and sanitary building and equipment designs, good manufacturing practices and good hygiene practices, and proper employee education and training. Employee education and training should be accomplished through development and implementation of specific standard operating procedures or job instructions for each activity. The management of the operations should also be educated to understand meat safety hazards and risks as well as the importance and function of HACCP because it provides

funding for materials, equipment, and adequate time for training and education of employees.

Microbial Testing

The development and widespread adoption and implementation of HACCP principles were based on the realization that traditional end-product microbiological testing is inadequate to assure meat safety. It is important to note that microbiological testing is essential for determination of hazards and risks, and for development, establishment, implementation, validation, maintenance, and verification of HACCP and other safety assurance systems. Limitations associated with finished product testing, as means of meat safety assurance, include the fact that foodborne pathogens are distributed sporadically and unpredictably, and/or occur at a low incidence within the product. Therefore, the number of samples that needs to be tested from a specific lot is unrealistically high if it is to provide meaningful information, and furthermore, the results obtained may provide a false sense of security. Thus, control of the microbiological safety of products should be based on the proper implementation of validated process management systems based on hazard prevention rather than end-product testing, which should be relied on for validation and verification of activities.

Conclusions

By nature, raw meat becomes contaminated with microorganisms, including some that are pathogenic to humans. Contamination is introduced during production in the field, harvesting, storage, distribution, further processing, retailing, preparation, and consumption, and it originates from soil, decaying material, and animal fecal waste, which contaminate water, air, animals, plants, processing facilities, equipment, and humans; leading to a complete contamination cycle. Pathogens present on meat may cause illness ranging from mild gastrointestinal discomfort to severe acute or chronic syndromes or death. Extent, prevalence, and type of contamination are controlled under sanitary and hygienic conditions during animal production, processing, and handling of the products at all stages of the chain. Control of pathogens and management of food safety risks should be based on an integrated effort and approach by all sectors involved, from the producer through the processor, distributor, packer, retailer, food service worker, and consumer. Interventions applied during processing include proper and adequate cleaning, effective sanitation, decontamination, heating, chilling, freezing, drying, fermentation, use of chemicals as acidulants or antimicrobials, packaging, proper storage and distribution, and appropriate handling. Proper application of control processes yields products that should be safe for consumption following proper cooking and serving. Consumers should be advised to handle and prepare all foods, including those of animal origin, properly, and to follow labeling instructions. They should store and handle foods under conditions that avoid cross contamination (clean and sanitary environment), and they should cook them properly (e.g., ground beef to 71 °C), and store or hold them at the correct temperatures (cold: under 4 °C; hot: above 60 °C) within the indicated length of time (<2 h). At-risk persons should be instructed to avoid or cook risky foods before consumption, and to avoid raw or unpasteurized products.

See also: Bacteria: *Listeria monocytogenes*; *Salmonella* Non-Typhi. Characteristics of Foodborne Hazard and Diseases: Microbial Stress Response; Sublethally Injured and Viable but Nonculturable Cells. Environmental Contaminants: Nitrate and Nitrite. Food Additives: Preservatives. Food Safety Assurance Systems: Building Design; Cleaning and Disinfection; Food Safety and Quality Management Systems; Good Animal Husbandry Practice; Hygienic Design of Equipment; Infestation Management in Food Production Premises; Microbiological Testing, Sampling Plans, and Microbiological Criteria; Personal Hygiene and Employee Health. Food Technologies: Biopreservation; Chilling; Cleaning and Disinfection Technologies (Clean-In-Place, Clean-Out-of-Place); Drying; Fermentation; Food Irradiation; Freezing; High Pressure Processing; Pasteurization; Pulsed Electric Field Technology; Pulsed Ultraviolet Radiation Processing; Sterilization. Foodborne Diseases: Overview of Biological Hazards and Foodborne Diseases; Prevalence of Foodborne Diseases in Africa; Prevalence of Foodborne Diseases in Europe; Prevalence of Foodborne Diseases in North America; Prevalence of Foodborne Diseases in South East and Central Asia; Prevalence of Foodborne Diseases in Western Pacific Region. Prions and Agents of TSEs: Bovine Spongiform Encephalopathy in Cattle. Public Health Measures: Food Inspections and Enforcement Systems. Safety of Food and Beverages: Probiotics and Prebiotics; Safety of Irradiated Foods

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http://www.fda.gov/
United States Food and Drug Administration.

SAFETY OF FOOD AND BEVERAGES

Poultry and Eggs

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Glossary

Logistic slaughter Flocks or animals with known or assumed low risk of microbial contamination are slaughtered before flocks or animals with known or assumed high risk of microbial contamination.

Microbial decontamination of foods Methods aiming at removal or inactivation of contaminant bacteria present in or on foods. These methods include physical, chemical, thermal treatments and other methods.

New York dressed poultry Poultry carcasses which are placed on the market in defeathered, but not eviscerated condition.

Organic food production Food production with intention to minimize the human impact on the environment; this includes, for example, restrictions in the use of synthetic pesticides in farming and pharmaceuticals in animal breeding, raising animals in open-air systems, a ban of genetically engineered organisms.

Introduction

Food of animal origin, its production, processing, transport, and preparation, has always been of concern for public health. This thematic issue remains topical through recent outbreaks of new zoonotic diseases, for example, influenza virus H5N1 or prion disease bovine spongiform encephalopathy and the possible transfer of such diseases to consumers. Nowadays, foodborne pathogens tend to spread more readily because of an increased worldwide trade in livestock and the foods of animal origin they produce: Especially globalization may make a primarily local foodborne disease a global issue.

In contrast to beef or pork, poultry meat is in demand by an increasing human population not only because it is considered to be healthy and its consumption is not prohibited by any religious codices, but also because it can be produced at lower cost and is therefore cheaper. Worldwide, poultry meat production amounts to approximately 95 million tons per annum (2010 data), second only to pork (approximately 108 million tons) and is steadily increasing, generally in farms producing under conventional conditions, which mode of production saw the highest increase in all food production systems over the past decade. This results in a high throughput of animals of similar genotypes, separation of breeding, fattening, feed production, and meat (egg) processing. At the same time, to be successful, all these facilities have to operate in an integrated way, i.e., in highly controlled, logistically and technically mature production units. Especially in Europe, the demand for organically produced poultry meat and eggs is increasing, for which specified protocols have been set up by the European community. These are stipulated by regulations including issues such as welfare, feeding, medical treatment, and environmental considerations. The access of production animals to outdoor facilities is probably the most important demand of organic meat and egg production. This bears the risk of disease transmission from wildlife to production livestock as well as the carry-over of epidemic diseases to (endangered) wildlife species. In this short overview, the authors focus on different poultry production systems and their implications for public health, on control measures already set in place, and on future mitigation strategies to minimize the consumers' risks for foodborne infections.

To this end, both conventional and organic poultry production are addressed.

Poultry Meat: Conventional Production

In conventional production, only a narrow selection of breeds are available for producers. Commonly used breeds include Ross, Cobb, Sasso, Hubbard, and Arbor, all of which gain a reasonable slaughter weight within 6 weeks under low feed conversion. The weight gain ranges from 35 to 62 g day^{-1}. The broilers leave the hatcheries and are delivered at the farm 1 day after hatching. Both stables with and without natural light prevail (scientific results on the benefits of natural light are still controversial). Many poultry farms have more than one stable house each of it giving room for up to 40 000 broiler chickens. Important factors include ventilation, temperature, lighting, and litter conditions. Different kinds of stable bedding can be used from straw to wood or corn shaving as well as sand or recycling paper. These broiler litters differ in chemical composition like pH and inorganic components. Thus, there are different advantages like moisture holding, dust reduction, or soil stability and soil biology for one or the other bedding. Also, bedding material influences the efficacy of insect and rodent control and thus the degree of colonization with foodborne pathogens such as *Campylobacter*. Good management practices (GMPs) include an effective ventilation to keep the moisture as low as 30% and the ammonia level in the air lower than

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25 ppm. Wet litter may not only cause pododermatitis and breast blisters, but is also known to adversely affect the respiratory system of chickens, which may lead to various bacterial or viral infections that need to be treated with antimicrobial substances. Temperature in poultry houses need to be such that chickens are able to maintain their body temperature between 40.6 and 42 °C. The birds' so-called thermo-neutral zone is between 18 and 24 °C and any lower temperature is associated with a higher feed conversion. Higher temperatures cause chickens to drink more and it leads to less feed uptake and ultimately higher humidity. This heat stress is a main problem in fast-growing breeds and can lead to high losses and an increased susceptibility to infectious diseases. In cold months heating is less of a problem for most poultry producers. Nevertheless, in recent years high prices for energy and feed have caused the increase in poultry production worldwide to stagnate. Great discrepancy exists regarding light management. Birds are very sensitive to light. It stimulates food intake and hormone regulation. But the day–night cycle is also important for developing good immunity. Thus, a dark period of at least 6 h is required for good health. Long light periods used in conventional poultry industry promote feed consumption and, consequently weight gain. With alternate dark and light periods birds are more active during the light period. Higher light intensity can induce activity but reduce weight gain. Whereas major zoonotic pathogens (*Campylobacter*, *Salmonella*) can colonize chickens, pathological signs like liver or myocardial necrosis are observed after infection with *Salmonella*. Finally, cellulitis or enterocolitis may be caused by toxin-producing *Escherichia coli* with a zoonotic potential.

Poultry Meat: Organic Production

Over eight million laying hens and over nine million broilers are kept under organic production in all of Europe, and still represent a rather modest part of the total production (e.g., in Austria, 3.5% in the case of broilers).

Organic broiler production is regulated in the European Union (EU) in Reg. (EG) 834/2007 which stipulates access to pasture, a minimum of 12 weeks of age before slaughter, feeding on organic feed produced mainly on the farm of the broiler production, more space per chicken weight, and the restrictive use of chemically synthesized allopathic veterinary medication (e.g., antimicrobial substances). Natural light and longer dark periods lead to less weight gain and higher activity. In most cases, organic poultry production is small scale (i.e., not more than 4500 chickens per flock). Good welfare indoors and the implementation of GMPs as regards temperature, ventilation, feed, water supply, and litter, are required to avoid disease contraction. Organic poultry production is important for protecting the environment, allowing sustainable agriculture and focusing on consumer interests. Most studies comparing conventional and organic poultry production have not found significant differences in colonization with zoonotic agents. Some studies report a reduced rate of antimicrobial resistance in bacterial isolates in poultry raised organically. The use of chemically synthesized allopathic veterinary medicinal products and antibiotics is prohibited for preventive treatment and can only be applied once in the case of disease.

Poultry Meat Processing: Transport, Stunning, Bleeding, Scalding, Plucking, Evisceration, Meat Inspection, Cooling Technology, and Packaging

Highly automated poultry meat processing systems have been developed over time. Both their efficiency and hygienic design have influence on slaughter hygiene and the degree of contamination of chicken meat with zoonotic agents. The observed differences in slaughter hygiene primarily depend on the age of birds at slaughter and cooling and packaging technologies, rather than on the region where they were produced. Nevertheless, consumer preferences on how to purchase and prepare chicken meat (e.g., with or without skin, fresh or frozen, breast meat or New York-dressed poultry) have an impact on processing technology, handling, and preparation. Provided good hygiene practices are applied, carcass contamination can be minimized as illustrated by the scalding process. For instance, washing carcasses pre-scald, the use of high scald temperatures approximately 60 °C, clean scald water, and water sprays instead of immersion will effectively reduce microbial loads on carcasses; however, 'hard' scald results in more crevices and porous structures in the skin, which will retain more water, and thus facilitate entrapment of contaminant bacteria in subsequent processing steps. The scalding process is important as it influences the degree of *Campylobacter* and *Salmonella* carcass cross-contamination. Other important operations for possible cross-contamination at poultry slaughter are defeathering and evisceration. Cleaning, disinfection, and removal of porous pluck fingers are major factors influencing the amount of microbial contamination. In particular, organic matter remaining on (porous) rubber pluck fingers, and the warm and moist environment allow accumulation and even multiplication of *Staphylococcus aureus* originally derived from poultry skin. An improved hygienic design should make such equipment more suitable for cleaning and disinfection. Evisceration technology has a high influence on fecal contamination and thus on contamination with zoonotic enteric bacteria like *Salmonella*, *Campylobacter*, *E. coli*, or clostridia. New systems with automatic cleaning and disinfection of knifes between each carcass evisceration reduce fecal contamination. Heterogeneity in broiler carcass sizes bears the higher risk of gut rupture and thus of fecal contamination. A possible new technology based on laser distance measurements followed by an automatic adjustment of the evisceration cutter to the carcass shape, may reduce or prevent gut rupture. Measures for reduction of microbiological loads on carcasses include cooling, decontamination, crust freezing, steam or hot water spray, and irradiation. Not all of these technologies are accepted in Europe or other parts of the world and they have diverse effects on the reduction or elimination of major zoonotic pathogens. Cutting and further processing of carcasses pose similar problems as known for other meats. In addition to the bacteria mentioned above, post-processing contamination by *Listeria monocytogenes* has been reported.

Egg Production: Laying Hens

Laying hens can be kept in cages, in so-called enriched cages, in floor pens, or free range (organic or conventional). Different breeds seem to behave differently under each of these conditions. Although the use of cages has been banned in Europe since 2012, not all European countries have implemented this regulation till today. Animal welfare and consumer preferences have – at the cost of economic disadvantages – been considered in this decision. The influence of housing systems on *Salmonella* infections has been seen as controversial. Some studies show an increase in transmission between birds in non-cage housing, whereas others demonstrate that *Salmonella* infections are seen significantly more often on farms keeping hens in cages. This has also been discussed by European Food Safety Authority (EFSA) in 2007 in their scientific opinion on a possible European ban of keeping laying hens in cages. Optimal biosecurity, cleaning, and disinfection have been characterized as protective against *Salmonella* in laying hens in existing housing system.

Eggs and Egg Processing

Eggs have arguably the most complex structure among the foods of animal origin. From the surface to the core, a thin layer (cuticle) is covering the porous shell, then two internal layers follow. In the center of the white egg (albumen), the egg yolk is situated. Microbial contamination can occur (1) in the ovarium, i.e., the egg yolk is contaminated, before formation of the albumen layer and egg shell or (2) in the cloaca, i.e., on the egg shell surface, or during/after laying when the egg comes into contact with litter, feces, etc. Generally, the cuticle and the inner membranes provide some protection against migration of bacteria in deeper structures, whereas the egg white has antibacterial properties due to lysozyme, high pH, and chelating compounds. Generally, eggs with damaged shells and protruding egg white are not considered fit for human consumption. In some countries, additional requirements apply to table eggs. Both eggs of best quality as well as eggs with damaged shell may be processed into egg products. Those basic products are whole eggs, yolks, whites, and blends including those with nonegg ingredients. Pasteurized egg products are available in a liquid or dried state, and (also unpasteurized) in a frozen state. Food industry and foodservice will predominantly use such products for bakeries, pasta, omlettes, and other dishes not only because of its convenience in storage and handling, but also because pasteurization will inactivate the common egg-borne pathogenic bacteria. Nevertheless, the egg-processing plants have to adhere to good manufacturing practices and implement hazard analysis critical control point (HACCP) to ensure overall microbiological quality and safety. The processing steps include transportation of eggs from primary production, breaking eggs, filtering, mixing, stabilizing, blending, pasteurizing, cooling, and possibly drying, freezing, and packaging. All of these processing steps are highly automated. Eggs are transported from hen stables in racks or pallets to the production facilities. Eggs as well as transport pens may carry microorganisms including pathogens that exist on the farm, for example, *Salmonella* and *Campylobacter*. To calculate the risk of a possible transfer from the primary production, it is relevant to distinguish between the various production systems, for example, conventional cage or free range. Thus, cleaning and disinfection of all racks and pallets is most important to prevent entry of pathogens from primary production into the processing facilities. Shell eggs are washed and sanitized before breaking to reduce the aforementioned risk. After separation of yolks and whites, the liquid egg is filtered and chilled before pasteurization. Pasteurization is an important critical control point to assure safety particularly against *Salmonella* spp. The processed egg should stay as close as possible to the primary product in flavor, color, or usability. The processing plant must adopt a sanitation concept particularly to avoid recontamination after the pasteurization process. A rodent and insect control is as important as the separation of the cooling, packaging facilities after pasteurization to guarantee a safe and proper way to use the product.

Risk and Control Measures

Although a variety of foodborne biological hazards are associated with poultry and eggs (Table 1), *Campylobacter* and *Salmonella* are the major pathogens implicated in foodborne diseases. In the EU, it is estimated that 44–68% of human foodborne cases of salmonellosis are attributable to the handling, preparation, and consumption of eggs, whereas a mere 4–7% is attributable to broiler and turkey meat. In contrast, 20–30% of human foodborne campylobacteriosis cases are attributable to handling, preparation, and consumption of

Table 1 Biological and chemical hazards transmissible to humans via poultry, meat, and egg (products) consumption or food preparation

Biological hazards	
Bacteria and their toxins	*Bacillus cereus* toxins
	(thermophilic) *Campylobacter* spp.
	Clostridium botulinum toxin
	Clostridium difficile
	Clostridium perfringens toxin
	E. coli, toxicoinfectious strains including verotoxin-forming *Escherichia coli* (VTEC)
	Extended-spectrum-β-lactamase/AmpC *E. coli* and *Salmonella*
	Listeria monocytogenes
	Salmonella sp., non-Typhi
	Staphylococcus aureus toxins
	Yersinia enterocolitica
Protozoa	*Toxoplasma gondii*
Helminths	Various trematodes
Chemical hazards	
Contaminants	Dioxins and dioxin-like polychlorinated biphenyls
Residues	Antimicrobials: chloramphenicol, nitrofurans, nitroimidazoles
Physical hazards	As in other meat species or foodstuff

Source: Adapted from EFSA (2012) Scientific opinion on the public health hazards to be covered by inspection of meat (poultry). *EFSA Journal* 10: 2741.

broiler and turkey meat (or 60–80%, when cross-contamination pathways occurring in the kitchen are considered).

Measures to control *Salmonella* in the poultry meat and egg chain start with the provision of *Salmonella*-free eggs for breeding layers or broilers, considering both intra-ovarian as well as shell-surface contamination. Preventing the introduction of the pathogen in flocks is a task of biosecurity measures, including feed and water. In addition, animal transport, slaughter, and further processing must be under control in the meat chain, while the cleanliness of the egg shell, integrity of the cuticle, and avoidance of temperature shifts are crucial for the safety of table eggs. From a regulatory view, the establishment of target prevalences is a key step that allows to assess if control measures have been effective. Safe handling and processing techniques must not only be implemented into good hygiene practice (GHP) systems of the food business operators, but also be communicated to the consumers. At farm level, vaccination of poultry as well as stamping out of positive flocks have been established as preventive or reactive measures. Intervention measures at slaughter may either be improvements in the common slaughter steps (scalding, defeathering, evisceration, chilling), or be separate measures ('microbial decontamination') aimed at removing bacteria from carcass surfaces (cold/hot water washes, or steam treatment), or to inactivate them (irradiation; dips, sprays, or rinses with organic acids or oxidizing compounds). Such inactivation treatments are not allowed in all countries and should be implemented only when GHP and HACCP is well established and functional. Temperature abuse may result in the multiplication of this pathogen, and breaching of other GHP principles may result in cross-contamination. It must be noted that also direct contact with live poultry has caused outbreaks of human salmonellosis, for example, in the USA in 2012.

As regards *Campylobacter*, there are few single measures that would substantially reduce the prevalence or concentration on poultry meat. The bacterium is sensitive to oxygen, dryness, and will not multiply under temperature conditions common in the meat chain. However, the structure of poultry skin and coexistence with oxygen-consuming *Pseudomonas* will allow *Campylobacter* to prevail in a viable state, albeit occasionally not culturable on selective media. Dry air chilling of eviscerated carcasses as well as retailing deep-frozen instead of chilled carcasses has an effect on the numbers of culturable *Campylobacter*. Whereas the latter approach was successful in Iceland, New Zealand fruitfully pursued a multistage approach. Slaughter of *Campylobacter*-negative flocks before positive ones (logistic slaughter) will have only limited effect

Table 2 Process criteria to ensure stop of growth or inactivation of selected biological hazards

	Freezing	*Heat treatment*	*Irradiation*	*Other*
Bacteria and their toxins				
(Thermophilic) *Campylobacter* spp.	n/a	50–57 °C for 0.8–13.3 min (D)	0.08–0.29 kGy (D)	Preservatives
Listeria monocytogenes	n/a	70 °C for 0.14–0.27 min (D)	0.34–1 kGy (D)	Preservatives, UV irradiation
Salmonella sp., non-Typhi	n/a	75 °C in the core of the food	0.2–11 kGy (D) dependent on Serovar and food medium	Preservatives
Staphylococcus aureus toxins	n/a	104–121 °C for 20–100 min, thus highly stable	n/a	Preservatives; more research is needed for conclusive results
Parasites				
Toxoplasma gondii (tissue cysts)	−20 °C, 11 days	58 °C, 9.5 min	0.4–0.7 kGy (D)	2% NaCl at 4 °C, 7 days

Abbreviations: D, time to reduce numbers of viable organisms to 1/10; n/a, not applicable; UV, ultraviolet.

Source: Adapted from International Commission on Microbiological Specifications for Foods (ICMSF) (1996) *Micro-organisms in Food 5: Characteristics of Microbial Pathogens.* London: Blackie Academic & Professional.

Table 3 Selected major product recalls or foodborne disease outbreaks attributed to poultry meat and eggs

Year	*Region*	*Disease outbreak, recall, ban*	*Food type*	*Source/reason*
1994	USA	Disease (established 22 400 cases), recall	Ice cream	Ice cream premix transported in a tank that had contained raw egg contaminated with *Salmonella*
1999	Belgium/Europe	*Post hoc*	Poultry and eggs	Feedstuff contaminated with dioxins/polychlorinated biphenyls (PCBs) in transformer oil
2011	Germany/Europe	Recall, ban	Poultry and eggs	Fat contaminated with dioxins/PCBs
2011	USA	Disease (136 cases), recall	Ground turkey	Undercooking
2011	USA	Disease (>30 cases), recall	Broiled chicken liver	Undercooking
2012	Germany/Europe	Recall	Eggs	Feedstuff contaminated with dioxins

on the overall carcass prevalence, as long as the flock prevalence is high; also, within the period from sampling of flocks to obtaining results and arranging the order of slaughter, conversion of previously negative flocks can occur. Although a number of intervention measures starting from the phase of microbial colonization of chicks have been proposed and studied and mathematical models have been set up to quantitatively describe the *Campylobacter* load along the poultry meat chain, the major players in poultry meat production and consumption still have to elaborate more harmonized risk control approaches to control this foodborne pathogen. A number of studies indicate that cross-contamination during food handling and processing contribute more to human foodborne campylobacteriosis than underheating of poultry meat.

Some process criteria for freezing and heating, as well as other treatments are given in **Table 2**; likewise, some major food recalls and foodborne disease outbreaks attributed to poultry meat and eggs are listed in **Table 3**.

Existence of commensal or pathogenic bacteria carrying resistance against a number of diverse antibiotics (multiresistance) or against an entire group of antimicrobial (e.g., due to extended spectrum β-lactamase production) is not unique in poultry and egg production, and the reader is referred to the pertaining articles of the Encyclopedia. Also, chemical hazards, in particular dioxine introduced via feedstuff or others introduced via litter, are dealt with in another article of this encyclopedia.

See also: Bacteria: *Campylobacter*; *Listeria monocytogenes*; *Salmonella* Non-Typhi; *Staphylococcus aureus*. Characteristics of Foodborne Hazard and Diseases: Drug Resistant Pathogens. Food Technologies: Chilling; Cleaning and Disinfection Technologies (Clean-In-Place, Clean-Out-of-Place); Freezing. Foodborne Diseases: Overview of Biological Hazards and Foodborne Diseases; Overview of Chemical, Physical, and Other Significant Hazards. Public Health Measures: Health Education, Information, and Risk Communication. Risk Analysis: Risk Assessment: Microbiological Hazards; Risk Management: Application to Biological Hazards

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www.efsa.europa.eu
European Food Safety Authority.

www.fsis.usda.gov
Food Safety and Inspection Service (in the U.S. Department of Agriculture).

www.who.int/foodsafety
World Health Organization.

SAFETY OF FOOD AND BEVERAGES

Milk and Dairy Products

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Glossary

Acquired immunodeficiency syndrome (AIDS) A disease of the human immune system caused by infection with the human immunodeficiency virus (HIV).
Biogenic amines Low molecular weight organic bases such as histamine, tyramine, cadaverine etc. formed in food by the microbial decarboxylation of amino acids that can result in consumers suffering allergic reactions.
Bovine somatotropin (BST) A metabolic protein hormone used to increase milk production in dairy cows.
Epitopes Allergenic segments or polypeptides of proteins capable of eliciting an immune response
Glycation The result of non-enzymatic covalent bonding of a protein or lipid molecule with a reducing sugar molecule, such as fructose or glucose.
Hemolytic uremic syndrome (HUS) A disorder that usually occurs when an infection in the digestive system produces toxic substances that destroy red blood cells, causing kidney injury.
Mastitis An inflammation of the mammary glands.
Milk Defined as the normal mammary secretion of lactating animals obtained from one or more milkings without either addition to or removal from it; intended for consumption as liquid milk or for further processing.
Milk products Also known as dairy products, are foodstuffs made from mammalian milk by processes such as homogenization, pasteurization, freezing, fermentation, evaporation and drying etc. Additional ingredients may be added.
Q-fever A global disease with acute and chronic stages caused by the rickettsial microorganism *Coxiella burnetii*. Cattle, sheep, and goats are the primary reservoirs.

Introduction

Potential foodborne hazards are undesirable substances or organisms in food that may constitute a health risk to the consumer. Three classes of such hazards in milk and dairy products are considered in the present article and include: (1) biological hazards, such as viruses, bacteria, and other microbial pathogens; (2) chemical hazards such as mycotoxins, residues of veterinary drugs used in lactating animals, pesticide residues, and a variety of industrial and environmental contaminants that might contaminate the feed of lactating animals; and (3) physical hazards such as metal, wood or glass, and any other foreign object that may have found its way into milk and dairy products, for example, hair, stones, and jewelry.

It is ironic that at a period in time when food is arguably safer than ever before, sporadic food safety incidents continue to undermine consumer confidence in the safety of the food supply. The illegal addition of melamine to milk in China is one of the more recent examples. Many of the potential health risks posed by contaminants in milk have been known for well over a century. Since that time, considerable efforts by both the public and private sectors have significantly reduced the prevalence of milkborne diseases. It is now common practice, at least in industrialized countries, that the production of safe and wholesome milk and dairy products includes control over all the factors that may impact on the safety of the final product at each step of the production chain. This procedure is often referred to as 'Integrated Chain Control for Food Safety.' This shared responsibility for food safety by all players along the production chain is recognized as the best means for assuring safe and wholesome milk and milk products. However, this approach first requires the identification of potential hazards and means for their control. These are discussed in the following sections.

Pathogens of Special Relevance

Milk is the ideal medium for the growth of both pathogenic and spoilage microorganisms. The fact that dairy products are consumed safely on a daily basis by most individuals in the world is a tribute to the efforts of all involved in ensuring the safety of dairy foods. Recent studies have indicated that dairy products account for a very small percentage of all foodborne illness. Although milk and milk products are among the safest foods worldwide, they have an inherent potential for causing illness as they are potentially the source of a very broad range of microbial, chemical, and physical

Table 1 Potential hazards and foreign bodies in milk and dairy products

Biological hazards	*Chemical hazards*	*Physical hazards*
● *Bacillus cereus* ● *Brucella* spp. ● *Campylobacter jejuni* ● *Coxiella burnetii* ● *Cronobacter sakazakii* ● *Cryptosporidium parvum* ● Pathogenic *Escherichia coli* ● Enterohaemorrhagic *E. coli* ● *Listeria monocytogenes* ● *Leptospira* ● *Mycobacterium bovis* ● *Mycobacetrium paratbuberculosis* ● *Salmonella* (non-typhi) ● *Shigella* spp. ● *Staphylococcus aureus* (enterotoxins) ● *Yersinia enterocolitica* ● Fecal-orally transmitted pathogens such as hepatitis A, *Salmonella* typhi and paratyphi, pathogenic *E. coli*	● Residues of antibiotics ● Antibiotics ● Pesticides ● Hormones ● Dioxin ● Aflatoxin M_1 ● Heavy metals ● Radionuclides Processing contaminants ● Packaging contaminants (bisphenol A) ● Melamine and other adulterants	● Metal fragments screws and rivets ● Machine filings ● Glass pieces ● Jewelry ● Stones ● Insulation/paint ● Plastic material fragments ● Personal effects such as jewelry, buttons, nail fragments, nail varnish, and dressings ● Hair, dust, and dirt insect parts/fragments

hazards. As such these hazards are a major concern to producers, processors, regulators, and consumers.

Normally milk is collected from a lactating animal (most commonly a dairy cow). Such milk can harbor a variety of microorganisms, many of which are pathogenic to humans. Principal sources of microbial contamination are endogenous, such as the cow itself, or exogenous, such as the environment (soil, water, manure, and domestic and wild animals). Unsanitary milking and/or processing equipment and contact by farmers or workers on the farm or in the factory are also considered exogenous sources. In general, milkborne diseases can be caused by bacteria, protozoa, or viruses. These are presented in Table 1.

Some of these infections cause disease in the dairy animal, whereas others do not. Obviously, asymptomatic animals make identification and control more difficult. Some of the microorganisms in Table 1 have highly specific symptoms in infected humans such as hemolytic uremic syndrome (HUS) caused by the enterohaemorrhagic *Escherichia coli* O157, Q-fever caused by *Coxiella burnetti* and tuberculosis caused by *Mycobacterium bovis*. Although most of the organisms cause self-limiting gastrointestinal disorders, some infections are associated with long-term sequelae. Persons falling in the so-called the Young, the Old, the Pregnant, and the Immunocompromised ('YOPI' group) are at greater risk of more severe and often lethal infections by some agents, such as *Listeria*.

Most milk is pasteurized or treated in some manner, thereby eliminating foodborne pathogens that may be present. In some countries, the practice of consuming untreated raw product has been increasing because of purported health benefits. Unpasteurized milk is often consumed directly by dairy producers, farm employees and their families, neighbors in the region, and raw milk advocates. It is also consumed directly by segments of the population in certain regions of the world through the consumption of raw-milk cheeses. However, the direct consumption of raw or unpasteurized milk has been unequivocally traced to outbreaks of disease in humans. In the ensuing Sections: Viruses, Rickettsiae, Protozoa, and Bacteria, these milkborne biological agents that can be eliminated by pasteurization or other treatment will be discussed. Prions, the agents of transmissible spongiform encephalopathies, will not be discussed because, except for the hypothetical case of bovine spongiform encephalopathy in small ruminants, presently there is no scientific evidence to suggest that milk and dairy products can present any risk.

Viruses

Viruses have emerged as significant causes of food- and waterborne diseases in recent years. In the US, the Centers for Disease Control and Prevention (CDC) reported on the ranking of various foodborne pathogens that were acquired domestically in 2011. Of these, norovirus was by far the leading cause of the estimated 47.8 million foodborne diseases that occurred that year. It was the second leading cause of the estimated 127 839 hospitalizations and the fourth leading cause of the estimated 3037 deaths. Foodborne hepatitis A virus is also important and was responsible for one of the biggest outbreaks ever documented when contaminated 'hairy' clams caused over 300 000 cases in China in 1988. Calicivirus, rotaviruses, and poliovirus are also important, particularly in developing countries.

Virtually all foodborne viruses are transmitted via the fecal–oral route and can be transmitted by raw- and improperly pasteurized milk that is directly or indirectly contaminated by fecal matter. Experiments have been conducted where various dairy products and pasteurized milk were artificially inoculated with a variety of viruses to assess their survival in these products. In inoculated pasteurized and boiled milk, poliovirus and coxsackievirus B5 could survive for at least 90 days at 4 °C and for up to 30 days at 25 °C. Yoghurt stored at 4 °C, supported the survival of poliovirus and coxsackievirus B5 for 90 days. However, pasteurization has been demonstrated to be an

adequate treatment to control any viruses that may have contaminated raw milk.

Regarding milkborne outbreaks, the first enteric virus associated with such an incident was the poliovirus in the period before the Second World War. The virus was transmitted through water and unpasteurized milk. Poliovirus is host-restricted to humans and therefore cannot infect cows, but inadequate milk handling practices by infected workers and lack of pasteurization sometimes allows outbreaks to occur.

In 1993, seven people were infected with the tick-borne encephalitis (TBE) virus after drinking unboiled goat's milk. Previous cases of alimentary TBE were recorded in 1984 (4 cases) and in 1989 (2 cases). Both of the latter outbreaks were associated with the consumption of unpasteurized goat's milk. TBE belongs to the flavivirus family and is the only enveloped virus known to be associated with foodborne infections. The virus infects dairy animals via the tick vector and infected animals shed the virus in their milk, which if ingested without pasteurization, may infect humans.

Protozoa

Over recent decades, parasitic protozoa have been recognized as having great potential to cause food- and waterborne diseases. The organisms of greatest concern in food production worldwide are *Cryptosporidium*, *Giardia*, *Cyclospora*, and *Toxoplasma*. Although other parasitic protozoa can be spread by food or water, current epidemiological evidence suggests that these four present the largest risks. Very little documented evidence is available in which milk or dairy products are implicated in cases of foodborne disease caused by these organisms. Of the four genera referred to above, *Cryptosporidium* appears to be the most significant in terms of milk and dairy products. Even in the case of *Cryptosporidium*, however, the public health risk of foodborne spread from the point of view of milk and dairy products (such as butter, cheese, and yoghurt), appears to be negligible. Consequently only *Cryptosporidium* will be discussed in more detail in this section.

It nevertheless remains important to prevent protozoan parasites from entering the food (and also the milk production) chain. Three main routes by which these parasites enter the food production process are:

- Contamination of food ingredients or raw materials on the farm;
- contaminated water use in the final product or resulting from processing, washing, or cleaning; and,
- transfer from infected food handlers in production, food service, or domestic settings.

Prevention and control methods should, therefore, be devised to cover these three routes whenever they could be of significance to the final product consumed. It is also important that analytical laboratories tasked with examining outbreaks of infectious intestinal disease should have the capability and expertise to diagnose the presence of parasitic protozoa in patients.

Cryptosporidium

Human cryptosporidiosis emerged as an important gastrointestinal infection in the 1990s, because of the ingestion of contaminated water and foodstuffs containing the protozoan parasite, *Cryptosporidium parvum*. When ingested, it is capable of causing a high degree of morbidity in healthy populations. It also can result in mortality in vulnerable populations such as immunocompromised persons infected with human immunodeficiency virus (HIV)/aquired immunodeficiency syndrome (AIDS) or cancer patients receiving chemotherapy.

There is no effective antimicrobial treatment to eradicate this agent from the gastrointestinal tract. Generally, cryptosporidiosis is a self-limiting disease presenting with various symptoms, including diarrhea, dehydration, abdominal cramps, vomiting, weight loss, and electrolyte imbalance. Cryptosporidial infections can, however, persist in persons infected with human immunodeficiency virus (HIV)/aquired immunodeficiency syndrome (AIDS) to the point of being life threatening. Although there are 10 recognized species of *Cryptosporidium*, human infection is mainly caused by *C. parvum*, which is an obligate intracellular parasite that infects the microvillus border of the epithelium in the gastrointestinal tract of humans and various animal hosts. Water has been found to be the most important source of *C. parvum* and because of its chlorine resistance, this organism has been a particular threat to otherwise safe drinking water supplies.

Cryptosporidium oocysts have been isolated from several foodstuffs, including fruit, vegetables, and shellfish. Several outbreaks of cryptosporidiosis have been associated with milk. In 1985, an outbreak of 22 cases of cryptosporidiosis occurred in Mexico in which contaminated milk was suspected. In 1995 50 cases of cryptosporidiosis were confirmed in the United Kingdom. Junior-level school children were infected after drinking milk that was distributed to the school by a small-scale local producer. The on-farm pasteurizer was found to be faulty and hence, the milk was not adequately pasteurized. In 1984, a mother and her 1-year old child were infected with *Cryptosporidium* after drinking unpasteurized goat's milk that had been purchased locally in Australia.

Cryptosporidium parvum cannot survive pasteurization of milk and 100% inactivation was achieved by heating milk to 71.7 °C for 5 s. *Cryptosporidium parvum* showed 0–5% viability after 48 h in ice cream stored at − 20 °C. However, prolonged storage of contaminated yoghurt for up to 240 h was not sufficient to destroy *C. parvum*. A decrease in viability of the organisms from 83% at time 0, to 61% after 240 h was noted.

Bacteria

Rickettsiae

Coxiella burnetti

Coxiella burnetti, the causative agent of Q-fever, is an organism that may infect the udder, probably by the hematogenous route. Consumption of, or contact with, the infected milk can also lead to human infection. This organism was found to be more heat resistant than *Mycobacterium tuberculosis* and in 1956, the recommended vat pasteurization temperature was raised from 61.7 to 63 °C (holding time 30 min) to ensure destruction of the organism. The current high temperature short time (HTST) pasteurization temperature of 72° for 15 s is also effective in this regard. Milk alkaline phosphatase, which serves as an indicator of pasteurization efficacy for

bovine milk, is more heat resistant than *C. burnetti* and is destroyed by the above pasteurization treatments.

Brucella

Brucella abortus and *Brucella melitensis* are zoonoses that can cause milkborne disease outbreaks in humans and are the causative agents of brucellosis in cattle. Brucellosis eradication programs in many countries have resulted in a decrease of outbreaks. In many parts of the world this eradication has been so successful that the organism no longer poses a hazard to human health. Where the disease still occurs, pasteurization of milk has minimized the number of human outbreaks by these microorganisms.

Mycobacterium

Mycobacterium bovis is a microorganism of concern to the dairy industry because it causes tuberculosis in cattle. It can also result in milkborne *M. bovis* infections of humans. Cattle immunization and pasteurization of the milk has limited the number of tuberculosis cases associated with the consumption of milk or dairy products.

Another *Mycobacterium* sp. that has generated increasing interest in recent times is *Mycobacterium avium* spp. *paratuberculosis* (MAP). This organism is the etiological agent of paratuberculosis (also known as Johne's disease), which is a global disease of ruminants that was first described in 1895. The disease itself is a slow developing colitis in which the intestinal macrophages are infected. In the process inflammatory reactions are induced in the host gut. This affects the ability of the gut to absorb protein from the diet resulting in clinical features that include diarrhea and chronic weight loss.

Although there are similarities between paratuberculosis in cattle and Crohn's disease in humans, the homology of the two diseases is still in dispute. However, sufficient evidence exists to indicate that MAP should be prevented from entering the human food supply. Milk may be contaminated by the natural shedding of infected macrophages or by fecal contamination. Although the infectious dose is reported to be as low as 1000 organisms, clinically affected animals may shed up to 5×10^{12} mycobacterial cells per day. Occurrence of the pathogen in milk producing animals is consequently a challenge to animal health, milk quality, and the safety of the milk supply. As Johne's disease in cattle is itself devastating for the animals and a cause of reduced milk productivity, the most efficient control measure is the eradication of the illness in cattle and the sourcing of milk from healthy animals.

Enterobacteriaceae

The presence of any member of the Enterobacteriaceae family is undesirable in pasteurized milk and dairy products. This is because of: (1) the inherent spoilage capacity of many genera in this family, (2) the fact that the presence of certain genera in water and food may be indicative of fecal contamination, and (3) the serious food safety implications that the presence in food or water of the many pathogens in this family may have.

Salmonella

The gastroenteritic form of nontyphoid salmonellosis was not clearly linked to raw milk consumption until the mid-1940s. Interest in milkborne salmonellosis has peaked twice since then. In 1966 several large outbreaks were traced to nonfat milk powder and again in 1985 one of the largest recorded outbreaks of foodborne salmonellosis involving more than 180 000 cases was traced to consumption of a particular brand of pasteurized milk in the Chicago area. The incident was caused by the postprocess contamination of pasteurized milk because of an error in equipment design allowing cross connection between raw and pasteurized milk lines. This outbreak also led to the discovery that reactive arthritis ('Reiter's syndrome') is a sequelae of salmonellosis, occurring in approximately 3% of patients. Today *Salmonella* and *Campylobacter* are generally recognized as the two leading causes of dairy-related foodborne illness in the US and Western Europe with rates of infection being particularly high in regions where raw milk is neither pasteurized nor boiled before consumption.

Raw milk can be a source of salmonellae and 32 of 678 raw bulk milk samples were reported to test positive in the US in 2003. Standard pasteurization destroys expected levels of salmonellae (i.e., < 100 cfu ml^{-1}), with a wide margin of safety. Inadequate pasteurization and postprocess contamination have occasionally resulted in milk and cream that test positive for *Salmonella* as evidenced by the reported outbreaks. *Salmonella* is not particularly tolerant of heat, refrigeration or salt, so the typical hurdles used in the dairy industry are effective in controlling this organism.

Escherichia coli

Escherichia coli is currently the best known indicator of fecal contamination, primarily, not only of water but also of raw food products. Its recovery from fresh dairy products consequently suggests that other organisms of fecal origin, including pathogens, may be present. *Escherichia coli* strains are commonly associated with the normal facultative anaerobic microflora of the intestinal tracts of humans and animals. Although many of these strains are harmless commensals, various *E. coli* strains have acquired genetic determinants (virulence genes) rendering them pathogenic for both humans and animals. These pathogens are responsible for three main clinical syndromes, namely enteric and diarrheal diseases, urinary tract infections, and sepsis/meningitis. On the basis of their distinct virulence properties and the clinical symptoms of the host, pathogenic *E. coli* strains can be divided into numerous categories or pathotypes. The extraintestinal infections are caused by three separate *E. coli* pathotypes, namely uropathogenic strains, neonatal meningitis strains and strains that cause septicemia in humans and animals. The diarrheagenic *E. coli* strains include enteroaggregative *E. coli*, enteroinvasive *E. coli*, enteropathogenic *E. coli* and enterotoxigenic *E. coli* (ETEC). Members of the latter group adhere to the mucosa of the small intestine and produce heat labile and/or heat stable enterotoxins. A group of ETEC strains produce cytotoxins called verotoxins or shiga-toxins and hence the acronyms VTEC or STEC. These colonize the intestinal tract of healthy domestic animals, chiefly cattle. The STEC serogroups, 0157, 026, and 0111, are also designated enterohaemorrhagic *E. coli* (EHEC). Only this latter group of organisms will be the subject of further discussion in the present article.

Several outbreaks of *E. coli* gastroenteritis have been traced to raw milk and dairy products. Infections with EHEC of the

serotype O157:H7 were first described in 1982. *Escherichia coli* O157:H7 has become a pathogen of major concern for the food and dairy industries because of its ability to cause severe ailments such as hemorrhagic colitis (HC), HUS, and thrombocytopenic purpura. These ailments affect all human age groups and the pathogen is exceptional in the severe consequences of infection, its low infectious dose and unusual acid resistance. Cattle are the main reservoir of *E. coli* O157:H7, although the most likely mode of transmission in foods is through fecal contamination. The organism has an oral infectious dose within the range of 10–100 cells, or even lower in the case of susceptible groups.

Its survival in acidic foods such as yoghurt and its survival in fermented as well as fermenting dairy foods for long periods of time make its hardy nature apparent. During the manufacture of cottage cheese (the most well-known and popular acid–curd cheese), low pH of the product did not halt the growth of *E. coli* O157:H7.

Prompt action taken in response to recent HC outbreaks associated with acidic foods has resulted in a large number of validation studies in many countries. In particular, the US mandates that food processors should guarantee a 5-log reduction of *E. coli* O157:H7 during processing of fermented sausages and fruit juices, but has not yet extended this to fermented milk products.

The behavior of *E. coli* O157:H7 was studied during the manufacture and ripening of raw goat milk lactic cheeses. When cheese was manufactured from raw milk in the laboratory and inoculated with *E. coli* O157:H7 to a final concentration of 10, 100, and 1000 cfu ml^{-1}, counts decreased to less than 1 $\log_{10} g^{-1}$ in curds just before molding. However, viable *E. coli* O157:H7 were found in cheeses throughout processing and even after 42 days of ripening. This implies that the presence of low numbers of *E. coli* O157:H7 in milk destined for the production of raw milk lactic cheeses can constitute a threat to the consumer. Consequently, it is imperative to ensure that milk used in the manufacture of such cheeses is of the highest microbiological quality.

Given the potential for contamination of milk by *E. coli* during milking, consumption of raw milk should be avoided. *Escherichia coli* O157:H7 is not heat resistant and, like most *E. coli* strains, is readily destroyed by the pasteurization process. If good manufacturing practices are followed, consumption of pasteurized milk poses little or no risk of contracting this microorganism. Although the organism is unable to grow at less than 10 °C, substantial growth can occur in temperature abused milk.

Yersinia

Yersinia enterocolitica and other yersiniae falling within the family Enterobacteriaceae, are often referred to as 'environmental strains.' *Yersinia enterocolitica* is, nevertheless, a well-established pathogen of humans, causing acute gastroenteritis, enterocolitis, and mesenteric lymphadenitis, as well as a variety of extraintestinal disorders. The symptoms of these ailments may be especially severe in children and individuals with underlying disease.

Yersinia enterocolitica is regarded as an unusual cause of milkborne illness because of the low incidence of human pathogenic strains in the raw milk supply and high susceptibility of the organism to pasteurization. Yersiniae contamination of raw milk is thought to occur through contact with feces or polluted water. Raw milk and inadequately pasteurized milk and dairy products have, nevertheless, been implicated in the transmission of *Y. enterocolitica* infections to humans. In 2005, the first recorded food-associated outbreak of yersiniosis occurred in New York, NY, USA where more than 220 individuals were stricken with acute intestinal illness after consumption of contaminated milk. In addition epidemiological studies have revealed that refrigerated food stored over prolonged periods of time pose an additional risk since *Y. enterocolitica*, as a psychrotrophic microbe, is able to grow at temperatures as low as 0 °C. *Yersinia enterocolitica* and atypical *Yersinia* spp. were also isolated from cheese samples in Turkey and results indicated that *Yersinia* spp. are more likely to be isolated from foods with a high level of coliforms than from foods with low coliform counts. This lends support to the hypothesis that *Yersinia* contamination of raw milk is of fecal origin.

Cronobacter sakazakii (previously *Enterobacter sakazakii*)

This is an opportunistic pathogen affecting infants and neonates causing sepsis, necrotic enterocolitis, and meningitis. It is ubiquitous in environment, but has particularly been associated with powdered infant formula. The organism is heat sensitive and is destroyed by heat treatment of milk. The major source of contamination is believed to be postprocess contamination, or contaminated ingredients in dry mix processes.

Campylobacter jejuni

Campylobacter jejuni has been recognized since 1909 as an important cause of abortion in cattle and sheep. Improved isolation strategies have also implicated this organism as a causative agent of human diarrhea. For the year 2011, CDC reported that *Campylobacter* spp. were the fourth leading cause of domestically acquired foodborne illness with 845 024 cases. They were also the third leading cause of hospitalizations and fifth leading cause of death. Although CDC is only beginning to compile food attribution data, a previous study indicated that many outbreaks of campylobacteriosis involved ingestion of raw milk. Similar reports linking raw- and inadequately pasteurized milk to 13 outbreaks in Great Britain from 1978 to 1980 helped to further substantiate *C. jejuni* as an important milkborne pathogen that has come to rival or even surpass *Salmonella* as an etiological agent of human gastroenteritis worldwide.

Campylobacter jejuni can be isolated from the feces of cattle infected or colonized with the organism and has been shown to cause asymptomatic bovine mastitis in which the organism is excreted directly into the milk by an infected cow. In most outbreaks, however, *Campylobacter* could not be isolated from milk after an outbreak. Nevertheless, a survey done in England in 1988 showed that 5.9% of milk samples tested were positive for *C. jejuni*. It was also found, in the latter survey, that there was a significant association between the presence of *E. coli* in milk and that of *C. jejuni*.

Campylobacter jejuni infections have been shown to be hyperendemic in developing countries, including South Africa, with an age-related decrease in the incidence of infections in humans. Acquired immunity could be important in preventing infection

or preventing illness after infection. Nevertheless, immunocompromised people are at risk of contracting the infection.

Campylobacter jejuni is killed by proper pasteurization, but outbreaks involving inadequately pasteurized milk have been described in England. Failure in the public electricity supply and a faulty pasteurizer were identified as the causes of the problem. *Campylobacter* is also acid sensitive and this suggests that the genus will not survive a normal fermentation in a product such as yoghurt.

Staphylococcus aureus

Staphylococcus aureus is a significant cause of mastitis in dairy cows throughout the world. The bovine mammary gland can be a significant source of enterotoxigenic strains of *S. aureus*. Enterotoxins produced by enterotoxigenic strains of this organism are classified according to serotypes into A–H groups and the presence of the so-called 'toxic shock syndrome toxin' (TSST). TSST was detected in *S. aureus* strains in milk from cows with clinical and subclinical mastitis and also from farm bulk tank milk. Various other studies also implicated bulk tank milk as a potential source of enterotoxigenic *S. aureus* in milk and milk products. Consumption of raw milk generally increases the chances of direct contact with food pathogens such as *S. aureus* and its toxins.

Workers involved in processing the milk and manufacturing dairy products can also be a source of *S. aureus* in the product. Various surveys that have demonstrated that from 4% to 60% of humans are nasal carriers of *S. aureus* and that between 5% and 20% of people carry the organism as part of the normal skin microflora.

Staphylococcus aureus was found to be the most frequent pathogen associated with cheeses from raw or 'unspecified' milk in toxi-infections alimentaires collectives (TIAC) reports in France. For TIAC, an outbreak is defined as the occurrence of two or more cases of a similar illness, usually gastroenteritis, caused by the same food. Although the majority of patients from the various outbreaks were hospitalized, no fatalities were reported. Other outbreaks of *S. aureus* enterotoxicoses have occurred. In 1985, 860 children were affected by drinking chocolate milk in Kentucky, USA. More than 14 000 people in Osaka, Japan, were affected by drinking contaminated pasteurized milk in 2000. The latter mentioned incident was because of the lack of cold storage as a result of an electricity failure.

Staphylococcal enterotoxin production can be prevented by keeping the raw milk refrigerated until the milk can be effectively pasteurized. Although pasteurization procedures will kill the cells of *S. aureus* in the milk, they will not destroy enterotoxin already present. Therefore, care should be taken to prevent postprocess contamination and maintaining the cold chain.

Listeria monocytogenes

Listeria monocytogenes has emerged in recent decades as a serious foodborne pathogen that can cause abortion and death in pregnant women and meningitis, encephalitis, and septicemia in newborn infants and the immunocompromised. Although the disease is rare resulting in 2.8 cases per 1 000 000 people per year in the USA in 2011 and accounts for only approximately 0.02% of all cases of foodborne illness, listeriosis is estimated to account for approximately 19% of the deaths resulting from foodborne illness in the USA. This severe mortality rate emphasizes the necessity to minimize the exposure of high risk individuals referred to above.

The variability in virulence of *L. monocytogenes* strains is gaining recognition and acceptance. Throughout the world, three serotypes (i.e., 4b, 1/2a, and 1/2b) account for 89–96% of cases of human listeriosis, providing additional evidence that certain strains are more likely to cause illness. Serovar 4b, for example, has caused several major outbreaks. One outbreak in Switzerland originated from soft cheese and involved 122 cases, resulting in 34 deaths during the period 1985–87.

It has been suggested that the infective dose of a virulent strain of *L. monocytogenes*, is in the order of 100–1000 cells. In general, foods that have been implicated in listeriosis have contained >1000 cfu ml^{-1} or per g. The most important property of *L. monocytogenes* is its ability to multiply in foods at refrigeration temperatures and most foods incriminated in listeriosis have been held under refrigeration. Any ready-to-eat food contaminated with *L. monocytogenes* that is kept refrigerated, may consequently yield population numbers that can present a threat to susceptible consumers. Milk and dairy products have been found to be sources of foodborne listeriosis and after outbreaks involving a large number of cases, *L. monocytogenes* has become a pathogen of great concern to the dairy industry.

Listeria monocytogenes is commonly encountered in the dairy farm environment. The most likely sources of the organisms in raw bulk-tank milk are, therefore, environmental in nature with fecal sources and manure playing major roles. Contamination from within the udder as a result of shedding because of bovine listeriosis or listerial mastitis is likely to be rare. The organism can be transmitted to cows via feeds such as improperly fermented silage and other feedstuffs, causing infection in the animal. Incidences of *L. monocytogenes* of 4.2%, 2.2%, and 2.6% have been reported in farm milk samples of bovine, ovine, and caprine origin, respectively.

Listeria has been isolated from many dairy products, including ice cream and various types of cheese. In soft ripened cheese, contamination is limited to the first few millimeters under the rind. Hard cheese such as Parmesan does not favor growth and other cheeses such as Colby, Swiss, Provolone, Munster, Feta, and Limburger show gradual die-off of the bacteria. In Mozzarella, the bacteria will survive the manufacturing process, but not the stretching temperatures. *Listeria monocytogenes* will not grow in cottage cheese, but can survive. Although the organism has been found to gain access to yoghurt as a postpasteurization contaminant, it will not survive at pH levels of less than 4.6.

Listeria monocytogenes is quite well adapted to dairy factory environments. It is generally more common in factories where conditions tend to be wet and cool with areas of pooled water or liquid. High organic load on the floors and in the drains can also contribute to the growth and survival of *Listeria*. The primary source of *Listeria* spp. in processing plants is probably floors and floor drains, especially areas around coolers or places subject to outside contamination. Because of the ubiquitous nature of the organism, all raw materials carried into the packaging area must be suspect. Cooling waters should also be considered as a possible source of contamination.

Work practices of factory personnel and the dispersal of the organisms in water sprays and aerosols have also been identified as significant sources of the organism throughout the factory.

Many researchers have demonstrated that once strains of *L. monocytogenes* become established in a food-processing facility, they can remain members of the resident microbial flora for many years. Examples of niches in which the organisms can become established are hollow rollers on conveyors, cracked tubular support rods on equipment, the space between close-fitting metal-to-metal or metal-to-plastic parts, worn or cracked rubber seals around doors, on–off valves, and switches for equipment as well as saturated insulation material.

Listeria monocytogenes can become established on steel and rubber surfaces in the form of biofilms. The organism was found to survive for prolonged periods on stainless steel and buna-*n* (acrylonitrile butadiene) rubber. Under favorable conditions, it can even multiply on stainless steel. Temperature, relative humidity, soiling, and the surface type affected the behavior of surface-associated *L. monocytogenes.* In addition, the attachment surface affected the efficacy of sanitizers.

In terms of prevention or control procedures, *Listeria* spp. can contaminate raw milk from many environmental sources and it is difficult to entirely prevent the presence of *Listeria* in raw milk. In most cases control of raw milk contamination should, however, be readily achieved by good sanitation and milking practices.

In controlling the presence of *L. monocytogenes* in the processing area and product itself, the following strategies can be followed:

- In the first place, steps should be taken to prevent entry of *L. monocytogenes* into plant areas: These steps basically include the isolation of the milk receiving area and associated personnel, from the processing and packaging area and prevention of any raw product from entering the processing area. Any product and container returns should similarly not be permitted back into the manufacturing or packaging area. There should be no unsealed openings to the manufacturing area from other areas.
- *Listeria* should be prevented from establishing and growing in niches or other sites that can lead to contamination of the processed food. This should include a thorough check of water and glycol cooling systems, cracks and crevices in storage tanks and other equipment components, and effective cleaning and sanitising. All drains must be properly constructed, cleaned, and sanitized each day. Floor drains should not be located under or near filling and packaging equipment.
- Effective pasteurization is the key step to control *Listeria* in processing. In the case of HTST pasteurization of milk, a minimum of 72 °C for 15 s is essential. Products containing higher fat or sugar levels require higher temperatures to ensure effective destruction of *Listeria* spp., such as 75 °C for 15 s to be safe.
- Cleaning and sanitizing programs are vital in ensuring that postpasteurization contamination does not occur. Absorbent items such as rags and sponges should be eliminated to reduce potential harborage and spread of the organisms. Separate brushes should be used for product contact and nonproduct contact surfaces.
- A sampling program should be implemented that can assess in a timely manner whether the food processing environment is under control. There should be a rapid and effective response to each positive sample obtained. Finally there should be verification by follow-up sampling that the source has been detected and corrected.

Bacillus cereus

Bacillus cereus is a well-known pathogen that may cause illness through the production of either an emetic (vomit-inducing) toxin or at least three diarrheal toxins or enterotoxins. Raw milk appears to be the major source of *B. cereus* in the pasteurized milk. Postpasteurization contamination along the milk processing line is possibly only a minor source. Nevertheless, *B. cereus* cells can attach to stainless steel surfaces and are capable of forming biofilms, a situation that can present a major problem for the food industry.

Spore formers such as *Bacillus* spp. which have survived the pasteurization process can cause a variety of spoilage problems in dairy products and *B. cereus* has been detected in ice creams, milk powders, fermented milks, and pasteurized milks. Although *B. cereus* is able to grow at low temperatures, pH is a definite hurdle with pH 5 being critical for its growth. It is expected, therefore, that growth in fermented dairy products with a pH lower than 5 will be minimal. Even where population levels of a toxigenic *B. cereus* strain in milk did attain levels of up to 9×10^7 cfu g^{-1}, the emetic toxin (cereulide) production was very low. Milk and milk products are consequently not likely to be sources of intoxication caused by emetic toxin-producing *B. cereus* strains.

Clostridia

Clostridia spp., for example, *Clostridium botulinum*, *Clostridium perfringens*, are widely distributed in soil, dust, water, sediments, sewage, and vegetation. Feeds and especially silage, can also be contaminated; under favorable conditions of water activity (aw), pH, and temperature, they can grow and contribute to the spread of spores. Raw milk can become contaminated during the milking process. Numbers of *Cl. botulinum* spores in raw milk are generally very low; in cheese production, during centrifugation and filtration steps, they can increase to 10 or more per gram of cheese. However, in favorable conditions of growth even low numbers of *Cl. botulinum* spores can be dangerous. Most outbreaks of *Cl. botulinum* intoxication are associated with proteolytic strains as they are more heat- and acid resistant than the nonproteolyitc ones. Outbreaks are often associated with pasteurized and heat-treated milk where competitive flora are killed. Outbreaks have been reported with cheese and yoghurt because of the addition of a contaminated ingredient. Contamination of infant formula with *Cl. botulinum B* spores has also been suspected to be a cause of infant botulism. Another potential concern is the consumption of milk derived from cattle or other animals affected by botulism.

Chemical Hazards

Mycotoxins

Mycotoxins are secondary metabolites produced by fungi of various genera when growing on agricultural products before or after harvest or during transportation or storage. Contamination by such fungi can occur at many stages during feed production, for example, during plant growth, harvesting, storage, and processing. Mycotoxins are found mostly on feed ingredients such as maize, sorghum, wheat, and groundnuts. Mycotoxins pose a risk to both animal and human health and depending on the chemical structure of the mycotoxin, pathology may result because of the carcinogenic, estrogenic, neurotoxic, dermonecrotic, or immunosuppressive activity of the toxin.

Fungal species of greatest concern in the dairy industry are *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nomius.* These species produce aflatoxin B_1 and related toxins under favorable conditions of temperature, water activity, and nutrient availability often found in subtropical climates. In recent years, concern has been expressed about the presence of aflatoxin M_1 in milk and milk products, which is an animal metabolite of aflatoxin B1.

The exposure of humans to aflatoxins has resulted in liver damage and cancer. Aflatoxin M_1 levels in dairy products are regulated in many countries. Codex has adopted a maximum limit of 0.5 $\mu g\,kg^{-1}$ of liquid milk, but has not set a level for animal feed. The European Union (EU) maximum limits (MLs) for different feed commodities vary between 0.05 and 0.005 $mg\,kg^{-1}$. Provided that these MLs for Aflatoxin B1 (and other mycotoxins) in feeds are observed, there should be no problems with harmful residues in milk.

Mycotoxins produced by fungal species other than *Aspergillus* and possibly *Penicillium* are of minor concern for dairy products. Nevertheless contamination of feed and forage with zearalenone (a *Fusarium* spp. mycotoxin) has been shown to result in residues of zeranol in forage fed cattle. Zeranol, approved in some countries as a hormonal growth promoter, is specifically prohibited from use in food animals in the EU. Hydrogenation of alfa-zearelenol, probably in the rumen, is responsible for the formation of zeranol. This finding of a 'natural' zeranol in cattle has complicated control measures. This makes it necessary to differentiate zeranol arising from feed and forage contamination from deliberate growth promoter abuse.

Once the feed has been contaminated, processing the feed by means of heat treatment has little effect in eliminating most mycotoxins. Continuous monitoring of levels needs to take place to ensure that contamination does not exceed acceptable tolerance levels. Preventing contamination at source is one of the most effective methods of reducing the risk of mycotoxin contamination. Suitable measures need to be applied during crop production, handling, storage, and processing. Proper drying is particularly important.

Antimicrobials

The most frequently and commonly used veterinary drugs associated with milk are antibiotics, employed to combat mastitis-causing pathogens in the dairy cow. National surveys in developed countries show that between 0.1% and 0.5% of tanker milk samples test positive for antibiotic residues. The occurrence of antibiotic residues in milk may have economic, technological, and even human health implications. In the first place, such residues can lead to partial or complete inhibition of acid production by cheese starter cultures. This can lead to inadequate ripening and maturation of the cheese, resulting in flavor or texture defects and substantial financial loss for the dairy industry. There has also been increasing public concern over the possible links between veterinary drug residues in milk and the transfer of antibiotic resistant organisms to humans as a result of veterinary and zootechnical use of antibiotics in food animals. A third concern raised was that sensitive individuals could exhibit allergic reactions to drug residues or their metabolites, especially in the case of β-lactam antibiotics.

As from 1990, maximum residue limits (MRLs) have been set in Europe for veterinary drugs in foodstuffs of animal origin like milk. Most dairy companies also use rapid tests to monitor all incoming milk for the presence of β-lactam antibiotics. Some of these companies are claiming compensation for the costs of disposing of the milk of a contaminated tanker load from the responsible farmer.

The solution to the problem of drug residues in milk lies in the application of the general principles of 'good farming practice.' These include the following principles:

- Good farm management should in the first place be directed toward the prevention of infectious diseases, such as (sub)clinical mastitis, in order to limit the use of veterinary drugs;
- in the process the farmer must keep his animals in sound physical condition by ensuring hygiene and good housekeeping practices and implementing sound farm management;
- in preventing mastitis, the use of properly functioning milking machines is of primary importance. The use of veterinary drugs, nevertheless, remains necessary, but this option should only be exercised after a correct diagnosis by a veterinarian. Only registered pharmaceutical products with known depletion patterns should be used;
- correctly administering the drugs is also very important in terms of prescribed dose, frequency, and route of administration;
- the keeping of reliable records of such drug use is also essential. It remains the responsibility of the milk producer to respect the prescribed withdrawal period. In the process, the treated animals need to be marked clearly to allow for correct identification (e.g., by taping a hind leg);
- treated cows need to be milked last and during the withholding period, the milk must be discarded in the proper way. The milking equipment should also be cleaned properly after contact with the contaminated milk;
- special care should be taken with the milk of cows that have been treated with long-acting dry cow products or with milk from cows that have been recently purchased; and
- good communication is also important. Everyone on the dairy farm should be informed of any treatment, although the number of people authorized to administer antibiotic

drugs should be limited. If in any doubt, the milk should be tested.

Other Veterinary Drugs

Many human drugs are contraindicated during lactation and a similar problem occurs in dairy animals. The treatment of animals with ectoparasiticides and endoparasiticides can result in residues in milk if the withholding period before returning to milk production is not observed. Endoparasiticides used to control helminths (including tapeworms, roundworms, and flukes) may be administered as feed additives, by injection or cutaneously. The most commonly used compounds in the past were levamisole and the benzimidazoles, but have now been largely supplanted by ivermectin. Studies on the excretion rate of these drugs indicate that a withdrawal time of 5 days is adequate after therapeutic treatment. Cutaneous treatment of animals against ectoparasites includes a wide variety of pesticides that are evaluated as veterinary drugs. In most cases, a 2-day withdrawal period is adequate for assuring that residues are within the safe limits. Consequently, for these types of drugs, the safety of the milk of treated animals depends on their proper use. However, the use of pesticides to control environmental problems, such as insects and rodents, also need to be handled with care to avoid contamination.

A number of hormones are also used in relation to dairy animals, such as oxytocin and prostaglandins. However, one of the more controversial is bovine somatotropin (BST) (sometimes referred to bovine growth hormone) and its genetically engineered counterpart recombinant BST (rBST). Adopted by a contentious vote in 2012, the use of rBST to 'freshen' cows is recommended by the Codex Alimentarius Commission and is approved in the US, but not in the EU because of animal health reasons. However, the milk from rBST-treated cows may be traded without restrictions. This is because both BST and rBST are not hormonally active in humans and if ingested, they are rapidly digested because they are protein hormones.

Cleaning Agents and Sanitizers

Cleaning and sanitizing are essential components of any good manufacturing practice in the food industry, but are particularly important for milk and milk products to remove any bacteria from food contact surfaces. However, it is also important to remove unsafe residues of cleaning agents and sanitizers to avoid contamination. At the farm level, maintaining hygiene of the udder is of critical importance to prevent contamination and various disinfectants have been developed to clean the udder before milking. At the plant level, the use of cleaning-in-place methods requires careful cleaning, sanitizing, draining, and rinsing procedures. Some sanitizers, such as certain iodophores, do not require rinsing and therefore, occur in milk and dairy products at very low concentrations as indirect food additives. The most commonly used sanitizers, including iodophores, chlorhexidine, and hypochlorites, contain iodine or chlorine as the active agents. In addition, hydrogen peroxide and quaternary ammonium compounds are also used.

Generally, only limited information is available on the toxicology of these compounds and their occurrence in food, with the exception of iodine. Iodine levels in milk have been increasing in a number of countries. Milk was found to be an important dietary source of iodine in New Zealand and as the industry moved to quaternary ammonium compounds, the government found it necessary to initiate the fortification of bread with iodine to prevent deficiencies of this micronutrient in their population.

Industrial and Environmental Contaminants

Pesticide Residues

Pesticides include insecticides, herbicides, and fungicides. The most common insecticides in turn include organochlorines, the organophosphates, and carbamates. The organochlorine pesticides enter the food chain as a result of their lipophilic properties, in this way causing a potential health risk for consumers. Milk is considered as one of the more convenient indicators for measuring the extent of persistent residues that have originated in contaminated animal feed. The main route of human exposure to many organochlorine pesticides is through food of animal origin of which milk is the most important product. Typical contaminants of milk are persistent fat soluble organochlorine pesticides such as hexachlorobenzene, dichlorodiphenyltrichloroethane (DDT), and to a lesser extent the cyclodiene compounds. However, most of these pesticides have been withdrawn from use, but remain as environmental contaminants as they are quite stable in soil. If the cow is grazing in contaminated pastures, the intake of soil with these pesticides can occur.

Organophosphate and carbamate pesticides are the most widely used insecticides today, but degrade rapidly in the environment and are further metabolized by animals. Codex routinely establishes MRLs for these and other pesticides on animal fodders as the result of their use on crops. In some cases, MRLs are also established for residues in milk and in other animal products, such as eggs.

Dioxins and Polychlorinated Biphenyls (PCBs)

The term 'dioxins' covers a group of 75 polychlorinated dibenzo-*p*-dioxin and 135 polychlorinated dibenzofuran congeners of which 17 are of toxicological concern. The most toxic congener is 2,3,7,8 tetrachloro dibenzo-*p*-dioxin which is a known human carcinogen, but also possesses other toxic properties. No adverse effects of dioxins were considered to occur in humans at levels below a certain threshold, but this is being challenged by epidemiology studies showing effects at low doses. The maximum level set in EU regulations for dioxins in milk and milk products, including butter fat, is 3 pg World Health Organization toxic equivalents (WHO-TEQ) per g fat.

The PCBs in turn are a group of 209 congeners which can be divided into two groups according to their toxicological properties. Twelve congeners exhibit toxicological properties similar to dioxins and are therefore termed 'dioxin-like PCBs.' The other PCBs have a different toxicological profile.

Dioxins and PCBs are extremely resistant to chemical and biological degradation and therefore persist in the environment and accumulate in the feed and food chain. Dioxins and PCBs arise during the production of chloroorganics and in emissions of industrial by-products and municipal incineration and other pyrolysis processes. Contamination of animal

feed occurs via particle-bound distribution on grass and other fodder plants. The accumulation of dioxins in animals is mainly from these contaminated feeding stuffs. Human foods of animal origin in turn contribute to approximately 80% of the overall human exposure to dioxins.

For these reasons feeding stuffs and in some cases soil, raise concern as potential sources of dioxins. Like the organochlorine pesticides, the dioxins and PCBs are fat soluble. Case studies that have involved these contaminants include the Belgian PCB/dioxin incident in 1999. Feedstuffs produced from a contaminated source were sent to 2500 farms and nearly every category of agrifood (pork, milk, chicken, and eggs) was affected.

Based on 13 797 food samples taken between 1995 and 2010, the European Food Safety Agency has estimated that the percentage of individuals exposed to dioxin and dioxin-like PCBs above the tolerable weekly intake of 14 pg WHO-TEQ per kg bodyweight was between 1.0% and 52.9%. The major contributor to total exposure was the food category of milk and dairy products for almost all groups of infants and toddlers, whereas for most of the groups of adolescents, adults, elderly, and very elderly, fish and shellfish were first, followed by milk and dairy products. For the total dioxins and dioxin-like PCBS, the upper bound estimate for 1422 samples of milk and milk products was 1.92 pg WHO-TEQ kg^{-1}. The corresponding EU maximum limit for milk and dairy products is 5.5 pg WHO-TEQ kg^{-1}. Of all samples, 0.5% exceeded this limit by a small margin. As the EU has implemented source directed measures, however, the exposure levels have been falling for many years.

Heavy Metals

The term heavy metals is a general term that applies to a group of metals and metalloids, including elements such as cadmium (Cd), chromium (Cr), nickel (Ni), copper (Cu), zinc (Zn), mercury (Hg), and lead (Pb). This sometimes includes arsenic (As) because of its toxicity.

The widespread distribution, contamination and multiple effects of heavy metals in the environment has become a global problem. Pb is one of the most common heavy metal pollutants and tolerable levels have fallen as the adverse effects of Pb, especially on the development of cognitive brain function in children, have been recognized. Pb is readily transmitted to milk as it is associated with calcium metabolism. However, in a 1999 study, the highest metal concentrations in dairy cattle feeds were for Zn and Cu. Mineral supplements contained higher concentrations of Ni, Pb, Cd, As, and Cr than did other feed components.

Allergens

Cow's milk allergy or hypersensitivity is commonly encountered with a prevalence of 2–3% in infants and 0.5–3% in adults, but this varies among different populations. This allergy in infants and children is in most cases a transient condition lasting from several months to a few years, after which tolerance is inclined to develop. The dietary management of this ailment includes strict avoidance of the allergenic proteins.

Detailed information on the allergenic epitopes of the major bovine milk proteins, namely α-s1-casein, β-lactoglobulin and α-lactalbumin, indicates that in individual patients, several proteins and per protein, several epitopes (allergenic segments or polypeptides), are often involved. Furthermore, multiple combinations of allergenic proteins and epitopes may occur. Allergenic epitopes are located in specific regions of the proteins, both on the hydrophilic surface as well as in hydrophobic regions, which become exposed on denaturation and/or digestion.

Because present knowledge of the exact mechanisms of sensitization and tolerance induction is still far from complete, it cannot be excluded that specifically fractionated and/or modified cow/s milk proteins, in combination with other bioactive components, will in future be useful in preventing sensitization and/or inducing tolerance.

Other Potential Chemical Hazards

The addition of water to milk is probably one of the oldest forms of economic adulteration of food. However, other materials, such as chalk, were often added to mask this fraud. The most recent variation of this practice was the addition of melamine to milk in China. This was done to avoid detection by the standard analysis of crude protein in milk, sometimes called total protein, which is used to monitor and control milk quality in the dairy industry. The Kjeldahl analysis measures the total nitrogen content of milk, which is then simply multiplied by 6.38 to express the result on a protein equivalent basis. Melamine is chemical substance rich in nitrogen, inexpensive and widely available as it is used in the manufacture of many laminates, plastics, coatings, glues, and kitchenware. In 2008, infant formula made with melamine-adulterated milk resulted in illness in a reported 300 000 infants, including over 50 000 hospitalizations and 6 deaths. A range of other products including, liquid milk, ice cream, and yoghurt were also contaminated. Because melamine can be present in milk and other products as a result of legitimate uses, the Codex Alimentarius Commission has recommended MLs to accommodate these uses at 1 mg kg^{-1} in infant formula and 2.5 mg kg^{-1} in other foods and animal feed.

Other contaminants that may be found in milk and milk products include radioactive isotopes whose sources are both anthropogenic and nonanthropogenic. For instance, background radiation in milk may vary from 40 Bq l^{-1} for potassium-40 to below 0.1 Bq l^{-1} for cesium-137. Radioactivity of food may increase following certain human activities such as weapons testing and nuclear accidents. The latter presents the greatest source of contamination as demonstrated by the Chernobyl accident in 1986 and the Fukushima disaster in 2011. Codex has developed derived intervention levels for milk and other foods for various radionuclides following such accidental releases.

Milk and dairy products may contain a range of other chemicals, usually at very low levels. In recent years, contaminants resulting from food processing and food contact materials have raised some concerns. In relation to infant formula, advanced glycation endproducts and 3-monochloropropandiol esters are being examined. Acrylamide may be a concern depending on what other ingredients, such as

chocolate, are added. In regard to food contact materials, bisphenol A, an endocrine disrupter, has been banned from baby bottles because of possible migration to milk and infant formula. Certain components of inks have been banned because printed cartons have contaminated milk. Cheese can be a source of biogenic amines, notably hisamine and tyramine, both of which cause intense, but transient intoxications. Furans have also been found in milk and dairy products, but at levels that pose little risk to the consumer. In this regard, the application of risk assessment to low-level contaminants in milk and dairy products is the best means to ensure that priorities for risk management are evidence-based.

Possible Procedures to Minimize the Risks of Feed and Milk Contamination

The production, processing, storage, transport and distribution of safe, suitable feed and feed ingredients is the responsibility of all participants in the food chain. These participants include farmers, feed ingredient manufacturers, feed compounders, transport contractors, etc. Each participant is responsible for all the activities under their direct control, including compliance with applicable statutory requirements. The Dutch Animal feed sector has opted for a quality assurance system based on the HACCP approach applied by the European food industry. This emphasizes that quality control in the animal feed industry and by preceding ingredient suppliers, which are integral role-players in the food chain, is essential.

Production of Feed and Feed Ingredients on the Farm

Adherence to good agricultural practices (GAP) is encouraged in the production of natural, improved and cultivated pastures, and forage and cereal grain crops used as feed or feed ingredients for food producing animals. Following GAP prescriptions will minimize the risk of biological, chemical, and physical contaminants from entering the food chain.

Crop residuals and stubble used for grazing after harvest should also be considered as livestock feed. The same applies to livestock bedding because most livestock will consume a portion of their bedding. Straw or wood shavings should therefore be managed in the same manner as animal feed ingredients. Rational grazing and dispersion of manure droppings should be applied to reduce cross contamination between animals.

Other factors should also be taken into consideration such as the proximity of the agricultural land to industrial operations where effluent or air emissions can lead to feed contamination. Similarly chemical fertilizers, manure, pesticides, and other agricultural chemicals should be stored, managed, and disposed of correctly.

Monitoring and Identification of Health Hazards

When purchasing feed ingredients from suppliers, such suppliers should be able to demonstrably guarantee product safety. Audit procedures can include inspection, sampling, and analysis for undesirable substances. Feed ingredients should meet acceptable and, if applicable, statutory standards for levels of pathogens, mycotoxins, pesticides, and other undesirable substances that may constitute a health hazard for the consumer. Any contaminated feed or feed ingredient is unsuitable for animal feed and should be discarded. Traceability of feed and feed ingredients, including additives, should be enabled by proper labeling and record keeping at all stages of production and distribution.

Processing, Storage, and Distribution of Feeds and Feed Ingredients

The effective implementation of GMPs and where applicable HACCP-based approaches should ensure that the following areas are addressed.

Premises

Buildings and equipment must be constructed to permit ease of operation, maintenance, and cleaning. Water should be of a suitable quality and effluent should be adequately disposed off.

Receiving, Storage, and Transportation

Feed and feed ingredients should be stored separately from fertilizers, pesticides, and other potential toxic materials. Processed material should also be stored separately from unprocessed ingredients. The presence of undesirable substances should be monitored for and controlled. The finished products should be delivered and used as quickly as possible. During storage special precautions should be taken to restrict microbial growth in feedstuffs and ingredients.

Personnel Training

Personnel should be adequately trained and aware of their role and responsibilities in protecting food safety.

Physical Hazards

Physical hazards, like microbiological and chemical hazards, can enter a food product at any stage in its production. There is a huge variety of physical items that can enter food as foreign material and most of these hazards pose risks of injury or choking. The main physical food safety hazards include items such as glass, metal (including metal particles resulting from friction between metal parts), stones, wood, plastic, dust, and hair. The main risk area for physical contamination of milk at the farm level is the stored milk in the bulk tank. Producers should, therefore, assess potential physical hazard risks in storage areas (e.g., breakable glass, loose material, etc.). Corrective action to be taken in the area housing the bulk milk storage tank is, for example, to use shatterproof light-covers to prevent glass contamination from taking place.

During the processing of milk, it is invariably subjected to procedures that will remove any physical contaminant. Examples of such procedures may include filtration, although centrifugal clarifiers are standard equipment in any commercial

milk processing operation. Glass fragments caused by glass bottle breakage may result in serious injury, and such breakage can be caused in a number of ways, including damage to the bottles in transit to the processing plant, or damage to bottles during mechanized handling (cleaning, filling, or capping) of the bottles. Similar to food beverages such as fruit juice, consideration of potential hazards associated with glass breakage should be part of a HACCP plan in any processing plant that may be packaging liquid milk products in glass. The most efficient control procedure is a glass-free policy in the plant and replacing glass bottles with other types of packaging.

If glass jars or bottles are preferred, on-line glass detection equipment such as X-ray detection, as a verification measure, may be used. Metal fractions may derive from equipment or from friction between metallic surfaces. Prevention consists of hygienic engineering of equipment and preventive maintenance. Additionally, sieving of milk powder or use of magnets can be used to remove any incidental presence of metal. Metal detectors can be also be used as a last resort, as a means of verification.

See also: Bacteria: *Bacillus cereus* and Other Pathogenic *Bacillus* Species; *Brucella*; *Campylobacter*; *Clostridium botulinum*; *Cronobacter (Enterobacter) sakazakii* and Other *Cronobacter* spp.; *Listeria monocytogenes*; *Mycobacterium avium* ssp. *paratuberculosis*; *Mycobacterium bovis*; Other Pathogenic Enterobacteriaceae – *Enterobacter* and Other Genera; *Pasteurella multocida*; *Salmonella* Non-Typhi; *Salmonella* Typhi and *Salmonella* Paratyphi; Shiga Toxin-Producing *Escherichia coli* and Other Pathogenic *Escherichia coli*; *Staphylococcus aureus*; *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*. Environmental Contaminants: Dioxins, Furans and Dioxin-like Polychlorinated Biphenyls. Food Technologies: Chilling; Pasteurization; Sterilization. Foodborne Diseases: Overview of Biological Hazards and Foodborne Diseases; Overview of Chemical, Physical, and Other Significant Hazards. Mycotoxins: Aflatoxins; Mycotoxins – General. Protozoa: *Cryptosporidium* spp.. Safety of Food and Beverages: Dairy Products: Cheese

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World Organization for Animal Health.

Dairy Products: Cheese

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Glossary

Cheese The product of casein coagulation in the milk followed by separation and removal of the whey from the curd.

Cheese made from pasteurized milk High temperature/short-time pasteurization: heat treatments of milk at 71.7 °C for at least 15 s or any equivalent combination; low temperature/long-time pasteurization: heat treatments of milk at 62–65 °C for 30–32 min, followed by cheese making.

Cheese made from thermized milk Cheese made from milk that has undergone a lower heat treatment than that of pasteurization – i.e., at >40 to <72 °C (typically 57–68 °C) for at least 15 s.

Cheese made from unpasteurized milk It may either be cheese made from raw milk or cheese made from thermized milk.

Hard cheese Cheese with 47–55% moisture content on a fat-free bases (MFFB).

Raw milk cheese Cheese made from milk that has not been heated beyond 40 °C.

Semihard cheese Cheese with 55–62% MFFB.

Semisoft cheese Cheese with 62–68% MFFB.

Soft cheese Cheese with more than 61% MFFB.

YOPI Vulnerable people, referred to as young, old, pregnant, and immunocompromised.

Introduction

In many parts of the world, the milking of dairy herd animals and cheese production has a long history. Before the 1980s, cows' milk cheese had caused several well-documented outbreaks (from hundreds to thousands of cases) due to different pathogenic microorganisms – mainly *Salmonella* spp. and *Listeria monocytoge*nes – in different countries (Tables 1 and 2). With the growing awareness of microbiological food safety problems from 1980s onwards, corrective actions have been taken, adequate control schemes adopted, and quality assurance and well-managed food safety systems implemented. In industrialized countries, these actions caused a shift toward outbreaks with lower numbers of cases due to cows' milk cheeses from industrial production. Nevertheless, outbreaks occur; however, nowadays they often cause fewer cases and either involve fresh, soft, or semisoft cheese or ewes' or goats' milk cheese. Furthermore, small-scale artisanal cheese production on farms, in mountain regions, or at home pose a higher risk of microbiological contamination. In outbreaks, pasteurized cheeses also are involved. Globally, the most frequently occurring pathogens in cheese are *Staphylococcus aureus* and its enterotoxins, *Salmonella* spp., *L. monocytogenes*, verotoxin-producing *Escherichia coli* (VTEC), and *Campylobacter* spp. VTEC and *L. monocytogenes* are the main vectors of severe illnesses and deaths. Raw milk cheeses have a long tradition in Europe (e.g., France, Italy, Spain, and Switzerland), and other countries (e.g., New Zealand) are allowing its production.

Numerous preconditions to food hygiene, food safety systems, and controls are essential for the production of adequately safe raw milk cheese. As an example, the correct manufacturing process of traditional Swiss raw milk cheeses encompasses several steps: (1) Raw milk may not contain more than 80 000 germs per ml, more than 350 000 somatic cells per ml, and may not contain inhibitors. These requirements are officially controlled 24 times per year and farm. (2) The processing of milk takes place within a maximum of 18 h after milking. (3) The continual heating period of the cheese curd at 53–60 °C lasts 30–60 min and is followed by several hours at temperatures above 50 °C under the press. (4) The acidification of the cheese mass by adding mesophilic or thermophilic starter cultures reduces the pH in the cheese mass to less than 5.3 within one day. (5) During ripening and maturation, the cheese is regularly salted. (6) The cheese is ripened for at least 60 days (up to several years). This leads to a significant loss of water (reduced water activity (a_w)).

The method developed to evaluate food safety is the risk analysis framework, with the scientific risk assessment being central to this process. For microbiological contaminations, a detailed description of this process is given in other articles of this encyclopedia and elsewhere. In brief, risk-based food safety covers the complete food chain. In chronological order, it involves raw materials at the farm level, the processes they undergo in production, transportation and storage of the finished products in retailer locations, transportation and storage in the homes of consumers, and, finally, the expiration date, the end of shelf life or the preparation and ingestion by

Table 1 Some foodborne disease cases due to *Salmonella* spp. in (cows' milk) cheese in different countries

Country	*Time period*	*Cases*	*Type of milk or milk product*	*Literature*
Canada	1982	?	Raw	De Buyser *et al.* (2001)
Canada	1984	>1700	Unpasteurized	De Buyser *et al.* (2001)
Canada	1994	35	Unpasteurized	De Buyser *et al.* (2001)
England and Wales	1989	42	Unpasteurized	De Buyser *et al.* (2001)
Finland	1985	35	Raw	De Buyser *et al.* (2001)
France	1990	277	Raw goats' milk cheese	De Buyser *et al.* (2001)
France	1993	273	Raw goats' milk cheese	De Buyser *et al.* (2001)
France	1995	25	Raw	De Buyser *et al.* (2001)
France	1996	14	Raw	De Buyser *et al.* (2001)
France	1997	113	Raw	De Buyser *et al.* (2001)
France	2001	215	Fresh cheese	MAF, 2011/58
France	2005	52	Unpasteurized fresh cheese	MAF, 2011/58
France	2006–07	23	Raw milk cheese	MAF, 2011/58
France	2008	25	Unspecified goats' milk cheese	MAF, 2011/58
Italy	1981	>100	Unspecified	De Buyser *et al.* (2001)
Switzerland	1985	215	Raw	De Buyser *et al.* (2001)
Switzerland	2006–07	82	Thermized semihard cheese	MAF, 2011/58
Netherlands	2006–07	224	Unpasteurized hard cheese	MAF, 2011/58
USA	1981	321	Pasteurized	De Buyser *et al.* (2001)
USA	1989	164	Pasteurized	De Buyser *et al.* (2001)
USA/Connecticut	2001	26	Unpasteurized soft cheese	MAF, 2011/58
USA/Pennsylvania	2007	29	Unpasteurized fresh cheese	MAF, 2011/58
USA/Illinois	2006–07	85	Unpasteurized Mexican-style cheese	MAF, 2011/58

Note: ?, Not specified.

Table 2 Some foodborne disease cases due to *Listeria monocytogenes* in cows' milk cheese in different countries

Country	*Time period*	*Cases*	*Type of milk or milk product*	*Literature*
Austria	2009–10	34 (3 countries)	Sour raw/milk cheese	MAF, 2011/58
Canada/Quebec	2002	17	Cheese made of thermized milk	MAF, 2011/58
Denmark	1989–90	26	Unspecified	De Buyser *et al.* (2001)
France	1995	36	Raw	De Buyser *et al.* (2001)
France	1997	14	Raw	De Buyser *et al.* (2001)
Japan	2001	86	Pasteurized	
Luxembourg	1989	2	Unspecified	De Buyser *et al.* (2001)
Sweden	2001	48	Raw milk cheese	MAF, 2011/58
Switzerland	1983–87	122	Taw, thermized	De Buyser *et al.* (2001)
Switzerland	2006	10	Raw milk soft cheese	MAF, 2011/58
USA	1985	142	Pasteurized	De Buyser *et al.* (2001)
USA/North Carolina	2000–01	12/13?	Mexican-style cheese	MAF, 2011/58
USA/Washington	2010	1	Fresh cheese	MAF, 2011/58

consumers. The general problem with microbiological hazards is that they often are present in the environment and are capable of multiplying in food production facilities as well as in foods and on food surfaces (like cheese rind). This means that slightly re- or cross-contaminated food (e.g., soft cheese) may develop considerable numbers of pathogenic microorganisms and its toxins until the time of consumption. However, food producers must guarantee the safety of their products during the entire shelf life. For this reason, food producers require substantial reductions of pathogenic bacteria in raw milk. Raw milk cheese production strongly relies on minimal microbiological contamination in the initial milk as well as the prevention of growth. Hurdles along the production and aging of cheese further minimize growth. Outbreaks related to cheeses made from unpasteurized milk can often be related to animals shedding pathogenic bacteria and inadequate temperature profiles during the storage of milk and the processing and ripening of cheeses. However, industrial production often applies a process step for eliminating present pathogenic microorganisms (i.e., pasteurization) and then avoids recontamination of the products. Outbreaks related to fresh, soft, or semisoft cheeses made from pasteurized milk can usually be linked to failure in pasteurization and inadequate postpasteurization hygiene resulting in recontamination.

Concerning chemical contaminants in milk and milk products, residues of medical, hormonal, or antibiotic treatments in animals and humans pose some problems and are, therefore, strictly controlled. Pesticide residues in feeds, as well as environmental contaminants such as dioxins, polychlorinated

biphenyls (PCBs) or polycyclic aromatic hydrocarbons (PAHs), and toxic metals, for example, cadmium or lead, can be found in milk and milk products. Naturally occurring plant toxins or mycotoxins from molds, especially aflatoxins, lead to problems in (dairy) herds; in addition, some carryover into milk takes place. For that reason, these contaminants must be controlled. The Chinese melamine scandal once again highlights the importance of controlling milk adulteration all over the world.

In-depth information on chemical risk assessment again is given in other articles of this encyclopedia and elsewhere. In brief, undesired substances in foods may enter the feed and food chain via numerous routes: Air, soil, and water may contaminate food and feed on the field; plants may form toxins; pesticides are applied for plant or crop protection, molds grow on plants and crops; farming animals are treated with medicines including antimicrobial agents; additives may be used in the production and storage of foods; and contaminants are formed in heat-treating processes during food production and food preparation. Of special importance to feed and food risk assessment are: (1) data in mammals on absorption, distribution, metabolism, and excretion of a chemical providing insight into its metabolic behavior and the kinetics of excretion; (2) data on acute and long-term toxic effects in animals; (3) the toxicological mechanism of action; (4) *in vivo* and *in vitro* testing of carcinogenicity, genotoxicity, neurotoxicity, reproductive, and developmental toxicity; (5) the intake of a chemical through food and feed; and (6) access to data from human epidemiological studies. This combined information usually allows for conclusions on the safety of ingested amounts of chemicals, toxins, and plant-inherent substances.

Microbial Contamination of Cheese

The risk of pathogens in cheese has been assessed in the three categories of high, medium, and low risk. The high-risk group is composed of *Salmonella* spp., *L. monocytogenes*, and enteropathogenic *E. coli*. *Salmonella* spp., widely spread in the environment, presents some species with low-infectious doses and heat resistance. Furthermore, *L. monocytogenes* grows at low temperatures, survives in some hard cheeses (e.g., Cheddar, Colby, and Swiss semihard cheeses) and multiplies in fresh and soft cheeses. Enterohemorrhagic *E. coli* (EHECs) are very virulent and may have low-infectious doses. The medium-risk group includes mastitis-causing agents like *Streptococcus* Groups A and C, *Brucella abortus*, *Mycobacterium bovis*, and *Coxiella burnetii*. The latter three are relatively heat tolerant; however, they are eliminated by pasteurization. The low-risk group is dominated by some viruses: *Clostridium perfringens* and *Bacillus cereus*, both with no or few outbreaks associated to cheese, and *S. aureus* and *Clostridium botulinum*, both well-known, and with effective control measures. Nevertheless, *S. aureus* and its toxins are often found in raw and recontaminated pasteurized milk cheese.

In Europe and the USA, milk and milk products were implicated in 2–6% of total bacterial foodborne outbreaks. In 2011, Batz *et al*. estimated the health impact in monetary cost of illness and loss of quality-adjusted life years in the USA for the food category dairy products at 8.7% and 8.8%, respectively. Hereby, 8.7% is the proportion of the total cost of all the estimated foodborne-related illness in the USA due to milk and milk products and the 8.8% is the proportion of the total loss of quality-adjusted life years from the total of foodborne diseases in the USA attributed to milk and milk products. Concerning proportions attributable to single pathogenic microorganisms coming from milk and milk products, De Buyser *et al.* found for France (from 1988–97) 24.9%, 1.8%, and 1.1% for *S. aureus*, *Salmonella*, and *C. perfringens*, respectively. Cheese, mainly fresh and soft cheese, was the responsible food vehicle, and milk accounted for another 10% of cases. However, Batz *et al.* found *Campylobacter* spp., *L. monocytogenes*, *E. coli* non-O157, and *E. coli* O157 responsible for 48.8%, 28.6%, 20%, and 6.5% of outbreaks attributable to milk products, respectively. The Centers for Disease Control and Prevention (USA) found severely higher rates of raw milk product-related outbreaks and that these led to much more severe illnesses and disproportionately affected people under the age of 20 in the USA. **Table 3** lists some cheeseborne disease outbreaks due to microbiological agents that are nowadays rarely found in cows' milk cheese. The responsible food vehicles now often are (raw) goats' and sheep's milk cheeses in Asia, Europe, and the USA. *Brucella* spp. and *Brucella melitensis* in cheese produced outbreaks in different countries well dispersed globally. The food vehicles were goats' or sheep's milk cheeses. The German outbreak was related to overseas infection by travelers. In Italy, five members of a family fell ill after consumption of sheep's milk cheese imported from a region with endemic *B. melitensis* presence. Neither outbreak resulted in fatalities. In the USA, the illegal importation of Mexican-style soft cheese was the cause of outbreaks due to *B. melitensis* as well as *M. bovis*, responsible for 35 human tuberculosis cases with one fatality.

Before the widespread use of pasteurization in the 1930s, the abovementioned (and other) agents were responsible for many disease outbreaks in humans and unpasteurized milk and milk products constituted an important vehicle for disease agents. In Austria, a tickborne encephalitis (TBE) virus caused an outbreak involving four patients and two asymptomatic persons. A shepherd in a mountain region produced homemade goats' and cows' raw milk cheese and fell ill. Three other family members, who had not been on the pasture, also exhibited flu-like symptoms and headaches. In recent years, TBE infections from dairy products were reported almost exclusively in Baltic countries.

The Most Threatening Pathogenic Bacteria in Cheese

Salmonella spp.

Salmonella spp., viruses, bacterial toxins, and *Campylobacter* spp. are the most relevant causes of foodborne disease outbreaks in Europe. Concerning *Salmonella* spp. in cheese, European Food Safety Authority (EFSA) data show that the vast majority of investigations were negative. Some positive samples of cows' and sheep's milk cheeses were reported; however, salmonellosis is mainly due to positive samples of other foods of animal origin. Moreover, salmonellosis, besides having a bimodal distribution within immunocompromised and elderly people

Table 3 Some foodborne disease cases due to microbiological agents nowadays rarely found in cows' milk and milk products

Country	*Time period*	*Microbiological agent*	*Number of cases*	*Type of milk or milk product*	*Literature*
Austria	2008	Tickborne encephalitis virus	6	Raw goats' milk cheese	MAF, 2011/58
Canary Islands	2003	*Streptococcus zooepidemicus*	15	Inadequately pasteurized fresh cheese	MAF, 2011/58
Egypt	2002–03	*Brucella* spp.	321	Raw milk products	MAF, 2011/58
Finland	2003	*S. zooepidemicus*	7	Fresh goats' cheese	MAF, 2011/58
France	1988–97	*Clostridium perfringens*	264	1.1	De Buyser *et al.* (2001)
Germany	2002–03	*Brucella melitensis* *Brucella suis*	30 1	Goats' raw milk cheese	MAF, 2011/58
Greece	1983–84	*Brucella* spp.	23	Goats' milk cheese	Teuber (2000)
Italy	1996	*Clostridium botulinum*	8	Sheep's milk cheese?	Teuber (2000)
Italy	2003	*B. melitensis*	29	Sheep's milk cheese	MAF, 2011/58
Italy	2005	*B. melitensis*	5	Sheep's milk cheese	MAF, 2011/58
Malta	1995	*B. melitensis*	135	Goats' milk cheese	Teuber (2000)
Scandinavia	1982	*Shigella sonnei*	>50	Soft cheese	Teuber (2000)
Spain	2002	*B. melitensis*	11	Goat raw milk cheese	MAF, 2011/58
Turkey	2005?	*B. melitensis*	4	Goat raw milk cheese	MAF, 2011/58
USA	1983	*S. zooepidemicus*	16	Raw milk cheese	Teuber (2000)
USA	1983	*Brucella* spp.	>30	Goats' milk cheese	Teuber (2000)
USA	1992	*B. melitensis*	11	Goats' milk cheese	Teuber (2000)
USA/NY City	2001–04	*Mycobacterium bovis*	35 Human tuberculosis cases	Mexican-style fresh cheese	MAF, 2011/58

Note: ?, Not specified.

on one hand, and infants and young children on the other hand, develops relatively mildly and rarely results in fatalities. Growth of *Salmonella* spp. is not observed in hard cheeses with $a_w < 0.95$. However, some *Salmonella* spp. are able to survive the fermentation of milk by lactobacilli and the maturation process of soft and other cheeses.

Canada also has had several reports of cheese-associated outbreaks of *Salmonella* serotypes (Table 1), where unpasteurized Cheddar or recontaminations by raw milk during processing were the vehicles of contamination. In 1982, the outbreak source was a single cow shedding 200 cfu ml^{-1} of milk. In some cheese lots, the pathogen survived under the press; in two lots, it survived the curing of the cheese for some days or weeks, and one lot tested positive after 125 days of maturation. In 1984, the largest-known outbreak caused between 1700 confirmed and 2700 estimated cases. The food vehicle again was Cheddar produced on a plant, where *Salmonella* spp. have sporadically been detected before the outbreak. The same *Salmonella* strain (*Salmonella typhimurium* phage type 10) was isolated from a cow's quarter, the cheese, a cheese trim bucket, and from infected employees. The source of salmonellae was two cows infected with different strains, and the workers might have served as reservoirs for recontamination of the produced cheese. Low numbers of *S. typhimurium* cells (1–6) and *Salmonella heidelberg* cells (100–500) in this outbreak led to many illnesses but no fatalities.

Campylobacter *spp.*

Campylobacter spp. was found mainly in sheep, and only occasionally in cows' milk cheeses throughout Europe. Again, human campylobacteriosis due to the consumption of cows' milk cheese seems extremely rare when compared to the number of positive samples of poultry and turkey meat or raw milk. Nevertheless, outbreaks of campylobacteriosis have been reported from many countries. *Campylobacter jejuni* is found in 1–12% of raw milk samples, where it rapidly decreases. At pH 5 it subsequently dies. In the USA, outbreaks before 1982 implicated raw milk consumption in 61%, and the outbreaks often involved high numbers of affected consumers. Three cheese-borne outbreaks were reported from 2004–10 related to soft cheese made from unpasteurized milk and to Mexican-style soft cheese illegally imported; together, they caused 79 illness cases without fatalities (Table 4). Batz *et al.* described the most important pathogen–food combination, with *Campylobacter* spp. in poultry occupying the first rank in the top-10 combinations with the greatest burden on public health. From 1998–2008, *Campylobacter* spp. as the causative agent of outbreaks involving dairy products was attributed to 48.8% of cases.

Table 4 Some recent foodborne disease cases due to *Campylobacter* spp. In cows' milk cheese in different countries

Country	*Time period*	*Cases*	*Type of milk or milk product*	*Literature*
USA/California	2003	11	Mexican-style soft cheese	MAF, 2011/58
USA/Kansas	2007	67	Unpasteurized soft cheese	MAF, 2011/58
USA/Nevada	2010	1	Mexican-style soft cheese	MAF, 2011/58

S. aureus

In France, *S. aureus* was by far the pathogen most frequently associated with illness outbreaks from milk and milk

products, causing 1439 illnesses from 1992 to 1997. The source of the pathogen is usually the udder, where the prevalence in quarter milk samples ranges from 5% to 22%. Animals with mastitis (infection of the mammary gland) may shed from 10^4 (subclinical) to 10^8 (clinical) cfu per ml milk. Other sources are the udder skin, employees, and contaminated starter cultures used in cheese making. Thirty percent and more of the *Staphylococcus* spp. may produce enterotoxins. These enterotoxins are heat stable (and thus withstand heat treatment processes) and may cause intoxications without viable *S. aureus* left in the cheese. Known *S. aureus* populations exceeding 100 000 cfu g^{-1}, mainly with the genes encoding for type A, D, and B, may start the production of sufficient amounts of enterotoxins to cause illness. The onset of symptoms is acute; however, fortunately, recovery takes place within a few hours or days. Death is very rare but may occur among the elderly, infants, and severely debilitated persons.

Whereas sheep suffer peracute mastitis, which almost always leads to the death of the animals, in dairy cattle, *S. aureus* often causes chronic subclinical mastitis or acute symptoms accompanied by fever, resulting in substantial financial losses. Genotype B, in particular, was related to high contagiosity and pathogenicity affecting whole herds, whereas genotype C resulted in 1–3 affected animals per herd, and other genotypes only caused infection of single cows. Enterotoxigenic strains are commonly found in raw milk intended for cheese manufacture in Norway, the USA, Germany, Japan, and Switzerland. In Switzerland, 95% of *S. aureus* strains associated with bovine mastitis were positive for at least one enterotoxin gene. Cooling of the milk before cheese making and correct acidification by starter cultures normally overgrow the less competitive *S. aureus* strains. The safety of raw and pasteurized milk products depends on the prevention of recontamination by employees. Both modes of transmission are exemplified by two outbreaks in Brazil, in 1999, involving 378 individuals (Table 5). In the first outbreak, 50 individuals became ill after eating Minas cheese, a typical fresh white cheese in South America. Illness symptoms (diarrhea, vomiting, dizziness, chills, and headaches) appeared within 2 h after ingestion. The second outbreak affected 328 individuals after consuming raw milk. The analysis of the products in the first outbreak showed the presence of *Staphylococcus* and the enterotoxins A, B, and C, whereas the raw milk in the second outbreak contained coagulase-negative Staphylococci exceeding 2×10^8 cfu g^{-1} and the enterotoxins C and D. Sources of contamination were the food handlers in the first and cattle mastitis in the second outbreak. Infected food handlers as sources of contamination of different pathogens were identified in 57% of outbreaks involving pasteurized dairy products in the USA from 1993 to 2006.

L. monocytogenes

Worldwide, healthy farming animals like goats, sheep, and cows shed *L. monocytogenes* at 2–50%. In addition, humans with *L. monocytogenes* in their feces may be asymptomatic; the microorganisms are simply shed through the intestinal tract. Nevertheless, the ingestion of foods exceeding 1000 cfu g^{-1} may lead to fever and diarrhea and is thought to be responsible for 90% of all listeriosis cases. Young, old, pregnant and immunocompromised (YOPI) are at particular risk of invasive listeriosis and perinatals, and may present severe illnesses and high mortality including abortion, death of newborns and handicapped newborns. This maintains *L. monocytogenes* among the most important causes of deaths from foodborne infections in industrialized countries. In Central and South America, as well as Asia, *L. monocytogenes* is related to fresh and soft cheeses with high moisture content, often including home- or farm-made raw milk cheeses (e.g., Minas Frescal and Mexican-style cheese). Other cheeses often implicated in listeriosis outbreaks are red smear and mold-ripened cheeses. Therefore, *L. monocytogenes* poses a serious problem to cheese producers in many continents, and the *Listeria*–dairy products combination ranks at fifth place in the top-10 combinations with the greatest burden on public health. It should be noted that this ranking is the only one dominantly involving milk products. In the European Union (EU), the highest percentage was reported for soft and semisoft cheese (2.8%) made from raw or low heat-treated milk as well as from pasteurized milk. *Listeria monocytogenes* rarely reached levels >100 cfu g^{-1}. Between 2004 and 2009, the EU's Rapid Alert System for Food and Feedstuff documented a total of 97 *Listeria*-positive milk products – 34 of them concerned pasteurized and 22 raw milk products.

Table 2 lists some outbreaks in America, Asia, and Europe. Probably the two best-documented outbreaks of listeriosis share several similarities. In 1985, there was an epidemic outbreak of listeriosis in Los Angeles County, USA. During the following 12 months, listeriosis cases were sporadic. From 1983 to 1987, an epidemic outbreak of listeriosis was also

Table 5 Some foodborne disease cases due to *S. aureus* in (cows' milk) cheese in different countries

Country	*Time period*	*Cases*	*Type of milk or milk product*	*Literature*
Brazil	1994	7	Unspecified	De Buyser *et al.* (2001)
Brazil	1999	50	Minas Gerais	Carmo *et al.* (2002)
Brazil	1999	328	Minas Gerais	Carmo *et al.* (2002)
Canada	1980	63	Unspecified	De Buyser *et al.* (2001)
England	1983	2	Pasteurized	De Buyser *et al.* (2001)
England	1988	155	Unpasteurized	De Buyser *et al.* (2001)
France	1983	20	Raw ewe cheese	De Buyser *et al.* (2001)
France	2009	23	Unpasteurized soft cheese	MAF, 2011/58
Scotland	1984	27	Raw ewe cheese	De Buyser *et al.* (2001)
USA	1981	16	Pasteurized	De Buyser *et al.* (2001)

observed in the Canton de Vaud, Switzerland. Starting in this time period, the Swiss authorities maintained a monitoring program. In consequence, there exist two pairs of epidemic outbreaks followed by sporadic outbreak cases, a coincidence essential for the quality of the observed data during the sporadic outbreaks. Both outbreaks were caused by soft cheeses: Jalisco, a Mexican-style soft cheese in Los Angeles County and Vacherin Mont d'Or, a traditional, smear-ripened soft cheese in the Canton de Vaud. Moreover, both outbreaks caused more than 120 cases and more than 30 deaths each. In the case of Jalisco, the industrial pasteurization process was inadequate and responsible for the *L. monocytogenes* contamination. Closing down the concerned production plant stopped the epidemic listeriosis outbreak. In the case of Vacherin Mont d'Or, the artisanal production process involved several traditional proceedings, maintaining opportunities for *L. monocytogenes* contamination (i.e., the use of wooden hoops, brushes to treat the rind, and wooden shelves in ripening cellars). Thermization of the cheese milk accompanied by a systematic controlling program was successful to eliminate *L. monocytogenes*, and Vacherin Mont d'Or was, subsequently, relaunched. Although *L. monocytogenes* was demonstrated to survive in some hard cheeses like Cheddar and Colby, the DHHS/FDA/CFSAN Draft Assessment of the Relative Risk to Public Health from Foodborne *L. monocytogenes* (2001) derived a safe pattern for the consumption of hard cheese: 3.2×10^{-13}–2.9×10^{-10} illness cases were estimated per portion of hard cheese consumed. Concerning listeriosis, this made long-ripened cheese the second safest out of 20 analyzed ready-to-eat food groups. Another assessment calculated the listeriosis risk of YOPI from 7.39×10^{-12}–1.85×10^{-8} illness cases per consumed food portion. The calculation was performed with up to 3160 cfu *L. monocytogenes* in the consumed food, thus including elevated contamination in foods others than hard cheese.

EHEC and Illness

Escherichia coli, a member of the Enterobacteriaceae family, occurs naturally in the intestine flora of humans, mammals, and birds. A subgroup of the verotoxigenic *E. coli* (VTEC or Shiga toxin-producing *E. coli* (STEC)) with more than 400 serotypes constitutes the EHEC, which may cause serious illnesses. The EHEC prototype *E. coli* O157:H7 is most frequently implicated in group illnesses and the development of the hemolytic-uremic syndrome (HUS). As few as 10–100 microorganisms may cause infection. The annual incidence of STEC illnesses in Europe is approximately 0.7 cases per 100 000 inhabitants. EHEC O157:H7 survives cold storage of foods and freezing. Some strains reproduce at temperatures up to 49 °C. Adaption to lactic acid as well as an enhanced ability to survive in the presence of other acids has been observed. Specific survival capability under extreme conditions in pathogenic and nonpathogenic strains of *E. coli* with extensive variability is inherent in individual strains. These properties make EHECs a real new emerging threat to raw milk cheese producers, not only for farm- and home-made cheese varieties but also for the manufacture of hard cheese, because EHEC O157:H7 was reported to survive in, for example, Cheddar cheese (Table 6).

Cattles are recognized as the main natural reservoir of VTEC, in particular, VTEC O157. Worldwide, the feces of milk cows show prevalences of 0.2–49% and 0.4–74% for VTEC O157 and non-O157, respectively. The huge differences are due to the measuring of the prevalence of virulence genes with significantly higher figures than the prevalence of cultivable isolates (that is, typically, 0–2%). Thus, milk contamination mainly takes place by the fecal route. The relative importance of dairy products as transmission vehicle associated with VTEC O157 and non-O157 outbreaks were 12.5% and 10%, respectively. Dairy-related outbreaks were caused by raw milk cheeses and raw milk, such as the major outbreak with 135 cases in the USA in 2007 at a correctional facility with an onsite dairy (Table 6). Ten inmates presented STEC O111 infection, and a retrospective prevalence study revealed the same infection in 14 out of 100 other inmates. The human STEC O111 isolates matched the STEC O111 isolates from cattle at the onsite dairy. It was suspected that employees at the dairy might have acquired STEC O111 infection on the job by animal-to-human transmission or transported fecal contaminated clothing into the main correctional facility and kitchen, thereby exposing other inmates. Raw milk cheese from America, Asia, and Europe harbored STEC isolates in 7.8% (Peru), 2% (Venezuela), 4% (Turkey), and 0.2–2.4% (EU), respectively. Within the EU, the differences in presence range from 0% to13.1% for isolates and 2% to 45% for the *stx* gene.

Table 6 Some past and recent foodborne disease cases due to *Escherichia coli* and enterohemorrhagic *E. coli* (EHEC), respectively, in cows' milk cheese in different countries

Country	Time period	Cases	Type of milk or milk product	Literature
Denmark	1983	?	Pasteurized	De Buyser *et al.* (2001)
Netherlands	1983	69	Pasteurized	De Buyser *et al.* (2001)
Sweden	1983	66	Pasteurized	De Buyser *et al.* (2001)
USA	1983	170	Pasteurized (plant of origin)	De Buyser *et al.* (2001)
Canada	2002–03	13	Unpasteurized hard cheese	MAF, 2011/58
France	2004	3	Unpasteurized goat milk cheese	MAF, 2011/58
France	2005	6	Raw milk soft cheese	Farrokh *et al.* (2012)
USA Wisconsin	1998	55	Fresh cheese	Baylis (2009)
USA Colorado	2007	135	Milk or milk product	CDC (2007)
USA	2010	388?	Raw milk Hard cheese	MAF, 2011/58

Note: ?, Not specified.

In Switzerland, *E. coli* O157 was isolated in 1.3% of fecal samples taken from healthy beef cattle. From 2006 to 2008, 1502 samples of Swiss raw unpasteurized semihard milk cheese were examined for STECs: 5.7% were polymerase chain reaction (PCR)-positive for STEC, and STEC isolates were extracted out of 29 samples (1.9%). None of the isolates was intimin positive; none belonged to the classic EHECs of serogroups O157, O26, O103, O111, and O145. Nine isolates carried the genes for EHEC-hemolysin, 17 isolates belonged to serotypes that are known to be human STECs; of these, in turn, 6 isolates belonged to serotypes O22:H8, O91:H10, O91:H21, and O174:H21, each of which can trigger HUS. Therefore, raw milk cheese producers must comply with good manufacturing practice, outstanding unpasteurized milk quality, and periodic controls. Swiss and other raw milk hard cheese varieties with elevated cooking temperatures ($>53\ ^{\circ}C$) as well as hard cheeses made from pasteurized milk are regarded as relatively safe and safe, respectively.

Mycobacterium avium Subspecies *Paratuberculosis* (MAP)

In the past two decades, there has been more evidence that MAP can cause Crohn's disease (CD); however, the direct link is still actively debated. The incidence of CD in Europe is approximately 5.6 cases per 100 000 inhabitants per year. It remains unclear as to whether there is a causal association or a secondary invasion following the onset of CD. A meta-analysis concluded that the association of MAP with CD seems to be specific; however, its role in the etiology of CD is yet to be defined.

MAP is found worldwide in numerous mammals, ruminants, and birds. In cattle, it causes Johne's disease, normally with symptoms like diarrhea, excessive weight loss, and lower milk production. In (bovine) herds, animals at different infection stages may spread and transmit MAP through feces, semen, colostrums, and milk. Many countries in Europe, Asia, and America run control or eradication programs for MAP in herds, where the prevalence may reach 70% (USA). However, the prevalence in single animals is low. As an example, the seroprevalence in Swiss herds was estimated to be between 6% and 8%, and single animal prevalence was estimated at 2% (culture positive). No viable MAP cells could be cultured from 143 raw milk cheeses; however, 4.2% tested positive with a real-time PCR system; evidently, MAP was present in the raw material. Because bovine herds constitute a main reservoir of MAP, it is recommended to significantly reduce the level of MAP in bovine herds, which serve as a main source of contamination of food of animal origin.

Pasteurization reduces MAP in drinking milk; however, survival was observed at time–temperature combinations of 15 s at 90 °C. In cheese, MAP is reduced mainly by the long-ripening time. *D* values are 45.5 and 27.8 days for Swiss hard and semihard cheese, respectively.

Antimicrobial Resistance

Since 2000, antimicrobial resistant *Campylobacter* spp., *Salmonella* spp., and *E. coli* have increasingly been reported in Europe and globally. It has been reported that extended-spectrum β-lactamases-producing Gram-negative bacteria pose a serious threat to public health in human medicine as well as increasingly in the veterinary context worldwide. β-Lactam antibiotics, such as the third generation cephalosporins, are a major drug class for the treatment of serious infections caused by *E. coli*. Concerning *E. coli*, β-lactamase production remains the most important mediator of β-lactam resistance. Extrachromosomal elements of *E. coli* can be laterally transferred to other species, thus contributing to inter- and intraspecies variability in genomic contents. β-Lactamases are bacterial enzymes that hydrolyze β-lactam antibiotics into treatment-ineffective compounds. The transmission of zoonotic multidrug resistant bacteria between food-producing animals and humans is reported, and there is a persistent fear of their transmission via the food chain.

Biogenic Amines

Very long-ripened raw milk cheeses especially may contain high concentrations of biogenic amines – low molecular organic compounds, mainly tyramine and histamine. These are formed by decarboxylation activity due to the proteolysis during the ripening and maturation. Microorganisms with decarboxylase activity may be present either in the native milk flora or, to a greater extent, in the starter cultures. Patients may suffer headache, migraine, hypertension, nausea, or even diarrhea and abdominal pain. The relevance of cheese consumption was ranked at third place for histamine and at second place for tyramine intake among different foods. Aged Swiss cheese, Cheddar, and extraordinarily-aged Gouda have caused reported outbreaks in England, France, and the USA. For the first time in March 2012, a histamine-contaminated Cheddar cheese was withdrawn from the British market. The cheese contained 1227 mg kg^{-1} histamine and caused illnesses in 38 children of a day nursery. Symptoms like red mouths and the break out of skin rashes arose shortly after consumption and disappeared within one day.

Presence of Chemical Residues and Contaminants in Cheese

Undesired substances in foods such as pesticides, their residues and metabolites, environmental contaminants, contaminants in plants and additives may enter the feed and food chain via numerous routes: Air, soil, and water may contaminate food and feed in the field, plants may form toxins, pesticides are applied for plant or crop protection, molds grow on plants and crops, farming animals are treated with medicines (including antimicrobial agents), food additives help in the production and storage of foods and contaminants are formed in heat-treating processes during food production and preparation. Moreover, migration from packaging materials like plastics, papers, and cans into foods may result in contaminations with monomers, catalysts and solvents, biocides, whitening agents, colors, and waxes, as well as can-coating resins. However, the migration of such

chemicals is typically very low, but might be, as seen with bisphenol A (BPA), of scientific and public health concern.

Pesticides

In Europe, Iceland, and Norway, in 2009, the majority of food of animal origin was free of detectable residues (99.7%). In total, 34 different pesticides were found in animal products; most of the pesticides were rather due to environmental contaminations with persistent pesticides that have been banned at EU level than actual uses of pesticides on feed crops. However, no milk or cheese was analyzed. Subsequently, in 2010, only 0.33% of noncompliant samples from food of animal origin including milk were reported.

At the Swiss national level in 2008, none of the milk samples analyzed exceeded any maximum residue level set to pesticide residues and antimicrobial substances. Such a positive outcome may be expected for organophosphate pesticides: because of their hydrophilicity, their presence in milk (and especially in fatty milk products like cheese) is not expected. However, this does not hold true for persistent organic pollutants (POPs), including, dioxins, furans, PCBs, and the organochlorine pesticides lindane, aldrin, and dichlorodiphenyl trichloroethane (DDT) as part of the 'dirty dozen.'

Organochlorine pesticides were used worldwide and intensively until the 1970s. Then, Europe and the USA restricted the application and other nations followed during the decades that followed. Meanwhile, these pesticides were still widely used in Africa, Asia, and Latin America because of their effectiveness in agriculture. Nowadays, especially countries with hot and humid tropical climate depend on these substances in disease vector control programs – for example, against malaria. In such countries, these lipophilic compounds may be found in cows' and buffalos' milk and milk products (like cheese) in amounts exceeding current international limits (i.e., those of the Food and Agricultural Organization of the United Nations (FAO)/World Health Organization (WHO)). Specifically, in African developing countries and countries in transition, DDT was found in milk at concentrations of 3.2–10.1 mg kg^{-1} (fat basis), thus exceeding existing actual international limits in a range of 100- to 1000-fold in the past. Measures of organochlorine pesticides in ultra high temperature milk of South American nations like Brazil and Argentina ranged from under to several-fold existing international limits. However, it has been reported that sterilization of milk (121 °C for 5 and 15 min) results in a certain loss of organochlorine pesticides. However, skimming of the milk was also shown to reduce these contaminants in cheese, milk, the contaminants would have been expected to be present in cheese at concentrations exceeding existing international limits (approximately 10- to 100-fold).

In Egypt, the different organochlorine pesticides were found in milk at concentrations exceeding existing international limits by approximately 10-fold. In the USA, despite the restricted use of DDT since 1972 (no more applications in agriculture, mainly cotton seed), its metabolite dichlorodiphenyldichloroethylene (DDE) almost persists (e.g., in ice cream and chicken), but significantly decreased in beef. In some parts of the world, adverse health effects in children may, at least in the past, have been expected.

Dioxins, Furans, and PCBs

In Europe, dioxins in foods and feeds have been the reason behind several scandals in the international food and feed industry. Dioxins, PCBs, dioxin-like PCBs (DL-PCBs), and nondioxin-like PCBs (nDL-PCBs) are widely present in the environment. Dioxins are aromatic hydrocarbons with polychlorinated dibenzo-*p*-dioxin (PCDD) and polychlorinated dibenzofuran (PCDF) congeners. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) is the most toxic compound and is classified as a known human carcinogen by International Agency for Research on Cancer (IARC). The congeners are unintentionally formed byproducts of some manufactured chemicals and in incineration and combustion processes.

PCBs, in contrast, formerly had been manufactured intentionally, and their properties (nonflammability, stability, and high dielectric constant) allowed applications in electrical equipment, plastic products, and building materials. DL-PCBs exert toxicity similar to dioxins (i.e., immunotoxicity, reproductive effects, teratogenicity, endocrine disruption, and carcinogenicity in humans). Moreover, they accumulate in the fat of living organisms throughout the food chain (biomagnification). Individual toxicity of congeners is assigned relative to the toxicity of 2,3,7,8-TCDD in order to obtain the toxic equivalency (TEQ) of mixtures. Till date, the toxicity of nDL-PCB remains unclear; however, it seems problematic for humans accumulating these substances very effectively when compared to experimental animals. At least 90% of the human exposure to dioxins, DL- PCB, and nDL-PCB comes from food of animal origin, with meat, dairy products, and fish as the main sources. Intake estimations vary from 0.4 to 1.5 pg TEQ (sum of 6 congeners) per kg bodyweight per day, 0.8 to 1.8 pg PCB-TEQ per kg bodyweight per day, and 10 to 45 ng per kg bodyweight per day for dioxins, DL-PCBs, and nDL-PCBs, respectively. For children and other specific subpopulations, no safety margin remains either to the WHO-tolerable daily intake (TDI) for dioxins and DL-PCBs (1–4 pq TEQ per kg bodyweight per day), nor to the tolerable weekly intake (TWI) (14 pq TEQ per kg bodyweight per week). Therefore, subpopulations exceeding these values exist. In Europe, milk and milk products contained between 0.95 and 2.4 pg g^{-1} fat of dioxins and DL-PCBs. Their contribution to the dietary intake was estimated to be 27% and 49% in the Netherlands and Switzerland, respectively. In Switzerland, 47 cheese samples showed a mean value of 0.92 pg g^{-1} WHO-TEQ in 2008, contributing to approximately 6% of the total intake of these substances from cheese consumption. In the USA, from samples taken in 1995–96, the mean intakes of 17 dioxin/furan congeners from butter, cheese, ice cream, and yogurt were estimated to be 0.5–11, 1.6–3.2, 4–19, and 0.8–28 pg per person per day, respectively. Butter samples from 24 countries showed WHO-TEQ for PCDD/PCDF >1 pg g^{-1} fat in China, Italy, and Spain and for PCBs values >2 pg g^{-1} fat in the same countries, as well as in India, Germany, the Netherlands, Czech Republic, and Tunisia. The Stockholm Convention,

aiming at eliminating or reducing POPs, was adopted in 2001 by the EU, the USA, and 90 other countries. The release of POPs since then has considerably diminished. However, certain groups of populations (i.e., children or ethnic groups depending on fish consumption) still ingest foods exceeding the internationally established maximum levels for different foods or their consumption of these toxic compounds exceeds existing international limits. Adverse effects on their health at low doses from milk and milk products are not expected.

Polycyclic Aromatic Hydrocarbons

PAHs, another class of organic compounds, also are formed in incineration and combustion processes and occur in mixtures. Fifteen of them, with benzo[a]pyrene (BaP) as the most toxic reference compound, are genotoxic as well as carcinogenic in animals. In 2008, the median dietary exposure in Europe was calculated both for mean and high dietary consumers, and varied from 3.9 to 6.5 ng per kg bodyweight per day for BaP and 28 to 51.3 ng per kg bodyweight per day for PAH8 (BaP and seven more carcinogenic PAHs). The major foods containing elevated concentrations of PAHs are meat and fish products, particularly grilled, barbecued and smoked products, oils and fats, cereals, and dry foods. High concentrations in soils and plants near areas of heavy transportation and industrialization have been reported. A Swiss study from 2006 evaluated the effects of the direct fuel oil method used to dry roughage fed to dairy cows. The sum of the PAHs in dry feeds amounted to $466 \pm 459\ \mu g\ kg^{-1}$ dry matter with low percentages of carcinogenic PAHs ($8.3 \pm 5.9\%$) and of BaP ($1.5 \pm 1.4\%$). Estimates showed milk and milk products to be responsible for the intake of 9% and 12% of the sum of PAHs and BaP, respectively. Meanwhile, the EU derived a margin of exposure (MOE) approach for BaP and PAHs, resulting in values approximately 10 000 for high level and $<20\,000$ for average consumers, thus MOEs of low concern for consumer health.

Similar results came from Australia. As for POPs, this will not hold true for all countries and all sites. Especially countries with ongoing open incineration and combustion processes (e.g., for plant oil production, land clearing, or energy production) may be confronted with higher intake values through food commodities. Additionally, in developing countries, residential heating and cooking is regarded as an important source of these contaminants. Subgroups of the population are expected either to have intakes passing the internationally established maximum levels for different foods or consumption of these toxic compounds exceeding existing international limits. Adverse effects on their health at doses from milk and milk products cannot be excluded without further studies.

Bisphenol A

The release of chemicals from packaging materials into food may be of human health concern (e.g., as seen with BPA from coating materials or sealants). BPA is suspected to be an endocrine disruptor and potentially carcinogenic in humans. The effects of long-time exposure at low levels are poorly investigated and remain controversial. In particular, canned (infant) food may show mean concentrations of <1–$2.6\ \mu g\ kg^{-1}$ food in Europe, Canada, and the USA, whereas the concentration may pass $15\ \mu g\ kg^{-1}$ food in Asia. In 2010, an FAO/WHO Expert Group estimated the mean exposure of adults and children to BPA at <0.01–0.40 and 0.1–0.5 μg per kg bodyweight per day, respectively. Assessments of WHO and other bodies support neither scientific nor statistical evidence on adverse human health effects, but may follow the precautionary principle and aim to lower or ban BPA release into (toddler and infant) food.

Melamine

In 2008, melamine in infant formula in China, Taiwan, Macau, and Hong Kong caused six deaths, 51 900 hospitalized infants and 294 000 illnesses. Food products containing melamine were exported internationally. In consequence, 68 nations recalled or banned Chinese products. In the USA, products recalled included biscuits, candy, coffee, cookies, milk, and flavored drinks; however, the melamine contamination in these products did not lead to health consequences. In China, melamine was added intentionally to animal feed at concentrations of 1.6–$410\ mg\ kg^{-1}$ and to milk at concentrations up to $6196\ mg\ kg^{-1}$ in infant formula. No action was taken in order to prevent a scandal before the Olympic Games. The maximal dietary exposure was estimated to reach 8.6–23.4 mg per kg bodyweight per day, a figure 40–120 times the TDI of 0.2 mg per kg bodyweight. The special susceptibility of infants is due to their higher concentration of naturally occurring uric acid when compared to adults. The combination of both substances is capable of forming kidney stones. Dairy cows fed melamine contaminated feed excrete approximately 0.5% of the dose into milk. Therefore, Chinese dairy products or products with high milk content contained 0.05–$3\ mg\ kg^{-1}$ melamine. This contamination alone would not have been expected to cause such a spectacular negative health outcome in Asia. Therefore, milk adulteration, especially the dilution with water and masking the lowered protein content with added nitrogen, is a fraud deserving control and combat all over the world.

Toxic Metals

Cadmium accumulates primarily in kidneys and liver and has a biological half-life of several decades. The toxic effect occurs in the kidneys; the TWI is currently set at 2.5 μg per kg bodyweight. Throughout Europe, the consumption of milk and milk products leads to an estimated intake of $0.22\ \mu g\ day^{-1}$ for adults (milk: 0.05–$0.4\ \mu g\ day^{-1}$; cheese: 0–$1.49\ \mu g\ day^{-1}$), contributing for approximately <1–4% and <1–2.3% of the TWI for milk and milk products, respectively. In the USA, cadmium concentrations in milk from uncontaminated areas was between 7 and $37\ \mu g\ kg^{-1}$ (ww) and intake estimates for whole diets ranged from 13 to $79\ \mu g\ day^{-1}$. Milk from foreign foods from contaminated areas was not analyzed. Plants, especially leafy vegetables, root crops, and cereals, incorporate cadmium more easily than they do other toxic metals. Because the intake of cadmium is

mainly via food and children occupy a much higher percentage (65%) of the TWI, the contamination of food with cadmium must be limited. Lead also accumulates in the body. The adverse health effect is on the development of the central nervous system in perinatals, with the consequence of diminished intelligence. A TWI is set at 25 mg per kg bodyweight. The mean contribution from milk and cheese is less than 1% of the provisional TWI. It has been shown that, over recent decades, the lead level in food has decreased significantly owing to source-related efforts to reduce the emission of lead, and improvements in quality assurance of chemical analysis. Lead is present at low concentrations in most foods. Offal and mollusks may contain higher levels. Contamination of food during processing or food production in contaminated areas is the main reason for enhanced lead intake via foodstuffs. Plants usually do not absorb lead. However, elevated contamination may occur if crops are grown at contaminated sites. Inorganic mercury exerts adverse health effects in the kidneys. A TWI is set at 5 mg per kg bodyweight for mercury. The intake through milk seems to be no cause for concern.

Mycotoxin Aflatoxin M_1 (AFM_1)

Industrialized countries have imposed strict regulatory and control actions in order to reduce the presence of mycotoxins in feed and food. However, in developing countries, chronic and even acute human health implications due to mycotoxin problems in food may be severe (e.g., due to tropical climates favoring the growth of mycotoxin-producing fungi). Evidence of acute aflatoxicosis in humans has been reported from many parts of the world (in Africa and Asia). Chronic exposure to various mycotoxins is of public health concern because of the carcinogenic property of mycotoxins such as aflatoxins and ochratoxin A. AFB_1 is a potent, naturally occurring carcinogen with the IARC-classification as a known human carcinogen. Mammal species (including humans) show similar clinical symptoms under acute aflatoxicosis, including vomiting, subcutaneous and pulmonary edema, hepatic injury, and lethargy. The target organ is the liver, but other organs may also show dysfunctions. Subacute doses of AFB_1 are metabolized, and most metabolites are excreted in the bile and urine within 24 h. Remaining metabolites are concentrated in the liver, where they are mainly found as deoxyribonucleic acid (DNA)-adducts. No description of a specific DNA repair mechanism for N^7-guanine–AFB_1-adducts was found in mammals. This fact might help to explain the high mutagenic potential of AFB_1-8,9-epoxide.

Food and feed commodities especially susceptible to AF contamination are cereals, oilseeds, spices, and tree nuts. Major food intake may result from the consumption of maize, peanuts, and figs; major feed contamination usually is due to maize, peanut-, and cottonseed cake. Milk and milk products may contain the AFB_1-metabolite AFM_1 and, therefore, substantially contribute to the aflatoxin intake in toddlers and small children. Data at the EU level show that the incidence of samples exceeding the limit of 0.05 μg AFM_1 per kg milk was 0.06% at the bulk milk level and 1.8% at the individual farm level. The carryover of AFM_1 into milk is usually 1–3%, but may reach up to 6% in high-yielding animals. In contrast, the carryover of other mycotoxins present in feeds, like ochratoxin A, deoxynivalenol, T2-toxin, zearalenone, and fumonisin B1 is approximately 100 times lower. The EFSA recommends monitoring not only AFs in known susceptible feeds and AFM_1 in cows' milk but also the milk of other species and AFM_1 in milk products of those species. The total dietary aflatoxin intake from Africa (Uganda, Swaziland, Kenya, and Mozambique), Asia (the Philippines, Thailand, and China), and South America (Ecuador) in the past showed a range from 3 to 222 ng per kg bodyweight per day and correlated well with the liver cancer incidence. The exposure in the USA is estimated at 2.7 ng per kg bodyweight per day. AFM_1 concentrations in milk were reported to be 0.05–0.37 (Brazil), >0.5 (Cuba), 0.05–0.18 (Greece), 0.1–3.5 (India), and 0–6.6 ng g^{-1} (Thailand). Most samples from the southwest and south of the USA showed AFM_1 levels between 0.05 and 0.5 ng g^{-1} and 8.1% >0.5 ng g^{-1}. AFM_1 in different Turkish cheeses was detected in 48–92% and exceeded established EU limits in 17.1–27.1%. Maximal contamination reached 7.2 ng g^{-1}. Cheeses from Japan and the USA showed maximal levels of 1.2 and 1 ng g^{-1}, respectively. Cheese samples from Egypt showed contamination between 0.05 and 0.5 ng g^{-1}. AFM_1 levels in milk and cheeses produced in tropical countries of Africa, Asia, and South America may exceed actual EU limits 10- to 100-fold and actual limits set in the USA by a factor of 10. In consequence, the systematic control of feedstuffs and milk and milk products for AFB_1 and AFM_1, respectively, would be desirable, but not of first order importance for public health in such countries.

Actual Concernment for POPs and Toxic Metals

New work discusses weight gain effects and diabetes in animal studies after exposure at low concentrations to a variety of chemicals, including, among others, organochlorine pesticides, organophosphates, PCBs, polybrominated diphenyl ethers, chemicals used in plastics such as phthalates and heavy metals such as cadmium and lead. The concern that chemicals in the environment may be partly responsible for the increasing occurrence of obesity in human populations is based on a significant and growing number of mechanistic studies and animal experiments, as well as on some clinical and epidemiological studies. The weight of evidence is compelling, although ethical and logistic factors have so far made it difficult to prove such associations in human studies. In the light of such possible implications of some POPs and trace metals on the human endocrine system, more attention will have to be paid to the research and management of low concentrations of these substances in basic food items such as fish, meat, and milk products.

See also: Bacteria: *Listeria monocytogenes*; *Mycobacterium avium* ssp. *paratuberculosis*; Shiga Toxin-Producing *Escherichia coli* and Other Pathogenic *Escherichia coli*; *Staphylococcus aureus*. Characteristics of Foodborne Hazard and Diseases: Drug Resistant Pathogens. Environmental Contaminants: Dioxins, Furans and Dioxin-like Polychlorinated Biphenyls. Mycotoxins:

Mycotoxins – General. **Processing Contaminants:** Polycyclic Aromatic Hydrocarbons (PAHs). **Risk Analysis:** Risk Assessment: Chemical Hazards; Risk Assessment: Microbiological Hazards. **Safety of Food and Beverages:** Milk and Dairy Products. **Toxic Metals:** Cadmium; Lead; Mercury

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http://www.who.int/en/
World Health Organization (WHO).

SAFETY OF FOOD AND BEVERAGES

Cereals and Derived Products

RL Beverly, Kellogg Company, Battle Creek, MI, USA

Glossary

Celiac disease A genetic disorder in which an autoimmune response causes damage to the small intestine when gluten proteins are contained in certain foods.

Cereal bars Processed grains and various edible additives that are assembled together with a sugar-based binding solution and compressed under low pressure.

Cereals or cereal grain Grasses cultivated for the edible components of their fruit seeds: the endosperm, germ, and bran. Cereal grains have been categorized as wheat, maize (corn), oats, rye, rice, barley, millet, and sorghum.

Dough A mixture of flour, milk and/or water, sugar, salt, and other ingredients that produces a batter dry enough to handle rolling, cutting, or sheeting. Baker's yeast is often added to the formulation.

Gluten A 33-amino acid protein that breaks down into smaller peptide units called the gliadins and glutenins.

Mycotoxins Secondary metabolites produced by molds that are capable of causing disease and death in humans and other animals.

Sponge A fairly stiff batter made of flour, sugar, water, and yeast. When mixed together, these ingredients are left to ferment for a set time and temperature, depending on the quantity of yeast used.

Introduction

Cereal grains supply humans and livestock with their primary and most efficient source of calories and nutrients. This is due to nutritional properties of the edible seed which comprises the endocarp, germ, and bran. The term 'cereal grains' includes a reference to wheat, barley, corn (maize), durum, millet, oats, rice, rye, sorghum, and soybeans. Cereal grains vary greatly in their processing and consumption. Rice is the main cereal grain for greater than half the world's population. Barley, rice, and wheat are used in the fermentation process for the production of beer. Most cereals are milled for flour or meal that is used in dough formation for baking breads, cakes, and pastries. Cereal grain products comprise flours, breakfast cereal, snack foods, corn meal, doughs, pasta, and a variety of dry mixes.

Although botanically classified as grasses, humans have cultivated this important energy source for greater than 9000 years serving as the first crop for agriculture. Maize, wheat, and rice account for 87% of all grains produced worldwide and serve as a vital source of calories for a hungry world. Other cereal grains produced in less quantities include barley, sorghum, millet, oats, rice, triticale, fonio, and buckwheat. Although some grains are consumed after cooking, most are dried to enhance the quality and are then further milled to create fractions, used to create flour or meal, for additional products. In more developed countries the harvest, drying, and milling occurs with precision, utilizing machinery and automation. However, in developing countries the process may be rudimentary and susceptible to climatic influences that may adversely impact grain quality and safety.

The growth of various spoilage microorganisms on cereal grains and the finished product held at improper temperatures can occur. Nevertheless, microbial growth can be controlled if good manufacturing practices (GMPs) are followed.

Effects of Processing and Storage

Nearly all cereal grains are allowed to dry in the field. Properly dried to below 14% moisture and stored, cereal grains are intrinsically resistant to spoilage due to their low water activity (a_w). In developing parts of the world the sun and wind are employed to dry the grains while in developed countries more automated mechanical equipment like mowers and threshing combines are used to dry and then maintain the low-moisture level of the grains during storage. When adequately dried and maintained under hygienic conditions, these grains provide the world with a stable and nutrient-dense food commodity.

The methods used to further process cereal grains into other products are added avenues for contamination. The additional sources of potential contamination of cereal grains are mills, conveyers, and processing equipments. The grains are typically milled to separate the bran and germ from the endosperm to produce flour and bran. The flour and bran are recombined to create a variety of nutritionally and functionally useful derivatives including graham flour and whole grain.

Although the grain cleaning and milling process would be expected to reduce the level of microorganisms, the process cannot be expected to eliminate spoilage or pathogenic microorganisms. The exposure to moisture during the milling process

is a possible source of contamination. Hence, the design of foods and food manufacturing processes must take into account the potential presence of pathogenic microorganisms in foods. However, spoilage microorganisms can thrive on cereal grains and further processed products if maintained at improper storage conditions.

Water Activity

Water activity (a_w) is just one of the many factors that determine the bioload on cereal grains. a_w is the measure of free moisture in a product as defined by the ratio of the vapor pressure of a substance to that of pure water at a specified temperature. It is the primary factor that regulates the growth of microorganisms in cereal grains especially during storage. Storage fungi are capable of growth at low a_w levels, whereas field fungi cannot. Few field fungi are able to grow when the a_w is below 0.90. A decrease in a_w restricts the temperature range for optimal microorganisms to grow. Mold does not have the ability to grow in cereal grain when the a_w is below 13%. Studies that have monitored a_w and the absence or growth of mold have demonstrated that dry grains stored below 13% have a shelf life of up to 1 year. This finding is however dependent on the type of cereal grain and the final moisture level.

Temperature Control and Heating

The external surface exposure of cereal grains to certain microorganisms is dependent on where the grains are grown and processed. The temperature is a factor that dictates the growth of the microorganisms during storage. Moderate to tropical ambient temperatures, 10–35 °C, provides the optimal temperature range for fungal growth. Temperature that is not controlled can lead to heating in stored grains. This heating allows the thermophilic fungi to grow and become dominate. This form of heating in grains is a direct result of fungal growth and metabolism. Even when cereal grain moisture is below 12%, increases in temperature is not safe for long-term storage. Nevertheless, in order for most grain-based foods to be considered edible, they must be given some type of heat treatment. Under the prescribed conditions, the heat supplied is sufficient to inactivate most microorganisms.

The use of low temperatures for grain storage aids in extending product shelf life. The harvest process for maize is done when the grain has greater than 20% moisture available for possible microbial growth. Storage for rice is done at refrigerated temperatures soon after harvest and drying is done in a slow methodical manner until the desired moisture level is achieved. However, this process must be continually monitored because some fungi can grow at these low temperatures.

Inherent Microflora

Cereal crops in their natural field state are exposed to a wide variety of microorganisms due to dust, diseased plants, insects, soil, fertilizer, and animal droppings. The nutritious composition and near-neutral pH of cereal grains make them the optimal substrate for microbial growth. The number and type of microorganism present will be influenced by temperature, sunlight, soil, and general climatic conditions during the growing and harvesting season. This diversity in microorganisms is compounded by the introduction of microorganism during grain transportation that likewise can be affected by these climatic influences and hygienic practices. For this reason cereals are considered as raw or nonprocessed agricultural commodity as one can expect the bioburden of the cereals to be a function of these natural conditions. These microorganisms may include both spoilage and pathogenic microorganisms and may originate in the feces of warm-blooded animals. In general, these microorganisms will either reside on the outside of the grain or invade the inner grain kernel. Although many microorganisms may be present, few are capable of invading the grain kernel without damage of the cereal crop due to the stresses of weather, mechanical injury, and insect infestation. The presence of these invasive microorganisms is inconsequential if the cereal grains are dried to a moisture level below 12% (0.70 a_w). The difference in grain varieties is commonly not a factor in the type of microorganism present.

Bacteria and Bacterial Toxins

Cereal grains may become contaminated with microorganisms originating in the field. However, further contamination is evident during harvest, distribution, storage, and processing. Although these microorganisms will not grow in cereal grains that are properly dried to less than 13% moisture, certain microorganisms will persist through grain cleaning and milling and may affect product quality or safety. Bacteria commonly found in cereal grains belong to the families *Pseudomonadaceae*, *Micrococcaceae*, *Lactobacillaceae*, and *Bacillaceae*. Observations have shown that bacterial populations may appear in numbers 10-times higher than mold populations. Bacterial populations in cereal grains may be present at levels of $10^6\ g^{-1}$. The extensive variety of species detected may include aerobic mesophilic sporeformers, lactic acid bacteria, coliforms, and pseudomonads.

The primary bacterial hazard of concern within cereal grains is *Salmonella*. The variation in seasonal conditions, processing, and distribution make an accurate estimate of the incidence of *Salmonella* contamination within milled cereal grains challenging. The incidence of *Salmonella* within wheat flour has been reported to be at 0.14%. Studies have demonstrated that viable *Salmonella* can be detected in dry flours after several months of storage. *Salmonella* contamination in low-moisture foods can be traced back to substandard sanitation practices, inferior facilities, poor equipment design, improper maintenance, and inadequate following of GMPs.

A number of foodborne illnesses are caused by intoxication. Bacterial intoxication is the ingestion of a toxin present in food before consumption. *Clostridium botulinum*, *Staphylococcus aureus*, and *Bacillus cereus* are the most common bacteria that cause bacterial intoxication.

Clostridium botulinum is a Gram-positive anaerobic spore that produces seven serologically distinct toxins. The toxin types are A, B, C, D, E, F, and G. The toxin types are associated with the strain based on the toxin produced. The species are further

subdivided according to the degree for proteolytic activity. Strain types A, B, and F, group I proteolytic strains, are of major concern in foods. In group II, nonproteolytic strains, B, E, and F are also a concern in foods. The deactivation of the *C. botulinum* spores is through a process that delivers a 12-D reduction, the heat required to inactive a theoretical contamination by 10^{12} *C. botulinum* spores.

Staphylococcus aureus, is a Gram-positive facultative cocci that has the ability to withstand high levels of salt. The enterotoxins of *S. aureus* are A, B, C_1, C_2, C_3, D, and E. Other strains of *S. aureus* are F, H, and G. Enterotoxin F and G have not been associated with food. Staphylococci are ubiquitous and their presence in foods at low numbers are not uncommon. *Staphylococcus aureus* is not heat resistant. Freezing and thawing have little effect on the viability of *S. aureus*. The use of heat is the most effective form to deactivate *S. aureus* in foods.

Bacillus cereus is a Gram-positive facultative spore-forming anaerobic rod. The *B. cereus* emetic toxin and diarrheal enterotoxin are causes of food poisonings. The deactivation of the *B. cereus* spores is dependent of the type of food it is in.

The consumer's kitchen and food manufacturing facility share the same hazard of the presence of *Salmonella* when it comes to handling various flours. Owing to the presence of *Salmonella* in wheat flour, handling and cooking practices must be clearly and effectively communicated to ensure the destruction of this organism. The importance of these handling and cooking practices was evident when *E. coli* O157:H7 was epidemiologically and analytically determined to be present in refrigerated cookie dough. Although the presence of this organism in wheat flour was unexpected, the consumption of raw cookie dough by consumers before baking was not. The linkage of foodborne illness to cookie dough illustrates the need for a farm to fork hazard analysis through revalidation of foods. This revalidation needs to consider the factors of consumer behavior and package communication language. Consumers must recognize that foods prepared with cereal grains that have not undergone further processing to eliminate pathogenic microorganisms may indeed contain these pathogens and must be adequately cooked before consumption.

Molds and Mycotoxins

Fungi capable of significance to grains stability and safety can be classified as field and storage molds. The field molds represent strains capable of survival and growth on cereals before harvest. These field molds following harvest and drying eventually die yet storage molds rapidly contaminate the grains and predominate. The storage molds in general are capable of growth under lower moisture levels, down to 0.70 a_w whereas the field molds do not. These storage molds reside within the grain handling, distribution, processing, and storage equipment and can be minimized within the stored cereal grains with diligent sanitation of all steps from the field through storage. Species of *Aspergillus* are common and an important storage fungi in grains. Damage of the cereal's natural barriers by mechanical, weather, and stress enables insects to invade the grain and damage the cereal grains. The destruction of the crop is exacerbated by vectoring of molds through insect activity through the germ or whole kernel.

The beneficial effects of some molds in fermented foods have been well documented. However, it is also known that some molds produce toxic chemicals. The primary spoilage microorganisms in cereal grains are molds in which growth at reduced a_w levels can occur. The invader of wheat grains are molds such as *Alternaria*, *Fusarium*, *Helminthosporium*, and *Cladosporium* species. Mycotoxins are typically found in obviously deteriorated kernels (dark color visible mold, broken pieces). Extensive mycotoxin reduction can be achieved through the sorting of grains to remove the dark and broken kernels. The ability of field and storage molds to produce mycotoxins in grains is common yet the factors and conditions controlling the production of these metabolites are not fully understood.

The potential to produce mycotoxins is the principal concern of spoilage caused by molds. The consumption of cereal grains with the presence of mycotoxins in flours, meals, and dry mixes is a greater health risk than the presence of *Salmonella* in the same products. The mold producing these mycotoxins may originate in the field and opportunistically invade the cereal grains when the moisture level exceeds 28–33% dry basis. The moisture level within stored grains that exceed 12% may produce other species of mold and may produce mycotoxins. Climactic and storage conditions may challenge the uniform drying of stored grains at moisture levels below 12% creating pockets of mold growth and mycotoxin production. Yeasts and molds may contaminate cereal grains to levels of 10^4 g^{-1}.

The production of mycotoxins, due to mold growth, is an important health hazard of cereal grain products. Some factors that affect mycotoxin production by molds are moisture, pH, and competitive and associative growth of other fungi and microorganisms. *Aspergillus*, *Penicillium*, and *Fusarium* are the primary molds of greatest concern that produce mycotoxins. At present, there are approximately 20 known mycotoxins found in nature from cereal products with sufficient frequency and large toxic amounts to be of concern for food safety. There are five toxin groups in which the 20 naturally occurring mycotoxins have been categorized into. These five groups are aflatoxins, ochratoxin, zearalenone, deoxynivalenol, and fumonisins. As the awareness of mycotoxins increases, patulin, cyclopiazonic acid, and moniliformin are toxins of emergence.

The consumption of any amount of mold growth on grains is a risk to humans and animals, yet animals may be more at risk given the likelihood they will eat larger quantities of moldy material. Mold contamination of raw cereal grains is not completely preventable. The awareness of the types of mold present in cereal grains are an early indicator of the types of mycotoxins that may be produced.

Heavy Metal in Cereal Grains

The bioavailability of metals in soil is important for the development and growth of cereal crops. Heavy metal contamination of soil is due to factors such as the use of sewage sludge and areas surrounding industrial activities. Heavy metals that are often reported are copper, lead, and zinc. Cadmium is a heavy metal that has high toxicity and bioavailability numbers. Among cereal grains, wheat accumulates much higher amounts of cadmium. However, these amounts

can be decreased with the use of fertilizers. There is a need to reduce the uptake of crops heavy in metal to crops based on amounts of heavy metal found in the overall human diet.

Mixed Weeds in Cereal Grains

The process of harvesting various cereal grains is one point of possible contamination due to mixed weeds or grasses. Weeds that are not controlled with effective control procedures compete with cereal grains for water, light, nutrients, and space, causing reductions to yield quality and quantity. The harvesting of weed grasses with cereal grains can cause grain contamination that may result in the crop being rejected at the mill or failing certification. This rejection and contamination of weed grass in cereal grains is less of a concern in industrialized countries.

Classification of weeds is usually in two common groups, grass weeds or broadleaved weeds. Weeds with established populations can cause approximately 25% yield losses. Grass weeds can carry the cereal disease ergot, caused by the fungus *Claviceps purpurea*. Ergot ruins the grains of developing crops and renders them toxic to both livestock and humans. *Claviceps purpurea* affects rye grains, but can also harm triticale, wheat, and barley crops. The florets of cereal grains are infected by the *C. purpurea* spore which mimic pollen grain growing cycles. Climbing weeds in the broadleaved weed group, such as cleavers, affect the profitability of a cereal crop by interfering with crop growth and harvest.

Acrylamide

Acrylamide, a naturally occurring reactive unsaturated amide with a double bond, is found in plant-based, high-carbohydrate foods after they are heated. It is a monomer used in water treatment facilities, in mining operations, and in the paper production industry. The Swedish National Food Administration along with the University of Stockholm announced in 2002 the high levels of acrylamide in processed and cooked foods that used high temperatures during process. Acrylamide in thermally processed foods has been linked to the Maillard reaction. During the Maillard reaction, reducing sugars and the amino acid asparagine are the major reactants. Acrylamide formation is also produced when decarboxylated asparagine is heated.

The findings that acrylamide is present in many processed foods caused many to have an apprehensive reaction due to the fact that the International Agency for Research on Cancer as a class 2A 'probable human carcinogen.' Toxicity studies of acrylamide demonstrate that, depending on the dosage and the length of exposure, neurotoxic, reproductive, and carcinogenic effects can occur.

Acrylamide is found in highest concentrations in french fries and potato chips, but it is also detected in diverse foods such as breads, cereals, cakes, and coffee, and cocoa. It is ubiquitous in the human diet. More than one-third of the calories we take each day come from foods with detectable levels of acrylamide.

Taeymans *et al.* (2004) describe several methods of detecting acrylamide in food. One method is gas chromatography–mass spectrometry. This method allows for the detection in certain foods that approach the range of 1–2 $\mu g\,kg^{-1}$. Other methods for detection are liquid chromatography–mass spectrometry and proton-transfer reactions mass spectrometry.

Cereal-Derived Products

The diversity in the types of microorganisms that can be indentified with various cereal grains is small in comparison to the diversity in the number of products that are produced from cereal grains. Products that are produced from cereal grains fall into several main categories of products: flours and starches, doughs, breakfast cereals and snacks food, baked goods, soy protein, pasta, and dry mixes. Each product categories has its own normal microflora associated with it.

Flours and Starches

Cereal grains that are processed into flour, meal, or grits carry the inherent risk of microbial contamination based on the conditions present in the fields and transportation. Grains that are milled have been bombarded with numerous cleaning and aspiration measures before tempering. This reduces the microbial load that is present as the grains are milled. The process of tempering the grains introduces moisture for a predetermined amount of time is a processing technique in which microorganisms can grow. The dry milling process for corn meal and corn grits avoids the addition of moisture to the grains. Processed flours that are properly handled should not be higher in microbial counts than levels in raw grains. In wheat flours, it is known that *Salmonella* maybe be present at levels up to 5%. Because of the *Salmonella* risk, some manufactures are using flour that has been heat treated to reduce the possible microbial load. These flours and starches then are used in a variety of sauces, doughs, and confections as the main ingredient.

Doughs

The microflora of doughs is due to ingredients such as flour, dry milk, eggs, sugar, spices, flavorings, yeast, and water use in the formulation. Yeast is the principal contributor to flavor in dough formation. However, moisture levels in dry cereal grain at 17% or higher will allow undesired yeasts to growth. *Saccharomyces cerevisiae*, one form of yeast, is important for flavor development during the proofing phase. The equipment used in the manufacturing of refrigerated or frozen doughs plays an important part in the finished product microbial load. The sanitation practices for this processing equipment must be followed to reduce potential contamination.

In some commercially produced dough products, processors have decided to use heat-treated flour. This added step is done as another safety measure to reduce any possible microbial loads present in the flour. However, in refrigerated doughs, bacterial growth is hindered due to the low a_w and pH formulated into the product. Likewise in frozen doughs, microbial growth does not occur when prescribed low temperatures are maintained during transport and storage.

Dry Mixes

Dry mixes are a combination of dried ingredients that are dry blended. These ingredients include items such as: flour, sugar, salt, dried eggs, and flavors. The microbial loads for the finished product of such manufacturing requires that diligence is given to various individual ingredients and manufacturing ingredients. The processing of dry mixes does nothing to reduce the microbial counts. Quality of the final product is affected if microbial load and the moisture level of the product are not controlled.

Baked Goods

The term 'baked goods' refers to breads and pastries. The process of baking or frying has been proven to destroy most microorganisms that are present. A common spoilage organism for breads and other baked goods is mold. Bread is targeted for spoilage and a short shelf life due to the high a_w of approximately 0.95 in the finished product.

Bacillus subtilis, a spore-forming bacterium, can survive the temperatures used for baking or frying. Spores of *B. subtilis* are known to cause 'rope.' Rope is a condition in which the bread exhibits a ropy and stringy texture of the product interior. The spores survive the baking process and begin to germinate. The bacteria produces amylases and proteases that breakdown the bread structure.

The use of various fillings supports additional spoilage microbial growth that is normally controlled in the baked part of the cereal product. Fillings, fruit, or custard have inherent ingredients like eggs or milk products that support microbial growth. The potential for growth is furthermore due to the processing parameters that may not supply enough heat and time to destroy microorganisms. The final filling product normally has a neutral pH and a high a_w which are contributing factors for microbial growth.

The prevention of mold spoilage is controlled when a_w is reduced. Another measure used to control spoilage in breads is the usage of preservatives such as propionic acid, sorbic acid, calcium propionate, or other weak acids. Spoilage of bakery products has been controlled by good sanitation procedures and bakery practices.

Soy Protein Products

Soybeans are not cereal grains, but a pulse. A pulse is a grain legume. Soybeans in nature need no industrial fertilizers, because they enjoy a natural symbiosis with a *Rhizobium* bacterium. This relationship provides organic proteins that are made directly from the surrounding atmosphere's nitrogen content, while eliminating the need for commercial nitrogen-based fertilizers. Grain legumes are cultivated primarily for their seeds, rich in both energy and protein. As a food, they provide humans with this protein and energy as well.

The finished food products using soy exhibit many of the same properties as cereal grains. The primary ingredient in soy protein products is soy flour. Soy flour is derived from soybeans that have been dried and ground.

Breakfast Cereals and Snack Foods

Breakfast cereal processing falls in the categories of flaking, puffing, or extrusion. In each of these processes, moisture is introduced. The introduction of moisture provides the opportunity for microbial growth that can be reduced by the heat applied during production. Post-heat contamination may happen as vitamins, minerals, sweeteners, colors, or flavors are applied to the grain. The addition of ingredients after postheat treatment must be free of microorganisms to maintain a contamination-free product. Processing equipment after post-heat processing must be maintained in sanitary conditions to avoid post-production contamination.

Owing to low-moisture levels of breakfast cereals, it was long considered that this would prevent any further microbial growth. However, it has been discovered that microbial contaminants may not grow in these low-moisture products, but they can survive. This discovery has been evident based on breakfast cereal recalls due to *Salmonella* contamination.

Under the heading of snack foods, the emergence of grain-based bars with or without filling or coating is ever increasing. These cereal bars are made using the process of agglomeration. Agglomeration is a process during which primary particles are fixed together to form larger, porous secondary particles. Cereal bars are made with flaked, puffed, or extruded grains that are assembled together with a sugar-based binding solution and compressed under low pressure.

Cereal bars are considered ready-to-eat products. The ingredients used to produce agglomerated bars require an added degree of diligence to ensure that the cereal grains and additive ingredients are free of microbial contamination. This is because there is no heat step used in the production of cereal bars to reduce the initial microbial counts that raw cereal grains inherently encompass. The heat used to mix the binding syrup is not sufficient to reduce the microbial load that may be present on contaminated cereal grain products. Added care must also be taken to ensure that cross-contamination does not occur during the processing of the agglomerated product from outside factors such as personnel or processing equipment.

Pasta

The manufacture of pasta does not include a heat step that may reduce the initial microbial load of durum wheat flour, water, enrichment nutrients, and/or egg ingredients commonly used. Microorganisms can proliferate during the mixing and drying process. The microbial load may also increase during the extrusion component of the process. There are two types of pasta productions: egg-based and macaroni-type. Egg-based pasta has an added bacterial risk of *Salmonella* contamination from the addition of fresh or dried eggs. Even when the egg-based pasta is dried, the potential for *Salmonella* to survive the drying process is a possibility. The initial microbial load is directly related to microbial load of the individual ingredients. As the pasta is being manufactured, it contains approximately 30% moisture which can support microbial growth. The potential for microbial growth is not inhibited until the product is dried below 13% moisture. Freshly dried pasta has had counts as high as $10^7\ g^{-1}$. This number was subsequently lowered during the drying process. Spoilage of dry pasta is rare during storage and distribution.

However, vegetative cells will be destroyed during normal boiling preparation with the staphylococcal enterotoxin remaining.

Gluten versus Gluten Free

Gluten is a protein that appears in foods processed from wheat and other cereal grains, including barley and rye. It gives elasticity to dough, helping it to rise and keep its shape, and often giving the final product a chewy texture.

Gluten, when dried and milled to powder and added to ordinary flour dough, improves rising and increases the bread's structural stability and chewiness. On digestion, the 33-amino acid gluten protein breaks down into smaller peptide units. There are two main groups of proteins in gluten, called the gliadins and glutenins.

The demand for cereal grain products labeled as 'gluten free' has grown over the past few years. One cause for the demand is the increase of people diagnosed with celiac disease. Celiac disease, also known as gluten-sensitive enteropathy, nontropical sprue, and celiac sprue, is a genetic disorder in which people are predisposed to an autoimmune response that causes damage to the small intestine when gluten proteins are contained in certain foods. Approximately three million people, 1% of the population, have been diagnosed with celiac disease.

Australia, Europe, and the USA have diagnosed celiac disease. The Food and Drug Administration (FDA) has proposed a gluten-free labeling rule for voluntary use in foods. The current standard proposed by FDA is that food mostly contain no more than 20 ppm to claim a gluten-free status. A gluten-free label means that the food being produced does not contain any species of prohibited grains such as, wheat, rye, barley, or a crossbred hybrid. In addition, it does not contain any ingredients that is derived from prohibited grains and has not been processed to remove gluten.

Manufactures that want to make 'gluten free' claims need to view gluten in the same manner as an allergen. Companies must design policies and systems within their facilities based on the presence or absence of gluten-containing products. Facilities that are not completely free of gluten-containing products increase the chance of not meeting the proposed labeling rule. However, systems can be designed to have the needed segregations and sanitation procedures. Plants must have segregated areas for receiving raw ingredients and develop systems to avoid cross-contamination. Processing equipment must be properly sanitized to ensure the absence of the gluten protein.

See also: Food Safety Assurance Systems: Management of Allergens in Food Industry. Foodborne Diseases: Overview of Biological Hazards and Foodborne Diseases; Overview of Chemical, Physical, and Other Significant Hazards. Processing Contaminants: Acrylamide

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SAFETY OF FOOD AND BEVERAGES

Oils and Fats

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Glossary

Coconut oil Oil derived from the kernel of the coconut (*Cocos nucifera*).
Crude oils Vegetable oil obtained by the oil extraction process before refining.
Maize or corn oil Oil derived from maize germ (the embryos of *Zea mays*).
Oil extraction Recovering of vegetable oil from the oil crop.
Oil Refining The purification of oils and fats to remove minor components like free fatty acids, phosphatides, and also color and off taste.
Palm kernel oil Oil derived from the kernel of oil palm fruit (*Elaeisguineensis*).
Palm oil Oil derived from the fleshy mesocarp of oil palm fruit (*Elaeis guineensis*).
Peanut or groundnut oil Oil derived from groundnuts (*Arachis hypogaea*).
Rapeseed oil Oil produced from seeds of the *Brassica* family.
Soybean oil Oil derived from soya beans (seeds of *Glycine max*).
Sunflower oil Oil derived from sunflower seeds (*Helianthus annuus*).
Vegetable oils Foodstuffs which are composed primarily of glycerides of fatty acids being obtained only from vegetable sources.

Introduction

Functionality

Oils and fats have been used from ancient times for food preparation as well as in non-food applications like lamp oil, lubricant, soap manufacturing, and skin care. This article will only deal with the use of oils and fats for food preparation or as an ingredient in food.

Oils and fats provide functionality in food preparation and use as well as nutritional benefits. They serve as the heat transfer medium at elevated temperatures (e.g., frying), improve the mouth feel (spreads and dressings) and give texture and flavor to a wide range of foodstuffs. A balanced intake of oils and fats is essential for human health. They supply a concentrated source of energy, deliver critical building elements for the body and act as a carrier for essential minor components like vitamins A and D.

Supply Chain

Oils and fats originate from plant and animal sources. The plant based oils and fats dominate in current food applications and will be the main focus of this article.

The supply chains of vegetable oils and fats consist of:

- The growing of oil seeds, fruits, kernels or nuts,
- Oil extraction to recover the oil; the by-product meal is mostly used as animal feed,
- Purification and modification to optimize the oil properties for its use,
- All transport from grower to end user.

Until the industrial revolution in the ninteenth century, rapeseed, linseed, olives and nuts were the main sources of vegetable oils. Today, the world market is dominated by palm and soybean oil, followed by rapeseed and sunflower oil. This also led to a change of the extraction and purification/modification processes. Originally, the oil extraction process consisted of cleaning, crushing, heating and pressing. From 1900 onwards, solvent extraction was also applied to recover the residual oil from the pressed cake or to replace the pressing process completely (e.g. for soybean oil). At more or less the same time, the oil purification process changed from a simple decanting and filtration to a combination of neutralization with caustic, bleaching with active clay and deodorization at high temperature under vacuum with steam. By this refining process, minor components could be reduced to improve taste and appearance while the removal of processing residues was required after the introduction of solvent extraction and nickel catalyzed hydrogenation.

Later, the introduction of improved analytical techniques showed that the refining process also reduces the levels of many of the contaminants present in the crude (=extracted) oil. This article gives an overview of the most important contaminants in the crude oil and the validation of the refining process for the removal of these components.

Contaminants in Crude Oils and Fats

Crude Oil Risk Hazard Analysis

The presence and levels of contaminants in crude oils depend on:

- Agricultural practices.
- Procedures of oil crop storage, drying, and handling.
- Oil extraction practices.
- Contamination and degradation during crude oil transport.

The risk of contamination and the type of contaminant will differ per oil type and origin. Information on the presence of contaminants in various oil types from different origins has been collected in three ways:

1. Visits of all steps of the crude oil supply chains.
2. Analyses of crude oils brought for further processing.
3. Sharing of contaminant information in industry organizations and during conferences.

The following contaminants were found at detectable levels: Pesticide residues, poly aromatic hydrocarbons, hydrocarbons of mineral origin, and mycotoxins. The following sections give the analysis of hazards associated with crude vegetable oils.

Pesticide Residues

Plant protection products or pesticides can be used during the cultivation and storage of oil seeds, fruits, kernels and nuts, to protect the crop during growing, to reduce weeds and to protect seeds during storage and transport. To guarantee pesticide use according to good agricultural practices and to protect consumer's health, authorities introduced limits for the residual levels of pesticides in the harvested crops; the so-called maximum residue limits (MRL). These limits are requested by pesticide manufacturers on the basis of residues found after pesticide use according to good agricultural practices (GAP). These MRL's are crop and pesticide specific and must be much lower than the harmful toxicological thresholds (see Figure 1). For crop/pesticide combinations for which a MRL has not been requested or the request has not been granted, the pesticide level in the crop has to be below the level of determination (LOD). For instance, in the EU directives the LOD is given for individual pesticides. Pesticides are considered to be chemical hazards if the level in the crop exceeds the MRL of the pesticide/crop combination.

Depending on their physical/chemical properties, the pesticide residues present in the oil crop will concentrate differently in the products of the oil extraction process:

- They will concentrate in the crude oil if they are oil or hexane (in case of solvent extraction) soluble. The maximum concentration factor, X(max), of oil- or hexane-soluble pesticides going from crop to crude oils is:

$$X(\text{max}) = 100\%/C_{\text{oil}}$$

  where C_{oil} is the fraction of oil in the crop (%).
- In palm oil extraction, they will concentrate in the sludge if they are water soluble.
- The concentrations in oil and meal fractions are both equal to the level in the crop if the pesticides are not soluble or equally soluble in oil and meal.

Hence, oil extraction may result in a pesticide level in the crude oil that is higher than the MRL of the oil crop.

The pesticides used in the seed oil supply chain are mainly organophosphorus insecticides. These are applied to protect

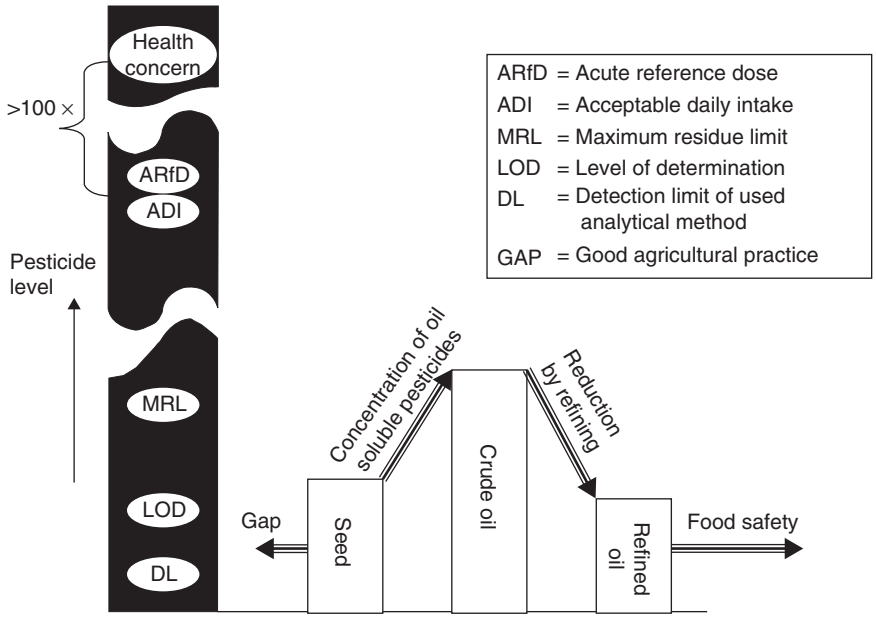

Figure 1 A qualitative picture of the change of a pesticide level by oil extraction and by subsequent refining. The vertical column shows a relationship with the different health levels, legal limit, and detection limit.

oil seeds during storage and transport after harvesting (post-harvest treatment). The following pesticides were found at detectable levels in crude rapeseed, crude sunflower, and crude soybean oil samples: Fenitrothion, Malathion, Pirimiphos-methyl, Parathion-methyl, Dichlorvos, Chlorpyriphos, Chlorpyriphos-methyl, and Endosulfan. Approximately 15% of the sunflower oil samples contained pesticide levels higher than the oil seed MRL valid in the EU; this percentage was much lower for rapeseed and soybean oil.

Oil palm fruits are processed within a few days and preferably within 24 h after harvesting for quality reasons. Post-harvest treatment of palm fruits is therefore not required and detectable pesticide levels in the crude palm oil samples were never found.

In the supply chains of palm kernels and coconuts, chemical crop protection is not applied, resulting in non detectable levels in the crude oils.

Poly-Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) constitute a large class of organic compounds that are composed of two or more fused aromatic rings. They are primarily formed by incomplete combustion or pyrolysis of organic matter. PAHs generally occur in complex mixtures which may consist of hundreds of compounds. Humans are exposed to PAHs by inhalation if they are smokers and by the consumption of contaminated food. Oil crop can be contaminated with PAHs by the absorption of these components from exhaust gasses, when these gasses are in direct contact with the crop during drying.

Oil mills set specifications for the moisture levels of oil seeds. At too high moisture levels, oil seeds need to be dried before arrival at the oil mill or by the mill itself. Drying by direct contact with exhaust gasses has been observed for soybeans in wood-fired packed bed dryers and for sunflower seeds in diesel-fired counter current dryers. Indirect dryers are used in the EU and the USA. In these dryers hot air is generated via a heat exchanger, this excludes contact between exhaust gasses and the product.

In the coconut supply chain drying is an essential operation, it avoids aflatoxin formation and releases the copra (coconut meal) from the shell. In the most commonly used method in the main producing country, the Philippines, halved coconuts are dried upside down on a grid over an open fire, burning coconut shells. Thousands of these drying installations are operated by small farmers.

Palm kernels are washed and dried after the removal of the shells in the palm oil mill by cracking and air separation. A majority of oil mills dry the kernels in indirect dryers, however, a minority use direct dryers. Oil palm fruit is not dried since it is processed shortly after harvesting while the fruit itself contains approximately 50% humidity.

Benz(a) pyrene (BaP), a PAH with 5 aromatic rings, is generally used as a marker for the presence of PAH in crude oils. Figure 2 shows the results of BaP analyses in various crude oils. This graph confirms that the contamination level and frequency of crude coconut oil is very high (maximum BaP level: 70 $\mu g\ kg^{-1}$, fraction of samples with BaP > 1 $\mu g\ kg^{-1}$ (frequency) was almost 80%). Crude sunflower oil was both high in maximum (40 $\mu g\ kg^{-1}$) and in frequency (12%), followed by crude rapeseed oil (maximum 10 $\mu g\ kg^{-1}$, frequency 9%), crude palm kernel oil (maximum 6 $\mu g\ kg^{-1}$, frequency 6%) and water degummed soybean oil (maximum 2 $\mu g\ kg^{-1}$, frequency 7%). Other oils which may contain PAHs are grape seed oil and cotton seed oil.

Hydrocarbons of Mineral Origin

Hydrocarbons of mineral origin consist mainly of alkanes of different chain length. They are manufactured from crude mineral oils in various refining steps such as distillation, extraction, and crystallization followed by purification. Mineral oil products can be divided in product groups on the basis of their carbon number and/or viscosity. Of importance for this

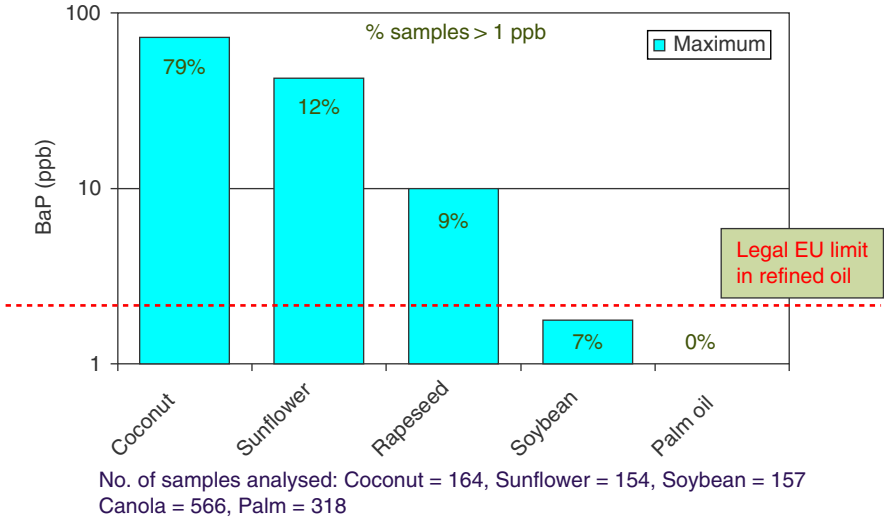

Figure 2 Results of BaP (benz(a)pyrene) analyses in crude oils. This graph shows the average of the samples containing more than 1 $\mu g\ kg^{-1}$ BaP and the maximum observed levels.

section are the following product groups (classified on basis of carbon number):

C6: Hexane, used as solvent for vegetable oil extraction.
C6–C10: Gasoline.
C10–C24: Mid-fraction, consisting of kerosene, diesel, and light fuel oil.
C20–C55: Medium and high viscosity oils such as grease oil, hydraulic oils, etc.
Carbon number >56: Solids.

Based on toxicity studies, the Joint FAO/WHO Expert Committee on Food Additives accepted different acceptable daily intakes (ADI) for mineral oil with high viscosity (ADI of maximum 20 mg kg^{-1} body weight) and for mineral oil with medium and low viscosity (ADI of maximum 10 mg kg^{-1} body weight for class I and maximum 0.01 mg kg^{-1} body weight for classes II and III).

Mineral oil products can be present in crude edible oils due to contamination during processing (lubricants and hydraulic oils), as residues from previous cargoes during transport and storage, and by fraudulent addition. However, their presence can also be the result of an allowed use as a processing aid, like hexane in solvent extraction, as a solvent for pesticides and as an anti dusting agent in oil seeds storage. It should be noted that long chain alkanes are synthesized by a large number of edible plants and animals, resulting in considerable levels of naturally occurring alkanes in crude edible oils (e.g., maximum 160 mg kg^{-1} in sunflower oil). Natural alkanes are characterized by a strong predominance of odd carbon numbers.

Oils and fats have to be free from contamination with hydrocarbons of a mineral origin. This can be ensured by supply chain auditing and by setting analytical limits. These limits should take into account the presence of mineral oil products from allowed practices, the presence of 'natural' alkanes, and the analytical detection limit. The following limits are industry standards based on good agricultural and manufacturing practices.

- Short chain hydrocarbons (shorter than C10) are volatile and are contractually limited by the flashpoint (temperature at which escaping gasses can be detected by flashing). The flashpoint has been introduced to exclude explosion risk during transport and storage. The contractual limit is minimum 121 °C.
- After an incident of diesel contamination in crude palm oil, the Dutch, Malaysian and Indonesian government agreed on a limit of 25 mg kg^{-1} diesel (expressed as C10–C24) in crude palm oil and palm products.
- In 2008, imported crude sunflower oil from the Ukraine had been contaminated with high viscosity mineral oil. The acceptable limit for imported crude sunflower was set at 50 mg kg^{-1} presence of total hydrocarbons (C10–C56). This is after correction for known amounts of natural alkanes. These known amounts follow from an historical analytical data base of non-contaminated samples.
- For all other vegetable oils an action limit was set at 300 mg kg^{-1} of total hydrocarbons (C10–C56). This limit includes an unknown level of natural alkanes and the allowed use of mineral oils as processing aids.

Table 1 Aflatoxin analyses in crude oil deliveries to Unilever

Samples taken from deliveries 1992–94

Aflatoxin B1		*Coconut oil*	*Groundnut oil*	*Rapeseed oil*
No of samples	–	42	11	3
Minimum	μg kg^{-1}	1	4	ND
Maximum	μg kg^{-1}	75	34	ND
Average	μg kg^{-1}	14	10	ND

Aflatoxin G1: Around 30% of B1 level.
Aflatoxin B2, G2: Not detectable.

Mycotoxins

Aflatoxin

Aflatoxins are mycotoxins that are produced by a number of different strains from the Aspergillus family (molds). They are found as contaminants in human and animal food as a result of fungal contamination during growing, and usually to a larger extent, post harvest storage. Carcinogenic effects of aflatoxins to humans are no longer doubted and legal limits for aflatoxins in foodstuff are very low. Aflatoxins are most commonly associated with groundnuts (peanuts), dried fruit, tree nuts (such as almonds, pecans, walnuts, pistachio, and brazil nuts), spices, figs, crude vegetable oils (peanut oil, coconut oil), cocoa beans and a range of agricultural products, the most important being maize, rice, cottonseed, and copra. The aflatoxins that may appear in oilseeds and vegetable oils are aflatoxin B1, G1, B2, and G2 of which B1 and G1 are the most common. In general, no more than 10% of the aflatoxins present in seeds, peanuts, and copra are transferred to the crude oil after pressing and extraction. This is due to the fact that aflatoxins are mainly protein bound.

An inventory of aflatoxin levels by Unilever in the mid nineties demonstrated the frequent occurrence of aflatoxins in crude coconut and peanut oil. The average aflatoxin B1 concentration for coconut oil was 14 μg kg^{-1}, with a maximum of 75 μg kg^{-1}. The average value for the peanut oil was 10 μg kg^{-1}, with a maximum of 34 μg kg^{-1}. The aflatoxin G1 levels in these oils were approximately 30% of the aflatoxin B1 levels, aflatoxin B2 and G2 were not detected (<1.0 μg kg^{-1}). Crude rapeseed oil showed no aflatoxin contamination (<1.0 μg kg^{-1}) (see Table 1).

Zearalenone in Crude Maize Germ Oil

The fungi (Fusarium species) producing the toxin Zearalenone (ZEN) are common soil fungi which mainly develop during flowering. The Fusarium fungi are usually found on cereals grown in the temperate regions of America, Europe, and Asia. The weather conditions during the two week flowering period have a determining effect on the toxin level. High ZEN levels are found at relatively low temperatures and high humidity. Various studies reported a negative effect of ZEN on the fertility of pigs.

In the maize milling process, where the germs are separated from the rest, ZEN is concentrating in the germs. The maize producer's industry organization (Association des Amidonniers et Feculiers (AAF)) estimates that the concentration factor is between 3 and 5. In the last ten year period, high ZEN levels were found in the European harvests of years

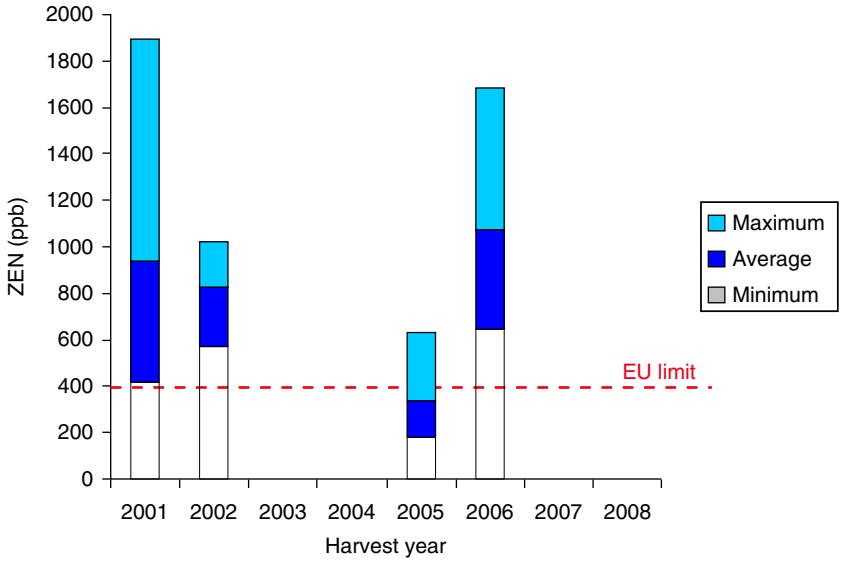

Figure 3 Results of Zearalenone analyses in crude maize oil. The x-axes shows the year of harvesting. The samples were taken from the crude oils produced from the harvested crop during the year following harvesting.

2001, 2002, 2005, and 2006. The other years showed very low ZEN levels (see Figure 3). The flowering period of summer 2006 was specifically unfavorable for ZEN formation (cold and wet). The maize producers found in 236 samples of unprocessed maize an average ZEN level of 370 μg kg^{-1}. With a concentration factor of 3 to 5, this results in an average level of 1100–1850 μg kg^{-1} in germs. Oil producer's data showed that the level in crude maize oil was almost equal to that in maize germs. Levels up to 1810 μg kg^{-1} were found in crude maize oil produced in the first quarter of 2007.

Residues of Previous Cargoes

The general principle is that the transport of both crude and fully refined oils and fats is only permitted in conveyances which are dedicated to foodstuffs. Conveyances include containers, road tankers, rail tank cars, river barges, coastal ships, deep sea vessels, land tanks, direct pipelines, and other handling facilities that may come into contact with the oils and fats. This general principle is applied in the EU and in most other countries.

Intercontinental transport of oils and fats in bulk is carried out in sea going vessels with a capacity of 30 000 to 70 000 ton. This bulk transport of oils and fats represents approximately 30% of the total bulk transport in this type of vessels. A restriction to foodstuff dedicated transport would result in an insufficient availability of ships to serve the overseas oils and fats trade. Therefore, the EU and the international trade have accepted a derogation of the general principle of foodstuff dedicated transport for this type of transport.

In the EU as example, this derogation was based on the following criteria:

1. It should not introduce toxicological concerns for which a threshold is difficult to establish (genotoxic or carcinogenic potential).
2. It is based on efficient procedures to clean ship tanks between cargoes.
3. The residue of the previous cargo after cleaning is diluted in the transported quantity of oil or fat.
4. The validated removal of the previous cargo residue by refining after unloading is considered a pre-requisite for crude and semi-refined oils.
5. It assumes the availability of analytical methods to verify the absence of residues of previous cargoes in the refined oils and fats.

These criteria led to the following set of rules for bulk transport in sea going vessels:

1) For crude and semi-processed oils and fats which are to be further processed before being used for human consumption (further processing has to be refining according to industry standards):
   a) The immediate previous cargo transported in that tank shall have been a foodstuff or a cargo from the list of acceptable previous cargoes, if the oil or fat is transported in a stainless steel tank, or a tank with epoxy resin coating. This list of acceptable previous cargoes is published by the EU and regularly updated.
   b) If the oil or fat is transported in a tank of a different material than those mentioned under (a), than the three previous cargoes transported in that tank shall have been a foodstuff, or a cargo from the list of acceptable previous cargoes.
   c) The buyer must obtain access to written information on the three previous cargoes carried in the relevant tanks.
2) For fully refined oils which are not further processed before being used for human consumption:
   a) If the ships tank is stainless steel or epoxy resin coated, the three previous cargoes transported that tank shall have been foodstuffs.

b) In all other cases, the transport must be dedicated to foodstuffs only.

The international trade of oils and fats is using standard contracts. In particular the standard contracts issued by FOSFA (Federation of Oils, Seeds, and Fats Associations Ltd, London, UK) contain conditions similar to those mentioned above.

Heavy Metals and Dioxins

The presence of heavy metals in crude oils and fats may originate from the oil crop due to uptake from the soil. Contamination risk during processing and transport of oil crop and during transport and handling of crude oil is very limited since heavy metals are not used in contact materials in this supply chain. Crude oil analyses confirmed that heavy metals are seldom present at detectable levels. Metals like iron and copper are commonly present in crude oils and fats, however, these metals only affect quality (they are catalysts for oxidation) but not health.

Monitoring programmes for dioxins, furans, and dioxin like PCB's showed levels well below the allowed level for oils and fats intended for direct human consumption. Only crude fish oil may contain relatively high dioxin levels due to concentration of dioxin in the fish feed chain.

Crude Oil Risk Matrix

The crude oil risk matrix, shown in Table 2, gives the risk classification (high, medium, or low) for presence of a contaminant in a crude oil, in case the origin of this crude oil is unknown. Knowledge of practices or procedures in dedicated supply chains may further reduce the risk classification in case these practices reduce contamination risk. The crude oil risk matrix can be used to determine the frequency of contaminant analyses in crude oils. The proposed frequencies are:

- High risk → check every delivery
- Medium risk → quarterly monitoring
- Low risk → annual monitoring

Crude oils and fats limits are set for pesticides, hydrocarbons of mineral origin, and previous cargoes.

- The pesticide level in the crude oil should not exceed the MRL for the pesticide/oilseed combination after correction by the concentration factor occurred during oil extraction (to be confirmed by updated EU regulation).
- The level of hydrocarbons of mineral origin should not exceed the limits defined by the industry (see above section on hydrocarbons of mineral origin).
- Previous cargoes are checked by comparing the previous cargo from the ship's logbook with the EU or FOSFA positive list of allowed previous cargoes, taking into account the construction material of the ship tanks. This activity is normally performed by an independent superintendent.

The other contaminants have no legal or industry limits in crude oil, but are regulated in the fully refined product. The

Table 2 Crude oil risk matrix. This shows the risk classification for contaminant presence in a crude oil, in case the origin of this oil is unknown. The matrix can be used to determine the frequency of analyses. Examples of limits are given for the EU

	Pesticides	*PAH*	*Mineral oil in edible oil imported in EU*	*Previous Cargoes in sea going vessels*	*Dioxins and PCBs*	*Aflatoxins*	*ZEN*
LIMIT	*> MRL*		*FEDIOL Code of practice*	*EC/4/2004*			
Soybean oil							
Sunflower oil							
Rapeseed oil							
Corn oil							
Palm oil							
Palm kernel oil							
Coconut oil							
Groundnut oil							
Fish oil							
Linseed oil							
Cottonseed							
Grape seed							
Olive							

		Occurrence	Monitoring frequency:
High risk		Regularly (> once a year)	Every batch
Medium risk		Occasionally (every 1–5 years)	Minimum once per quarter
Low risk		Seldom (< once every 5 years)	Maximum once per quarter
Regulated		Not applicable	Every batch

crude oil analytical results are therefore the input for the process validation for contaminant removal.

Refining Process Validation for Contaminant Removal

The levels of most contaminants are regulated for fully refined oils but not for crude oils. The refining process validation will assure that the contaminant level in the fully refined oil is below regulated limit, even for the crude oil feedstock with the highest observed contaminant level. The validation process is as follows:

1) The refinery is informed in case of a crude oil delivery with a contaminant level higher than the refined oil limit (or the highest level used in previous process validations) and the contaminated lot is blocked.
2) A minimum batch of contaminated oil is processed in the refinery using the standard refining recipe. The contaminant levels are analyzed in deodorized end product (and preferably also after the intermediate refining steps).
3) The crude oil is de-blocked and the whole lot can be processed if the contaminant level in the deodorized oil is below regulated limit. The validation process needs to be repeated with modified process conditions if the contaminant level in the deodorized oil is still too high. Alternatively, the crude oil can be sold for non-food application (feed or bio-fuel) in case removal is technically or economically not feasible.
4) This validation process needs to be repeated for every delivery of crude oil with a contaminant level higher than the levels used in previous process validations.

Pesticide Residues Removal

Pesticide concentrations will reduce during the refining steps. The effect of each step on residual level depends on the physical/chemical properties of the pesticide.

Water soluble pesticides dissolve in the alkaline solution during neutralization and are removed with the soap stock.

Some pesticides (e.g., Pirimiphos-methyl) are absorbed by acid-base interactions onto the bleaching earth.

Volatile pesticides are removed with the steam during the deodorization process. All organophosphorus insecticides have a higher volatility than free fatty acids and will be removed at increased temperatures (220–270 °C).

Figure 4 gives an example of a process validation experiment for pesticide removal.

Poly-Aromatic Hydrocarbons Removal

Poly-Aromatic Hydrocarbons (PAH) are removed by active carbon dosing in the bleaching process. Volatile PAH will be additionally reduced during high temperature deodorization. The volatility depends on the number of aromatic groups in the PAH compound; 4 or less are called light PAH, while 5 or more are called heavy PAH. The tracer compound BaP has 5 aromatic groups and is a heavy PAH. For example: The current EU regulation sets limits for PAH levels for oils and fats intended for direct human consumption or used as ingredient in food. The current EU limits are: A limit of 2 ppb for BaP and an additional limit of 10 ppb for 4 specific PAH including BaP.

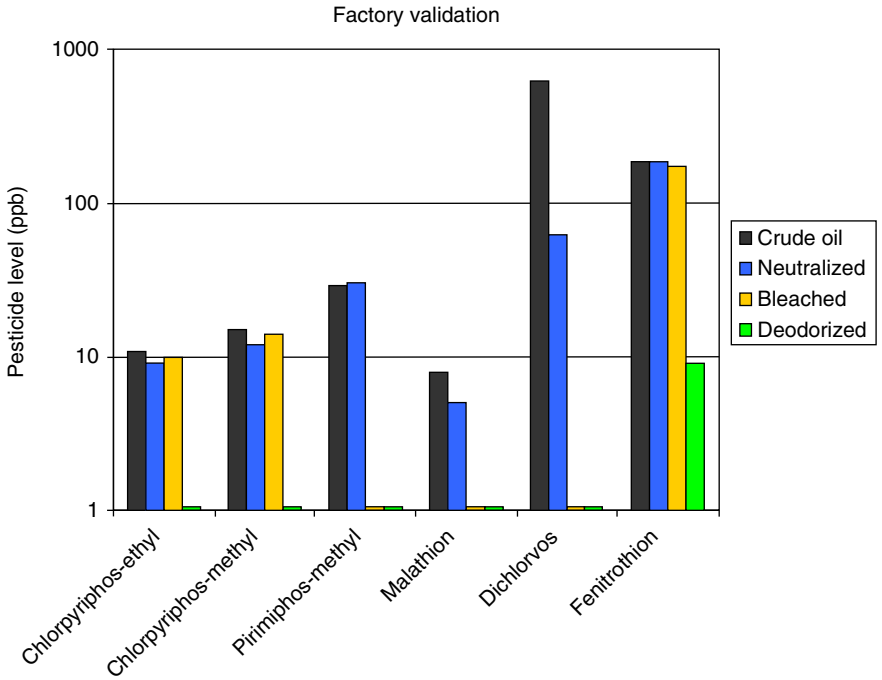

Figure 4 An example of process validation of pesticide removal. The graph shows the levels of the pesticides present in the crude oil and the levels after neutralization, bleaching, and deodorization.

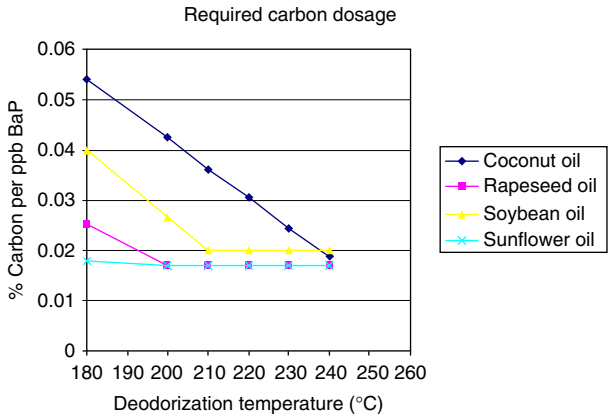

Figure 5 Process validation for BaP removal. The graph shows the percentage of active carbon, per percentage of BaP in the crude oil, needed to reduce BaP below the legal limit of 2.0 μg kg^{-1} and the total of 13 tested PAH below 25 μg kg^{-1}. The horizontal axis gives the deodorization temperature; this mainly influences the light PAH.

Light PAH will be partly removed during deodorization, the degree of reduction depends on deodorization temperature (approximately 50% reduction at 180 °C, up to 90% reduction at 240 °C).

There are two ways for determining the required active carbon dosage for PAH removal:

a. Measure the BaP content in every incoming parcel of oil and add active carbon according to experience from previous process validations. Figure 5 shows the results of process validations performed in Unilever refineries.
b. If BaP analysis is not available (on time), the dosage should be based on a realistic worst case BaP level in the crude oil. The standard active carbon dosage will then be the dosage of the successful process validation performed with this worst case crude oil.

Removal of Hydrocarbons of Mineral Origin

Hexane (C6) is partly removed by the vacuum systems of neutralization and/or bleaching whereas the remainder is removed in the deodorizer to a level below the detection limit (0.1 mg kg^{-1}).

Gasoline has never been detected as chemical hazard in crude oils and fats. Therefore, the refining process has never been validated for gasoline removal. However, experiences with compounds of a similar or even lower volatility indicate a complete removal during deodorization.

The upper limit for presence of mineral oil mid fraction (C10–C24, including kerosene and diesel) in crude palm oil is 25 mg kg^{-1}. Process validation has shown that this level can be reduced to below detection limit (10 mg kg^{-1}).

Mineral oil fractions with a carbon number above C24 will hardly be removed in the refining process. The physical and chemical properties of these components differ insufficiently from the properties of edible oils and fats to enable separation.

Mycotoxins Removal

Aflatoxin Removal

Both the chemical and physical process sequence will reduce the aflatoxin levels to below the detection limit (1.0 μg kg^{-1}). The processes that are responsible for aflatoxin reduction are neutralization with lye (in chemical refining) and bleaching. The activated bleaching earth is responsible for the removal during bleaching; addition of active carbon will hardly increase the separation efficiency. Deodorization, even at high temperature, gives only a modest contribution to aflatoxin removal. The EU limits for aflatoxin in oils and fats intended for direct human consumption or as ingredient in food are: Maximum 2.0 for aflatoxin B_1 and maximum 4.0 for the sum of aflatoxins B_1, B_2, G_1 and, G_2.

Zearalenone Removal in Maize Oil

The refining process will largely reduce the Zearalenone (ZEN) content in maize oil, however, a complete removal will not be obtained under standard refining conditions. Additionally, the removal efficiency depends on the refining process used (chemical or physical). Chemical refining will remove 80–98% of ZEN whereas the removal efficiency of physical refining varies between 70% and 80%. The EU limit for refined maize oil is 400 μg kg^{-1} based on ALARA principle.

Other Contaminants

Residues of Previous Cargoes

The validated removal of substances by a standard refining process is one of the criteria for the acceptance of these substances as allowed previous cargo. Further refining process validation is therefore not required for the substances on the allowed (positive) list. Oils and fats cannot be accepted for food use (even after refining) when the previous cargo is not on the positive list.

Heavy Metals

Heavy metals are seldom present at detectable levels in crude oils and fats. Therefore, the refining process cannot be validated for heavy metal removal except in exceptional cases where heavy metals are present in the crude oil. Metals like iron and copper are effectively removed by neutralization and bleaching with acid pre-treatment. It is assumed that also heavy metal levels will be reduced by these processes. The EU has only regulated the level of lead in oils and fats (maximum 0.1 mg kg^{-1}).

Dioxins

The dioxin level in fish oil is reduced by active carbon addition during bleaching followed by deodorization at moderate temperature (maximum 190 °C to limit isomerisation). The EU sets limits for the sum of dioxins and dioxin like PCBs, taking into account the toxicity equivalents of the individual compounds. The limit for the sum of dioxins in vegetable oils and fats is 0.75 pg g^{-1} (WHO PCDD/F-TEQ) and 1.5 pg g^{-1} (WHO PCDD/F-TEQ) for the sum of dioxins and dioxin like PCBs. The limits for fish oil are higher: Sum of dioxins is 2.0 pg g^{-1} (WHO PCDD/F-TEQ) and 10.0 pg g^{-1} (WHO PCDD/F-TEQ) for the sum of dioxins and dioxin like PCBs.

Table 3 The refining link table. This table summarizes process validation experience of contaminant removal. Examples of limits are given for the EU

Contaminants	*Hydrocarbons < C20*	*Hydrocarbons > C20*	*PAH (BaP)*	*Pesticides*	*Aflatoxin B1*	*Zearalenone*
Legal or industry	*LOD*	*LOD*	*2.0 μg kg⁻¹*	*MRL or LOD*	*2.0 μg kg⁻¹*	*400 μg kg⁻¹*
Crude oil						
Degumming Neutralization					93%	
Bleaching					77%	
Deodorizaton						

= Chemical refining.
= Physical refining.
= Chemical and physical.

Refining Process Validation Experience

The refining process validation experience can be summarized in a refining link table (see **Table 3**). This link table shows the contaminants, the regulated or industry limits in refined oil, and the process step which reduces the concentration of this contaminant in the product. This link table gives a quick reference for process optimization and trouble shooting. It can also be the basis for the application of the hazard analysis and critical control points system in an oil processing plant.

Conclusions

Contaminant levels below the legal limit or industry standard can be assured by the combination of preventive measures (good agricultural practices, crude oil analysis, and validated refining processes). This system should be regularly checked by internal and third party (customer) audits and by monitoring of the contaminant levels in the refined product. Crude oil supply hazard analyses should be periodically repeated by a combination of supply chain visits and analyzing of crude oils for a wide range of potential oil soluble contaminants.

At the end, consumer good manufacturers are responsible for the quality and food safety of the products they put on the market. They may not own the supply chains of their ingredients but they need to assure the food safety by participation in determining and controlling the preventive measures.

See also: Environmental Contaminants: Dioxins, Furans and Dioxin-like Polychlorinated Biphenyls. Food Safety Assurance Systems: Hazard Analysis and Critical Control Point System (HACCP): Principles and Practice. Foodborne Diseases: Overview of Chemical, Physical, and Other Significant Hazards. Mycotoxins: Aflatoxins; Zearalenone. Processing Contaminants: Polycyclic Aromatic Hydrocarbons (PAHs). Safety of Food and Beverages: Nuts

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MVO specifications for refined vegetable and marine oils excluding olive oil.

SAFETY OF FOOD AND BEVERAGES

Spices and Seasonings

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Glossary

Water activity Water activity (a_w) is an equlibrium parameter defined as $a_w p_{food}/p_{water}$, where p_{food} is the partial pressure of water over a food at a given temperature and p_{water} is the vapor pressure over water at the same temperature.

Definition and Classification

Spices are a large number of plant products or blends thereof used for flavoring, improving fragrance, and other sensorial properties of foods. From a food safety point of view, the importance of spices and seasonings/condiments depends on the dietary habits of the communities. Although in certain countries they are only used as minor food ingredients, in other areas they constitute an important part of the daily menu for some societies and are consumed in considerable quantities. The so-called ethnic foods which feature a variety of spices, seasonings, and condiments become more-and-more a part of the mainstream consumption pattern. Many spices are of tropical or subtropical origin. Certain spices improve color qualities or may extend shelf-life of foods by their antioxidant and antimicrobial activities. Some dehydrated vegetable seasonings (e.g., onion and garlic powders) can be discussed collectively with spices.

Spices and seasonings are a chemically and histologically heterogeneous group of commodities which are important items of the international trade. Their extracts and oleoresins are also generally recognized as safe (GRAS) or approved food additives. The spice industry is global and the regulations and standards differ around the world.

Many spices from developing the countries are sourced from thousands of farmers who grow, harvest, and dry spices on small plots of land. Some spices grow wild and are gathered in many different areas from the source country. Most of these countries lack the infrastructure for modern food production, have problems with pollution, and lack clean potable water.

Spices are classified according to the parts of the plants from which they are derived, such as seeds (e.g., anise, caraway, and coriander), fruits (e.g., capsicums and cardamom), berries (e.g., peppers and allspice), flowers (e.g., camomile), leaves (herbs) (e.g., bay, basil, peppermint, oregano, and thyme), roots (e.g., horseradish), and rhizomes (e.g., ginger). Various spices require widely diverse harvesting and post-harvest methods which affect the microbial content in different ways. A list of some frequently used spices is given in Table 1.

Table 1 List of some frequently used spices

Common name	*Parts used*	*Form used*
Allspice (pimento)	Fruit	Whole or ground
Anise	Fruit	Whole or ground
Basil (sweet)	Leaves	Whole or ground
Bay (Laurel)	Leaves	Whole or ground
Capsicum (chili)	Fruit	
Caraway seed	Seeds	Whole
Cardamom seed	Fruit	
Cassia	Bark	Ground
Cayenne	Fruit	Whole or ground
Celery seed	Fruit	
Chervil	Leaves	
Cinnamon	Bark	Ground
Cloves	Flower bud	Whole or ground
Coriander seed	Fruit	Whole or ground
Cumin seed	Seed	Whole or ground
Dill seed	Fruit	Whole
Fennel seed	Fruit	Whole or ground
Fenugreek	Fruit	Whole or ground
Garlic	Bulb	Chopped, dried, powdered
Ginger	Rhizome	Dried, ground
Horseradish	Root	Ground
Mace	Aril	Whole or ground
Marjoram	Leaves (part of tops permitted)	Whole or ground
Mustard	Seed	Whole or ground
Nutmeg	Seed	Whole or ground
Oregano	Leaves	Whole or ground
Paprika	Fruit	Whole or ground
Parsley	Leaves	Dried
Pepper (black)	Berry	Whole or ground
Pepper (white)	Berry	Whole or ground
Peppermint	Leaves	
Poppy	Seed	Whole
Rosemary	Leaves	Whole or ground
Sage	Leaves	Whole or ground
Savory	Leaves and tops	
Sesame	Seed	
Taragon	Leaves and tops	Whole or ground
Thyme	Leaves and tops	Whole or ground
Tumeric	Rhizome	Whole or ground

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Selected Spices and Seasonings with Particular Importance for Food Safety and International Trade

Berries

Pepper is the most widely used spice. It is a small globular berry of the pepper vine *Piper nigrum* (Piperaceae). The major producers of pepper are Brasil, Malaysia, Indonesia, and India. Black pepper is a dried, unripe fruit of this tropical plant. When the harvested fruits are boiled for a short time in water and dried, they become brownish or black, with coarse wrinkled surface; whereas the seed is almost white adhering to the pericarp. The pepper fruits are aromatic and pungent. White pepper is prepared by soaking the berry and then removing the pericarp and the pulpy covering before drying. White pepper can also be prepared from black pepper corn by grinding off the outer parts. Owing to its irregular surface, black pepper holds more surface dirt than the smoother white pepper, and usually carries larger microbial load.

Bulbs

Garlic (*Allium sativum*) products used as seasonings are garlic flakes, dehydrated garlic powder, and garlic paste. Garlic has a long history of folk use in a wide range of ailments. Its active components are the various sulfur-containing compounds (e.g., alliin, allicin, etc.).

Fruits

Capsicum spices are produced from the dried fruits of the genus *Capsicum* (Solanaceae).

Cayenne pepper and red pepper are the two synonyms of *Capsicum frutescens*, whereas paprika or chili is derived from *Capsicum annuum*. Diverse groups of *Capsicum* cultivars encompass pungent and nonpungent fruits. They also have a large variability for fruit size/shape. The pungency is caused by alkaloids called capsaicinoids whereas the color originates from the carotenoid pigments. The commercial value of paprika and its oleoresin depends mostly on their coloring capacity. Drying of fruit should be performed at a relatively low ($<60\ ^{\circ}C$) temperature. The fruit moisture content of approximately 8% is considered to be optimal because more than 11% moisture content allows mold growth and less than 4% moisture causes extensive color loss. Selecting cultivars with high antioxidant content (Vitamin E as fat soluble and Vitamin C as water soluble) has an effect on reducing color loss. Appropriate cultivars, standardization, and adoption of proper drying and storage conditions are the important quality management factors.

Cumin is the dried, ripened fruit of *Cuminum cyminum* (Apiaceae). The fruits have bristly hairs along the ribs. The crop is threshed and after partial drying, winnowing, sieving, and cleaning, is dried. The principal volatile components are cuminaldehyde and p-cymene.

Coriander is derived from the dried ripe fruits of *Coriandrum sativum* (Apiaceae). The major component of the essential oil of coriander seed is linalool.

Leaves

The olive green to brown bay (laurel) leaves are derived from the plant *Laurus nobilis* (Lauraceae). They are characteristically fragrant when crushed, but taste bitter and aromatic.

Oregano consists of the dried leaves and the flowering tops of *Oreganum vulgare* (Laminaceae). They have a strong camphoraceous odor and a warm, pungent bitter taste. The plant is collected when in flower and dried at less than 35 °C.

Peppermint consists of the dried, dark green leaves and the flowering tops of *Mentha piperica* (Laminaceae). Peppermint has a characteristic aromatic odor and taste, with a cooling sensation while drawing-in breath. The plant is harvested when in bloom and the harvested herb is allowed to dry. After attaining the desired brittleness, the leaves are stripped from the stem, completely dried, and fragmented.

Rosemary is the dried leaves of *Rosmarinus officinalis* (Lauraceae). The upper surface of the leaves is dark green, whereas the lower one is wooly white. The odor of rosemary is aromatic, the taste is pungent, aromatic, and bitter. The rosemary extract shows good antioxidant activity that makes it useful as a food additive.

Sage is the dried leaves of *Salvia officinalis* (Lauraceae). The leaves are gathered for use while the plants are in flower.

Thyme consists of the dried leaves and the flowering tops of *Thymus vulgaris*. Its volatile oil is rich in linalool and terpene hydrocarbons: thymol and carvacrol.

Rhizomes

Ginger is derived from the underground plant part, rhizome, of *Zingiber officinale* (Zingiberaceae). It is harvested in different stages depending on the requirements of the user. There are three primary products of ginger rhizome: (1) fresh or green ginger, (2) preserved ginger in syrup or brine, and (3) dried ginger. Dried ginger is used directly as a spice and also for the preparation of its extracts, ginger oleoresin and ginger oil. Principal volatile constituents are sesquiterpene hydrocarbons, predominantly zingiberene.

Turmeric is obtained from the rhizomes of *Curcuma domestica/Curcuma longa*, also a member of Zingiberaceae. The rhizomes when dried and ground provide a brownish-yellow and flavored powder, which is an important constituent of curry powder.

Curcumin is the principal curcominoid polyphenolic compound that gives turmeric its yellow color. Curcumin acts as a free radical scavenger and an antioxidant, inhibiting lipid peroxidation and oxidative DNA damage. Some studies suggest that curcumin may be useful for prevention and treatment of several diseases, although further studies seem to be necessary to establish the benefit/risk profile of the compound.

Seeds

Fenugreek is the dried seed of the leguminous plant *Trigonella foenum-graeceum* (Fabiaceae). The pods split easily and are threshed to free seeds, winnowed, cleaned, and further dried. Their characteristic components are trigonellin and δ-cadinene.

Nutmeg is the dried, large, oval kernel (nut) and mace is the dried aril surrounding the seed of *Myristica fragrans* (Myristicaceae). The fruits are harvested when split on the tree, or gathered after they have fallen to the ground. Nutmegs are dried in their shells at low (35–40 °C) temperature. Nutmeg is sold as whole nutmeg or further ground to adjusted mesh size for spice powder. Its major volatile constituents are safrole and myristicin.

Sesame is the seed of *Sesamum indicum* (Pedaliacae). The hulled seeds are cream or pearly white in color. The seeds possess a faint nutty odor and taste.

Barks

Cinnamon is the inner bark of an evergreen tree (*Cynnamomum zeylanicum*) whereas cassia is made from the entire bark of a related species (*Cynnamomum aromaticum*). They are used especially in the powdered form.

Production and Primary Processing Methods

Various spices obtained from plants require diverse cultivation conditions, harvest or postharvest methods, as well as primary processing techniques. The main methods of primary processing are cleaning, fermentation, drying, grinding, and extraction. Most spices, like other raw agricultural materials must be dry at harvest or dried quickly after harvest to prevent their spoilage by molds. Fermentation is used for a few products, for example, for cassia bark to facilitate removal of the outer layer and for allspice berries to develop color and appearance.

Spice Oleoresins and Essential Oils

Oleoresins are the concentrated extracts of spices obtained by using organic solvents and evaporating the solvents from the extract in vacuum. Oleoresins contain all of the volatile and nonvolatile flavor components and the natural antioxidants of the spices. Certain extraction methods use extraction with water or aqueous alcohol. The process used for extraction depends on the nature of vegetable matter, and depending on its thermal instability, the operating temperature ranges from ambient to the boiling point. Vibrating the material by means of ultrasonic treatment facilitates, greatly, the dispersion. The CO_2 extraction system uses high pressure and applies either liquid carbon dioxide or supercritical carbon dioxide. This extraction technique is based on good solubility of most odorants and vegetable matters in carbon dioxide and has the advantage that it does not leave residual solvent. It is performed at low process temperature (30–50 °C) and is energy efficient.

Oleoresins are hygienic and can be standardized. However, despite these advantages over ground spices, the sensitivity of oleoresins to light and oxygen is a disadvantage. One approach to overcome this is microencapsulation by spray-drying with specific carrier materials, which convert the oleoresins into a more stable and free-flowing powder.

Oleoresins are free from enzymes, superior to native spices in microbial control, and some of them with high antioxidant capacity may reduce lipid oxidation when used in meat products. However, the product acceptability may be decreased by their too strong taste/flavor.

Essential oils are the volatile aromatic substances prepared by steam-distillation of ground spices. They are comprised of two basic groups of compounds which are hydrocarbons including terpenes, sesquiterpenes, and diterpenes, and oxygenated hydrocarbons such as alcohols, esters, aldehydes, ethers, and ketones.

Oleoresins and essential oils are free from the microbial burden of the natural spices.

Antimicrobial Properties

The composition and content of essential oils, and their antimicrobial activities, vary from spice to spice and even within the same spice depend on agricultural practice, geographic and climatic conditions during the growing season. The major antimicrobials present in some spices and herbs are listed in Table 2.

Spices and herbs that contain the most inhibitory essential oils are cloves, thyme, oregano, cinnamon, allspice, cumin, and caraway. Onion and garlic juices also contain antimicrobial components. Concentrations of essential oils in some spices and antimicrobial activity of active components are given in Table 3. However, these data must be considered with caution because they are derived from different investigations and different test conditions, and many flavor-intense herbs and spices possess too little antimicrobial activity when used in food at organoleptically acceptable concentrations. In general, yeasts are more readily inhibited than bacteria.

Microbiological Contamination and Safety

Microbiological Ecology and Sources of Contamination

Initially, spices and herbs contain those microorganisms which are indigenous to the soil and plants from where they originated and survive the drying process. Dust, insects, fecal material from birds and rodents, and the water used in some processes (e.g., soaking peppercorns in the preparation of white pepper) may be the sources of contamination. Closed drying systems reduce the risk of contamination. Microbial counts are a function of original bioload, proliferation, and die off. Counts frequently decrease during storage of dry spices.

The viable microbial cell counts of spices and herb samples of various origin might show deviations in several log cycles and extremely large variations occur in the microbial count of different lots of the same spice. Black

Table 2 Major antimicrobials present in some spices

Spices	*Antimicrobials*
Allspices	Eugenol
Anise	Anethol
Cassia	Cinnamic aldehyde
Cinnamon	Cinnamic aldehyde
Cloves	Eugenol
Cumin seed	Cuminaldehyde
Garlic	Allicin
Oregano	Thymol, carvacrol
Rosemary	Ursolic acid
Sage	Thymol
Savory	Carvacrol
Thyme	Thymol, carvacrol

Table 3 Concentration of essential oils in some spices and antimicrobial activity of active components

Spice	*Essential oil in whole spice (%)*	*Antimicrobial compounds in distillate or extract*		*Antimicrobial concentration (ppm) lab media*	*Target organisms*
		Compound	*%*		
Allspice (*Piementa dioica*)	3.0–5.0	Eugenol Methyl eugenol	73–79 9.6	1000 (G) 150 (I)	Yeast, *Acetobacter* *Cl. botulinum*
Cassia (*Cinnamomum cassis)*	1.2	Cinnamic aldehyde Cinnamyl acetate	75–90	10–100 (G)	Yeast *Acetobacter*
Clove (*Syzgium aromaticum*)	16.0–19.0	Eugenol Eugenol acetate	72–92	1000 (G) 150 (I)	Yeast *Cl. botulinum* *V. parahaemolyticus*
Cinnamon bark (*Cinnamomum zeylanicum*)	0.5–1.0	Cinnamic aldehyde Eugenol	65–76 4–10	10–1000 (G) 100 (I)	Yeast, *Acetobacter* *Cl. botulinum* *L. monocytogenes*
Garlic (*Allium sativum*)	0.3–0.5	Allyl sulfonyl Allyl sulfide		10–100 (I)	*Cl. botulinum* *L. monocytogenes* Yeast, bacteria
Mustard (*Sinapis nigra*)	0.5–1.0	Allyl isothionate	90	22–100	Yeast, *Acetobacter* *L. monocytogenes*
Oregano (*Origanum vulgare*)	0.2–0.8	Thymol Carvacrol	60–85	100 (G) 100–200 (I)	*V. parahaemolyticus* *Cl. botulinum* A,B,E
Paprika (*Capsicum annuum*)		Capsicidin		100 (I)	*Bacillus*
Thyme (*Thymus vulgaris*)	2.5	Thymol Carvacrol		100 (G) 100 (I)	*V. parahaemolyticus* *Cl. botulinum* Gram + bacteria *Asp. parasiticus* *Asp. flavus* aflatoxin B_1 and G_1

Abbreviations: G, germicidal; I, inhibitory.
Source: Courtesy of Blackie Academic and Professional, UK.

pepper, turmeric, paprika, allspice, and marjoram are the spices most highly contaminated with bacteria. The aerobic plate count of these spices may sometimes reach the 80–100 millions g^{-1} level. Anaerobes are less numerous than aerobic bacteria.

For certain sectors of the food processing industry (e.g., canning), the contamination of ingredients with heat-resistant bacterial spores is especially troublesome. In some canned foods, the microbiota of the major components consists of relatively heat-sensitive organisms. However, if even a minor ingredient, for example a natural spice, contributes highly heat-resistant microorganisms such as bacterial spores in terms of the process applied, then this item becomes the single most important component determining the bacteriological quality of the product, and control over the finished product involves control over the spore load of this ingredient. Thus, a major concern of the food processors is that the microbial load of ingredients and processing aids do not contribute to spoilage of foods they produce and do not diminish their microbial safety. In many spices, the major part of the microbial load is composed of spores of mesophilic (aerobic) sporeforming bacteria (e.g., *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus megaterium*, *Bacillus pumilus*, *Bacillus brevis*, *Bacillus polymixa*, and *Bacillus cereus*), because many vegetative cells die during the drying stage whereas spores survive. Thermophilic anaerobes and aerobes are found occasionally, sometimes in moderate numbers. Thus, some spices are potential sources of highly heat-resistant bacterial spores, including thermophilic 'flat sours,' 'putrefactive anaerobes,' and 'sulfide stinkers.' Regarding nonsporeforming bacteria, coliforms are also often found. Generally, psychrotrophic bacteria (growth at 7 °C) are less numerous in spices and herbs than mesophilic ones.

Several seasonings which are used as herbal products/nutraceuticals have been investigated for prevalence of antibiotic-resistant bacteria and a considerable number of the bacterial isolates proved to be resistant to multiple antibiotics.

Mold contamination can also be an important contaminant. White pepper, black pepper, chili, and coriander seem to be most heavily contaminated with molds. The *Aspergillus glaucus* group, *Aspergillus niger* and *Penicillium* spp. are normally prevalent. Molds may grow on spices and herbs before drying or during drying, storing, and shipping. Yeasts have been found in spices in low numbers only.

From the point of view of microbiological stability and eventual mycotoxin formation (see Mycotoxins in Spices), the mold contamination of spices, herbs, and dry vegetable seasonings is of importance. Fungal spoilage of these commodities may occur at high relative humidity and elevated temperature, or, if localized wetting occurs.

Microbial Risks and Outbreaks

Excessive numbers of various bacterial spores introduced with spices into food can induce spoilage if further food processing is not sufficiently severe to inactivate them. Moldy spices may introduce off-flavors and unwanted enzymes, for example, pectinases.

Spices are not the major contributors of foodborne diseases, however, they occasionally contain bacteria that can cause foodborne infections (see Pathogenic Bacteria).

Mold counts of spices may reach the 10^5 propagules per gram level, and a relatively high incidence of toxigenic molds has also been found. Climate changes and global warming is expected to increase the problem of mycological safety of spices.

According to the European Community's Rapid Alert System for Food and Feed (RASFF), 1179 (10%) of the 11 403 hazard notifications were related to the product category 'spices and condiments' during the 4-year period of July 2003 to June 2007. Regarding 'microbiological hazards,' 229 notifications were concerned with this product category.

Pathogenic Bacteria

Among the spore-formers, *B. cereus* and *Clostridium perfringens* that are capable of causing gastroenteritis when ingested in large numbers frequently occur in spices but usually in low numbers. In extreme cases, however, *B. cereus* counts up to 10^5 CFU g^{-1} have been reported. Even when *B. cereus* and *Cl. perfringens* may be present in spices and herbs at counts below 10^3 CFU g^{-1}, they may multiply to high levels (10^5–10^6 CFU g^{-1}) in food to which the spice is added, if the food is inappropriately handled. Thus, rapid cooling of food in shallow containers is important for microbiological safety of seasoned commercial meals.

Several other *Bacillus* spp. that are opportunistic pathogens are more frequently isolated from spices. Because bacterial spores may survive cooking temperatures, ingredients harboring these spores must be considered as a potential health hazard.

Conditions of the growing regions of most spices make them susceptible to contamination by Enterobacteriaceae, including, albeit infrequently, salmonellae and *Escherichia coli*. However, depending on the temperature and water activity, salmonellae can remain viable in spices and condiments for considerably long storage periods. Their presence is of special concern when spices are used in foods that are consumed raw, or when the spices are added to food after cooking. According to the 2005 Food Code, the USA Food and Drug Administration (FDA) considers spices by nature to be ready-to-eat (RTE) products. The general public would not consider spices to be a safety risk and frequently uses them without the benefit of a lethality step, such as cooking at the appropriate temperature and time by boiling, baking, etc.

Salmonella spp. are the most common bacterial pathogens associated with product recalls and outbreaks caused by spices. During the period of 1970–03, the FDA monitored 21 recalls involving 12 spice types contaminated with bacterial pathogens. In all but one instance, the recalled spices contained *Salmonella*. A wide variety of countries were the sources of the recalled spices.

Recent knowledge gathered about microbiology of various low-moisture foods shows that *Salmonella* cells appear to be more tolerant to inactivation procedures and their infectious dose in low-moisture conditions may be very low (often less than 10 CFU g^{-1}), as evidenced by outbreak investigations. In this sense, contamination of dry spices and herbs by *Salmonella* may also present a considerable human health hazard.

A European Commission Recommendation required the Member States in 2004 to perform a co-ordinated program of sampling and testing of dried spices and herbs from the manufacturers, packing centers, and retail premises to gain insight on prevalence of contamination by *B. cereus*, *Cl perfringens* and *E. coli*. Such an assessment has been executed in the United Kingdom and the results have been published. *Salmonella* spp. were detected in 1.5% (2/132) of production batches and 1.1% (31/2833) of retail samples. Overall, 3.0% of the herbs and spices contained high counts of *B. cereus* (1%, $\geq 10^5$ CFU g^{-1}), *Cl. perfringens* (0.4%, $\geq 10^3$ CFU g^{-1}), and *E. coli* (2.1%, $\geq 10^2$ CFU g^{-1}).

Outbreaks

It is not easy to link spices to outbreaks of illnesses because spices are used in so many ways and people do not recall eating them. In spite of this fact, in relation to their contamination, spices, particularly black and white peppers, have been implicated in serious salmonellosis outbreaks in the past decades of the twentieth century in several countries. In 1993, a nationwide outbreak of salmonellosis occurred in Germany following ingestion of paprika-powdered potato chips contaminated with a great variety of *Salmonella* serovars. The majority of cases were in children aged 14 years or less. This largest documented outbreak (an estimated 1000 cases), due to contaminated spices, proved that even extremely low numbers of salmonellae adapted to the dry state were able to cause illness. (A quantitative analysis performed on a sample of the paprika powder yielded 2.5 salmonellae per gram.) The infective dose was estimated at 4–45 organisms with an attack rate of 1 in 10 000 exposed persons. Other outbreaks of salmonellosis have been also linked to the consumption of chili, turmeric powder, aniseed, and curry powder.

Multistate outbreaks of salmonellosis illnesses in 2009/10 in the US connected with white and black peppers and crushed red pepper sparked large recalls of these spices as well as the salami products made with them.

Chemical Safety

Chemical safety of spices and seasonings requires the necessity to minimize the potential for contamination by heavy metals (particularly lead and cadmium), pesticide residues, and mycotoxins.

Heavy metals are usually toxic in low amounts and they can arise, for example, from soil that the crop is grown in. Once they become incorporated into the food they cannot be removed.

Pesticide residues found in spices and herbs are mainly organo-chlorine compounds, organo-phosphorus compounds, and carbamates.

Adulteration of certain spices, i.e., the intentional addition of an adulterant to make the product seem more attractive or valuable, may occasionally also be a public health issue when a toxic substance is added to them as an adulterant (e.g., Sudan Red pigment to curcuma/turmeric).

In 2003, chili powder and related products in the European market, which originated in India, turned out to be contaminated with Sudan I–IV. This chili powder was incorporated into various sauces, which were then used as ingredients in a range of ready meals. The withdrawal of more than 1000 products involved a significant cost for the food industry.

More detailed aspects of chemical safety can be found in other articles of this encyclopedia. However, mycotoxin contamination and certain naturally occurring toxicants are briefly dealt with below.

Mycotoxins in Spices

Mycotoxins are produced by certain molds that can grow on foods such as cereals, nuts, dried fruits, spices, and legumes under certain environmental conditions – usually at high moisture levels – at various points within the supply chain. Different mycotoxin-producing molds exist in temperate climates and tropical climates. Mycotoxins can be produced both before and after harvest and most mycotoxins are stabile and survive food processing. Products of countries with a warm and humid climate and poor storage conditions are especially susceptible to fungal contamination and development, which can lead to accumulation of mycotoxins. The most commonly observed mycotoxins include the aflatoxins and ochratoxin A. Permitted legal limits for aflatoxins and ochratoxin A have been established, for example, by the EU legislation. The problem is that mycotoxins are seldom uniformly distributed throughout food or feed commodities. Therefore, appropriate sampling procedures are needed to obtain an estimation of mycotoxin contamination and the measurement uncertainty has to be taken into account when comparing the observed levels of mycotoxins with legal limits.

By the 1970s, several authors had found aflatoxins in a range of spices (black pepper, ginger, turmeric, celery seed, and nutmeg) although the levels of aflatoxins recorded were generally low. However, pepper was implicated as the source for the toxigenic *Aspergillus flavus* and for high level of aflatoxins in sausages and pepper cheese. Aflatoxins, which are carcinogenic compounds, are produced by *Asp. flavus* and *Aspergillus parasiticus*.

Although certain spices and herbs, especially cinnamon, cloves, and possibly oregano and mustard, inhibit mycelial growth and subsequent toxin production, others, particularly sesame seed, ginger, and rosemary leaves appear to be conducive to aflatoxin occurrence.

Besides aflatoxins and ochratoxin A, zearalenone, fumonisins and trichotecenes have also been found in certain spices. In the 2003–2007 period, 161 notifications were related to mycotoxin hazards in the 'spices and condiments' product category in the frame of the European Community's RASFF rapid alert reporting system.

Other Naturally Occurring Toxicants

Phytochemicals present in commonly consumed plant foods are normally nontoxic, some of them even have the potential for preventing chronic diseases. Some spices have been used historically from ancient medicine even up to current clinical trials. However, besides mycotoxins, potential risks and adverse effects of some spices are also studied. There are a number of naturally occurring compounds in spices, as in other food items, that are carcinogenic or have some other toxic or detrimental effect. These compounds are present in low concentrations, therefore a grossly exaggerated consumption of the food, usually over an extended period of time, is required before their toxicity can be translated into a hazard.

Coumarin is a natural flavoring that is found in small concentrations in many plants. It occurs in higher concentrations (up to 5%) in the types of cinnamon grouped together under the name 'cassia cinnamon'; in contrast 'ceylon cinnamon' only contains low levels (0.45%) of coumarin. According to the risk assessment by the Federal Institute of Risk Assessment (BfR), Germany, there is some risk of moderate liver inflammation of particularly sensitive individuals by consuming relatively small amounts of coumarin. However, the effects are reversible. Thus, it is advisable that, for example, cinnamon biscuits which contain considerable concentration of this flavoring should be eaten in moderation. The BfR and the European Food Safety Authority established a tolerable daily intake (TDI) as 0.1 mg coumarin per kilogram body weight per day.

Safrole is a compound naturally occurring in a variety of common spices (e.g., star anise, cumin, black pepper, ginger, cinnamon, and nutmeg) and herbs such as basil. This compound has been shown to be carcinogenic in rodents and several of its metabolites are mutagens. However, mutagenicity of some spices due to presence of safrole can be destroyed during drying, or during cooking or irradiation. In the US, safrole was once widely used as a food additive in root beer and other common goods, but was banned by the FDA after its carcinogenicity in rats was discovered.

Nutmeg is widely used as a traditional medicine in the Middle East and Asia. However, nutmeg contains myristicin, a hallucinogen that is not toxic in small amounts but can cause death when taken in excess. Thus, children may be at risk where nutmegs are widely available as a cooking additive, or in the course of its use in traditional medicine if overdose occurs. However, based on the available data, it seems unlikely that the intake of myristicin from essential oils and spices in foods, estimated to a few mg per person per day, would cause adverse effects in humans.

Anethol, the major volatile oil component in anise and fennel has been found to have estrogenic activity. Sage and parsley have also been shown to have estrogenic property.

Some spices (mustard, celery, and sesame seeds, and, even coriander) have been classified as having potential allergenic

properties. Certain consumers have allergenic reactions to the presence of sulfur dioxide traditionally used within the spice industry either to improve the visual appearance of spices or as a pest prevention method. In the EU, if a spice contains more than 10 ppm of sulfur dioxide residues then it has to be clearly labeled as such.

Control Measures

Managing the supply chain is a key to ensuring clean, safe spices using good agricultural practice (GAP), good manufacturing practice (GMP) (including pest management), and hazard analysis and critical control point (HACCP) programs.

Herbs and spices should be grown on soils where heavy metal levels are low, and any possible contamination with heavy metals during preharvest and postharvest operations should be avoided.

Prevention of microbial contamination of spices and seasonings and outbreaks thereof lies in the application of good hygienic practices during growing, harvesting, and processing 'from farm to fork' and an effective decontamination as risk mitigation. The International Organisation of Spice Trade Association (IOSTA) issued General Guidelines for Good Agricultural Practices for Spices in 2008.

Although testing of spices for pathogens, including *Salmonella*, may be useful to screen for high rates of contamination, it cannot eliminate risk because negative results do not always ensure safety. The International Commission on Microbiological Specification for Foods (ICMSF) has published more information and a decision tree on this subject.

Microbial Decontamination Methods

All the problems described above have led to attempts to reduce the viable cell counts of spices and seasonings by a decontamination treatment. A 'Guide for the Microbiological Quality of Spices and Herbs Used in Processed Meat and Poultry Products' has been prepared for the Codex Committee on Processed Meat and Poultry Products. This Code contains recommended procedures for the decontamination treatment together with the end-product specifications of spices and herbs to improve and assure their utility in processed meat and poultry products.

Fumigation with ethylene oxide routinely, or, to a lesser extent, propylene oxide, was practised for many years but it has been banned in many countries because the fumigants are now recognized as potential carcinogens.

Satisfactory decontamination of certain spices is possible by various heat treatments, particularly, 'steam sterilization' under superheated steam and subsequent drying and cooling, but many spices and seasonings may suffer serious loss of quality (flavor, color) from less sophisticated methods of heat processing.

Efficient treatment of spices and seasonings by ionizing radiations on commercial scale is also a reality in numerous spice-producing and spice-importing countries. Radiation decontamination is dealt with in another article ('Food Irradiation') of this Encyclopedia.

See also: Foodborne Diseases: Overview of Biological Hazards and Foodborne Diseases; Overview of Chemical, Physical, and Other Significant Hazards; Overview of Emerging Food Technologies

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SAFETY OF FOOD AND BEVERAGES

Oilseeds and Legumes

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Glossary

Cross-contamination Cross-contamination occurs when microbes or pesticides are transferred from a contaminated surface to a one which is not contaminated.

Genetically modified organisms (GMOs) GMOs or genetically engineered organisms (GEOs) are organisms whose genetic material has been altered using genetic engineering techniques.

Maximum residue levels (MRLs) The upper legal levels of a concentration for pesticide residues in or on food or feed based on good agricultural practices and to ensure the lowest possible consumer exposure.

Introduction

Vegetable oils are a kind of oil made from many different plants. They are used in food and for cooking. Some kinds of plant oils that people use are African oil palm, maize, olive, peanut, rapeseed, soy, and sunflower. Margarine is an artificial butter made from vegetable oil. Oilseed crops are grown primarily for the oil contained in the seeds. The oil content of small grains (e.g., wheat) is only 1–2%; that of oilseeds ranges from approximately 20% for soybean to over 40% for sunflower and rapeseed. The major world sources of edible seed oils are soybeans, sunflowers, rapeseed, cotton, and peanuts. Edible vegetable oils are used as salad or cooking oils, or may be solidified to make margarine and shortening. These products supplement or replace animal products (e.g., butter and lard), to meet the needs of an increasing world population. Although there are many uses for industrial vegetable oils, total world production is only approximately 3% of that of edible oils. This summary report provides risk factor associated with oilseeds and legumes. All commonly known oilseed plants include soybeans, sunflower seeds, cottonseeds, flaxseeds, sesame, and canola seeds.

Legumes have been widely grown and seeds of legumes are used as human food, to provide calories and protein. The legumes used by humans are commonly called food legumes or grain legumes. The food legumes can be divided into two groups: the pulses and the oilseeds. Pulses consists of dried seeds of cultivated legumes, which is being consumed from a long time. The oilseeds consists of those legumes used primarily for their oil content that may be extracted by pressing or by solvent extraction. The oil is known as vegetable oil and is used as cooking oil. The residue, called 'oil cake', normally has high protein content and is used as food and animal feed. Generally, legumes can be classified into those that are relatively low and high in edible oil. Several crops from both the groups may be eaten raw as cooked or green vegetables, but invariably are harvested as dried grains. The most widely cultivated legumes are the two principal oilseeds: soybean and peanut. In 2008, they represented approximately 75% and 16%, respectively, of the total world production of grain legume crops. The other dried legume grains, mung bean, chickpea, pigeon pea, cowpea, etc. represent only 9% in the same year (Tables 1 and 2).

Legume cultivation in developed countries is mechanized, starting from planting, harvesting, handling, storage, and processing. But legume cultivation in developing countries use traditional methods and they incur postharvest losses of approximately 20–25%. The traditional processing and utilization of legumes also require further research attention.

Sources of Oilseed and Legume Contamination

There are four main types of food contaminants: microbiological, chemical, physical, and genetically modified (GM) foods. Foods can become contaminated during growth and harvesting of raw materials, storage and transport to the factory, and processing into finished products. The final product may then become recontaminated during subsequent storage and

Table 1 World oilseed production

Oilseeds	*Production quantity (million metric tons)*
Dry bean	18.82
Faba bean	3.26
Chickpeas	8.28
Cowpeas	5.19
Lentils	2.07
Pigeon peas	3.75
Soybean in West Africa	0.62
Total	41.99

Source: Food and Agriculture Organization of the United Nations (2008) FAOSTAT statistics database. Available at: http://faostat.fao.org

Table 2 World legume production

Oilseeds	*Production quantity (million metric tons)*
Soybeans	251.5
Rapeseed	60.8
Cottonseed	46.6
Sunflowerseed	38.9
Peanut	35.5
Palm Kernel	13.4
Copra	5.8
Total	452.5

Source: Food and Agriculture Organization of the United Nations (2008) FAOSTAT statistics database. Available at: http://faostat.fao.org

transport to shops, and during storage and preparation by the consumer. The main sources of contamination are the environment, animals, and people. The main transmission routes (vectors) of contamination are contaminated surfaces, air, water, people, and pests. Processing, packaging material and equipment, and transport vehicles may also act as vectors. Public health concern with foodborne diseases emerged around the 1880s. This was after the microorganisms had been found to be infectious agents. Pathogenic microorganisms are the major safety concern for the food industry. The vast majority of outbreaks of food-related illness are due to microbes.

Beside the microorganism contamination, transgenic plants are another aspect concerning food safety. The term GM foods or GM organisms (GMOs) is most commonly used to refer to crop plants created for human or animal consumption using the latest molecular biology techniques. These plants have been modified in the laboratory to enhance desired traits such as increased resistance to herbicides or improved nutritional content. The enhancement of desired traits has traditionally been undertaken through breeding, but conventional plant breeding methods can be very time consuming and are often not very accurate.

The development of the modern molecular plant breeding methods that employ DNA technology and DNA-mediated transformation provided breeders with a powerful tool for crop improvement. Over the past dozen years, transgenic crops that are resistant to insects, viruses, fungi, bacteria, and herbicides have increased yields and profitability of agriculture, and reduced the environmental impact of agriculture, in both developed and developing countries. Plant breeders have also used the technology to improve the nutritional value of crops designed to reduce malnutrition and improve health. To date, transgenic high-lysine maize and oilseeds with modified oil content are being planted; many nutritionally enhanced crops are undergoing development and testing. The key areas of controversy related to genetically engineered (GE) food are food safety, the effect on natural ecosystems, gene flow into non-GE crops, and corporate control of the food supply. It is not possible to make general statements on the safety of all GM foods.

A Food Safety Perspective on Novel Foods

Transgenic crops are subjected to rigorous premarket safety assessment, in spite of the fact that they can be less GM than crops produced by other modalities of breeding. They pose no new or different risks to humans or animals. Precautionary regulation was triggered because these crops were considered to be novel foods (foods that humans had not previously consumed). This definition is itself debatable because it is fair to ask if an organism into which one or two genes have been added to 20 000 or 30 000 genes in the plant genome makes the plant a novel food. Some degree of care and safety consideration should be taken before one consumes a food that one has never seen before and which is not commonly eaten.

Mycotoxins and Food Safety

Mycotoxins found primarily in grains, tree nuts and groundnuts, oilseeds, legumes, juices, and wine, can also be passed through animals into products such as milk. It has recently been suggested that the adverse health effects of two classes of mycotoxins, fumonisins and aflatoxins, have been seriously underestimated, particularly in many developing countries where products that are prone to mycotoxin contamination make up for a large portion of the diet.

The Safety Issue of Pesticide Residues in Food

A pesticide is any product that kills or controls various types of pests. A pest is defined as a plant or animal that is harmful to man or the environment. Pesticides may be used in a variety of different ways during the production of food. Sometimes, small amounts of pesticides used in these ways can be found in or on foods. The pesticides found in or on foods are called 'residues.' Some pesticides, even though no longer used, persist and remain in the environment. Residues of these pesticides are sometimes found on food grown on contaminated soil, or in the fish that live in contaminated waters. The consequences of using pesticides for food production and the realization that some foods do contain pesticide residues are of paramount importance to today's health-conscious consumer. Specifically, the public continues to voice its concerns by ranking pesticide residue as one of the top five food safety issues. Pirimiophos-methyl was also the most commonly detected substance in sunflower seeds more than the quantification lower limit in 20–39% of samples. The mean concentration of pirimiophos-methyl in sunflower seeds is 19–25 $\mu g\ kg^{-1}$. Dichlorvos and malathion were detected in excess amounts of the maximum residue levels (MRLs) in 18% of samples of sunflower seeds and 18% of samples of rapeseed, respectively.

Pesticides Residues Arise from Cross-Contamination at Storage

Pesticide residues such as organophosphate insecticides (pirimiophos-methyl, malathion) are found in oilseeds that are mainly used in empty storage facilities and for direct application to stored cereal grains. These residues arise from cross-contamination from storage bins and grain handling equipment of grain stores, and not from illegal use. This uptake

of insecticide residues from their storage environment by oilseeds may lead to residue contents that exceed regulatory limits. The insecticides that were most commonly detected were pirimiophos-methyl, malathion, dichlorvos chlorpyriphos-methyl, and deltamethrin in sunflower. Cross-contamination could have occurred when cereal grains were treated on receipt, when rapeseed was delivered, especially when treatments were done systematically to the cereal grains.

To reduce cross-contaminations, the storage managers had been advised to avoid sharing same receipt circuits when cereals are systematically treated, and to avoid accumulation of risky situations. It is also very important to periodically verify the proper use of insecticide treatment equipment. storage companies should be aware about insecticide cross-contamination, and to understand how cross-contaminations can occur in their silos and how to avoid them. MRLs of pesticides allowed by European Communities Commission (ECC) are 0.05 mg kg^{-1} for pirimiophos-methyl, 0.05 mg kg^{-1} for chlorpyriphos-methyl on rapeseed, and 0.1 mg kg^{-1} for deltamethrin in rapeseed. The MRL for malathion in oilseeds is 0.02 mg kg^{-1}.

Oilseeds

Soybean

Soybean (*Glycine max* L. Merr.) is an Asiatic leguminous plant cultivated in many parts of the world for its oil and proteins, which are extensively used in the manufacture of animal and human foodstuffs. Soybean rank fifth in the world production of major crops after wheat, maize, rice, and potato. The major use of soybean as food is its oil, whereas the 96% of soybean meal is used as animal feed.

Soybean Mycotoxin Contamination

Soybean is often attacked by fungal infections during cultivation or postharvest. These fungi can be potential mycotoxin producers. The most frequently studied mycotoxins are produced by species of *Aspergillus*, *Penicillium*, *Fusarium*, and *Alternaria*. The occurrence of fungi in soybean seeds has received far more attention than the occurrence of fungi in pods and flowers. This is understandable from a practical standpoint: Infected seeds and infected seedlings developing from them represent greater economic risks in soybean production, and seed contamination with mycotoxins represents a health risk to humans and animals. The *Fusarium* species identified are: *F. equiseti*, most frequently recovered from flowers, pods, and seeds (40%), followed by *F. semitectum* (27%) and *F. graminearum* (11%). Members of *Fusarium* genus are known to produce a broad spectrum of toxins including trichothecenes A and B. Among B-type trichothecenes, deoxynivalenol (DON) and nivalenol are important mycotoxins produced by members of the *F. graminearum* species complex. DON is the most distributed *Fusarium* mycotoxin and occurs worldwide in crops from temperate regions. Most *Alternaria* species are opportunistic plant pathogens. *Alternaria* species are also well known as postharvest pathogens. Some *Alternaria* species are well known for the production of toxic secondary metabolites, some of which are powerful mycotoxins that have been implicated in the development of cancer in mammals. Among these metabolites with mammalian toxicity are alternariol (AOH) and AOH monomethyl ether (AME). Recently it has been reported that AOH and AME possess cytotoxic, genotoxic, and mutagenic properties *in vitro*. Tenuazonic acid (TA) is a mycotoxin and phytotoxin, produced primarily by *A. alternata*. TA has been shown to be more toxic than other mycotoxins produced by *Alternaria* species such as AE, AOH, and AME. These mycotoxins have been demonstrated to be produced by *Alternaria* species on wheat, tomato, sorghum, pecans, sunflower, and cotton.

Natural Contamination of Alternaria *Mycotoxins on Soybean Seeds*

Alternaria toxins have recently received much attention, both in research programs and in risk-assessment studies. From a survey of 50 soybean seed samples evaluated for AOH and AME contamination, it was found that 44% of them were contaminated with AME. AME was found in levels ranging from 62 to 1.153 ng g^{-1}. Although a limited number of samples were evaluated, this data were the first report on the natural occurrence of *Alternaria* toxins in soybean seeds and is relevant from the point of view of animal public health. Also the results showed that AOH and AME are produced on soybean seeds at harvest time. These data agree with other studies in which it has been demonstrated that the environmental conditions optimum for growth and mycotoxin production by *A. alternata* on soybean-base media were similar to those occurring during soybean development in the field until harvest.

Fate of Fungal and Mycotoxin Contamination During Soybean Meal Production Process

Hygienic safety of soybean and by-products depends on fungal contamination among other microorganisms. With respect to fungal contamination, the levels of fungal propagules in all points of the process were no higher than 104 cfu g^{-1}, a value considered safe. However, several potential toxigenic fungi were detected, especially species belonging to the genera *Fusarium* and *Aspergillus*. Among *Fusarium* species, *F. verticillioides* was most frequently recovered (60% of isolates), followed by *F. oxysporum*, *F. subglutinans*, *F. proliferatum*, *F. semitectum*, and *F. graminearum*. The genus *Aspergillus* was the second most frequent genus isolated and the dominant *Aspergillus* species identified belong to the sections *Flavi* (*A. flavus*) and *Nigri* (*A. niger* aggregate). According to the species identified, the natural occurrence of aflatoxins (produced mainly by *A. flavus* and *A. parasiticus*), fumonisins (produced by *F. verticillioides* and *F. proliferatum*), and DON and zearalenone (produced mainly by *F. graminearum*) were analyzed at six points in soybean meal-production process. This result showed that DON contamination of soybean seed occurred at field stage previous to harvest.

The Effects of Isoflavone-Rich Soy Products on Fertility and Hormonal Imbalance

There has been considerable investigation into the effects of isoflavone-rich soy products and isoflavone supplements on hormone levels in both men and women. Decreases of testosterone and estrogen levels might indicate a possible role of soy in reducing prostate and breast cancer, respectively.

However, the proposed chemopreventive effects of soyfoods appear to be due to some other mechanism as the vast majority of studies have shown no effects on hormone levels in response to the intake of soy protein or isoflavones in men or women. Nor is there any evidence that isoflavones contribute to infertility in humans, despite the effects seen in some animals when exposure to isoflavones is very high.

Soyfoods and Breast Cancer Risk

The estrogen-like effects of isoflavones form the concern that soyfoods might be contraindicated for women at increased risk of breast cancer or for those with estrogen-sensitive tumors. More importantly, human data suggest that isoflavones do not exert stimulatory effects on breast tissue. The National Cancer Institute states that, for breast cancer survivors, 'soyfoods, as a part of a healthy diet and in moderate amounts, are safe to consume'.

Soy, Isoflavones, and Thyroid Function

Concerns about antithyroid effects of soy are based primarily on animal studies involving isoflavones. Preliminary results indicate that soy also has no adverse effects on thyroid function in people who are marginally hypothyroid.

Effects of Soy on Mineral Status

Soyfoods are frequently used in place of animal proteins, many of which are good sources of iron and zinc, and of dairy foods, which provide calcium. Consequently, there is a need to understand the impact of soy on mineral status. The consumption of relatively little red meat meets daily iron and zinc requirements, so questions about the effects of soy on the status of these two minerals pertains mostly to those eating a predominately plant-based diet.

Soybeans, like other legumes and whole grains, are high in phytate, which reduces the absorption of some minerals; especially divalent cations. Zinc absorption from soyfoods is only modestly lower than that from other sources. But because soybeans are not naturally rich sources of this mineral, unfortified soyfoods are not particularly good sources of zinc. Because zinc status is difficult to assess, vegetarians are advised to identify good plant sources of zinc in their diet or take a zinc supplement. In contrast to zinc, soyfoods are relatively high in iron. However, new research using improved methodology indicates that iron absorption from soy may be higher than previously thought because most of the iron in soy is in the form of ferritin. Finally, in addition to phytate, soybeans are high in oxalate, another inhibitor of calcium absorption. However, calcium absorption from soy is good despite the presence of oxalate and phytate.

Allergic Reactions to Soy Protein

Soy protein can cause allergic reactions in sensitive individuals. But allergy to soy protein is actually relatively rare. Approximately 1 out of 2500 adults reported having doctor-diagnosed allergies to soy protein. The rate is undoubtedly higher in children.

The Risk of Soybean Transgenic Plant

Transgenic crops are spreading more rapidly than any other agricultural technology in history, suggesting that farmers perceive important advantages in growing them. Developing countries now account for 38% of global transgenic crop area despite continuing controversy surrounding them. The most extensive *ex post* studies of transgenic crop adoption in developing countries have been conducted for insect-resistant (IR) cotton in Argentina, China, India, Mexico, and South Africa. Transgenic herbicide-tolerant (HT) soybeans are being grown in Argentina, Brazil, Paraguay, and elsewhere, but Argentina is the only developing country for which peer-reviewed studies have been published. Some developing countries also produce HT and/or IR maize, but the only peer-reviewed *ex post* analyses of their impacts published so far are for Argentina and South Africa.

Pesticide Residue: Safe Preharvest Interval after Cypermethrin Application

Cypermethrin is a synthetic pyrethroid used as an insecticide on a large scale. Cypermethrin is highly toxic to fish, bees, and aquatic insects, according to the National Pesticides Telecommunications Network. It is found in many household ants and cockroach killers, including Raid and ant chalk. Cypermethrin 10% emulsifiable concentrate (EC) when sprayed at the concentration of 100 g ai ha^{-1} showed a residue level of 0.71 mg kg^{-1} at 0 day followed by 0.52 mg kg^{-1} at 1 day in dry season, which were higher than the recommended MRL of 0.5 mg kg^{-1} set for beans with pods. The average residue level of any insecticides depends primarily on the quantity of its active ingredient. A preharvest interval of 3 days might be considered for dry season. But in case of early wet season where crop canopy was smaller allowing higher deposition of cypermethrin residues on pods, it showed a preharvest interval of 10 days for the safe use. So vegetable soybean should be harvested after 10 days of cypermethrin application to avoid any health risk of consumers.

Peanut

The peanut, or groundnut (*Arachis hypogaea* L.), originated in South America and is now grown throughout the tropical and warm temperate regions of the world. Peanut, an important oil and food crop, is currently grown on approximately 42 million acres worldwide. It is the third major oilseed of the world next to soybean and cotton. China, India, and the USA have been the leading producers for over 25 years and grow approximately 70% of the world crop.

Peanut Food Allergy

Food allergy affects approximately 6–8% of children younger than 4 years of age and approximately 2% of the population beyond the first decade of life. Food allergy accounts for approximately 30 000 anaphylactic reactions, 2000 hospitalizations, and 200 deaths each year in the USA. Allergies to peanuts and tree nuts account for the majority of fatal and near-fatal anaphylactic reactions. A national survey indicated that approximately 1.1% of people are allergic to peanuts, tree nuts, or both. More than 80% of patients were not given appropriate information to avoid accidental, food-induced

allergies. The cause of the rising prevalence of peanut allergy is the growing demand for highly nutritional, 'quick-energy' foods in the diet. Breastfeeding is increasingly common, and peanut products have increasingly been promoted as excellent nutritional sources for pregnant and lactating women. In patients with peanut allergy, approximately 85% had been breastfed and more than 70% had had their first allergic reaction after their first apparent contact with peanuts. Currently, treatment of peanut allergy consists of teaching patients and their families on how to avoid the accidental ingestion of peanuts, how to recognize early symptoms of an allergic reaction, and how to manage the early stages of an anaphylactic reaction. There is some findings about inverse association between consumption of peanuts in the first year of life and the development of peanut allergy. This means that children who eat peanut-containing foods before 1 year of age appear to be protected against peanut allergy.

Mycotoxin Contamination in Peanut

The plant pathogens are of concern not only for their ability to destroy several ergonomically important food crops, but also due to their ability to produce several mycotoxins. These mycotoxins are associated with specific species or subgenera of the *Aspergillus* and are in general toxic to livestock, poultry, fish, and humans. *Aspergillus flavus* is an important storage fungus associated with the deterioration of wheat, corn, rice, barley, bran, flour, soybeans, peanut, and other seeds. It has also been reported as a pathogen of humans and animals and as a plant pathogen. Aflatoxin, a natural toxin and carcinogen, is produced by certain strains of the mold *A. flavus* and *A. parasiticus*. Crops with the highest risk of aflatoxin contamination are corn, peanuts, and cottonseed, but aflatoxins are detected occasionally also in milk, cheese, nuts, almonds, figs, spices, and a variety of other foods and feeds. Milk, eggs, and meat products are sometimes contaminated due to feeding animals aflatoxin-contaminated peanut, corn, and cottonseed feed. Aflatoxins often occur in peanut crops in the field before harvesting. Postharvest contamination can occur if crop drying is delayed and during storage of the peanuts, when excessive moisture produces fungal growth.

Salmonella *Contamination in Peanut*

Salmonella poisoning has dramatically increased since the 1990s. Every year, approximately 42 000 cases of salmonellosis are reported in the USA. It is estimated that approximately 400 persons die each year with acute salmonellosis. The actual number of infections may be many times greater. Children are most likely to get salmonellosis. The rate of diagnosed infections in children less than 5 years old is higher than the rate in all other persons. Young children, the elderly, and the immunocompromised are the most likely to have severe infections. Most persons infected with *Salmonella* develop diarrhea, fever, and abdominal cramps 12–72 h after infection. The bad thing about peanut butter is that because of its fat, the *Salmonella* bacteria can survive for a long time. Some American company recalled their peanut butter, after the Food and Drug Administration and the Centers for Disease Control and Prevention linked 29 *Salmonella* illnesses in 18 states to peanut butter.

Flaxseed

Flax (*Linum usitatissimum*) is a member of the genus *Linum* in the family Linaceae. Almost all domestic flaxseed produced is used for extracting linseed oil. Flax grown for fiber is a different type, and is not suitable for oil production. Flaxseed yields approximately 36% of its weight in linseed oil, with the residual linseed cake or meal used for feeding livestock. The value of linseed oil obtained from flaxseed represents three-quarters of the combined value of the linseed oil and the linseed meal. Linseed oil can be used only for inedible purposes. Flaxseeds are also used as birdfeed. Study on mycoflora of flaxseed in birdfeed market showed that *Aspergillus* section *Nigri*, *Flavi*, *Circumdati* and *Aspergillus* followed by *Fusarium*, *Nigrospora* and *Penicillium* are the most prevalent fungi associated with seeds. Most of these fungi are mycotoxin producers.

Sesame

Sesame seed (*Sesamum indicum*) is grown chiefly in tropical countries; none is grown domestically. Sesame can be used whole, or it can be crushed for oil and meal. In the USA, the whole seed is used primarily as a topping for bakery products, principally in competition with poppy seed, and as a filling in pastries and candy. Some low-grade sesame seed is also used as birdseed. When crushed, sesame seed yields an exceptionally high proportion of superior quality oil (approximately 47% of the weight of the seed).

Allergic Reaction to Sesame

Some people are allergic to sesame seeds and sesame oil. Even some infants have been found to exhibit allergies to sesame. In Australia, the occurrence of allergy to sesame seed was estimated to be 0.42% among all children, whereas in the UK the allergic reaction was found to affect 0.04% of adults. Prevalence of sesame allergy varies per country. While it is one of the three most common allergens in Israel, sesame allergy prevalence is considered small relative to other allergens in the USA. Some experts consider sesame allergies to have 'increased more than any other type of food allergy over the past 10–20 years' in the USA. Such increasing prevalence led Canada to issue regulations that require food labels to note the presence of sesame.

Contamination of Sesame Seed and Tahini with Salmonella

Foods of nonanimal origin, which have been shown to be contaminated by *Salmonella* spp. include coconut, barley, cereal powder, yeast, cottonseed, chocolate, peanut butter, soybean sauce, cider, watermelon, white and black pepper, and watercress. Tahini, a product made from crushed sesame seeds, has been contaminated with *Salmonella* spp. and caused a number of outbreaks worldwide, including New Zealand and Australia. Growing sesame seed crops can be exposed to *Salmonella* from many sources such as soil, manure, irrigation water, wild birds, and animals. Seeds may also become contaminated during the drying process. If the seeds are infected thus, there is a high risk that the Tahini produced also contains the bacteria; not forgetting the risk of contamination if processing was not well controlled and properly monitored.

Thus, illness can be prevented for ready-to-eat seeds by hygienic manufacturing applications in the field, during harvesting and during processing of seeds.

Tahini, or sesame seed paste, is a known vehicle for foodborne bacteria such as *Salmonella*. The substance is high in fats, and therefore provides an ideal home for bacteria, much as peanut butter does. Products that are made with imported sesame pastes have been shown to be associated with *Salmonella* outbreaks and that they should be considered as possible sources of foodborne illness.

Sunflower

Sunflower (*Helianthus annuus* L.) is one of the few crop species that originated in North America. Commercially available sunflower varieties contain from 39% to 49% oil in the seed. Sunflower accounts for approximately 14% of the world production of seed oils. One of the world's major oil-bearing seeds is obtained from the sunflower, a hardy drought-resistant plant well suited to the colder or arid areas where many other oilseed crops cannot be grown. Although primarily used as a source of vegetable oil, sunflower seed is also eaten as an edible snack nut and used in bird feed mixtures. Typical sunflower seed yields oil equivalent to approximately 40% of the weight of the kernel and hull. Consumption of sunflower seed oil was used primarily for salad and cooking oil and secondly for frying and shortening. There are two distinct types of sunflower seed: oil stock and confectionery. The oil stock sunflower seed used to produce oil and meal ('crushed') accounted for approximately 61% and confectionery sunflower seed accounted for 39%.

Allergic Reactions to Sunflower

Allergic reactions to sunflower seeds are rare, but those who are allergic can experience symptoms worse than a peanut allergy. People who are allergic to nuts should make sure their sunflower seeds were not packed in a nut facility.

Sunflower Seed Mycotoxigenic Flora

The following mycotoxigenic fungi and the related mycotoxins were isolated from sunflower seeds: *A. flavus*, *A. flavus* var. *columnaris* (aflatoxin), *A. aureolatus* (sterigmatocystin), *A. quadrilineatus* (sterigmatocystin), *A. melleus* (ochtratoxin A), *A. ustus*, and *Penicillium*.

Pesticide Residues in Sunflower

MRLs of pesticides allowed by European regulations according to ECC are 0.05 mg kg^{-1} for pirimiophos-methyl, 0.05 mg kg^{-1} for chlorpyriphos-methyl on sunflower, and 0.05 mg kg^{-1} for deltamethrin in sunflower. The MRL for malathion in oilseeds is 0.02 mg kg^{-1}.

Cottonseed

Pesticide Residues in Cottonseed

Few people think of cotton as a food. If you drink milk or eat beef or processed foods, you are likely an indirect consumer of a crop that is grown and regulated with slight attention to its importance as a foodstuff. Chemicals that have been banned for food crops are still being used on cotton. Some extremely toxic pesticides are sprayed on cotton than on any other crop. The industry view is that pesticide residues are removed from cottonseeds during their chemically intensive processing: The seeds are washed with caustic lye or sodium hydroxide and the oil is extracted with hexane, a highly volatile solvent, and is then filtered through sulfuric acid-laden clay. Cottonseed oil is sometimes extracted with a mixture of hexane and benzene, a known carcinogen that is very difficult to remove. Residues from pesticides such as the defoliant have frequently appeared on cottonseed and other cotton by-products. The most direct entry for pesticides into the human food supply is through the spent seed hulls and gin trash that are regularly fed to cattle. Estimation of the risk of pesticide residues in cotton products and meat consumed from pastures adjacent to cotton fields showed that the following parameters affect the residues: rate of application, frequency of usage, timing of application, partitioning into lipid (fatty) phases from water, and persistence in plants. Study the residues of different insecticides in lint and cottonseed showed that endosulfan was detected in all samples of lint for the first and second picking, respectively. Low levels of cypermethrin were also detected in some lint samples. The residues of organophosphate insecticides were detected in 12% and 33% lint samples from first and second picking, respectively. Chlorpyriphos was detected in 30%, quinalphos in 20%, triazophos and profenophos each in 8%, and malathion in 1% of the samples. Analysis of cottonseed from the same samples showed low levels of endosulfan residues in nine and seven samples ranging from Tr-0.070 μg g^{-1} to Tr-0.081 ng g^{-1} for the first and second picking, respectively. Cypermethrin (0.06 μg g^{-1}), chlorpyriphos (Tr), and quinalphos (Tr) each were detected in only one sample of cottonseed.

Cottonseed Aflatoxin Contamination

Aflatoxins are primarily produced by the fungi *A. flavus* Link and *A. parasiticus*. Optimal thermal conditions for fungal growth are 36–38 °C, whereas maximum toxin production occurs at 25–27 °C. Growth in storage facilities is favored by humidity above 85%. Because suitable conditions for its growth and toxin production occur in most areas, aflatoxin contamination of food is a universal problem across the world.

Transgenic Cotton

There are concerns relating to the safety of transgenic food for human consumption. Worldwide, most transgenic crops are grown in developed countries (76% of total area) and 24% in the developing countries. Four countries, the USA, Argentina, Canada, and China, grew 99% of the global total of transgenic crops in 2000. Most of this area is divided among four crops: soybean (58%), maize (23%), cotton (12%), and oilseed rape (7%). China, where some 7.5 million small farmers are growing IR cotton, represents the most successful case so far in terms of productivity, farmer incomes, equity, and sustainability.

Legumes

Legumes are plants in the family Fabaceae (or Leguminosae), or fruits of these specific plants. Farmed legumes can belong to

many agricultural classes, including forage, grain, blooms, pharmaceutical/industrial, fallow/green manure, and timber species. Well-known legumes include alfalfa, clover, peas, beans, lentils, Mesquite, soy, and peanuts.

Pea

Risk Assessment for GM Pea

GM field peas underwent a number of tests during development: (1) laboratory and glasshouse tests; (2) performance studies in the field; (3) feeding trials; and (4) immune response tests. Scientists and their collaborators modified the gene that produces the protective protein. Using gene technology they then introduced the gene into the pea. Field trials showed that the GM peas were 99.5% resistant to pea weevils. Results showed the alpha-amylase inhibitor GM peas provided 99.5% protection against the pea weevil with yields comparable to non-GM field peas.

Feeding Trials Test of GM Pea

The bean alpha-amylase inhibitor protein has been studied extensively over many years and has shown no health risk to humans or animals. However, as part of its risk assessment, Commonwealth Scientific and Industrial Research Organization (CSIRO) worked with other research groups to conduct feeding trials in chickens and pigs. In two feeding trials, chickens were fed GM and non-GM field peas incorporated into a maize–soybean diet. In the first trial, birds were fed the diet from 3 to 17 days posthatching. In the second trial, the birds were fed the diet for 40 days. In another trial, pigs were fed wheat mixed with either non-GM or GM field peas for 15 days. Results of each study showed that whereas protein digestion was the same in pigs and chickens fed GM or non-GM field peas, starch digestion was reduced in animals eating the GM field peas. This indicated that the alpha-amylase inhibitor protein also affected digestion of starch. Mice fed alpha-amylase inhibitor GM-peas showed evidence of an immune response after 2 weeks, with the response increasing at 4 weeks. The reaction in mice was inflammation in the lungs and increased serum antibody levels. The reaction to the pea alpha-amylase inhibitor protein was compared to bean alpha-amylase inhibitor protein when administered to the mice lungs.

Gene Flow from GM Plant to Wild Plant

Peas are self-pollinating and it is unlikely that gene flow would occur between GM and non-GM peas. However, CSIRO conducted gene flow studies to test this assumption. The results of the work showed that gene flow did not occur between GM and non-GM field peas. According to these results, CSIRO made the decision to discontinue developing the alpha-amylase inhibitor GM peas.

Bean

Food Allergy to Bean

As a member of the legume family, the green bean frequently associated with food allergy. However, allergic reactions caused by skin contact or by inhalation of vapors from boiling legumes are rare. Test results indicate that the patient has type I hypersensitivity to raw green bean antigen(s). This case is of interest because it demonstrates that a food allergen, when inhaled, can induce respiratory symptoms in sensitized patients and may even be the source of primary sensitization.

A bean allergy is an adverse reaction by the body's immune system to beans or food containing beans. This type of allergy is rare and serious reactions are very rare. The body's immune system produces immunoglobulin E (an antibody) and histamine in response to contact with the allergen. The specific symptoms that can result vary considerably among patients, for example, skin, respiratory, and behavioral symptoms.

Occupational allergy to castor bean has been found in workers in a felt-manufacturing plant. Of the workers, 37% complained that they were affected by the felt. Of these, 50% were considered to have occupational allergy. The presence of castor bean allergens in the felt was suggested by the correlation between the radioallergosorbent test (RAST) scores to the felt and castor bean and confirmed by RAST inhibition experiments. The RAST results correlated well with the results of skin prick tests to felt and castor bean extracts.

Bean Seedborne Fungi

The average percentages of seedborne fungi in the bean is approximately 50%. The most-frequently isolated fungi are *Alternaria*, *Aspergillus*, *Eurotium*, *Fusarium*, *Rhizoctonia*, *Penicillium*, *Rhizopus*, *Sclerotinia*, *Gliocladium*, and *Curvularia*. Based on these profiles, *Fusarium* and *Aspergillus* were identified as the most-probable mycotoxin-producing fungi in the bean. The infected beans contained the *Fusarium* toxins diacetoxyscripenol (DAS), DON, T-2 toxin, and fumonisin B1, aflatoxins B1, B2, G1, and G2. *F. avenaceum*, *F. culmorum*, *F. graminearum*, *F. moniliforme*, *F. oxysporum*, and *F. solani* were isolated from moldy bean. The incidences of mycotoxin contamination have a correlation to seed discoloration. Several mycotoxins were detected in naturally infected moldy navy beans (*Phaseolus vulgaris* L.) with distinct whitish gray to pink discoloration. Three types of bean samples were analyzed for mycotoxins: (1) healthy beans without any apparent discoloration; (2) beans with pink discoloration; and (3) a mixture of beans with whitish gray and pink discoloration. The results indicated that DAS, DON, T-2 toxin (T-2), and fumonisin B1 (FB1) were present in type B and C samples but not in the healthy type A samples.

Chickpea

The Effect of Food Processing on Pesticide Residue

The effect of food processing on pesticide residues on dry chickpeas has been studied in a supervised trial. Food processing offers a suitable approach to deal with the current scenario of poor quality and unsafe food prevalent in the developing countries. Food legumes are widely consumed over the world on account of their nutritive value. The effects of common domestic processing techniques like soaking and germination on pesticide residue showed germination (day 1) resulting in almost complete elimination of chlorpyrifos and its metabolites in chickpea, hence germination of legumes is a simple, cost-effective, suitable process for addressing the

concerns of food quality and safety especially for rural populace of developing countries. The residue of triadimefon and triadimenol 2 weeks after the last application, were at or about the limit of determination.

Mycoflora and Mycotoxigenic Fungi in Chickpea

High production of crops requires using high quality of seeds. However, seedborne fungi play an important role in deterioration of seed quality, which leads to high economic losses in crop yield. Poor harvesting practices, improper storage, and less than optimal conditions during transportation, marketing, and processing can also contribute to fungal growth and increase the risk of mycotoxin production. Many fungal species such as, *A. porri*, *A. alternata*, *A. amstelodami*, *A. flavus*, *A. fumigatus*, *A. nidulans*, *A. niger*, *A. sydowi*, *A. wentii*, *Botrytis cinerea*, *Cladosporium macrocarpum*, *Curvularia lunata*, *F. equiseti*, *F. moniliforme*, *F. oxysporum*, *F. semitectum*, *Macrophomina phaseolina*, *Myrothecium roridum*, *Penicillium notatum*, *Rhizoctonia* sp., and *Rhizopus arrhizus* have been reported from chickpea.

Mycotoxins attract worldwide attention because of the significant economic losses associated with their impact on human health, animal productivity and trade. *Aspergillus*, the most common genus, emerged from 100% of Fabaceae seed samples tested; *A. flavus*, *A. niger*, and *A. fumigatus* were the most prevalent; and *A. parasiticus*, *A. flavus* var. *columnaris*, and *A. ficuum* emerged in moderate or low frequency of occurrence in Fabaceae. Mycotoxin with different toxins and varying degrees of toxicity were found in 26.7% of the tested samples. No mycotoxins tested were detected in any chickpea seed samples investigated. Aflatoxins (B_1, B_2, G_1, and G_2), ochratoxin A, sterigmatocystin, simple microcyclic trichothecenes (DAS, T-2 toxin, and HT-2 toxin), patulin, fumigillin, and zearalenone could not be detected in most of roasted chickpea seed samples tested, but unfortunately the isolated fungi exhibited potentially mycotoxin production in favorite conditions.

Lentil

The genus *Aspergillus* is the most prevalent genus in seed of lentil (*Lens esculenta*) followed by *Rhizopus*, *Penicillium*, *Fusarium*, and *Chaetomium*. Natural contamination with aflatoxins B1, B2, G1, and G2 (14.3 $\mu g\,kg^{-1}$) were reported.

Lentil Allergy

Peanuts and soybeans are the major legumes involved in human food allergy; however, scarce data exist on adverse reactions of lentils. Some people have symptomatic allergy to lentils. The most frequent symptoms were oropharyngeal symptoms and acute urticaria. Some people also have allergic symptoms when they were exposed to steam from cooked lentils. Allergic reactions to lentils started early in life, usually below 4 years of age. Allergic reaction to lentil frequently associated with allergic reaction to other legumes such as chickpeas, peas, and green beans; oropharyngeal symptoms and acute urticaria are the most common symptoms through ingestion.

Risk of Pollen Migration in Transgenic Alfalfa

Alfalfa (*Medicago sativa* L.) is a perennial legume widely cultivated to provide high-quality forage in the form of hay, silage, and to a lesser extent as a grazing crop, as well as for improving soil fertility. The availability of alfalfa lines that can be easily transformed with *Agrobacterium* and regenerated in tissue culture, makes this plant attractive for genetic engineering and provides the opportunity to develop new uses for alfalfa with value-added products of commercial interest. Alfalfa is the most important forage crop in North America. It is currently grown on approximately 26 million acres in the USA. Because alfalfa is easily genetically transformed, it is often considered as the ideal plant to use in large-scale production of transgenic products. Pollen can function as a vehicle to disseminate introduced genetically engineered genes throughout a plant population or into a related species. Concerns have been raised over the release of GE plants into the environment, because of the risk of inadvertent dispersal of engineered genes into the cultivated crop, or into related weed species. Factors germane to that risk include the rate of pollen-mediated gene spread, the maximum dispersion distance of pollen, and the spatial dynamics of pollen movement within seed production fields, none of which are known for alfalfa (*M. sativa* L.), an insect-pollinated species.

Hay

Hay is grass, legumes, or other herbaceous plants that have been cut, dried, and stored for use as animal fodder, particularly for grazing livestock, such as cattle, horses, goats, and sheep. Hay is also fed to pets, such as rabbits and guinea pigs. The long drying periods with high humidity allowed field growth of mold on the hay. Mold will grow on hay without preservative at moisture levels above 14–15%. Molds commonly found in hay include *Alternaria*, *Aspergillus*, *Cladosporum*, *Fusarium*, *Mucor*, *Penicillium*, and *Rhizopus*. These molds can produce spores that cause respiratory problems, especially in horses or other animals fed in poorly ventilated areas and, under some conditions, will produce mycotoxins. Mycotoxins affect animals and subsequently human life in the following ways: (1) intake reduction or feed refusal; (2) reduced nutrient absorption and impaired metabolism, including altered rumen fermentation and microbial growth, diarrhea, intestinal irritation, reduced production, lower fertility, lethargy, and increased morbidity; (3) alterations in the endocrine and exocrine systems; (4) suppression of the immune system, which predisposes livestock to many diseases and may increase milk somatic cell count. A suppressed immune system may also cause lack of response to medications and failure of vaccine programs; and (5) cellular death causing organ damage.

Mycotoxins may cause acute health or production problems, which are most common in horses and other nonruminants. In cattle, mycotoxins more likely will contribute to chronic problems including a higher incidence of disease, poor reproductive performance, or suboptimal milk production. Ruminants are somewhat protected from acute toxicity because the rumen destroys a large portion of most mycotoxins.

The mycotoxins of greatest concern are those produced by *Aspergillus* (aflatoxin, gliotoxin, fumitremorgens, and fumigaclavines), *Fusarium* (DON, zearalenone, and T-2 toxin), and *Penicillium* (PR toxin, mycophenolic acid, roquefortine C, and patulin), but other mycotoxins can be present. There are approximately 400 different known mycotoxins. Increased attention to field and storage management may help reduce the incidence and concentration of mycotoxins in forage. Heavily contaminated forage may need to be discarded. Lightly contaminated feed can be diluted and used for animals under less stress.

Another trend contributing to the frequency of mycotoxin contamination of feeds is improved global grain transportation systems and global trading of agricultural commodities. This allows more extensive shipping of grains and other feed components throughout the world. As a result, complete feeds are likely a more complex blend of feedstuffs with more widely varying geographic origins than was seen in the past. The potential for aflatoxins and mixtures of *Fusarium* mycotoxins to be cocontaminants in feeds is, therefore, enhanced.

Feed samples (alfalfa hay, wheat straw, shrubs, pasture, and concentrates) and milk were investigated for contamination with pesticide residues. The results showed that the summation operator endosulfan was the main pesticide residue that was detected in all the concentrate samples at a mean concentration of 5.36 mg kg^{-1}, which is much higher from the MRL. In addition, the summation operator endosulfan was also detected in all the alfalfa hay samples but at a mean concentration of 0.10 mg kg^{-1} which is lower than the MRL. In the wheat straw, shrubs, and pasture samples no pesticide residues were detected. No pesticide residues were also detected in milk samples of sheep and goats. Thus, this milk from the farms sampled presents no human health risks.

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Nuts

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Introduction

The tree nut industry consists of almonds, brazil nuts, cashews, hazelnuts, macadamias, pecans, pinenuts, pistachios, and walnuts. Tree nuts are produced in more than 40 developed and less-developed countries (Tables 1 and 2) and consumed worldwide. Millions of hectares of land are devoted to tree nuts, providing economic livelihood for hundreds of thousands of producers and small families.

Table 1 Summary of global tree nut production and area harvested in 2010

Tree nut	*Area harvested (hectares)*	*Production quantity (metric tons)*
Almonds (in shell)[a]	1 675 284	15 007
Brazil nuts	–[b]	2 514 022
Cashews	4 404 105	3 585 807
Hazelnuts	620 383	888 328
Macadamias (kernel basis)	–[b]	28 714
Pecans	–[b]	91 861
Pinenuts (kernel basis)	–[b]	9 702
Pistachios	461 955	912 379
Walnuts	846 059	2 545 388

[a]Data are based on information gathered from producing country government statistics and industry sources and FAO production database (Brazil nut, pistachio, walnut, almond, hazelnut, and cashew data from FAO production database).
[b]There are no data.

Worldwide Tree Nut Consumption

Tree nuts are widely consumed in both raw and processed forms. Unlike groundnuts, which are predominantly used for oil and feeding stuff, tree nuts are primarily consumed as whole foods, as ingredients in foods, or in medicinal preparations. For example, in several Asian cultures, almonds play a significant role in Ayurvedic preparations, a philosophy which for thousands of years has promoted the interrelationship of nutrition/diet with healing, prevention, and longevity (Table 3).

Based on data contained in the 2007 FAO Food Balance Sheets, it is apparent that tree nut consumption varies both among and within the regions where there is tree nut production.

Sources of Nut Contamination

There are three main types of nut contaminants:

- Microbiological,
- chemical, and
- physical.

Table 2 Tree nut-producing countries

Almonds (AL)	*Amazonias Brazils (BR)*	*Cashews (CA)*	*Hazelnuts (HA)*	*Macadamias (MA)*	*Pecans (PE)*	*Pinenuts (PIN)*	*Pistachios (PI)*	*Walnuts (WA)*
Afghanistan	Bolivia	Brazil	France	Australia	Australia	China	Greece	Argentina
Australia	Brazil	China	Greece	Brazil	Israel	Italy	Iran	Chile
Chile	Equador Peru	India	Italy	Costa Rica	Mexico	Portugal	Italy	China
Iran		Indonesia	Spain	Guatemala	South Africa	Spain	Syria	France
Israel		Guinea basal	Turkey	Israel	USA	Turkey	Turkey	Greece
Italy		Kenya	Russia	Malawi			USA	Hungary
Greece		Mozambique	USA	South Africa				India
Morocco		Nigeria		Thailand				Iran
Portugal		Tanzania		USA				Italy
Spain		Thailand						Maldova
Tunisia		Vietnam						North Korea
Turkey								Turkey
USA								Ukraine
								USA

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Table 3 Worldwide nut consumption

Location	*Per capita consumption*	
	kg per year	*Range*
Africa	1.0	0.0–7.3
North and Central America	2.3	0.0–3.3
South America	0.5	0.0–6.4
Asia (including Middle East)	1.0	0.2–13.6
Europe	2.8	0.6–9.9
Oceania	2.6	0.1–3.4
World	1.3	0.0–13.6

Source: Data from FAO (2007) *Food Balance Sheet 1961–2006*. Rome: FAO.

Nuts can become contaminated during growth and harvesting of raw materials, storage and transport to the factory, and processing into finished products. The final product may then become recontaminated during subsequent storage and transport to shops, and during storage and preparation by the consumer.

Pathogenic microorganisms are the major safety concern for the nut industry. The vast majority of outbreaks of nut-related illness are due to microbial pathogens, rather than to chemical or physical contaminants. As they are generally undetectable by the unaided human senses (i.e., they do not usually cause color changes or produce 'off'-flavors or taints in the food) and they are capable of rapid growth under favorable storage conditions, much time and effort are spent in controlling and/or eliminating them.

Pistachio

Pistachio (*Pistacia vera* L., Anacardiaceae) originates from Central Asia. Domestication occurred less than 2000 years ago and traders introduced them throughout the Middle East and the Mediterranean area. Today the major production areas are located in the Middle East, North America, and Europe. Iran is the world's largest pistachio producer and it accounts for 44% of world production.

Change in hull color is closely connected with shell splitting and so it is important to harvest nuts when they are fully mature to ensure maximum shell split.

Smaller shake and catch harvesters mostly operate with a catching frame not unlike an inverted umbrella that can be opened under the tree canopy when the tree is shaken and closed for transport to the next tree.

Whether the nuts are destined for the fresh or dried pistachio market they are harvested by the shake and catch method. In both cases, the harvested nuts must be quickly transported for processing. Fresh pistachios are sold in the hull and so do not need hulling. The fresh nuts are cooled immediately after harvest in a cool room.

Nuts that are destined for the dried pistachio market are quickly transported to the hulling and drying plant. If hulls are not removed within 24 h, the nuts generate heat and the shell can become stained. The sooner the nuts are hulled and dried, the better quality the nut will be.

The nuts are then slowly dried using ambient air to retain kernel quality.

Heat during drying can damage the kernel. The nuts are stable in storage when the moisture content is approximately 6%.

Depending on the conditions of production, processing, transport, and distribution, pistachios can become contaminated with different hazards. These range from chemicals such as pesticide residues, toxic metals (lead and cadmium), or microbial hazards such as *Salmonella*.

The biggest concern for pistachio has so far been contamination with toxigenic molds, for example, *Aspergillus flavus*, and growth of these and subsequent formation of aflatoxins (AFLs). Other mycotoxigenic fungi that are ochratoxin and fumonisin producers were identified from pistachio, but these toxins were not detected in pistachio fruit.

AFL in Pistachio

The Codex Alimentarius adopted standards for AFL in pistachios for ready-to-eat (RTE) and for further processing. In 2010, the European Union (EU) which previously had stringent regulation on AFL (2 and 4 ppb, respectively, for AFL B_1 and total AFLs) relaxed its requirements and aligned its requirement with that of Codex Alimentarius.

Codex Alimentarius has also adopted a sampling plan consisting of 2×10 kg of sample for RTE nuts (including pistachio, hazelnut, and almond) and 1×20 kg sample for pistachios for further processing. From February 2010, EU has adopted the sampling plan recommended by Codex Alimentarius.

Contamination of pistachio nut by *Aspergillus* species and their mycotoxins are the most serious challenge to pistachio production, consumption, and exportation in the world.

Factors influencing infection of pistachio nuts include: cracking of pistachio nuts (especially early hull splitting pistachios), environmental factors, cultural practices, frequency and time of irrigation, plant litter, animal manures, frequency of toxigenic strains, distribution of AFL in pistachio bulks, and harvesting date. These factors have been shown to be critical in infection especially in early splitting cultivars where the hull (pericarp) gets split exposing the kernel to molds and insects increasing chances of AFL production and contamination. Whereas the molds such as *Aspergillus* spp. may cause direct contamination resulting in AFL production, insects may play the role of spreading fungal spores, which in turn infect exposed kernels.

Pistachio nuts are characterized by a split in the shell at the calyx end of the nut. This split normally occurs on the tree about a month before harvest. The hull (mesocarp) of the pistachio usually encloses the shell and remains intact through harvest, serving as protection for the kernel. On normal nuts, there is space between the hull interior and shell exterior, so the shell can split open without splitting the hull. However, approximately 1–4% of the time, the hull will adhere tightly to the shell and the hull will split open along with the shell. These nuts are called 'early splits.' The split in the hull allows an unobstructed passage to the kernel for airborne mold spores and insects or other small animals, such as mites, that

might be carrying mold spores. Insects and small animal infestation rates on early split nuts are much higher because of the easy access to the kernel. The mold, *A. flavus*, has been found in pistachio nuts before harvest.

Salmonella in Pistachio

Salmonellosis is an infection with bacteria called *Salmonella*. Most persons infected with *Salmonella* develop diarrhea, fever, and abdominal cramps 12–72 h after infection. The illness usually lasts 4–7 days, and most persons recover without treatment. However, in some persons, the diarrhea may be so severe that the patient needs to be hospitalized. In these patients, the *Salmonella* infection may spread from the intestines to the blood stream, and then to other body sites and can cause death unless the person is treated promptly with antibiotics. The elderly, infants, and those with impaired immune systems are more likely to have a severe illness.

On March 26, 2009 the US Food and Drug Administration (FDA) informed Centers for Disease Control and Prevention (CDC) that multiple samples of pistachio nuts and pistachio-containing products collected over several months from a single company were contaminated with several serotypes of *Salmonella*, including Montevideo, Newport, and Senftenberg. Since that time, CDC has been actively investigating whether this contamination is linked to human illness. In addition to the pistachios, some of these deoxyribonucleic acid (DNA) fingerprints have been associated with other foods, and a fingerprint match does not mean that the illnesses are necessarily linked to pistachios. CDC is collaborating with state and local public health agencies to interview persons with *Salmonella* strains having DNA fingerprints that match those from the pistachio products to determine whether they had eaten pistachio nuts or pistachio-containing products before their illnesses. To date, one patient in Connecticut infected with a *Salmonella* strain with a matching DNA fingerprint has reported consuming a pistachio-containing product.

Salmonella that contaminates food, including nuts, can cause human illness and is a public health concern. CDC estimates that cases of salmonellosis reported to the public health system represent approximately 3% of the illnesses that actually occur, because many ill persons do not seek medical care or have a specimen cultured. Even if no reported illnesses are related to a contaminated product, it is possible that some illnesses might occur.

Pre and postharvest pistachios are exposed to environments that impose some level of risk of contamination with foodborne pathogens. Pistachios that have fallen to the ground several days preceding harvest may absorb water. The water may be contaminated with *Salmonella* or other pathogens originating from wild or domestic animal feces, inadequately composted manure, irrigation water, or run-off water. Cleaning pistachio may involve immersion in water and cross-contamination with foodborne pathogens. Wetting of inshell nuts may result from leaks in roofs or walls of storage facilities, thereby resulting in potential contamination or development of high-moisture environments that favor the growth of mycotoxigenic molds and foodborne pathogens. Conditioning pistachio also creates an environment that may result in cross contamination.

As with most horticultural crops, the implementation of appropriate good agricultural practices is considered important to reduce the opportunity for foodborne pathogens to contaminate the crop.

The potential to recontaminate a finished nut product is high if adequate good manufacturing practices are not in place. Facility design, product flow (separation of raw from finished product), equipment and facility maintenance, cleaning and sanitation, as well as human hygiene should be adequately controlled to prevent contamination. Cleaning and sanitation may be challenging in dry areas of the facility where moisture should usually be restricted; however, dry cleaning and sanitation programs are available and widely used in the food industry. The efficacy of the cleaning and sanitation program should be monitored through a robust environmental monitoring program that includes assessment for *Salmonella* or other pathogens, if appropriate.

Pistachio Allergies

Pistachios are relatively commonly reported tree nuts to cause food allergy symptoms, and are cross-reactive to cashews and mangoes. Hay fever to the pollen from the *Parietaria* weed, found in Europe, appears to predispose to pistachio allergy.

Pistacia vera, which likewise cause allergic reactions in sensitized individuals, have a major and several minor IgE-binding proteins. Pine nuts (*Pinus edulis*) contain at least three proteins that bind human IgE.

Almond

The almond tree grown today originated from wild almonds growing in deserts and low mountain forests of central and south-west Asia from northern Syria and Turkey to Iran and Iraq. From the bitter-seeded wild forms a sweet nut was selected and cultivated some 4000 years BC. Thousands of years later in the mid-1800s almonds were first cultivated as a commercial nut crop.

Today the US accounts for approximately 80% of world almond trade and produces more than half of the world almond supply. The next highest producer is Spain.

Harvesting is done by shaking the tree to ensure all nuts are down, allowing the nuts to dry, sweeping the nuts into windrows, and picking up the nuts with a harvester. In preparing the orchard for harvest, the floor is mown short or sprayed with a knockdown herbicide and cleared of branch debris and stones. Dry weather throughout the harvest operation is needed to avoid nut deterioration. When the hulls are splitting open and dry, the trees are ready for harvest.

Harvested crops are not safe to stockpile unless the moisture content is down to approximately 5%. At this stage t he kernel will snap in half. At moisture levels higher than this the nuts will deteriorate due to mold and spontaneous combustion can occur. The stockpile is left uncovered unless temporary covers are needed for rain protection. Almond crops are bulky because of the amount of hull in the har

vested crop. However, hulls are a marketable byproductand valued as a high-protein stock feed. Harvest is a dusty operation and when all is complete, the trees are irrigated.

Varieties that are marketed in shell are hulled and those marketed as kernel are hulled and cracked. After hulling, shear–roll machines crack the nuts and the shell is separated out and the kernel is then graded.

AFL in Almond

Insect-feeding damage is a principal factor leading to preharvest fungal infection of nut kernels of almonds, pistachios, and walnuts which may lead to subsequent AFL contamination. Wounds to the protective layers surrounding nut kernels (hull, shell, and seedcoat) provide avenues for infection by windborne spores of aflatoxigenic aspergilla. The principal insect pests of tree nuts are larvae of the navel orangeworm (NOW).

Amyelois transitella Walker (Lepidoptera: Pyralidae), which infests kernels of almonds, walnuts, and pistachios; the peach twig borer, *Anarsia lineatella* Zell. (Lepidoptera: Gelechiidae), which infests meristem leaf shoots, husks, and kernels of almonds.

There is considerable evidence that almonds, pistachios, and walnuts show a differential resistance to contamination by AFLs. For example, in 2005, no walnut shipments to the EU were the subject of alerts or notifications for AFLs, although one shipment was the subject of an alert for mold. In contrast, 28 almonds and 13 pistachio shipments were identified. Although this is circumstantial evidence, it reflects market realities and conforms to the position of the industry groups, in which walnut producers are primarily concerned with spoilage microorganisms such as *Rhizopus*, *Penicillium*, and *Aspergillus niger*, whereas almond and pistachio producers are greatly affected by the economic impact of AFL contamination.

Such differences cannot be accounted for merely by differences in physical protection of the nuts by the hulls or shells because both codling moth and NOW are major pests of almonds, pistachios, and walnuts, wounding the external tissues and providing many avenues for entry of fungal spores into the edible portion.

Salmonella in Almond

Nuts have been associated with *Salmonella* outbreaks in the USA and Canada several times over the past 10 years. Almonds were responsible for the first outbreaks, in 2001 and then again in 2004, when contamination with *Salmonella enterica* triggered a recall of 13 million pounds of California almonds. A requirement was implemented in 2007 that all almonds sold on the domestic market be pasteurized. A court recently upheld that requirement.

The rule is a response to two outbreaks of *Salmonella* poisoning, which sickened more than 100 people in Canada in 2001 and 29 people in the USA and Canada in 2004. One person died in the 2004 outbreak and a costly lawsuit against a major almond processor ensued. The *Salmonella* outbreak of 2004 was traced to Paramount Farms, the world's largest supplier of pistachios and almonds, with 9000 acres of nut crop production, although the source of the bacteria was never identified. (*Salmonella* is directly associated with manure and other fecal matter.) The most common method of sterilizing almonds is propylene oxide (PPO) fumigation. Other pasteurization methods include steam heating, oil roasting, and blanching. Organic 'raw' almonds will not be fumigated, but will undergo the steam pasteurization, so that they are no longer truly raw.

Both PPO and steam treatments kill bacteria with a 5-log reduction, meaning that if 100 000 harmful pathogens are present on an almond before treatment, only one will remain afterward.

Almond Allergies

Almonds are the most popular tree nut consumed in the US, and the third most common tree nut allergy. Almonds are commonly found in food processing, and are unexpected ingredients in breakfast cereals, granola bars, and baked goods. Almond allergy may predispose a person to other tree nut allergies, especially pistachio nuts.

Ingestion of tree nuts, the edible kernels of the seed of several trees, may also cause immunological illness. Sweet almonds (*Amygdalus communis*) and bitter almonds (*Prunus amygdalus*) comprise 22% protein and contain multiple IgE-binding proteins that may trigger severe allergic reactions.

Brazil Nut

Brazil nut '*Bertholletia excelsa*' is a member of the Lecythidaceae family and grows in the tropical forests of Amazonia. These Brazil nut forests are known as 'castanhais' and occur in the Guianas, Amazonian Brazil, south-eastern Colombia, southern Venezuela, eastern Peru, and northern Bolivia.

Most of the collected Brazil nuts are then subjected to minimal processing (selection, classification, cleaning, drying, and shell cracking among the main processing steps) for export either in shell or shelled (as a kernel), mainly to Europe and the USA. Bolivia is presently the largest Brazil nut producer and exporter, followed by Brazil and Peru.

Brazil nuts are harvested almost entirely from wild trees during a 5–6 month period in the rainy season. The fruits, which weigh from 0.5 to 2.5 kg and contain 10–25 seeds, are gathered immediately after they fall in order to minimize insect and fungal attack of the seeds, and to control the number of seeds carried away by animals. The number of capsules produced per tree ranges from 63 to 216.

After collecting the fruits from the forest floor, the gatherers carry them to local dealers then to peeling and bagging factories before export. The harvested fruits are split open, washed, and dried. In Brazil the nuts are dried in rotary driers, whereas in Bolivia and Peru nuts are dried on slatted floors. The nuts are shelled either by the autoclave process using steam to expand the shell and loosen the inner skin, or by soaking for 24 h to expand the shell to ease manual cracking. The nuts are graded and then oven dried or sundried before storage.

AFL in Brazil Nut

The highest levels of AFL occurred in nuts when the relative humidity of the storage environment approached 97% and

was accompanied by temperatures in the range of 25–30 °C. At 10 °C or at a relative humidity level below 75% toxin was not produced. Warm temperatures and a high relative humidity are characteristics of the production areas in the Amazon, especially during the harvesting season, which occurs from January to April, and reducing relative humidity and or temperature is not economically viable; however, reducing the moisture in the nuts to lower than 5.0% could prevent AFL production especially during storage. Therefore, air and or mechanical drying of the nuts following harvesting can be used to control mold growth, thereby limiting toxin formation. Despite that, when their shells are cracked either when pods fall on the ground or during pod opening for nut extraction (done by an axe) and exposed to high moisture and temperature of the tropical forest, fungi may grow leading to nut spoilage. If fungi are toxigenic, they may produce AFLs. Nevertheless, the Brazil nut production chain is confronted with problems of contamination by AFLs, which are toxic secondary metabolites produced by *A. flavus*, *Aspergillus parasiticus*, and *Aspergillus nomius* when the nuts are kept under conditions that favor the development of these fungi and toxin production.

Beside *A. flavus, A. nomius* is an important producer of AFLs in Brazil nuts and that its occurrence, and possibly other B and G AFL producers.

The higher selenium (Se) content present in the Brazil nuts leads to less fungi proliferation, growth rate in the plate, and AFL production that could be caused by its toxic effect to the fungus provoking stress and activating AFL production.

Selenium Toxicity and Radioactivity of Brazil Nut

Se is an essential micronutrient that, once incorporated into selenoproteins, performs important functions in the human body, participating in antioxidant defense, in the immune system, and in the regulation of thyroid function. Glutathione peroxidase is a selenoprotein that acts as an antioxidant enzyme in plasma and is associated with slowing the aging process, boosting the immune system, and protecting against heart disease and certain forms of cancer. However, at doses above the recommended daily intake of 55 μg per day, Se can be toxic. Brazil nut trees absorb the natural element Se from soil as along with radionuclides, such as 226Ra. The concentration of radioactive elements in Brazil nuts can reach quantities up to 1000 times greater than in other foods. Owing to the high concentration of radionuclides, studies report that Brazil nuts are radioactive.

Perhaps radiation is the most bizarre risk associated with Brazil nuts. The trees are so large, their extensive root systems bore deep into the ground and soak up unusual amounts of radium from the soil. This radium gets transferred into the nuts, making them radioactive. It is believed Brazil nuts have 1000 times more radium than the next most radioactive food. Although extensive studies are yet to be conducted, the amount of radiation in a Brazil nut is still small when compared to radiation encountered in everyday life, and it is not believed to pose any serious health risk, regardless of the quantities ingested. The occurrence of cancer is among the risks associated with the ingestion of radioactive foods, and monitoring is a form of health protection. The Brazil nut is native to the Amazon region and is economically important. It is collected in indigenous regions and in small communities during the rainy season, and transported to processing plants, to undergo treatment involving the following stages: sorting, drying, breaking, and sorting by size. The initial stage of manual/visual sorting is important to remove moldy and stained nuts, and precedes classification by size. At the end of the drying and cooling processes, the product is subjected to vacuum packaging and hot sealing.

Brazil Nut Allergies

Allergy to Brazil nut is uncommon, probably because these are not commonly eaten tree nuts. It is possible that allergy to Brazil nuts will increase in the future, because genetically modified soybeans accidentally have proteins similar to those found in Brazil nut allergen. People with Brazil nut allergy may also be allergic to walnuts.

Brazil nuts (*Bertholletia excelsa*, *Bertholletia myrtaceae*), ingestion of which has triggered allergic reactions in children and adults, contain 14% protein, including a methionine-rich protein (Ber e 1) that constitutes the major allergen.

Cashew

Cashew (*Anacardium occidentale*) belongs to the Anacardiaceae family. Pistachio and mango also belong to this family and the cashew tree foliage is very similar to pistachio foliage. Cashew trees are evergreen and grow rapidly to form a large much-branched tree of some 15 m in height.

The cashew or monkey nut is native to tropical America and north-eastern Brazil, in particular. Cashew trees thrive in warm humid climates where there is an annual rainfall greater than 1000 mm and they grow from sea level to an altitude of 1000 m. Cashew is cultivated primarily in India, Brazil, Vietnam, Tanzania, and Mozambique. Plantings have also been established in West Africa and Australia. Cashew trees are robust fast-growing evergreen trees that tolerate periods of drought. The nut that forms at the base of the cashew apple is highly valued as a roasted snack nut, in confectionery, and in cooking, but the apple also has value. Cashew apple juice is a popular drink in many communities and it can be fermented into a Madeira-like wine. The fruit pulp can be made into preserves, jelly, candied fruit, and syrup. However, the cashew apple harvested from commercial plantations is discarded when the nut is removed due to lack of markets for apple products. It has been reported that in India alone approximately 1.25 million tons of cashew apple is wasted each year.

The liquid enclosed in the shell is called cashew nut shell liquid (CNSL) and it is caustic. It contains cardol and anacardic acid and acts as a vesicant producing burn-like blisters when in contact with human skin. However, CNSL has many industrial uses because of its polymerizing and friction-reducing properties. It also has a use as a waterproofing agent and preservative. When distilled and polymerized, the oil is used in varnishes, cements, tiles, lubricants, and inks. It has also been used in tropical medicine.

Dry weather is essential before and during harvest. As mentioned above, fertilizer applications can be adjusted to

ensure fruit drop completes before the arrival of the wet season. In dry weather during nut drop, the apple remains firmly attached to the nut at harvest but if rain occurs during nut drop, the apple may rot on the ground before harvest.

The orchard floor is cleaned before nut drop. Traditionally cashew fruit is harvested by hand and the crop can lie on the ground to dry in fine weather, but it must be harvested if the weather is wet.

After harvesting, the cashew product is transported to a cleaning belt for removal of debris and it is then conveyed into driers. Ambient air is used in the drying process and drying can take a few days. The dried product is then stored in a dry atmosphere protected from vermin, usually in silos, until it is processed to remove the nut from the cashew apple. The dried product will remain in good condition for a year under good conditions.

Cashew Allergies

Cashews are the second most common tree nuts to cause food allergies. The oil found in the nutshell of the cashew is known to cause contact dermatitis, and is related to the oils found in the leaves of poison oak and in the skin of mangoes. Cashew allergens are similar in structure to palm oil, *Macadamia* nuts, peas, soybean, and walnut. Therefore, people with a walnut allergy may experience allergic reactions to these other foods.

Tree nut allergies, including allergic reactions to cashews, affect 1.2% of the children. Although 9% of children outgrow this allergy by the time they reach 6 years of age, the reaction triggered by eating cashews may be severe. A study published in the August 2007 issue of the journal *Allergy* indicates that breathing and cardiovascular issues are common, especially when compared to the allergic reactions triggered by eating peanuts.

AFL in Cashew

There is limited information as to the degree of the suitability of cashew nut as a substrate for mycotoxin production, especially AFLs by *A. flavus*.

In a study, 12 samples of cashew were tested for AFL and mold. The incidence of molds in cashew nuts in the first testing period was between 91.67% and 31.25%, and in the second period it was between 89.58% and 62.5% for sales points 1, 2, 3, and 4. The incidence of *A. flavus* and *A. parasiticus* in cashew nuts was 5.74% and 0.49%, respectively, and the differences were not significant. The concentrations of AFLs recovered from cashew nuts were between 20.67 and 11.33 ppb, for all sales points.

Walnut

Walnuts belong to Juglandaceae, the same family as pecans. Although all temperate species are deciduous, subtropical species tend to be evergreen in their native habitat.

The species cultivated for its sweet nut is *Juglans regia*, commonly known as English walnut but it is also called Persian walnut and in years past, the royal nut of Jupiter. This species is believed to have originated near the Caspian Sea in Iran and the native habitat extends east from Turkey and Iran to valleys in western China and the eastern Himalayas. A distinctive characteristic of this walnut is that the hull separates readily from the shell.

Cultivars of English walnut are grown for commercial nut production in southern Europe (particularly France, Germany, Italy, and Turkey), in the USA (California and Oregon), and in China, India, South Africa, Australia, and New Zealand.

Walnuts are ready to harvest in autumn when the hull cracks to release the nut and the oil content of the kernel is high.

Tree shakers are the most valuable piece of harvest machinery to own because nut fall can occur at the optimum time and this makes pick-up most efficient and produces greatest nut quality. When approximately 80% of the hulls are cracking, trees to be harvested that day are shaken.

Walnuts must be picked up and dried as soon as possible after nut fall preferably within hours. Failure to do so will result in discolored shell and moldy kernel in moist weather, heat-affected nuts in hot weather, and potential vermin damage.

The moisture content of nuts at harvest can vary from 20% to 40% depending on the weather and locality. The aim is to reduce the moisture content down to approximately 8%. Large capacity driers heat the air pumped through the nuts to reduce the drying time, whereas small-scale operations may use ambient air and heat only when required to reduce humidity. The dried nuts are then stored in a cool room or cool, vermin-proof silo, or store room.

AFL in Walnut

There also are metabolites in walnuts, which have potential as endogenous constituents of tree nuts as inhibitors of growth of *A. flavus* or aflatoxigenesis. The primary barrier to attack by external organisms is the hull of the walnut, which is known to contain a structurally related series of naphthoquinones, including 1,4-naphthoquinone, juglone, 2-methyl-1,4-naphthoquinone, and plumbagin. All of the quinines can delay fungal spore germination at lower levels and can completely inhibit growth at higher levels. The most potent compounds were 2-methyl-1,4-naphthoquinone and plumbagin, which were similar to each other in activity, i.e., they delayed germination for 40 h at 20 $\mu g\, g^{-1}$ and inhibited growth at 50 $\mu g\, g^{-1}$, so that walnuts are considered at lower AFL risk. However, in a study of walnuts, 75% of the walnut samples contained AFL in the range of 15–25 $ng\, g^{-1}$.

Control of insect pests in walnut orchards is a major strategy to lower AFL contamination, because insect feeding damage can lead to fungal infection and subsequent mycotoxin contamination. Through breeding and genetic engineering, new varieties of walnuts have been developed which are resistant to insect attack.

Penitrems in Walnut

Penitrems are a group of six related tremorgenic mycotoxins produced by *Penicillium. crustosum*, of which penitrem A ($C_{37}H_{44}NO_6Cl$) is the most toxic and the most studied

member. At low doses it was found to cause tremors in several animals. Dogs were reported to be victims of poisoning via consumption of moldy foods, such as walnuts contaminated with *P. crustosum*.

Walnut Allergies

Walnuts are the most common tree nut allergy, especially English walnuts. Many people are allergic to walnut pollen, which causes symptoms of allergic rhinitis, but this does not mean that the person is also allergic to the tree nut. Walnut allergens are similar in structure to those allergens found in pecans, cashews, Brazil nuts, castor beans, cottonseed, and mustard. Therefore, people with a walnut allergy may experience allergic reactions to these other foods. *Juglans regia* have also been etiologically implicated in human anaphylaxis.

Pecan

Pecan trees are also known as hickory trees and they belong to the walnut family Juglandaceae. In fact, the pecan grown commercially for nuts was originally classified as a species of walnut, *Juglans illinoensis*, but was renamed *Hicoria pecan* and finally *Carya illinoensis*.

Pecan trees are native to North America, growing in woodlands in the Mississippi Valley from Indiana and Illinois to Kansas and Texas, and at higher altitudes south of central Mexico. Pecans are also grown commercially in Australia, Brazil, China, Israel, and South Africa.

Nut maturity coincides with a peak in kernel oil content. The first visual sign of nut maturity is when the husk or shuck begins to split open.

The sooner the nuts are harvested and dried after they fall from the shuck, the better the nut quality will be. Trees are shaken with a tree shaker and most harvesting machinery is fitted with trash removal equipment that separates out leaves, husks, and twigs from the nut crop. The nuts are then transported straight to the dryer. Efficient drying is critical to nut quality. Nuts that lie moist on the ground for days, or nuts that are dried too slowly or at too high a temperature may develop off-flavor, darkened color, mold, and may soon become rancid.

The moisture content of nuts at harvest may be approximately 20% and they are dried to approximately 8% moisture content. They may then be dried further down to 4.5% for long-term storage.

AFL in Pecan

Leaves of the pecan *C. illinoensis* (Wangenh) K. Koch, another member of the Juglandaceae but in a different subfamily from *Juglans*, contain the naphthoquinone juglone, which inhibits mycelial growth of *Cladosporium caryigenum* (Ellis & Langl.) Gottwald (*Fusicladium effusum* G. Winter), the causative agent of pecan scab.

The natural occurrence of alternariols was reported in pecans. However, their production was limited to discolored pecan kernels, which are removed from shelled pecans during processing, which led to the suggestion that such pecans would be rejected by buyers of inshell pecans.

In a study, 148 isolates of *A. flavus* and *A. parasiticus* were isolated from 5608 pecans obtained from Chicago and Georgia markets. The percentage of internal contamination by these species was 7.3% in the pecans in Chicago market and 1.7% in those from markets in Georgia. Of the 148 isolates, 93% of the *A. parasiticus*, but only 54% of the *A. flavus*, were capable of producing AFL. Overall, 57% of the isolates were potentially aflatoxigenic. *Aspergillus parasiticus* isolates generally produced a greater amount of AFLs than *A. flavus*.

Salmonella in Pecan

Pecans thought to be contaminated with *Salmonella* were recalled from the US market in 2009.

Pre- and postharvest pecans are exposed to environmentsthat impose some level of risk of contamination with foodborne pathogens. Pecans that have fallen to the ground several days preceding harvest may absorb water. This water may contain *Salmonella, Escherichia coli* O157:H7, or other pathogens originating from wild or domestic animal feces, inadequately composted manure, irrigation water, or run-off water. Cleaning pecans may involve immersion in water and cross-contamination with foodborne pathogens. Wetting of inshell nuts may result from leaks in roofs or walls of storage facilities, thereby resulting in potential contamination or development of high-moisture environments that favor the growth of mycotoxigenic molds and foodborne pathogens. Conditioning pecans also creates an environment that may result in cross contamination.

Studies showed that *Salmonella* can survive on dry inshell pecans and pecan halves for several weeks. Survival was enhanced by storing nuts at freezing or refrigeration temperatures. Studies also showed that aqueous extract of pecan shucks is toxic to *Salmonella*.

Escherichia coli Contamination of Pecan

Escherichia coli is a common shell contaminant, especially for nuts harvested in orchards where animal grazing is practiced. Contamination of nutmeats may occur if shells are broken during harvest or if shells are not properly sanitized by addition of chlorine to heated soak-water just before cracking. Because of high amounts of organic material on the surface of pecan shells, chlorine should be monitored and replenished as needed to maintain the desired concentration.

Pecan Allergies

Pecans are a common food in the southern USA, but less common elsewhere in the world. Pecan affects approximately 1 in 10 people with tree nut allergies. People with pecan allergy are at risk for allergies to walnut, given the similarities between the allergens in these tree nuts.

Hazelnut

The hazelnut trees planted in commercial orchards (*Corylus avellana* cvs.) are smallish deciduous trees growing to

approximately 3 m tall. Turkey is the world's main producer of hazelnuts with a 75% share of the total world production and 70–75% of the total world's export.

Hazelnuts mature from late summer to early autumn depending on the variety and the location. As the husks yellow, nuts begin to fall and are ready to harvest. Some varieties fall free of the husk and others fall in the husk and require dehusking.

To ensure best quality, hazelnuts should be harvested from clean dry ground and transported to the dryer as soon as possible. In dry weather, nuts can remain on the ground for some days without loss of quality; however, both kernel and shell deteriorate rapidly when left on the ground in moist conditions.

The harvested nuts are dehusked and cleaned by hand or mechanically using dehuskers, trommel tables, trash-removing drums, or hand work via a conveyor. Where possible, blank nuts and nuts with shriveled kernel are blown or floated off. The cleaned nuts are immediately placed in a dryer.

The moisture content of harvested nuts can be as high as 20% depending on the weather and locality, and the aim is to reduce moisture content down to 8%. The dried nuts are stored in a cool room or cool situation in a vermin-proof silo or store room.

AFL in Hazelnut

A limited survey for the presence of AFLs in tree nuts from local markets and supermarkets in Valencia was carried out. The samples containing AFLs were hazelnuts, with AFL B1 and G1 levels up to 0.42 and 0.52 mg kg^{-1}, respectively.

In another study of hazelnuts, 90% of the hazelnuts were AFL positive (25–175 ng g^{-1}). One sample was also contaminated with zearalenone. The dominant fungal flora during storage is *Penicillium* and *Aspergillus* spp. *Aspergillus flavus* capable of producing AFL has been isolated from hazelnuts in storage. Reduction of inshell moisture content to below 10%, and nutmeat moisture content to less than 6% is an effective means deterring mold growth. Sanitation with chlorine dips may also be effective in reducing the incidence of mold infestation by reducing the amount if innoculum is carried into postharvest storage. Because of the high amounts of organic material on the surface of shells, chlorine concentrations should be monitored and replenished as necessary to maintain chlorine at concentrations necessary to kill microorganisms.

Romularia in Hazelnut

The most common decay found in hazelnuts is molds, with *Romularia* spp. most prevalent throughout nut development and the major pathogen associated with kernel tip mold. Although *Romularia* spp. appears to infect hazelnuts during nut development and may be quiescent before maturity and storage, many molds require breakage of the shell to contaminate the nut and thus intactness of the shell offers some natural defense against mold infestation.

Hazelnut Allergies

Allergy to hazelnut is more common in Europe than in the US. Hazelnut pollen is a common cause of seasonal hay fever, and it appears that a person with hazelnut pollen allergy is at risk for food allergy to the tree nut itself. Hazelnut is also related to birch pollen, and therefore people with birch pollen allergy may experience oral allergy symptoms with eating hazelnuts. People with hazelnut allergy may also be allergic to coconut, cashews, peanuts, and soybean, given the similarity between the allergens in these foods.

Macadamia Nut

The family Proteaceae includes approximately 10 species of the genus *Macadamia*, two of which produce edible nuts: *Macadamia integrifolia* and *Macadamia tetraphylla* or hybrids of these. The major species of commerce is *M. integrifolia*. *Macadamia* nuts are native to Australia and are produced there as well as in Hawaii, Central and South America, and parts of Africa. Nearly all production consists of grafted trees of cultivars developed in Hawaii or Australia. The edible kernel is enclosed in a thick, hard shell that, in turn, is enclosed in a husk that separates from the tree at about the time the seed is mature. The kernel is nearly spherical, consisting of joined equal-sized halves, i.e., cotyledons.

Macadamia nuts should be husked within 24 h of harvest, after which the drying process should be initiated. Freshly fallen nuts contain approximately 25% kernel moisture, although nuts that have remained on the ground for extended periods may have as little as 10–15% moisture. Drying should begin with ambient air, followed by a gradual increase in temperature that will not exceed 60 °C (140 °F) in the final stage of drying. Drying may be completed in shell (to 1.5% kernel moisture) or the nuts can be partially dried in shell (to approximately 5–6% kernel moisture), followed by cracking and finish drying of kernels alone to 1.5% moisture. It is important to protect the dry kernels from moisture and O_2, so that packaging in a material that is impervious to moisture is important. Vacuum packaging or nitrogen flush offers protection from O_2. Exposure to moisture results in loss of crispness and shelf life. Likewise, prolonged exposure to O_2 results in rancidity. Cold storage is normally not necessary for short-term storage, but might be desirable for extended periods. Frozen storage (-18 °C, 0 °F) can be very effective in extending shelf life.

Extended harvest intervals may result in mold, yeast, or bacterial contamination in the field as well as insect damage. Southern green stinkbug (*Nezara viridula* (L.)), tropical nut borer (*Hypothenemus obscurus* (F.)), koa seed worm (*Cryptophlebia illepida* (Butler)), and litchi fruit moth (*Cryptophlebia ombrodelta*) are the major insect pests causing damage to *Macadamia* nuts in Hawaii, resulting in an unmarketable kernel. Delay in husking the nuts following harvest can also result in mold growth and fermentation. Husking should occur within 24 h of harvest. The thick shell provides considerable protection for the kernel, but cracked shells and open micropyles can provide entry of fungi and bacteria as well as ants. When the nuts fall from the tree, the kernels contain approximately 25% moisture. At harvest, moisture level can range from approximately 10% to 25%. Once the nuts have been dried to 1.5% kernel moisture, the water

activity is 0.3, which is insufficient to support the growth of mold or bacteria.

Macadamia nuts are common foods in Hawaii and the tropics, and are a novelty food elsewhere in the world. There is significant cross-reactivity between the allergens in *Macadamia* nuts and cashews, hence a person is likely to be allergic to both of these tree nuts.

See also: Foodborne Diseases: Overview of Chemical, Physical, and Other Significant Hazards

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Water (Bottled Water, Drinking Water) and Ice

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Chemical Safety of Drinking Water

Minerals

Risk Associated with a Lack of Minerals

Minerals are essential for human life, being involved in a number of biochemical reactions, and as constituents used by the body. The drinking water, because of its essentiality, is one valuable source. The mineral content of water defines its hardness, and several studies have investigated its potential health effects: most of them were ecological epidemiological studies, which founded an inverse relationship between hardness and cardiovascular mortality.

In 2006, an expert meeting was organized by the World Health Organization (WHO) to address the possible role of drinking water containing calcium and/or magnesium as a contribution to the daily intake of those minerals. Indeed, the major source of micronutrients such as calcium and magnesium comes from the diet, but due to food habits, and the needs which can vary considerably among individuals and life periods, some people from various countries fail to reach the recommended intakes of these nutrients. Finally, a consensus was stood between the experts for the benefits linked to the calcium and magnesium intakes through the drinking water consumption. Regarding calcium, inadequate intakes are mainly associated with increased risks of osteoporosis and nephrolithiasis (kidney stones). Magnesium has also a significant impact on the health, with low magnesium levels being linked to endothelial dysfunction and hypertension.

Fluoride is another mineral found in most drinking waters. Demineralization and some other treatment processes will reduce its levels. However, where dental caries risk is high or increasing, authorities may consider addition of fluoride to the demineralized public water supply to between 0.5 and 1.0 mg l^{-1}, but other factors should also be considered. In countries where dental health awareness among the public is very high and alternative vehicles for fluoride, such as fluoridated toothpaste, are widely available and widely used, the absence of fluoridation would likely be of little consequence. However, in developing and developed countries where public dental health awareness might be much lower, addition of fluoride in drinking water would be important. The WHO drinking-water guideline value for fluoride is 1.5 mg l^{-1}.

Some treatments can have also a negative impact on the mineral wealth of drinking water. Point-of-entry ion exchange devices (water softener) are used in some households to remove hardness from water. Each divalent ion in the water is replaced by two monovalent sodium ions. Softening will not only have several esthetically beneficial effects inside the home, such as reducing scaling in pipes, fixtures, and water heaters and improving laundry and washing characteristics, but it also increases the sodium content of the drinking water. Point-of-use reverse osmosis and distilling devices remove virtually all the minerals from the input water, functioning as a final barrier against potential trace-level contaminants that may be present, as well as removing nutrients. The resultant drinking water is devoid of all minerals. Use of these devices may result in the reduction of the overall intake of nutrient minerals by the consumers.

Risk Associated with High Amount of Minerals

Nutrients are a good example of a risk – risk assessment: at low levels, there is a health risk linked to an inappropriate intake such as osteoporosis, cardiac malfunction, dental health, etc. However, at high doses, another health risk will occur: excessive intake of fluoride cause crippling skeletal fluorosis and possibly increased bone fracture risk. Ingestion of excess fluoride during tooth development, particularly at the maturation stage, may also result in dental fluorosis. These effects may be mitigated by coexposure to some minerals, such as calcium or magnesium.

Another example of nutrients that can lead to a negative health impact with high exposure is nitrates. They are widely encored in the nature, as part of the global nitrogen cycle. Moreover, they have been extensively used as fertilizers in agriculture, and as food additives (e.g., conservatives for meat and fish). Both groundwater and surface water can be contaminated by nitrates as a result of agricultural activities. Levels of nitrate contamination are usually significantly higher in groundwater, particularly in shallow wells, in which concentrations that exceed the current WHO guideline value of 50 mg l^{-1}, and exceptionally exceed 500 mg l^{-1}, have been reported in intensive agricultural regions. Nitrites are not frequently detected in drinking-water; when they are present, their concentrations rarely exceed 3 mg l^{-1}. Nitrates per se are relatively nontoxic, but their metabolites and reaction products, for example, nitrite, nitric oxide, and *N*-nitroso compounds, have raised concern because of implications for adverse health effects such as methemoglobinaemia and carcinogenesis. Dealing with methemoglobinaemia, infants are a critical subpopulation due to their higher gastric pH inducing

a higher rate of reduction of the nitrates to nitrites. Nitrites react with oxidized hemoglobin to form nitrates and methemoglobin.

Carcinogenicity is another worry on nitrates exposure: ingested nitrates and nitrites are classified into group 2A (probably carcinogenic to humans) by the International Agency for Research on Cancer (IARC) since 2006. Their toxicity comes from their reduction from nitrates to nitrites, and by endogenous nitrosation to *N*-nitroso compounds such as nitrosamines.

Current Issues

Environmental Contaminants

Heavy Metals

Heavy metals are ubiquitous and many foodstuffs are contaminated. Drinking water follows this rule, but heavy metals can come from anthropogenic or natural sources (soil, rocks crossed by water, etc.). The WHO sets guideline values for most of them, and both the US Environmental Protection Agency (EPA) and the European Commission also defined legal maximal limits for heavy metals.

Among them, lead is associated with some consumer products (e.g., paint, toys, and leaded fuel), and its exposure has dropped since the 1990s, especially thanks to the forbidden use of lead tetraethyl as fuel additive. Lead induces neurobehavioral and intellectual troubles, especially in young children, and recent studies discussed the relevance of a threshold level for its neurotoxic effects at low doses. Children are more sensitive to its health effects, due to a bigger water intake/mass weight ratio, a specifically sensitive window for neurobehavioral development, and a higher gastrointestinal absorption of lead (4–5 times more than adults). Currently, the US Centers for Disease Control and Prevention (CDC) set a maximal safe limit of the blood level to be at 10 $\mu g\,dl^{-1}$. Lead is very persistent in soil and can therefore leach in water by the erosion of natural deposits. However, its main origin in drinking water would come from pipes, solder and plumbing products. The leaching level varies depending on the several factors: presence of chloride and dissolved oxygen, pH, temperature, water softness, and standing time of water, soft, acidic water being the most plumbosolvent. Some focus was made in scientific journals, which summarize the recent overexposure to lead via drinking water in North America. Indeed, many water treatment plants now use disinfectants called chloramines, combinations of chlorine and ammonia. However, in some water systems this switch has coincided with an increase in lead in water, perhaps because chloramines cause lead to leach from pipes, fixtures, and solder. The US regulation of lead in drinking water is zero, with an action plan set at 15 $\mu g\,l^{-1}$, whereas the guideline value from the WHO for drinking water is 10 $\mu g\,l^{-1}$.

Arsenic, another heavy metal associated with drinking water safety, is known since antiquity as both a remedy and a poison. In the nineteenth century, the Fowler's solution was one famous tonic containing the arsenic derivate, and organoderivatives of arsenic were used to cure syphilis. Nevertheless, arsenic is even more known as one of the most toxic metals. Arsenic exists in oxidation states of -3, 0, 3, and 5, mainly in organic forms in food and inorganic form in drinking water, the latter showing the highest toxicity. It has been classified as evident carcinogen in humans by both the IARC and the National Toxicology Program for its ability to induce skin, bladder, and lung cancers following oral intake. Although there is substantial epidemiological data showing an association between skin cancers and arsenic exposure through drinking water, uncertainty of arsenic risk remains high for low doses. The available data on its mode of action cannot provide evidence for using linear or nonlinear extrapolation. Moreover, its toxicity is influenced by both endogenous and exogenous factors (genetics, malnutrition, etc.). Currently, neither the European Food Safety Authority nor the Joint FAO/WHO Expert Committee on Food Additives (JECFA) has determined a safe threshold value for inorganic arsenic. JECFA previously did it, but the Committee has recently considered that the previous provisional tolerable weekly intake is no longer appropriate. The maximum likelihood estimates, using a linear extrapolation, for bladder and lung cancer for the US populations exposed to 10 $\mu g\,l^{-1}$ of arsenic in drinking water are, respectively, 12 and 18 per 10 000 population for females and 23 and 14 per 10 000 population for males. The WHO has retained the level of 10 $\mu g\,l^{-1}$ as the arsenic guideline value. Unfortunately, its presence in drinking water is a daily reality for millions of persons living in West Bengal and in Bangladesh. It is estimated that of the 125 million inhabitants of Bangladesh between 35 and 77 millions are at risk of drinking contaminated water. According to a study conducted in 1998 by the British Geological Survey, 35% of the sampled tube-wells in Bangladesh showed arsenic concentration levels greater than 50 $\mu g\,l^{-1}$, and 8.4% showed greater than 300 $\mu g\,l^{-1}$, well above the WHO guideline value.

Others

Other chemical contaminants in drinking water, the presence of which express safety concern, are organic contaminants such as pesticides, dioxins and polychlorobiphenyls, polycyclic aromatic hydrocarbons, and so on. The phytosanitary product family encompasses a wide variety of chemicals, from agricultural activities and from the water treatment used for the control of disease-carrying insects in the drinking water. The third edition of the WHO guidelines for drinking water quality quotes five pesticides, all of them being planned to be used as a mosquito larvicide in containers in order to control the dengue fever. Even if the WHO considers that it is not appropriate to set a formal guideline value, the maximal dosage for each pesticide is specified as assessed by the WHO Pesticides Evaluation Scheme. Apart from these, the WHO sets guideline values for almost 40 active substances: for herbicides, such as alachlor (CAS number 15972-60-8), atrazine (CAS number 1912-24-9), and for insecticides like aldrin (CAS number 309-00-2), chlordane (CAS number 57-47-9), DDT (CAS number 107917-42-0), etc. In terms of comparison, the European regulation on drinking water (Council Directive 98/83/CE) does not consider pesticides individually but as a whole: the maximal limit per pesticide is 0.10 $\mu g\,l^{-1}$ and for the total pesticides 0.50 $\mu g\,l^{-1}$. Here the term pesticide means organic insecticides, herbicides, fungicides, nematocides,

acaricides, algicides, rodenticides, slimicides, related products (inter alia, growth regulators) and their relevant metabolites, degradation, and reaction products. In the case of aldrin, dieldrin, heptachlor, and heptachlor epoxide the parametric value is 0.030 $\mu g\,l^{-1}$.

Process Contaminants

Disinfection Byproducts (DBPs)

Although disinfection of drinking water is indisputably beneficial for human health, the chemical reactions between oxidative agents (chlorine, ozone, etc.) and natural organic matter lead to the formation of DBPs. Among the hundreds of DBPS, only half of them are known, even less with chloramine treatment. The best known are chloroform (CAS number 67-66-3), bromoform (CAS number 75-25-2), bromodichloromethane (CAS number 75-27-4), and dibromochloromethane (CAS number 124-48-1) from the family of the trihalomethanes (THMs), and mono-, di-, and trichlororacetic acids (CAS numbers 79-11-8, 79-43-6, and 76-03-9, respectively) and mono- and dibromoacetic acids of the haloacetics acids group (CAS numbers 79-08-3 and 631-64-1, respectively).

To date, the European Commission regulated the presence of four THMs, chlorite (CAS number 7758-19-2), and chlorate (CAS number 7775-09-0). The US EPA also included five haloacetic acids and bromate (CAS number 15541-45-4). The last edition of the WHO guidelines for drinking water includes other DBPs such as 3-chloro-4-dichloromethyl-5-hydroxy-2(5H)-furanone (MX, CAS number 77439-76-0) or *N*-nitrosodimethylamine (NDMA, CAS number 62-75-9). In parallel, recent studies performed on emerging DBPs highlighted that a classification for toxicity potential can be addressed with the following hierarchy: iodo-DBP > bromo-DBP > chloro-DBP. However, despite that the experimental toxicity data showed genotoxic and reprotoxic potentials, the epidemiological studies on the incidences of bladder cancer, colorectal cancer, or stillbirths and congenital malformations failed to find a strong relationship with the level of DBPs, always expressed by the concentration of THMs. New studies are planned in Europe to dig the link between the exposure to chlorinated water and the occurrence of pregnancy issues. In these studies, all sources of exposure should be assessed, including the inhalation one (during the shower), the oral exposure (both the water drank and the water used for the preparation of food), and the dermal way (during all recreational activities in swimming pool).

Ozonation is another good means to disinfect water and to eliminate the micropollutants. However, ozonation also generates toxic byproducts. In the presence of bromide ions, these are oxidated to hypobromous acid and further the hypobromite ion is oxidated into bromate. As the second step requires the ionic form, and does not proceed with the protonated form, the rate of reaction increases with increasing pH. In terms of toxicity, bromates are mutagenic and carcinogenic, and potassium bromate is classified into group 2B by IARC. In male rats, bromates induce multisite tumors in the kidneys, the thyroid gland, and in the peritoneum. Only renal adenomas and carcinomas were seen in female rats. For the WHO , the weight of evidence from the rat bioassays clearly indicates that bromate has the potential to be a human carcinogen. Owing to insufficient data on the mode of action for carcinogenicity of bromates, the International Programme on Chemical Safety developed both a threshold and a linear extrapolation model. Applying the multistage linearized model, the concentration of bromate in drinking water associated with an upper-bound excess lifetime cancer risk of 10^{-5} is 2 $\mu g\,l^{-1}$. The health-based value was raised from 2 to 10 $\mu g\,l^{-1}$ by the WHO for analytical and technological reasons. A provisional guideline value of 10 $\mu g\,l^{-1}$ is currently recommended.

Materials in Contact with Water

Conditioning of water, including central softening and stabilization, may be necessary to reduce corrosion of piping materials and/or scaling effects in installations and to improve consumer acceptability. Corrosion and scaling can be associated with adverse effects on health (from leachates such as lead) and the environment (from leachates such as copper if the water is not conditioned) and reduced the lifespan of the distribution network and appliances using water. Soft waters are aggressive and can corrode water pipes leading to matter release, but any new water treatment can enhance pipe corrosion. In several counties in the USA, the switch from chloride to chloramine treatment has been accompanied with a huge peak in lead concentration in drinking water. Indeed, the addition of chloramine in water modifies the configuration of lead, increasing its solubility in water.

The topic of packaging is also of particular concern for bottled waters. The polyethylene terephtalate (PET, CAS number 25038-59-9) is now extensively used for the bottling of water in small volume. It is known that the use of such material is associated with the migration of antimony trioxide (CAS number 1309-64-4), a catalyst used in its manufacture for nearly 30 years. However, the migration levels recorded in bottled water samples are very low. These levels are well below the health standards set by the US EPA (6 $\mu g\,l^{-1}$), by the European Food Safety Agency (40 $\mu g\,l^{-1}$), or by the WHO (18 $\mu g\,l^{-1}$). Now, the PET is under a new evolution with the increasing incorporation of a recycled PET with the virgin material to reduce the carbon footprint of bottled water.

The Home and Office Delivery service implies the reuse of bottles, which need to be built with a rigid, resistant, and crystal material for volumes of approximately 5 gal. Such bottles are made with polycarbonate, made with bisphenol A (CAS number 80-05-7), which can migrate from the packaging into the water. There is a strong scientific debate on toxicity of bisphenol A, with endocrine disruption effects observed at both high and low doses, following a U-curve. The reliability of tests, the endpoints observed and the extrapolation of experimental data to humans are the key points currently discussed by risk assessors for allowing the best risk management for public health.

Emerging Issues

Endocrine Disruptors

The WHO gave a definition of endocrine disruptors: "An endocrine disruptor is an exogenous substance or mixture that

alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations.'' Their mode of action can be direct or indirect. For example, the pharmaceutical estrone (CAS number 53-16-7) and the industrial contaminant alkylphenols polyethoxylates are able to directly link the estrogen receptor and activate it. Traces of these substances have been detected in rivers, and then can be detected in the drinking water. Thankfully, water treatments (activated charcoal, ozonation, etc.) are efficient enough to remove most of these molecules. Another example of an endocrine disruptor chemical found in water is perchlorate (CAS number 14797-73-0). It exerts an antithyroid activity by an indirect mechanism. Thyroid gland produces hormones: triiodothyronine (T3) and thyroxine (T4), under the control of the thyroid-stimulating hormone, which is synthesized in the anterior pituitary gland. These hormones are essential for good biological development, and the role of iodine in their synthesis is primordial. In humans, perchlorate can interfere with iodine uptake by the thyroid gland, causing changes in the levels of the thyroid hormones. These changes can lead to problems in growth and development of the fetus and infant and may impair the function of many organs in humans of all ages.

Pharmaceuticals and Personal-Care Products

A recent research has focused on this class of contaminants with many articles published on it. The consumption of drugs is associated with a specific disease, and once cured, pharmaceuticals are often disposed of down toilets. In parallel, those absorbed are metabolized and eliminated by the body. The steps of metabolism, especially with the enzymes of the second phase, increase their aqueous solubility to enhance their natural elimination. Taken all together, both parent molecules and metabolites can be found in wastewater and then in the environment. The first difficulty the authorities faced was the definition of a limited list of pharmaceuticals to be looked for in the environment and the drinking water. The priorization was made to take into account the volume of production, the level of consumption, the biodegradability, and their physicochemical properties. Some classes of pharmaceuticals, with representative molecules, have been identified. Among them, the most often detected substances are carbamazepine (CAS number 298-46-4), an antiepileptic drug, diclofenac (CAS number 15307-86-5), ibuprofen (CAS number 15687-27-1), and other nonsteroid antiinflammatory medication. Caffeine (CAS number 58-08-2) and aspirin (CAS number 50-78-2) can also be detected but their origin is subject to caution: aspirin under the form of salicylic acid can be naturally found in the environment and analytical cross-contamination can occur for caffeine at low levels of quantification. Nevertheless, at the levels usually found, none of these compounds are susceptible to exert toxic effects. Two questions are now rising: What is the impact of these mixtures at low doses and what is their behavior in the environment (e.g., development of antibiotic resistance) and during the drinking water treatments. Indeed, triclosan (CAS number 3380-34-5), an antibacterial agent, can lead to the formation of dioxin under ozone oxidation.

Assessing the Risks

Principles

The level of contaminant in drinking water is usually expressed in milligrams per liter, even in micrograms per liter for the most toxic chemical hazards. In toxicological risk assessment, comparison is made with toxicological reference doses, expressed for oral toxicity in mass unit per body weight (in kg) and, for nonacute exposure, per exposure time (usually given by day). These toxicological reference doses are most often based on *in vivo* experimental data, with safety factors applied to extrapolate the experimental data to a safe exposure for humans. The risk results from the exposure to a hazard: indeed, the risk must be identified (what is the hazard) and characterized (what is the level of exposure to such hazard). To be able to compare contaminant concentrations in drinking water with toxicological reference doses, an assumption of the quantity of water drunk every day must be set. A global approach is to consider a drinking water consumption of 2 l per day for a 60 kg adult. This assumption is declined for toddler (1 l per day for a 20 kg child) and for infant (0.75 l per day for a 5 kg baby). It must be specified that this assumption can be adapted and modified by national agencies in order to be closer to the scope of their assessment. Thus, the US EPA considers in its assumption a 70 kg body weight as an average weight for American adults, whereas the WHO uses preferentially 60 kg body weight as the standard for worldwide adults.

This methodology is used for chemicals for which a toxicity threshold exists. This is not the case for substances which are both genotoxic and carcinogenic. For those, no safe dose can be established and the recommendation is to reduce the exposure as low as reasonably achievable. However, for risk management, a maximal level must be specified, and it is done through a linear extrapolation of the dose–effect relationship observed at high doses from the epidemiological data in humans or from the experimental data in animals. This is a conservative approach as it considers that the organism answers in a similar manner at low doses than at high doses. The linear extrapolation model gives a correlation factor between the additional cancer risk and the hazard concentration. An 'acceptable' risk corresponds to an additional risk of cancer for one million of individuals (10^{-6}).

Example

Benzene is an evident human carcinogen leading to acute nonlymphocytic leukemia and a variety of other blood-related disorders in humans following occupational exposure. Its oral carcinogenicity has been observed in experimental animals. Additionally, changes in blood and bone marrow consistent with hematotoxicity are recognized in humans and experimental animals. Although it is not mutagenic in standard bacterial assays *in vitro*, its genotoxicity (chromosomal aberrations) has be shown *in vivo*. Two different approaches were made by both the US EPA and the WHO.

The EPA states in 1998 that ''there is [currently] insufficient evidence to deviate from using an assumption of a linear dose-response curve for benzene, hence, the Agency's past approach of using a model with low dose linearity is

Table 1 Major outbreaks linked to the consumption of drinking water, bottled water, and ice

Germs involved	*Type of organism*	*Number of cases/ deaths*	*Year and localization*	*Water involved*	*Reference*
Cryptosporidium parvum	Parasites	403 000/0	1993 Milwaukee, USA	Public water supply	Mac Kenzie *et al.* (1994)
Escherichia coli O157 and *Campylobacter*	Bacteria	2000/7	2000 Walkerton, Canada	Public water supply	Holme (2003)
Hepatitis A	Viruses	107/0	2006 Sichuan Province, China	Ice snacks made with untreated well water	Zhang *et al.* (2008)
Legionella	Bacteria	449/9	2001 Murcia, Spain	Hospital cooling towers	Garcia-Fulgueiras *et al.* (2003)
Noroviruses (Calicivirus)	Viruses	41/0	2005 Pugia area, Italy	Ice?	Rizzo *et al.* (2007)
Salmonella	Bacteria	46/0	2006 Gran Canaria, Spain	Bottled water	Palmera-Suarez *et al.* (2007)
Campylobacter, *Giardia*, and enteric viruses	Parasite Bacteria Viruses	8453/0	2006 Nokia, Finland	Public water supply	Laine *et al.* (2007)

still recommended.'' In 2000, the EPA extrapolated data on cancer risk associated with inhalation exposure to oral risk. The inhalation unit risk range is first converted to unit of dose (μg per kg body weight per day). Then, assuming a 100% oral absorption and a standard intake of 2 l per day, the unit of risk is expressed in $\mu g\,l^{-1}$. The WHO built its assessment of the risk estimates on the basis of a 2-year oral study in rats and mice. As for the EPA, a robust linear extrapolation model was used, data having been unfit for the linearized multistage model. The estimated range of benzene level in drinking water corresponding to excess cancer risks in female mice and male rats are similar to those derived from the epidemiological data. Thus WHO and EPA found the same relationship between benzene concentration in drinking water and excess lifetime cancer risk: 10^{-4}, 10^{-5}, and 10^{-6} excess risk at 100, 10, and 1 $\mu g\,l^{-1}$, respectively. The US EPA set a maximal limit at 5 $\mu g\,l^{-1}$, whereas the WHO guideline value is 10 $\mu g\,l^{-1}$.

Microbial Safety of Drinking Water

Microbial contamination is one of the major risks drinking water suppliers have to face, in developing and developed countries as well. Outbreaks of waterborne diseases affecting thousands of people worldwide are reported from countries at all levels of development and from all types of water supply.

Impact of Drinking Water-Related Diseases

The global impact of waterborne and water-related diseases is difficult to assess. The main reasons are a global lack of data especially in developing countries, the difficulty to find the origin and the agent responsible for the infections, seasonal as well as geographical disparities, secondary transmissions from person to person, etc.

Gastroenteritis is the most frequent water-related outcome. The precise ratio of water-linked enteric illnesses is not precisely estimated but the WHO quotes 1.7 million deaths associated with unsafe water, sanitation, and hygiene mainly among young children under the age of 5 years and elderly.

In addition, the exact health impact of the microbiological quality of drinking water is also not easy to estimate. 'Drinking water' that ''should be suitable for human consumption and for all usual domestic purposes including personal hygiene'' according to WHO includes waters not only from piped sources, water from springs and wells but also packaged and bottled water and ice.

More complete epidemiological data have been reported by the US CDC and EPA. During 2007–08, 36 drinking water-related outbreaks were reported in the US leading to 3 deaths and 4128 infectious cases. Of these, 61.1% were gastrointestinal infections, 33.3% were linked to respiratory illness (Legionellosis), and others were related to skin injury or hepatitis. More than half of the described outbreaks were associated with untreated or inadequately treated groundwater.

Indeed, the microbial quality of water resources as well as the different types of disinfection applied are both essential to ensure the safety of drinking waters.

Surface waters and groundwaters under direct influence of surface waters are more 'vulnerable' to microbial contaminations from environmental or animal origins than well protected groundwaters. Disinfected waters – through physical and/or chemical processes – are also less susceptible to microbial contaminations than untreated, poorly protected drinking waters.

However, reports of major outbreaks linked to the consumption of drinking water, packaged water, and ice are well documented (Table 1), proving that microbiological safety of drinking water is a real challenge.

Waterborne Pathogens

Main Waterborne Pathogens

Unfortunately, germs potentially causing diseases and transmitted through water include a large range of organisms from viruses, bacteria, protozoa, and even helminthes.

It can be considered that mainly most of the enteric pathogens can be transmitted through water. Water as food is one of the common transmission ways of fecal–oral transmitted diseases.

However, waterborne pathogens are not strictly limited to enteric ones and other organisms, transmitted for instance through inhalation or contact (during bathing or washing) should not be omitted.

Microbial contamination can occur via introduction of human and animal fecal matter into drinking water but additionally organisms occurring in the environment such as *Legionella* spp. and *Acanthamoeba* spp. can lead to severe infectious diseases. Infections spectra is large from mild gastroenteritis to severe diseases targeting the intestinal tract as cholera or dysentery or hepatitis, to respiratory illness such as Legionellosis and skin or brain disorders (Table 2).

Usually, organisms are classified according to the epidemiological evidences of being water transmitted:

- Organisms for which clear epidemiological data of water transmission are available (such as *Vibrio cholerae*, *Cryptosporidium parvum*, noroviruses, etc.).
- Organisms for which water transmission is conceivable (such as *Helicobacter pylori*, *Isospora belli*, etc.) but not yet confirmed.
- Known pathogens for which there is no evidence of water transmission.

Toxin producers (e.g., cyanobacteria) are also to be included as waterborne pathogens.

Host–germ interactions play an important role in waterborne infections and diseases. For instance, asymptomatic infections are poorly reported and the precise part of water-related contaminations, i.e., with hepatitis A virus, are not easy to distinguish in large endemic areas.

Waterborne Pathogen's Behaviors in Water

The occurrence of an organism in drinking water cannot only justify its significance regarding human health. Indeed, the survival of the target organism in water (i.e., inactivation rate) as well as the infectious dose greatly impacts the probability of infection through water ingestion/inhalation.

Some pathogens can both survive and multiply in water. Most of them are from environmental origin as *Legionella pneumophila*. Others harbor long survival abilities in water keeping their infectiosity as parasites like *Cryptosporidium parvum*, *Giardia lamblia*, and also enteric viruses. Some seem to have a more limited survival in water as *Campylobacter* spp. Qualitative information on the survival of waterborne pathogens' in drinking water are available in Table 2.

Under certain living conditions as starvation, bacteria may enter into a viable but not cultivable (VBNC) state: cells are metabolically active, while being unable to grow on its usual growth medium. *Pseudomonas*, *Campylobacter*, and *Escherichia coli* are the known VBNC bacteria. VBNC cells could persist in the water and may keep growth capability and infectivity.

Influence of Industrial Processes on Waterborne Pathogens

Among 84 outbreaks occurred in Europe between 1990 and 2004, Smets *et al.* found that a large part of them was due to insufficient treatment or treatment failure irrespective of the origin of the water, whether surface or groundwater. Inadequate treatment to deal with rare events of high and punctual contaminations of the water source was also cited.

Drinking water treatments indeed are key for providing safe drinking water except in the case of natural mineral waters, where no microbiological treatment is allowed.

In addition to the process dedicated to remove organisms, some process mainly designed for particle removal can also have an impact on pathogenic organisms. The combination of all process steps has to be combined to assess the overall microbial reduction as shown with the multibarrier approach.

Monitoring drinking water storage and distribution (tanks, pipes, and bottles) is also required to protect water from postcontaminations and to guarantee its microbial safety until it reaches the consumer.

Disinfection

Chemical disinfection consists of adding a disinfectant (generally a strong oxidant) to the water, which reacts with the organic matter and microbial organisms. Most frequent chemical disinfection compounds are chlorine dioxide, chlorine, and chloramines on one hand and ozone on the other hand. Depending of the water, the chemical disinfection efficiency can be lowered, for instance, at higher pH when using chlorine or with high organic matter concentrations. Byproducts originating from oxidative reactions can also be generated by chemical disinfection. Some may have adverse health effects.

Microorganisms adsorbed on particles are more resistant to chemical disinfection than free living organisms. Chemical disinfection is generally used as the primary microbial removal step and can be completed by another disinfection to ensure microbial quality during the distribution.

Efficiency of disinfection is defined using the Ct value which is defined as the product of disinfectant concentration (milligram per liter) and the contact time (min). Temperature and pH both influence the Ct value.

Chlorine is a strong disinfectant regarding vegetative bacteria in a range from 0.05 to 200 mg-min l^{-1} depending on the organic matter load. Chlorine dioxide is also a strong oxidant and disinfectant more effective than free chlorine at basic pH. Ct values for chlorine dioxide are less than 1 mg-min l^{-1} for most vegetative bacteria and viruses (Table 3).

However, some parasites as Cryptosporidium are known to show high resistance to chlorine-based oxidation with Ct values of approximately 15 mg-min l^{-1} for *Giardia* cysts.

Table 2 General overview of the main waterborne organisms that can cause or be suspected of causing illnesses

Organism	*Reservoir*	*Transmission route*	*Clinical features*	*Infectious dose*	*Survival in water*
Campylobacter	Animals	Fecal–oral	Gastroenteritis Guillain–Barré syndrome Meningitis	Low	Moderate (VBNC)
Pathogenic *Escherichia coli*	Animals Humans	Fecal–oral	Bloody diarrhea Hemolytic uremic syndrome	Low	Low to moderate (Biofilms?)
Legionella	Environment (surface waters)	Inhalation Ingestion	Legionellosis Pontiac disease	Moderate	High (Amoebae)
Salmonella typhi/ paratyphi	Humans	Fecal–oral	Typhoid	Low	ND
Other Salmonella	Humans Animals	Fecal–oral	Acute gastroenteritis	Moderate	Low to moderate (VBNC)
Shigella	Humans	Fecal–oral	Dysentry Acute gastroenteritis	Low	Low (VBNC, amoebae?)
Vibrio cholerae	Environment Humans	Fecal–oral	Cholera gastroenteritis	Low	High (water temperature $>20\ ^{\circ}C$)
Yersinia enterocolitica *Yersinia. pseudotuberculosis*	Animals (swine and rodents)	Fecal–oral	Gastroenteritis Mesenteric adenitis	High	Unknown
Aeromonas	Environment (surface waters)	Inhalation Ingestion	Respiratory tract infections Gastrointestinal illness	Unknown	High (regrowth)
Burkholderia pseudomallei	Environment Animals (sheep, goats, and swine)	Inhalation, ingestion, person-to-person?	Melioidosis Acute respiratory infections	Unknown	Moderate? Regrowth?
Cyanobacteria	Environment (surface waters)	Ingestion of cyanotoxins Skin contact	Gastrointestinal and, respiratory disorders, liver damage, neurotoxic reactions	$1\ \mu g\ l^{-1}$ for microcystin LR	High (regrowth)
Helicobacter pylori	Humans, Environment?	Person to person Fecal–oral	Gastritis, stomach ulcers	Unknown	Low to moderate (VBNC)
Nontuberculosis mycobacteria	Environment Animals	Inhalation Ingestion Contact	Pulmonary and gastrointestinal diseases Lymphadenitis Skin infections	Unknown	High (regrowth)
Pseudomonas aeruginosa	Environment	Inhalation Contact Ingestion?	Acute respiratory infections	Unknown	High (regrowth)
Adenovirus	Humans	Fecal–oral Inhalation Contact	Gastroenteritis Respiratory infection and conjunctivitis	Low	High
Calicivirus (Norovirus)	Humans	Fecal–oral	Gastroenteritis	Low	Moderate to high
Enterovirus	Humans	Fecal–oral	Poliomyelitis Encephalitis Meningitis Myocarditis	Low	Moderate to high
Hepatitis A	Humans	Fecal–oral	Gastroenteritis	Low	Moderate to high
Hepatitis E	Humans Animals	Fecal–oral	Gastroenteritis	Low	Moderate
Rotavirus	Humans	Fecal–oral	Gastroenteritis	Low	Moderate to high
Cryptosporidium	Humans Animals	Fecal–oral	Cryptosporidiosis	Low	High
Giardia	Humans Animals	Fecal–oral	Giardiosis	Low	High
Cyclospora	Humans	Fecal–oral	Cyclosporiasis	Low	High
Acanthamoeba	Environmental	Inhalation Contact	Encephalitis Keratitis	Unknown	High

Abbreviations: ND, not detected; VBNC, viable but not cultivable.

Source: Adapted from WHO (2011) *Guidelines for Drinking-water Quality*, 4th edn., ch. 7, p.119.

Infectious dose: low <1000 organisms; moderate $<10^6$ organisms; and high $>10^6$ organisms.

Survival: low <3 weeks; moderate <4–6 weeks; and long $>$several months.

Table 3 Ct values for virus disinfection

Disinfectant	*Units*	*2 Log inactivation (99%)*	*4 Log inactivation (99.99%)*
Chlorine	mg.min per l	3	6
Chloramine	mg.min per l	643	1491
Chloride dioxide	mg.min per l	4.2	25.1

Source: Adapted from OEDC/WHO (2003) *Assessing Microbial Safety of Drinking Water*. London: Organization for Economic Co-operation and Development and the World Health Organization.

Table 4 UV fluence required for microorganisms removal

	Required fluence (mJ.cm^2)	
Targeted inactivation rate (Log removal)	*1 Log*	*4 Log*
Adenoviruses	42–56	167
Feline Calicivirus (Norovirus surrogate)	9	38
Rotavirus	10	39
Bacillus subtilis	56	222
Clostridium perfringens	45	–
Legionella pneumophila	8	30
Enterococcus faecalis	9	30
Escherichia coli	5	18–19
Vibrio cholera	2	9
Cryptosporidium	3	12 (3 Log removal)
Giardia	2	11 (3 Log removal)

Source: Adapted from Smets P, Rietveld L, Hijnen W, Medema G, and Stentröm TH (2006) Efficacy of water treatment processes. *Microrisk, Microbiological Risk Assessment: A Scientific Basis for Managing Drinking Water Safety from Source to Tap*. EU Project.

Ultraviolet light (UV) is also widely used for water treatment. UV lamps are placed in a flow-through contact compartment that is continuously operated. UV fluence corresponding to the UV light intensity and the contact time (function of water flow rate) directly impacts the disinfection efficiency.

Both flow patterns and the type of lamp (medium to low pressure mercury lamps) influence the removal of organisms. Inactivation of adenoviruses by UV treatment requires higher fluencies than that for other organisms (Table 4).

In addition to disinfection, special attention should be paid to ensure the absence of recontamination posttreatment. Chlorine, for instance, is widely used to protect drinking water safety along the water supply to the point of use.

Filtration

Filtration covers a wide range of processes from microfiltration to nanofiltration and reverse osmosis. Removing not only the particles but also organic compounds and colloids that can be present in water is the main aim of filtration. Nevertheless, depending on the membrane porosity and/or molecular weight cut-off, filtration can offer an alternative process to disinfection (Table 5).

There are mainly two classes of membrane treatment systems:

- The low pressure membrane systems, such as microfiltration and ultrafiltration (UF), are commonly used to remove particulate and microbial contaminants.
- The high pressure membrane systems, such as nanofiltration and reverse osmosis (RO), are typically applied for the removal of dissolved constituents.

Operating filtration devices with the aim of pathogen removal requires, however, application of good hygienic practices and strict maintenance conditions. Another drawback of such systems compared with disinfection treatment as UV or chlorine can be water losses that are generated especially for UF and RO filtrations.

Coagulation and Flocculation

Coagulation and flocculation first aim to remove organic matter and particles that can be present in source water as surface waters. Addition of chemical coagulants causes flocs to form that are further removed by sedimentation and clarification or filtration. Microbial germs, either as free living organisms or adsorbed on particles, can be partly embedded in coagulation flocs and/or naturally settle or be retained by filters.

Efficiency of coagulation and clarification depends obviously on water composition and pH; organic matter concentration; type of coagulants used and its concentration; process design including clarification, settling or filtration steps, and operating conditions (e.g., increased flow rates, increased turbidity, etc.).

Average microbial removal for coagulation/sedimentation/filtration processes ranges from

- 0.7–3.5 log for bacteria,
- 0.85–3.05 log for enteric viruses, and
- 0.2–3.5 log for parasites

depending on water turbidity (inlet and outlet), filter types, pH, and flow rates.

Bottling Process

Many different processes can be applied to packaged and bottled waters with the exception of natural mineral waters.

Disinfection treatments such as UV or ozonation are widely used as well as filtration systems depending on the source of the water (surface water and vulnerable groundwater). Maintaining the sanitary conditions of bottling operations are key to avoid microbial contaminations from catchment to bottle and especially postdisinfection. It implies, in addition to the

Table 5 Filtration systems and their potential removal of microbial organisms

Technology	*Operating pressure*	*Pore size*	*Primary applications*	*Microbes removal*
MF	1–2 bar	0.02–10 μm	Removal of particles and turbidity	Algae, protozoa and some bacteria (function of porosity)
UF	<5 bar	20–200 nm (5000–500000 Da)	Removal of dissolved nonionic solutes	Algae, protozoa, most bacteria, and viruses
NF	5 bar	1–10 nm (200–10000 Da)	Removal of divalent ions (softening) and dissolved organic matter	
RO	15–50 bar	<2 nm (50–300 Da)	Removal of monovalent ions (desalination)	

Abbreviations: MF, microfiltration; NF, nanofiltration; RO, reverse osmosis; and UF, ultrafiltration.
Source: Adapted from LeChevallier M, and Au K (2004) *Water Treatment and Pathogen Control*. London: IWA Publishing/WHO.

protection of the borehole, strict hygiene from plant environment to the design of tanks, pipes, pumps, and other process equipments. Filling area is of particular concern regarding potential microbial contamination. Air as well as operators can be possible vectors of microorganisms.

Packaging materials, bottles, containers, and caps are additional ways of contamination. Dedicated cleaning and disinfecting processes are usually applied for packs more than ever for glass bottles and reused jars (home delivery service). The Codex Alimentarius code of hygienic practice for packaged drinking waters CAC/RCP 48-2001 defined the minimal requirements to be applied by bottled water manufacturers.

In spite of packaged treated waters, natural mineral waters are not allowed to be disinfected (in line with the European Standards) as they originate from well protected groundwaters. At source, natural mineral waters must be free of any pathogenic organism. Specific recommendations have been provided by Codex Alimentarius in its code of practice for collecting, processing and marketing of natural mineral waters (CAC/RCP 33-1985).

Freezing

Freezing is considered to have a low impact on microbial survival. Avian Influenza viruses remained infectious in frozen environmental waters and a large outbreak of viral gastroenteritis was reported in 1987 by CDC due to ice made with water originated in wells that had been flooded by a creek during heavy rains.

Microbial Indicators

The Role of Indicators

As in food industry, the management of microbial risks in drinking water relies on both 'index organisms' and 'indicators.' Index organisms indicate the presence of ecologically similar pathogens (potential health risk) whereas indicator presence shows inadequate processing for safety (process failure). Basically, indicators must be present when pathogens are present and must be easy and fast to detect and quantify.

The detection of each pathogenic organism cannot be associated with the indicator concept as the presence of one pathogen does not mean the presence of others (e.g., there is no link between the presence of Legionella and Cryptosporidium) and its absence the absence of other contaminants. Moreover, some pathogens may be poorly detectable because of lack of reliable and sensitive methods or because of their physiological state in drinking water (VBNC for instance).

No indicators, however, can indicate the presence of environmental pathogens as Legionella and mycobacteria.

Fecal and Quality Indicators

Microbial indicators are commonly classified as fecal indicators and quality indicators.

- *Escherichia coli* is a well recognized bacterial indicator of fecal contamination being directly linked with the feces of warm-blooded animals. As such, it shows the potential presence of other enteric pathogens and a potential health risk.
- Coliforms can be of fecal origin (as *E. coli* which belongs to the coliforms group) or of telluric origin (soil and environment). Their presence in drinking water must at least be considered as indicative of microbiological quality weakening: treatment failure, loss of disinfectant products, addition of contaminated water into the water supply or the resource, etc.
- Enterococci (previously fecal streptococci) belongs to the fecal indicators group too as they originate from the gut of animals. They are widely used for recreational waters due to their resistance into the environment.
- Sulfite-reducing anaerobes (clostridia) are organisms that form spores that are environmentally resistant and their presence may indicate soil contamination, although some species may be associated with corrosion of distribution pipes. *Clostridium perfringens* is associated with fecal contamination. Their presence postdisinfection treatment may indicate a failure of efficiency as spores are more resistant than other vegetative bacteria as the *E. coli*.
- *Pseudomonas aeruginosa* is a ubiquitous environmental organism. Its presence is constant in surface waters and it can

easily grow in all types of freshwaters, even in pure water. As it is an opportunistic pathogen, its presence can either show a vulnerability of resources to surface waters/environmental contaminations or a vulnerability of the process environment. *Pseudomonas aeruginosa* is widely used in bottled water industry as a process indicator.

Enteric Viruses

Among waterborne pathogens, enteric viruses play an important role. Their detection remains uneasy as some are still uncultivable. Genomic techniques (deoxyribonucleic acid (DNA)/ribonucleic acid (RNA)) allow detection of viral genomes in waters but do not indicate if the detected viruses are infectious or not. Usual bacterial indicators (*E. coli*) seem not to be reliable for monitoring viral contamination because of

- a lack of correlation between the presence of fecal indicators and enteric viruses;
- enteric viruses are naturally more resistant to disinfection; and
- viral particles transfer through soil and even filters are totally different from bacteria.

Bacteriophages, which are viruses that infect bacteria, and especially somatic and F-RNA coliphages, seem good candidates as they harbor the same structure and size, surface charges and to a less extent similar resistance toward treatments.

Heterotrophic Plate Count (HPC)

HPC bacteria represent microbes isolated by a particular method that may vary regarding growth medium, time, and temperature incubation. Other names are used such as ' total viable count,' 'total flora,' and 'autochtonous flora.' The detection method largely impacts the number of cultivable bacteria and the species types that are isolated.

The number of HPC bacteria in drinking water varies widely depending on the source water, the applied treatments, disinfection residuals, condition of storage (even in distribution system), etc and, of course, on the HPC method that is used.

Even if, "there is insufficient clinical evidence that the addition of a maximum limit on HPC populations would provide a higher level of public health protection" as stated by Allen *et al.*, HPC counts are useful tools to monitor disinfection efficiency, defining adequate storage and distribution conditions, monitoring bacterial regrowth and for natural mineral waters assessing the stability and protection of the resources.

Assessing Microbiological Risks

Principles and Examples

Risk assessment is the first component of the risk analysis process (recent information is available on the US Department of Agriculture guideline for food and water, 2012). It is based on a four-step framework which includes

- hazard identification,
- hazard characterization,
- exposure assessment, and
- risk characterization

as described in the 'Principles and Guidelines for the Conduct of Microbiological Risk Assessment, from Codex Alimentarius.

Quantitative Microbial Risk Assessment (QMRA) is now largely applied in drinking water field.

Identifying water-related microbial hazards consists of prioritizing for each waterborne pathogen the range of potential human health outcomes associated with exposure. The occurrence and severity of disease have to be considered and also the host characteristics (age, immune status).

Assessing exposure is a complex task. The amount of the targeted microorganism in the drinking water, the volume ingested, the frequency and duration of exposure (e.g., every day) have to be included. The survival of the germ in water and its resistance toward treatments and the inactivation rates are key components of exposure assessment.

Dose–response mathematical models are widely used to define the probability of developing an illness (or infection) depending on the concentration of the pathogen. Data from clinical studies using volunteers are sometimes available but can be limited depending on the germ. The two main models – exponential and beta-Poisson – define the probability of infection function of the dose–response curve and the number of ingested (or inhalated) pathogenic organism.

The last step, characterizing the risk, integrates all the previous data to have an estimation of the potential risk toward a population. Simulation using Monte-Carlo tools are always needed in order to include the distribution of probabilities among all situations from worst case scenario to realistic situations. Assumptions as percentage of infectious organisms, removal of the target organisms by water treatments, water intakes (usually 2 l per day per person) may have also an impact on the risk estimate.

Quantitative microbial risk assessment focuses mainly on exposure assessment and as such continuously evolves thanks to new epidemiological data, new detection methods, better knowledge on microbial survival and resistance to drinking water treatments. QMRA processes are involved in risk management strategies defined either by health authorities as well as by drinking water suppliers.

Water Safety Plans (WSPs)

The WSP approach is applicable throughout the whole water supply from catchment to the consumer to ensure safe drinking water. It has been largely described in the WHO Guidelines for Drinking Water Quality and relies on the classical principles of microbial risk assessment. WSP relies on five related steps.

- Health-based targets based on public health protection and disease prevention.
- System assessment to determine if the water supply chain (up to the point of consumption) as a whole can deliver water of a quality that meets the defined targets.
- Monitoring the steps in the supply chain that are of particular importance in securing safe drinking water.

- Management plans documenting the system assessment and monitoring extreme events, including documentation and communication.
- Systematic independent surveillance that verifies that the above are operating properly.

Besides risk assessment, WSP takes account of each specific drinking water supply including its operational design and monitoring. It should also contain emergency action plans to ensure that the water supply is safeguarded even in case of rare events.

Concluding Remarks

Microbial safety of drinking water remains a public health concern, not only in developing countries, and should remain as a priority for water and health community. Future challenges should address better monitoring of water resources, especially regarding climate changes and water scarcity, as well as better monitoring and prevention of rare contamination events.

See also: Foodborne Diseases: Overview of Biological Hazards and Foodborne Diseases; Overview of Chemical, Physical, and Other Significant Hazards. Public Health Measures: Modern Approach to Food Safety Management: An Overview

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SAFETY OF FOOD AND BEVERAGES

Soft Drinks and Fruit Juices

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Glossary

Brix The sugar content in aqueous solution. (One degree Brix is 1 g of sucrose in 100 g of solution and represents the strength of the solution as percentage by weight (% w/w). If the solution contains dissolved solids other than pure sucrose, then the °Bx only approximates the dissolved solid content.

Fruit juice The unfermented product obtained from fruit which is sound and ripe, fresh or preserved by chilling, of one or more kinds mixed together, having the characteristic color, flavor, and taste typical of the juice of the fruit from which it comes.

Soft drink A beverage that may be made from natural and artificial flavors and sweeteners, carbonated and noncarbonated water, vegetable and fruit juice extracts, and other ingredients, but which contains no alcohol.

Introduction

Perhaps the most widely consumed beverages in the world (outside of water) are soft drinks and fruit juices. These two categories of beverages include a variety of products but do not include alcoholic beverages (wine, spirits, beer, cider, etc.) and natural mineral and bottled drinking water, which are covered in other articles.

What Constitutes a Soft Drink?

For the purpose of this article, a soft drink is defined as water-based flavored drink usually with added carbon dioxide (CO_2) and with nutritive, nonnutritive, and/or intense sweeteners with other permitted food additives. This definition also includes gaseosa (water-based drinks with added CO_2, sweetener, and flavor), and sodas such as colas, pepper types, root beer, lemon–lime, and citrus types, both diet/light and regular types. These beverages may be clear, cloudy, or may contain particulate matter (e.g., fruit pieces). Soft drinks also include so-called 'energy' drinks that are carbonated and contain high levels of nutrients and other ingredients (e.g., caffeine, taurine, and carnitine).

Recipes for soft drinks can be formulated so that their composition is controlled and be maintained as a trade secret. Most recipes contain an extract, sugar, water, and various additives, such as acidity regulators, colors, and citric acid. Some additives are prohibited by specific legal regulation.

Categories of Soft Drinks

The most important categories of soft drinks are:

1. *Ready-to-drink essence-flavored beverages*: These are practically always carbonated. The essences used in them may be of natural or of synthetic origin and the sweetener used may be nutritive, nonnutritive, and/or intense.
2. *Ready-to-drink beverages containing fruit or fruit juice*: These can be made from fruit juice or concentrated fruit juice with the addition of sweetening agents, citric acid, and water. The normal juice content is not generally sufficient to impart sufficient flavor, and therefore, essences are added.
   a. This class is not therefore entirely distinct from class (1), as soft drinks are made with many differing ratios of fruit juice to essence. Ready-to-drink beverages can also be made by macerating whole fruit producing a fruit comminute. These beverages are composed of fruit juice and tissue with natural peel oil emulsion, thoroughly homogenized (i.e., with uniform dispersion of the solid matter throughout the drink).
   b. This class includes both carbonated and noncarbonated beverages. Similar in some ways to these are the so-called 'nonalcoholic wines' and other fermented beverages similar to alcoholic ones, but with a very low alcohol content.
3. *Concentrates of beverages intended for drinking after dilution with water at home*: These fountain syrups (e.g., cola syrup), fruit syrups for soft drinks, frozen or powdered concentrate for lemonade and iced tea mixes. These are noncarbonated and usually have a fruit base, though flavoring essences may be added as well. They include citrus 'squashes' containing fruit juice with cells, sugar syrup, essences, and citric acid, and intended to be diluted four or five times by volume before consumption. This also includes concentrated comminuted citrus drinks, similar to those described in class (2). This class also includes cordials containing noncitrus juices and flavored cordials, as well as syrups.

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The largest single ingredient of soft drinks is water, and their manufacture depends on a supply of safe, good quality water. In addition to the water and the fruit juice or flavoring, most soft drinks require sweetening agents. Nutritive, nonnutritive, and/or intense sweeteners can be used for this purpose. There is a certain demand, from diabetics and consumers wishing to achieve weight control, for soft drinks that contain nonnutritive sweeteners.

Soft drinks, *per se*, are not of great importance in international trade. Their bulk is large due to their water content, and most countries have their needs for soft drinks supplied by local manufacturers. Consequently, there is a considerable trade in concentrates, particularly those used for the cola-type drinks, fruit juice-based and comminuted fruit drinks. Similarly many countries import considerable quantities of fruit juices for manufacture into soft drinks. National trade statistics in general do not distinguish between these various classes.

The typical soft drink of the US is known as soda water. This phrase is to some extent, a misbranding, it is so called because originally the CO_2 gas with which it is carbonated was derived from the carbonate or the bicarbonate of soda. The name has become so firmly attached to waters of this kind that it probably will be accepted in the future as distinctive. Water is mixed with syrup which contains the sugar. A flavoring material is included to give it the character of the drink. For example, quinine is added to produce tonic water.

Manufacture of Soft Drinks

The most important element in the manufacture of a soft drink is the formulation of the syrup. The syrup is made to a predetermined strength using extracts, flavors, colors, sweeteners, and additives, such as preservatives, according to the recipe, which is usually considered a trade secret. As noted above, this syrup is made at a central location and is then shipped to distributors who add water in a fixed proportion to produce the drink. The drink is then is cooled, carbonated and filled into containers, the lid or cap is applied, sealed, check weighed, and packed off.

In addition to the fruity flavor, taste, and aroma imparted by the product, the worldwide acceptance of carbonated beverages is also due in part to the taste and sparkle imparted by CO_2. CO_2 derives from three sources, namely, fermentation, heating limestone, and burning carbon.

In the manufacture of carbonated drinks, CO_2 also destroys or inhibits growth of bacteria. This preservative action increases in line with the level of carbonation specified in the soft drink. In some beverages, such as ginger ale and tonic water, higher levels of carbonation are present so that the product can be used as a mixer with noncarbonated components, such as whiskey or gin.

The level of carbonation is normally described as the gas content or degree of carbonation or the volume(s) of CO_2. Depending on the form in which it is supplied and stored, the CO_2 should be sited convenient to its delivery area for reasons of safety and convenience. All the pipework in the case of liquid CO_2 must be able to withstand high pressure and be of stainless steel and preferably jointed seamlessly.

In typical practice, the syrup is pumped from the syrup tank across to a 'carbo cooler,' which is designed to produce a final product with a consistent brix and level of carbonation. To incorporate the CO_2 into the finished drink a large surface area of liquid is required and this will be reflected in the diameter of the carbonating vessel or container which must also be designed to withstand high pressure. In the carbocooler the chosen brix of a product is achieved by use of a specific orifice design to allow a small amount of syrup to be added to a designated quantity of water on a continuous basis. The resultant beverage is pumped across to a filler. In the filling of cans, the product is filled down the internal sides of the can allowing air to escape at the top of the filling valve. The cans are discharged from the filler and immediately enter the can seamer where the lid is seamed on to the can. As the can contains cooled beverage, it next passes into a can warmer to bring the product to ambient temperature. The can is then passed under an X-ray detection device to ensure correct fill. Finished cans are then conveyed to the packing unit for tray forming, can collation, shrink wrapping, and palletization.

Health – Negative Effects of Consumption

Because of the carbonation, soft drinks are generally considered safe from a microbiological perspective. In fact the World Health Organization recommends soft drinks when travelers are in locations where the quality of the drinking water is suspect. Most of the negative health effects associated with most soft drinks relate to their sugar content. Consumption of sugar has been shown to increase dental carries and excessive consumption of sugar has been linked to obesity. However, high-energy drinks have been associated with insomnia, headache, agitation, rapid heartbeat, and seizures. The US Substance Abuse and Mental Health Services Administration report found that the number of people who attended at the hospital emergency room after consuming energy drinks had more than doubled from just over 10 000 in 2007 to nearly 21 000 in 2011. In 42% of the cases, people reported consuming the energy drinks in combination with other drugs. Health Canada has proposed new regulations to limit the amount of caffeine in a single serving of an energy drink to 180 mg from more than 500 mg. A 237 ml cup of coffee contains about 135 mg of caffeine.

What Constitutes a Fruit Juice?

Fruit Juice is the unfermented but fermentable product obtained from fruit which is sound and ripe; fresh or preserved by chilling; of one or more kinds mixed together; and having the characteristic color, flavor, and taste typical of the juice of the fruit from which it comes. Fruit juices are expressed from fruits, which are among nature's most bountiful and healthy foods.

Fruits are pleasing to the senses of sight, taste, and smell. Their color, texture, and tartness add to their enjoyment as fruits. There are hundreds of different kinds of fruits. Some like apples and berries grow in temperate climates whereas others, such as citrus and exotic fruits, grow in tropical climates. Many

fruits have prominent roles in culture and religion and many have been used for their 'medicinal' value. This is not surprising because they are rich in health-sustaining vitamins and minerals, energy-giving fruit sugars, and health-protecting components such as antioxidants. With modern day technology, their juices give the same pleasure and benefits.

Categories of Fruits

Fruits can be divided into a number of main categories. For each category, a few examples of the possibilities are given below:

1. Citrus fruits: oranges, mandarins, grapefruit, lemons, and limes.
2. Soft fruits: strawberries; raspberries; gooseberries; blackberries; and white, red, and black currants.
3. Stone fruits: peaches, apricots, plums, and cherries.
4. Pome fruits: apples, pear, and quinces.
5. Exotic fruits: comprise a disparate group of fruits which do not form part of the other main categories including bananas, pineapples, pomegranates, melons, mango, kiwis, passion fruits, and figs.
6. Mixed fruit juices: combinations of the above. Note that rhubarb though a vegetable is often blended with fruit juices.

Primary Processing of Single Strength Juice

During processing, the juice is separated from flavor components, pulp and cells, but these may be restored to the juice in the final formulation. In the case of citrus fruits, the fruit juice must come from the interior fleshy portion (endocarp). Lime juice, however, may be obtained from the whole fruit, by suitable production processes whereby the proportion of constituents of the outer part of the fruit is reduced to a minimum.

Fruit juices may be considered in various categories depending on market requirements. For example, fruit from which the juice is extracted just before consumption is the only juice that may be classified as both pure and fresh. In the EU, only physical treatments are allowed for this juice. In the case of freshly squeezed juice, chilling is optional but is not regarded as a process. All other juices undergo processing to a greater or lesser extent.

Primary Processing of Concentrated Juice

Fruit juice from concentrate may be defined as the product obtained by replacing the water in the concentrated fruit juice that was extracted from that juice during concentration. If appropriate, pulp and cells that were removed during the process of producing the fruit juice, may be added back. In the reconstitution process, the water used must possess appropriate characteristics, particularly from the chemical, microbiological, and organoleptic viewpoints, to guarantee the safety and essential qualities of the juice. The final product obtained must display the basic organoleptic and analytical characteristics at least equivalent to those of its fresh counterpart.

Primary Processing of Nectars

Fruit nectar is the unfermented but fermentable product obtained by adding water with or without the addition of sugar, honey, syrups, and/or sweeteners to fruit juice, concentrated fruit juice, fruit purees or concentrated fruit purees, or a mixture of those products. Aromatic substances, volatile flavor components, pulp, and cells, all of which must have been recovered from the same kind of fruit and obtained by suitable physical means, may be added. Products may be based on a single fruit or fruit blend. Fruit nectars typically contain 25% to 50% fruit. Under the requirements of Annex IV of the EU Fruit Juice Directive, the addition of sugars and/or honey is permitted up to 20% of the total weight of the finished product. Where fruit nectars are manufactured without added sugar or with low energy value, sugars may be replaced wholly or partially by sweeteners, in accordance with Directive 94/35/EC of the European Parliament and of the Council of 30 June 1994 on sweeteners for use in foodstuffs.

Secondary Packing of Juice and Juice Products

Juices may be packed in cartons, polyethylene terephthalate (PET) bottles, glass, or cans. The carton, however, dominates the market worldwide and is based on the form, fill, and seal principle. Juices packed in the carton system are generally packed aseptically. The carton consists of a seven layer wall with polyethylene on the inside in contact with the juice. There normally are few issues with regard to shelf life. The biggest offender is damage caused by handling abuse.

Tetra Pak and Elopak are two of the major suppliers of form, fill, and seal packaging systems. To pack juices using either of these systems the heat exchanger and filler are presterilized. Juice is subsequently pasteurized in the presterilized heat exchanger and fed directly into the presterilized filler. Asepsis is maintained throughout the system.

A typical juice from concentrate preprocessing line consists of a drum dumper feeding juice concentrate to a juice/water proportioner automatic in line brix controller, juice tankage in which the juice is thoroughly mixed; pumped or gravity fed to a pasteurizer; then optionally fed to a deaerator; and from there it is fed to the carton packing machine.

The individual cartons are conveyed from the filler to case packers or in the case of smaller cartons to the drinking stray applicator, multicarton, packer. The larger 1 l packs go directly to the case packers where they are subsequently shrink wrapped and placed on pallets for despatch to warehousing.

Forms Under which Juice is Marketed

Citrus fruit juice and in particular orange juice dominates the fruit juice market. According to the United Nations Commodity Trade Statistics Database, international trade in orange juice exceeds US$16 billion a year. This trade involves orange juice in various forms. Single strength orange juice should have a brix value of at least 10% (or 10°Bx) measured at the time of picking. This juice is known as 'Not from Concentrate (NFC)' in the trade because it has not been concentrated and reconstituted with water. NFC juice may be pasteurized, chilled, or frozen before shipment to the packing house.

Juice from concentrate, however, is initially concentrated by evaporating the water resulting in a concentrate of 60° or 65°Bx. Concentration may be achieved using a thermally accelerated short time evaporator (TASTE). This type of evaporator is mainly used in the large citrus plantations of Brazil and USA. An important parameter in regard to process fruit juice is the degrees brix, which is the sugar content of in the aqueous portion of the juice. One degree brix is equivalent to 1 g of sucrose in 100 g of juice. If the solution contains dissolved solids other than pure sucrose, then the degrees brix only approximates the dissolved solid content.

Unlike NFC, citrus juice concentrate has a minimum value of 11.2°Bx because the concentrate plants mix concentrates from early, mid, and late seasons together to give the concentrate and subsequently its reconstituted juice the flavor, texture, and taste that meet the preferences of the market that the company is targeting. Certain food additives may be used to enhance the juice. For example, ascorbic or citric acid may be added to that which is naturally present in orange juice to give it more tartness. Ascorbic acid may also be added to improve the vitamin C content. Other nutrients, such as calcium, vitamin D, and omega-3 fatty acids may also be added. In addition, the producers of reconstituted orange juice may add a mixture of compounds derived from orange peels to give the juice the aroma of fresh-squeezed juice as these are lost during the concentration process.

Health – Benefits of Juice Products

As the consumption of fruits and vegetables have been shown in many studies to be associated with reduced risk of many cancer types, stroke, and Alzheimer's disease, the consumption of fruit juices has increased significantly in recent years. Most consumers believe that the health benefits of fruit juice are equal to fresh fruit, but some nutritionists have noted that juice lacks fiber of fresh fruit.

Health – Negative Effects of Consumption

Juice may naturally contain high levels of sugar, which poses the same risks of dental carries and obesity as refined sugar. In this regard, juices may contain added sugars, including high-fructose corn syrup.

Fruits can frequently be contaminated with biological hazards, such as viruses and bacteria. As a consequence, processed fruit juices are pasteurized to minimize this potential problem. However, fresh-squeezed juices are typically not and may present a health risk unless the fruit is decontaminated and sanitation of the equipment is maintained. In addition, fruit can contain residues of pesticides, which may be transferred to the juice. Controls should be directed at the source of the fruit to assure that levels do not exceed those allowed by regulation.

See also: Safety of Food and Beverages: Alcoholic Beverages; Fruits and Vegetables; Water (Bottled Water, Drinking Water) and Ice

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Relevant Website

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SAFETY OF FOOD AND BEVERAGES

Alcoholic Beverages

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Glossary

Alcohol by volume (ABV) The percent of alcohol in a beverage based on the volume of alcohol present.
Bead Size of bubbles.
Brut Very common sparkling wine term – literally 'dry' but in practice not bone dry.
Cru Vinyard.
Distillation The technique of extracting alcohol from a fermented liquid by heating it and condensing the vapor, which is enriched in alcohol.
Fermentation yeasts The microorganisms used to convert sugar into alcohol and carbon dioxide.
Fusel oils The undesirable higher order alcohols and related compounds formed during the fermentation.
Malt The product produced when grain, usually barley, has been stimulated artificially into germination and then halted by drying.
Mash The sweet liquid after hot water has been flushed through the grist.
Nose The aroma of a wine or spirit.
Rose A wine made by blending red and white wines.

Introduction

The production of alcoholic (ethanol containing) beverages, namely, beers, wines, and spirits has provided mankind with sustenance and refreshment since prehistoric times. The experience of drinking alcoholic beverages is made all the more satisfying when consumed with foods. There has always been a close relationship with bread making and fermentation of alcoholic drinks owing to their common ingredients, namely, grain, water, and yeast. The Sumerians baked barley loaves called bappir that could be either eaten as bread or mixed with malted barley to form a mash for brewing. Some have speculated that bread and beer increased prosperity to a level that allowed time for development of other technology and contributed to the building of civilizations.

As with the discovery of other foods, it can be assumed that alcoholic beverages were discovered by accident. Indeed, from the earliest times the art of preparing alcoholic beverages depended on local ingredients, such as grapes, barley, or honey, and on traditional fermentation methods. Over the centuries these beverages became rooted in the physical environment and culture. In Europe, for example, some vineyards have been cultivated for over 2000 years. In many cases, the great traditional beers, wines, and spirits have assumed national identities that draw their characters from the melding of environmental and cultural landscapes.

In achieving an appreciation of beers, wines, and spirits, it is necessary to understand the industrial processes and technology involved in their manufacture. Of obvious importance is the alcohol content. The percent of alcohol by volume (ABV) is now the harmonized measurement for alcoholic content. In the USA, the strength of a spirit was previously expressed as 'proof,' which was equivalent to 7/4 the alcohol content. According to US Federal regulations, the required labeling of alcohol content of wines and spirits is now ABV. However, the alcohol content of malt beverages, including beer, is optional unless required by State regulations.

Beer

Beers are alcoholic beverages brewed from germinated barley (malt), hops, yeast, and water. Examples include ale, brown beer, weiss beer, pilsner, lager beer, oud bruin beer, Obergariges Einfachbier, light beer, table beer, malt liquor, porter, stout, and barleywine. Beer is truly international probably because it is the oldest alcoholic beverage. Almost any substance containing sugar can naturally undergo alcoholic fermentation. It is likely that many cultures, on observing that a sweet liquid could be obtained from a source of starch, independently invented beer.

Historical Development of Beer

Beer is one of the world's oldest prepared beverages, possibly dating back to approximately 9500 BC, when cereals such as barley and wheat were first grown. Beer production is recorded in the written history of ancient Egypt and Mesopotamia. The available evidence suggests that barley beer or cervisia was made and consumed over many millennia. Barley beer was

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initially made domestically by women who were described as 'brewsters.' Besides sources of nutrients, beer might have also been purer than water in some regions.

The Ebla Tablets, discovered in 1974 in Ebla, Syria, and dating back to 2500 BC, reveal that the city produced a range of beers, including one that appears to be named 'Ebla' after the city. A fermented beverage using rice and fruit was made in China approximately 7000 BC. Barley beer, however, is not the true ancestor of the beer which is consumed today. The difference between barley beer and beer brewed from the fifteenth century is that what is today referred to as beer contains hops as an integral ingredient in the recipe.

Some of the earliest Sumerian writings found in the north African/Middle East region contain references to a type of beer; one such example, a prayer to the goddess Ninkasi known as 'The Hymn to Ninkasi,' served as both a prayer and a method of remembering the recipe for beer in a culture with few literate people. Much later monks in monasteries took up the brewing of beers and some monasteries continue that tradition today.

In 1516, William IV, Duke of Bavaria, adopted Reinheitsgebot (purity law), perhaps the oldest food-quality regulation still in use in the twenty-first century, according to which the only allowed ingredients of beer are water, hops, and barley malt. The development of thermometers and hydrometers changed brewing by allowing the brewer more control of the process and greater knowledge of the results.

It is interesting to note that in the case of Guinness, which was founded in 1759 that the head brewer did not have a scientific qualification until 1902. The art of brewing had been mastered by thousands of brew masters down the centuries and that art had been intuitively crafted to produce excellent beers since the dawn of civilization. Although the chemistry and microbiology were not articulated in modern scientific terms, the brewers had always followed a method that enabled them to achieve the required result time and time again. It was not until the mid-nineteenth century that the work of Louis Pasteur unlocked the secrets of fermentation.

Brewing of Beer

Brewing is the process by which beer is made from the recipe ingredients including malted barley, water, hops, and yeast. Initially the wort is made by mixing the crushed malted barley, also known as grist, in a mash tun with hot water, also known as liquor. This takes approximately 2 h. In a process called 'sparging,' the sweet wort is separated from the grist or grains by washing and collecting the maximum wort. In separating the wort from the grains, the grains themselves form the filter bed and this part of the process is called 'lautering.' Filter frames may also be used depending on how many 'runnings' are needed to give paler beers.

The sweet wort is then transferred to a kettle known as the 'copper' and boiled for approximately an hour. Hops are then added as a source of bitterness, flavor, and aroma. The longer the wort containing the hops is boiled the greater the bitterness but the less flavor and aroma because these volatiles are flashed off.

The hopped wort is then cooled and is ready for the addition of yeast. The yeast ferments the hopped sweet wort and produces beer after approximately a week. The length of time may be longer depending on the type of yeast used and the strength of beer required. During this time fine particulate matter and the yeast in the wort settles out leaving a clear beer. A secondary fermentation may be undertaken depending on the storage requirements, whether stored in casks or kegs, cans, or bottles. In addition to barley, other sources of starch, maize or rice may be used and these are termed adjuncts.

The chemical quality of the water gives beers a distinctive character. Hard water suits the production of stouts, such as Guinness, whereas soft water in Plzen suits the production of pale lagers, such as Pilsner Urquell. The initial stages of beer production are similar to spirits production. Different roasting times and temperatures are used to produce different colors of malt from the same grain.

Brewer's yeast is mainly *Saccharomyces cerevisiae*, which is a top-fermenting yeast. However, *Saccharomyces uvarum* is a bottom-fermenting yeast used in the production of lagers and grows best at lower temperatures.

Brewers may add clarifying agents to beer to give the beer a bright and clean appearance. Particulate matter and proteins are precipitated out, removing the cloudy appearance, especially in beers made from wheat.

Although there are many types of beer brewed, the basics of brewing beer are shared across national and cultural boundaries. The traditional European brewing regions – Germany, Belgium, England, and the Czech Republic – have local varieties of beer. Top-fermented beers are most commonly produced using *S. cerevisiae* at temperatures between 15 and 23 °C. At these temperatures, the yeast produces significant amounts of esters and other flavor and aroma products, and the result is often a beer with a slight 'fruity' taste.

'Pale Ale' is a beer, which uses a top-fermenting yeast and a predominantly pale malt. It is one of the world's major beer styles. Mild ale has a predominantly malty palate. It is usually dark colored with an alcohol content of 3.0–3.6% ABV, although there are lighter hued milds as well as stronger examples reaching 6% ABV.

Lager is the English name for cool fermenting beers of Central European origin. Pale lagers are the most commonly consumed beers in the world. The name 'lager' comes from the German 'lagern' for 'to store,' as brewers around Bavaria stored beer in cool cellars and caves during the warm summer months. These brewers noticed that the beers continued to ferment and to also clear off sediment when stored in cool conditions.

Lager yeast is a cool bottom-fermenting yeast *S. uvarum* and typically undergoes primary fermentation at 7–12 °C and then is given a long secondary fermentation at 0–4 °C (called the lagering phase). During the secondary stage, the lager clears and mellows. The cooler conditions also inhibit the natural production of esters and other byproducts, resulting in a cleaner tasting beer.

Assessment of Beer

Bitterness of Beer

Beer is measured and assessed by its bitterness, strength, and color. Bitterness is due to the inclusion of hops in the recipe.

Hops are used as a flavor and as a preservation agent by eliminating undesirable bacteria. The bitterness balances the sweetness of the malt and aids in the retention of the head in a poured glass of beer. Perceived bitterness is measured by the International Bitterness Unit, defined in cooperation between the American Society of Brewing Chemists and the European Brewing Convention.

Color of Beer

Beer color is determined by the quality of the malt and its roasting. The most common color is a pale amber produced from using pale malts. Pale lager and pale ale are terms used for beers made from malt dried with coke. Coke was first used for roasting malt in 1642, but it was not until approximately1703 that the term pale ale was used.

Strength of Beer

Beer ranges from less than 3% ABV to approximately 14% ABV. The alcohol content of beer varies by local practice or beer style. The pale lagers that most consumers are familiar with fall in the range of 4–6% ABV. Some beers, such as table beers, are of low alcohol content (1–4% ABV). Exceptionally strong beers with very high alcohol content are also made.

Consumption – Positive and Negative Effects on Health

The moderate consumption of alcohol, including beer, is associated with a decreased risk of cardiac disease, stroke, and cognitive decline, including accidental injuries. However, excessive consumption can lead to addiction, cancer of various organs, neurologic problems, social problems, diseases of the liver, including alcoholic hepatitis and cirrhosis. In the USA, an estimated 80 000 deaths are caused by excessive alcohol consumption with an estimated cost of US$223.5 billion in 2006.

In particular, pregnant women or women who are trying to become pregnant should not drink any alcohol. Alcohol can cause miscarriage and stillbirth among pregnant women, and a combination of physical and mental birth defects among children that last throughout life. In addition, any person who is driving, planning to drive, or participating in other activities requiring skill, coordination, and alertness should avoid alcohol. Also people who have certain medical conditions or who are taking medications should also avoid alcohol.

Brewer yeast is known to be a rich source of nutrients; therefore, as expected, beer can contain significant amounts of nutrients, including magnesium, selenium, potassium, phosphorus, biotin, chromium, and B vitamins.

Wine

Wines are alcoholic beverages obtained exclusively from the partial or complete alcoholic fermentation of fresh grapes, whether crushed or not, or grape must. Wines can also be made from fruits other than grapes such as apples and pears and from other agricultural products, including grain as in the case of sake made from rice. However, the production of wine from grapes is by far the largest. Consequently, this section only addresses wine made from grapes. Finished bottled wine can be still or sparkling and can be red, white, or rose. The color of red wine is due to contact with grape skin, which also enriches the wine with tannins and antioxidants.

Varieties

The common varieties of the European species *Vitis vinifera* are Pinot Noir, Chardonnay, Cabernet Sauvignon, Gamay, and Merlot. When one of these varieties used is the predominant grape (usually at a level of 75–85%), the resultant wine is said to be a 'varietal' as opposed to a 'blended' wine. Blended wines are not considered inferior to varietal wines but they utilize a different style of winemaking; some of the world's most highly regarded wines that come from regions such as Bordeaux or the Rhone Valley are blended from different grape varieties.

Wines are made from a single variety or from a blend of varieties. In 'Old World' countries, wine laws prescribe and proscribe certain varieties for each of their designated quality wine areas based on what grows best there. Consequently, only certain grape varieties or blends of grapes may be used to produce the wine in a particular area with its specific appellation control.

Sometimes flavor differences in freshly picked grapes, such as Reisling or Gewurztraminer, are more pronounced in the resultant wines. Some varieties perform best when used as single varieties, such as Burgundy, Pinot Noir, and Chardonnay. Most varieties are ensemble players and are usually found in blends principally Cabernet Sauvignon, Merlot, and Cabernet Franc.

In the 'New World,' wine production rules such as these are almost nonexistent. The advantage of this system is that the winemaker is free to experiment, but it could also be the case that little-known grapes such as Gros and Petit Manseng, which are used to make the traditional and little-known wine of Jurancon, in France, could disappear. Some form of appellation control is desirable irrespective of the country of origin.

It should be noted that most varieties of European grapes have been grafted onto the North American species' rootstock due to their resistance to phylloxera, a root louse that eventually kills the vine plants. In the late nineteenth century, most of Europe's vineyards were destroyed by this infestation.

Soils

Grapes grow well in a variety of soils such as gravel, granite, chalk, and slate to mention but a few. A large number of vineyards are situated on the sides of river valleys in well-drained gravel deposits ('graves' means gravel). Some vines grow better in poor well-drained soils. This makes the vine roots extend deeper to source water, trace elements, and other plant nutrients. The Bordeaux region has gravelly soils, which are responsible for the fame of the wines of this region and it particularly suits growth of the Cabernet Sauvignon grape variety. Other soil types that underlay the gravel can also have an effect. Where the soil is clay, the wine will have less acidity than it would if it were limestone, for example.

Châteauneuf-du-Pape and Tavel rose are produced in the southern Rhone in granite country. This area is littered with

'pudding stones' making cultivation almost impossible. Once the stock has taken root, the granite stones reflect heat back onto the vine. This results in the production of big, high in alcohol, reds, and France's most famous rose. In Beaujolais, granite suits the Gamay variety in which its chemical properties reduce the wine's natural acidity.

Slate is another soil type and is typical of the alluvial deposits on the banks of the Rhine and Mosel rivers, which suit some vines in particular. This soil is responsible for the delicate fragrance of the fruity local wines. The great advantage of slate is that it retains heat during the colder days, which compensates for the cooler day temperatures in which the grapes have to ripen.

Another soil type is chalk, which provides very good drainage. This alkaline soil suits predominantly white grape varieties. The Chardonnay forms part of the inimitable blend for Champagne. Grapes grown on this type of soil are characteristic for their acidity and this is what links the Champagne, Chablis, and Sancerre.

Winemaking

Winemaking can be a simple operation. The grape is the basis of the global wine industry, although there are separate categories of wines made from fruits such as apples. All the ingredients necessary to make wine are contained in or on the surface of grapes, namely, the fruit sugars and the yeast. If grapes are crushed in a vessel, after a few days in a warm place the mixture will turn into an alcoholic liquid. This process called fermentation is due to the action of the yeasts. There are millions of yeast cells on the skins of grapes, which can ferment the natural sugars in the grapes. Although the resultant liquid can be described as wine, it may not look or taste good. Open vat fermentation is an unpredictable process, which can produce aldehydes and ketones that can taste bad and cause adverse reactions. To deal with this problem, the Romans often sweetened their wine with lead acetate, which had even more serious health effects.

Winemaking can also be complex when one considers the variety of grapes available, the viticulture, soil types, and climate. Soil types must have the ability to retain or reflect heat as well as providing nutrients for growth of the vines. Harvesting, fermenting, and maturing practices are also extremely important in producing a fine wine.

In winemaking, there are several stages commencing with the picking operation in the vineyard. Picking, sorting, stem removal is undertaken before transfer to fermentation vats. At this point, the type of wine to be made, that is, red or white, will determine whether skins are separated or allowed to remain in contact with the wine as is the case for making red wine. Cultured yeasts may be added in dried form to aid fermentation, although normally wild yeast either from the vineyard or the winery itself is sufficient to enable the fermentation to proceed to success. If cultured yeast is used, the wine is first treated with sodium sulfite to destroy the wild yeast.

As with all raw materials, however, the quality of the grapes is extremely important. In the vineyard the grapes on the vine must be picked when they have reached the optimal stage of ripeness. Grapes that are not ripe enough, or are overripe will affect the quality of the resultant wine. The grapes as they are harvested contain the potential of the wine. You can make a bad wine from good grapes, but not a good wine from bad grapes.

Harvesting of Grapes

Depending on the nature and size of the vineyard, the picking operation will normally be done by either hand or machine, ideally in good weather conditions. In the case of hand picking, the pickers pick bunches of grapes that are loaded into bins for transfer to the winery. In the case of mechanically harvested grapes, the operation results in individual grapes being harvested. Increasingly, grapes are being machine harvested. This is more cost-effective, and in warm regions quality can be preserved by picking at night, when it is cooler for improved quality. Hand-picked or machine-harvested grapes are sorted for quality. Sorting grapes enables rotten or 'raisined' grapes, leaves, and petioles to be removed. Sorted grapes go to the de-stemmer, which removes the stems. They may then be crushed.

Differences in Making Reds, Whites and Rose

This is where red winemaking differs from whites. Red wines are pressed and fermented in their skins, whereas white wines are pressed, separating juice from skins, before fermentation. The fermentation vessel may be a shallow stone lagar. The grapes are foot trodden, so that the juice can extract color and other components from the skins or in other wineries the grapes are loaded and taken by conveyor to a tank, from where they are being pumped into the fermentation vessel depending on whether the winery uses traditional methods. Fermentation begins naturally after the grapes have been foot trodden. Skins and juice are mixed by hand and this process is repeated many times a day to help with extraction, and also to prevent bacteria from growing on the cap of grape skins that naturally would float to the surface.

Sometimes red grapes are fermented in stainless steel tanks. During fermentation, carbon dioxide is released so the surface can be exposed. On other occasions, however, fermentation takes place in vented closed tanks to facilitate carbon dioxide escape.

Smaller tanks are used in which the skins can be punched down using a robotic cap plunger. In some wineries this is done by hand, using poles. An alternative to punch downs is to pump wine from the bottom of the tank back over the skins. Here, fermenting red wine is being pumped out of the tank, and then pumped back in again. The idea is to introduce oxygen in the wine to help the yeasts in their growth. At other stages in winemaking, care is taken to protect wine from oxygen, but at this stage it is needed. White wines are fermented in stainless steel containers.

Once fermentation has finished, most red wines are then moved to barrels to complete their maturation. Barrels come in all shapes and sizes. The most common size is 225–250 l. The source of the oak, and whether or not the barrel has been used previously, is important in the effect it has on the developing wine. Sometimes much larger, older oak barrels are utilized, imparting virtually no oak character to the wine. This suits some wine styles better than smaller barrels.

Once fermentation has completed and the young wine has been drained of the skins, the remaining skins and stems are pressed to extract the last of the wine that they contain. This is termed a bladder press, used for some reds and almost all whites. A large bladder fills with air, pressing the contents gently and evenly, with gradually increasing pressure. In smaller wineries, basket presses are employed. The marc is what is left at the end and is usually used to make compost. Red wines are generally matured in oak barrels.

As the wine industry developed, the science of enology came to the fore and which in turn augments the art of winemaking. Enologists are trained to classify wines by appearance, taste, and aroma. Once the wine has fermented and matured for its specified period of time, an enologist will sample it to determine if it is ready for bottling. After a wine is bottled and labeled, it is then aged for a designated period of time to reach its peak taste. Certain wines may be fine to sell immediately, whereas others are aged for a considerable period of time. Enology is generally concerned with winemaking and it can also include wine marketing. Enologists often meet with buyers to promote their wines. Those who work in the marketing aspect also arrange wine tastings for potential customers.

Vintage Wine

A 'vintage wine' is made from exceptional quality grapes that were grown in a particular year, and labeled as such. Some countries allow a recognized vintage wine to include a small portion that is not from the vintage year. Variations in a wine's character can include subtle differences in color, palate, nose or bouquet, body, and development. High-quality wines can improve in flavor with age if correctly stored. It is not unusual for wine enthusiasts and traders to save bottles of an especially good vintage wine for future consumption. For consistency, some nonvintage wines can be blended from more than one vintage to assist winemakers sustain a reliable market image and maintain sales even in nonvintage years.

Positive and Negative Effects on Health

Consumption of wine, especially red wine, is good because it is rich in antioxidants. The main active ingredient of wine is alcohol and therefore, the health effects of alcohol mentioned in the above section Consumption – Positive and Negative Effects on Health for beer also apply to wine.

White wines have been adulterated with ethylene glycol, a toxic chemical found in antifreeze. High levels of lead were found on wine that had lead casks covering the cork. Although the industry no longer uses lead, old bottles of wine with lead casks should be carefully cleaned before removing the cork.

In 2008, researchers in the UK reported finding high levels of toxic metals in wine. The sources of the contamination was not identified, but the researchers called for a study of wine production, including grape variety, soil type, geographic region, insecticides, containment vessels and equipment, and seasonal variations that may have an impact on metal ion uptake.

Beneficial cardiovascular effects from the consumption of one glass of red wine (but not white wine) have been reported. However, a recent study of beer consumption indicated that moderate consumption of beer resulted in a similar decline in cardiovascular risk as red wine. This raises questions about the nature of the beneficial agent because wine and beer are quite different in composition.

Spirits

Spirits includes all distilled spirituous beverages derived from grain (e.g., corn, barley, rye, and wheat), tubers (e.g., potato), fruit (e.g., grapes and berries), or sugar cane that contain greater than 15% alcohol. Spirits are produced in many countries and are often referred to as the national drink. Examples include bagaceira belha from Portugal made from pressed skins, seeds, and stalks of the grapes; grappa from Italian made from the residues of pressed wine; schnapps from Germany usually derived from rye; mistelle from France; jeropico from South Africa; ouzo from Greek and flavored with aniseed; rum from the Caribbean; and cachaça from Brazil made from fermented distilled sugar cane juice; vodka from Russia made from potatoes; and whiskey from Ireland and Scotland derived from barley. Whiskey is an excellent example of a spirit beverage in that it encompasses all the processes necessary to make alcohol, all of which need not necessarily occur on the same manufacturing site. Whiskey undergoes several processes such as malting, mashing, fermentation, distillation, maturation, and blending in its manufacture. Consequently, the following relates to the production of whiskey because it would not be possible to discuss every spirit.

History of Distillation

Distillation of fermented brews for alcoholic consumption is thought to have been introduced to Ireland and Scotland in the sixth century by missionary monks returning from the Mesopotamia regions of the Middle East and Egypt where they observed the distillation of perfume using the Alembic Still. The monks modified the Alembic Still to create a Pot Still designed to make 'aqua vita' (Latin for water of life), which is known today as whiskey. The first written record of whiskey comes from 1405 in Ireland. The production of whiskey from malted barley is first mentioned in Scotland in an entry on the 1494 Exchequer Rolls, which reads "Eight bolls of malt to Friar John Cor, by order of the King, wherewith to make aqua vita." The first license to produce whiskey was granted to Bushmills distillery in 1608 in the reign of James I of England. The Pot Still, which is basically a batch still, was used until continuous stills were introduced in the nineteenth century to increase economies of scale and meet commercial demand. The monks in other European countries created many different spirits, such as gin, brandy, vodka, and rum, as well as spirit-fortified wines, beers, and stout/porter, which survive in production to this day.

Barley as a Crop

Barley is a cereal crop that grows well in the more temperate regions of the world. It is one of the most widely distributed of all cereals. Barley grows well in cool and semi-arid climates and on fertile well-drained soils. It prefers higher soil pH but is

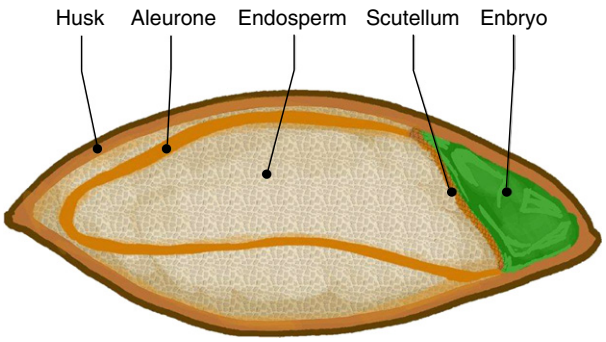

Figure 1 The mature barley grain.

most susceptible to fungal attack of all the cereals. There are two types of barley, two-rowed and six-rowed, with the six-rowed the most commonly grown. Barley can form part of a crop rotation, sometimes after a cultivated crop such as potatoes or sugar beet. Barley also makes an excellent cover crop planted with a legume to help with weed control. The requirements, however, for malting barley are very ridged, and special management and environmental control are necessary for the production of good malting barley.

Barley as Raw Material

In the normal crop life cycle of barley, as the temperature rises barley seeds are planted and over time the crop sprouts, develops roots, and grows to maturity. In the production of alcohol, man learned how to simulate the germination of barley under controlled but artificial conditions to make alcohol.

To appreciate the process of germination, it is essential to understand the nature and composition of the barley kernel (Figure 1). Barley, after harvesting, is dried to approximately 14% moisture so that it can be stored without risk of deterioration until it is required for use.

The mature barley grain comprises the embryo and endosperm, which provides a store of carbohydrates, mainly starch and protein to support the initial growth of the germinating embryo. The endosperm is a nonliving storage tissue, surrounded by a living nonstarch cell layer called aleurone. The grain is protected by an outer husk.

A grain or kernel of barley contains within it two main parts, namely, the embryo and endosperm. The embryo is the most important part of the grain as it contains all the organs necessary to enable the kernel to germinate and develop into a plant. The embryo is made up of three parts, the cotyledon or seedleaf, epicotyl or shoot, and radicle or root. The barley kernel is classified as a monocot because it consists of only one cotyledon. The endosperm contains the food source needed for germination by the embryo, that is, starch and protein.

Starch exists in two forms in the endosperm, amylose and amylopectin. The following enzymes degrade the starch into smaller polysaccharides: α-amalyse, β-amalyse, α-glucosidase, and phosphorylase.

The most significant enzymes are α-amalyse and β-amalyse. α-Amalyse is synthesized once germination begins, whereas β-amalyse is present in the grain in an inactive form, which subsequently becomes active during germination. α-Amalyse is involved in modifying the starch, cleaving it into polysaccharides known as dextrins. Maltose is produced by the action of β-amalyse on the starch.

Malting

Malting begins by soaking the barley in water. In this process, the raw barley is transferred into tanks filled with sufficient warm water where it remains for 2–3 days soaking up the water. The source of the water in this and subsequent stages is very important. The mineral and chemical properties of the water ultimately help to define the nature of the whiskey. Soft water is best. Soaked barley is then transferred to a huge room where it is spread out evenly on the floor over the heating source. This floor has ducts in it through which the air is circulated sometimes with the assistance of a fan. It is thus aerated and held at approximately 15 °C. It is regularly turned to prevent the temperature from rising too high and to ensure even germination. The grain continues to sprout but turning prevents the grain from putting down roots. This continues for approximately 5 days or so. At this stage it is termed 'green' malt. When the sprouts have reached a certain length, it is ready for drying and is transferred to a malt kiln. Drying stops germination due to denaturing the β-amalyse enzyme.

Mashing

When the malt is dried, it is then ground by rollers to form grist. The grist is then transferred to the mash tun, a large tank, and hot water added to dissolve the sugars and starches to produce a sweet liquid called wort. The wort is then drawn off, and this process cycle is repeated two more times. The water from the third mashing produces 'watery' wort, which is used to begin the mashing of the next batch. The grist itself, once it has gone through this process three times is removed from the mash tun and sold to farmers as a nutritious cattle feed.

Fermentation

Once the liquid wort has cooled, yeast is added to convert the sugars into alcohol. This is called the fermentation process. Yeast, similar to most organisms, can break down monosaccharides, such as glucose and fructose, and disaccharides, such as sucrose and maltose, because they are water soluble. It also develops other enzymes, such as proteases, which break down the proteins in the grain into forms that can be used by yeast. When fermentation is completed, a liquid called wash is produced, ready for distilling. It has an alcoholic content approximately the same as beer.

Distilliation

The alcohol from the fermented liquid is separated by boiling the wash in a large copper pot still or continuous still. The shape and size of the still determines the final character of the whiskey. Alcohol boils or vaporizes at a lower temperature than water and so is drawn off first leaving much of the water and other impurities behind. Although the resulting spirit is

much purer and higher in alcohol, the process is repeated a second time before the 'middle cut' or final spirit is collected by the operator of the still who decides on the exact moment to begin and end drawing off the precious 'middle cut.' At this stage, typically the colorless spirit is approximately 70% ABV.

Aging or Maturation in Casks

The spirit from the distillation process is diluted with water, invariably from the same source used in earlier stages, until its alcohol content is approximately 64%, then decanted into casks to begin the long, slow process of aging. Unlike wine, whiskey does not continue to mature in the bottle. The alcohol commences to draw flavors and color from the casks, which are made of oak. How it does this is not fully understood but over a minimum of 3 years, the colorless alcohol becomes a golden amber color and the taste becomes mellow. During this aging process/maturation, approximately 2% of the whiskey evaporates. This suggests that the oak casks or barrels 'breathe' and this loss of alcohol is termed the 'Angels share.'

In Ireland, the casks used have already been used in the making of sherry, although other casks used in winemaking are more recently being used as the distillers create new whiskeys to differentiate the market. In Scotland, the distillers use American oak casks that have already been used in the making of bourbon. The US law expressly requires several types of American whiskey, especially all American whiskey labeled as malt, rye malt, rye, wheat, bourbon, or straight whiskey to be aged in new oak casks. The selection of casks can affect the character of the final whiskey. To ensure continuity of supply of used oak casks, some Scottish distilling groups own oak forests in the USA and rent the new barrels to the US Bourbon producers for first fill use. Bourbon casks impart a characteristic vanilla flavor to the whiskey.

The spirit must be aged for at least 3 years in Ireland and in Scotland before it can be called whiskey. However, most distillers allow much longer times and part of the reason these whiskeys cost more is that they lose more of the 'Angels share' over time.

Whiskey may be produced as a single-malt whiskey or as a blend of different malt whiskeys. Single-malt whiskeys are generally made using pot stills and blends may combine the output of two or more different pot stills or by using continuous distillation. Single malts are typically associated with single-malt Scotch, although they are also produced in various other countries. Under the UK Scotch Whisky Regulations, a 'Single-Malt Scotch Whisky' must be made exclusively from malted barley, must be distilled using a pot still, and must be aged for at least 3 years in oak casks of a capacity not exceeding 700 l (150 imperial gallons; 180 US gallons).

Negative Effects of Over-Consumption of Whiskey

Because whiskey contains a large amount of alcohol, the health effects are a particular concern.

Whiskeys may also contain fusel oils. The term fusel comes from the German meaning 'bad liquor' or 'potato oil' in Europe. These are higher order alcohols, that is, alcohols with more than two carbon atoms, formed during fermentation. These fusel oils can also be present in other fermentations, such as cider, mead, beer, wine, and other spirits to varying degrees.

Alcoholic beverages can also contain ethyl carbamate (urethane), which is genotoxic and carcinogenic. The highest levels are found in stone-fruit brandies.

It is also vital that the compounds used in the sealing disks in the caps used in the bottling process or the sealing compounds do not introduce contaminants. For example, traditional Chinese spirits were reported to have high levels of phthalate esters from contact with certain plastic materials.

Spirits are also adulterated with other alcohols, but especially methanol, which can cause blindness and even death.

See also: Safety of Food and Beverages: Soft Drinks and Fruit Juices

Further Reading

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Coffee, Tea and Herbals, Cocoa and Derived Products

RH Stadler, Nestlé Product Technology Centre, Orbe, Switzerland
G Hughes, Nestlé Product Technology Centre, York, UK
O Guillaume-Gentil, Nestlé Product Technology Centre, Orbe, Switzerland

Glossary

Conching A process carried out under temperature, which permits the removal of volatiles and aids the even coating of sugar crystals in fat.
Enrobing The coating of a confectionery product in a thin film of chocolate.
Hazard analysis critical control point (HACCP) A systematic and science-based approach to the identification, assessment, and control of food safety hazards.
Prerequisite programs (PRPs) These programs are defined as basic conditions and activities that are necessary to maintain a hygienic environment throughout the food chain suitable for the production, handling, and provision of safe products.
Quenching A process (water or air) used to cool down the coffee beans after roasting.
Tempering A process of heating and cooling chocolate to ensure that the cocoa butter in chocolate forms stable crystals that are able to harden in a uniform crystal structure.
Winnowing A separation of cocoa shells from the cocoa nib.

Introduction

Coffee is one of the most popular beverages consumed worldwide, with a global production output of *ca*. 7 million tons in 2010, compared to 6.7 million tons in 1998–2000. Coffee plants belong to the family Rubiaceae and develop a pulpy fruit known as the 'cherry,' which contains two seeds otherwise termed coffee beans. Two coffee species are used successfully in commercial cultivation, namely *Coffea arabica* (Arabica) and *Coffea canephora robusta* (Robusta). The former accounts for *ca*. 64%, whereas Robusta accounts for *ca*. 35% of the world coffee production. Both species are chemically distinct and characterized by different amounts of minerals, polyphenols, and caffeine.

Arabica or Robusta beans are used either alone or as blends to impart to the finished product its unique organoleptic properties and sensory attributes. In fact, the botanical origin is frequently stipulated on the product label. Moreover, the sensory properties are not only influenced by the coffee species but also, within the same species, by the coffee variety. In essence, a coffee manufacturers' main aim is to deliver to the consumer a product of high quality and continuously superior in-cup taste and aroma. The latter are clearly influenced by the geographic and botanical origin, as well as the quality of the green beans.

Manufacture

Green Coffee Bean

Green coffee is processed by either the dry method (natural) or the wet (washing) method. In the dry method, where water is scarce, the ripe cherries containing the beans are shaken from the tree and laid on trays and sundried. They are then hulled (or shelled) and the beans washed. In the wet method, the cherries are passed in a flow of water into a pulping machine. The machine removes the outer skins, leaving the mucilage surrounding the bean exposed. After being fermented for approximately 1.5 days, the mucilage is then easily washed away, revealing the parchment covering the beans. In this state, still in water, the beans are divided into first and second quality, depending on their weight. They are then removed from the water, thoroughly dried, hulled, and graded by size.

An important factor to control for quality and safety is the green coffee bean moisture that in most cases ranges between 8% and 12.5%. The moisture content of the bean affects several parameters such as the roasting characteristics, sensory attributes of the finished product, susceptibility to insect attack, mold growth, and economical aspects.

Coffee arrives at the factory or facility for further processing either in smaller units, such as jute sacks, or in bulk via containers (train and truck), or one ton big bags. All arrivals should be subject to initial inspection, for example, evidence of moldy beans, integrity of the bags, signs of infestations, and wet damage. Quality approval of incoming lots is a key process before releasing the material for production. Coffee consignments are first sampled following a predefined sampling plan and then subject to physical testing and sensorial analysis. These tests enable a grading of the coffee (confirmation that delivery complies with a specification) and include quality characteristics such as defects, moisture (according to the International Organization for Standardization's (ISO's) standard ISO 6673), and, if specified, screen size. In the case of decaffeinated coffee,

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the caffeine content in the green beans may be checked periodically, depending on the level of supplier confidence.

Roasting and Grinding

Green coffee beans are heated between 180 and 240 °C for *ca*. 6 to 15 min, depending on the degree of roast required. The longer the coffee bean is roasted, the darker it becomes. The roasting process can be described in different phases, i.e., preheating, endothermic and exothermic reactions, and finally a cooling stage. A multitude of chemical, in particular pyrolytic reactions take place during roasting and lead to changes in the cellular structure of the bean that expands considerably. Roasting is also characterized by bean dry matter losses of approximately 10% but is dependent on the thermal input. At higher temperatures and toward the end of the roast cycle, the reaction becomes exothermic that contributes to further raising the temperature of the roaster. Time–temperature conditions (roasting profile) are decisive for the generation of aroma compounds during coffee roasting. Many different roaster designs are in the present day commercially available, and the majority used in industry are hot air fluidized bed roasters with varying features (e.g., batch or continuous) and load capacity. At the same degree of roast, physical properties and concentrations of aroma compounds can differ considerably as a function of the applied time–temperature combinations. When coffees with different initial water content are roasted, differences in the evolution of the degree of roast and aroma formation are observed in light roasts because of the slower temperature increase in coffees with high moisture content.

At the end of the roasting process, water is usually sprayed on the beans (quenching water) to enable cooling. Quenching of coffee can also be carried out with air. When coffee is water quenched, water uptake is possible. In turn, the increased moisture content affects bean firmness and grinding behavior, accelerates degassing in whole coffee beans, and influences loss and degradation of aroma compounds. Roasting in commercial plants is typically followed by storage of the roasted whole beans of up to several hours before grinding – with the maximum tolerable storage time depending on individual quality criteria.

European roast coffees cover a broad range of flavor profiles according to different regional preferences and are designed to meet specific consumer groups' acceptances. The individual flavor profiles are controlled by specifying the green coffee blend, the degree of roast (typically measured by light reflectance or color measurements), and roast time.

To ensure a consistent brew performance and extractability, the roast whole beans are ground to controlled mean particle sizes. This increases the surface area and facilitates the extraction of soluble materials. The grinding process opens the cell structures of the beans, and it is known that grinding potentially causes significant losses of volatile aroma compounds. Very fine grinds may impact taste and facilitate extraction of carbohydrates and oils into the brew. The level of aroma loss depends on specific process conditions. Typical European drip filter grind sizes are in the range of 350–500 mm.

Carbon dioxide is generated in coffee beans as part of the roasting reactions. Roast and ground coffees intended for vacuum packs need to be degassed 2–6 h before packing. This is to allow carbon dioxide to escape from the beans in order to avoid this taking place inside the vacuum bag, which would result in a buildup of pressure and softening of the pack. The required degassing times are mainly dependent on bean type, roast conditions, moisture, and particle size. The amount of time depends on the grind.

Filling and Packing

Coffee is usually packed in a material that provides a gas (oxygen) and moisture barrier (metalized film or flexible laminate) that serves as a preservation of aroma and product characteristics. Packing is ideally under vacuum to maintain freshness and ensure an extended shelf life. In the present day, there exist a number of different packaging possibilities for roasted coffee beans and roast and ground coffee, such as simple pouches, sachets, vacuum packs, foil lined cartons, cans, glass jars, pods, and capsules, the latter for placement in an extraction chamber of a coffee dispensing machine.

Hazards and Control Measures

To help explain the system of managing safety, reference will be made to prerequisite programs (PRPs) and critical control points (CCPs) as defined by the Codex Alimentarius Commission. A food safety study must also consider primary production practices, manufacturing, utilities, transport, storage, and the distribution chain up to the consumption of the final product. In this section, only the significant hazards identified at the raw material and manufacturing stages will be addressed. These are usually defined as hazards that must be considered in a Hazard analysis critical control point (HACCP) plan, independent of the processes or set up of the manufacturing lines. It is assumed that the potential hazards, but nonsignificant are controlled through the PRPs.

Physical Hazards

Before further processing (roasting and grinding), the green beans are passed through a magnet to not only remove metallic contaminants but also as a protection of the coffee grinder at a later stage of processing. Other foreign matters, such as pieces of string (from bags), stones, wood, glass, plastic, etc. are removed with a bean cleaner and the green coffee conveyed to the intermediate storage silos.

Control Measures

Contamination of product with foreign bodies such as glass or metal is serious and can lead to product withdrawals or even public recalls. This may occur even in the presence of metal detectors and magnets and relying solely on detection systems, which does not suffice. In many cases, foreign bodies originate from process equipment and installations, and to mitigate contamination, appropriate measures at factory level must be in place to eliminate all potential sources of foreign bodies.

Important sources of foreign body issues are contact points along the line, for example, metal-to-metal friction and glass jars in case of glass filling lines. Points to consider are, for example, tote bins – lids, latches, and lid holders, tank or mixers with metal blender shafts, screw feeds, sieves, and fluid bed parts. In fact, anywhere in equipment where there is a risk of metal-to-metal friction and where there is mechanical movement, there is a risk of creating metal particles.

Depending on the final packaging, different physical hazards may need to be considered. In the case of filling in glass jars, an efficient procedure to avoid as far as possible glass breakage must be in place. In case of deviations or breakage, root cause analyses must be conducted and measures identified and implemented to avoid reoccurrence. It is important to emphasize that detection/rejection systems (e.g., X-ray and metal detector) have their limitations and will not solve issues without a solid foreign body prevention program in place. Detection systems need to be carefully maintained and fully validated, understanding their limitations in detecting a defect, and of course keeping the number of false rejects to a minimum.

Chemical Hazards

Green coffee may be treated with pesticides and fumigants, the hazard being the presence of unacceptable amounts of residues in the green beans and hence potentially the final roasted product. The European Commission has established a comprehensive database per crop and pesticide and lists >440 compounds and maximum residue limits (MRLs) for coffee, albeit the majority at the lower limit of analytical determination (Table 1).

The possible contamination of coffee beans with mineral oil was raised at the beginning of the 1990s. Investigations showed that jute and sisal fibers were softened with mineral oils before spinning and weaving. Hydrocarbon residues containing a high proportion of polycyclic aromatic hydrocarbons (PAHs) were found in commodities transported in such bags. This contamination was due to the use of low-grade mineral oils. Hydrocarbon residues were found in green beans and in the roasted coffees at 230 and 10–150 mg kg^{-1}, respectively.

However, PAHs have also been reported to be formed adventitiously during the roasting process and are present particularly in dark roasted coffee, albeit at the very low part-per-billion level. Further, analyses showed that PAHs were not released in both home brewing and industrial extraction. The evidence to date confirms that the risk of PAH formation in coffee is negligible.

Other food processing contaminants that have recently received much attention are acrylamide and furan. Coffee is considered a major contributor to both acrylamide and furan exposure via the diet. Several studies have been reported on the formation of acrylamide in coffee, and some have summarized the challenges of finding appropriate mitigation options. The content of acrylamide in coffee reaches a peak early in the roasting process, and toward the end acrylamide drops off sharply and consequently darker roasted coffees contain relatively lower amounts of acrylamide. However, the Maillard reaction also leads to the production of melanoidins, compounds with potent antioxidant activity. Coffee is said to contribute 64% of an average Norwegian's antioxidant intake and has been linked to a reduced risk of certain disease, especially liver disease and diabetes. More intense roasting to obtain darker colored beans not only reduces acrylamide levels but also negatively affects the ability of the brew to scavenge free radicals.

Another factor that affects the level of acrylamide in roasted coffee is the Arabica/Robusta ratio, with Robusta on average giving relatively higher levels. Studies on roast and ground coffees have also shown that the storage condition and time have an impact on acrylamide, with clear reduction at ambient storage. The acrylamide in roasted coffee is largely extracted into the brew and stable within the usual time of consumption. As these main factors also substantially affect the organoleptic properties of the brew and as modifications of the process have to comply with the consumer-accepted boundaries of taste profiles, only small effects in terms of acrylamide reduction are expected to be achievable. Laboratory and pilot studies employing the enzyme asparaginase, that converts the precursor asparagine into aspartic acid, have been reported, albeit with only marginal reductions ranging from 10% to 30% and characterized by a major impact on the sensorial properties of the final product. Further work with asparaginase, for example, using steam treated or water extracted beans, is ongoing, and data are expected to be published in the near future. Recently, the European Commission (EC) issued a recommendation on acrylamide in different foodstuffs and has set 'Indicative Values,' stipulated for roast and ground coffee and soluble coffee at 450 ppb and 900 μg kg^{-1}, respectively. Certain light roast coffees will be above this level, and currently there are no mitigation options available to reduce levels in coffee, without negatively impacting the organoleptic properties of the brew.

Several studies have been reported on the formation of furan, including coffee. The reduction of furan formation in food seems to be more challenging compared to, for example, acrylamide, particularly because the formation is based on multiple precursors and different reactions mechanisms. Recently, the 'European Coffee Cooperation Task Force' published a pilot study on the different stages in coffee manufacture that may contribute to the formation (or loss) of furan, including the consumer handling aspect. In the case of furan, which is highly volatile, accurate intake assessments are lacking, and the brewing technique has a major impact on the final furan exposure. Health authorities are also extending furan studies to include as well alkylfurans (2- and 3-methylfuran) that may exhibit similar toxicity profiles. For adults, coffee clearly tops the range with an average intake assessment of 0.43 μg per kg bodyweight per day for total furans, with the methylated furan analogs contributing considerably to the overall exposure. As recently stated by the European Food Safety Authority (EFSA), before considering remediation recommendations, there is a need not only for more detailed exposure assessment data but also for better toxicological information on which to base a comprehensive risk assessment.

Coffee consumption, in particular boiled coffee (Scandinavian, Turkish, and French press types), has been associated with elevated blood cholesterol and increased

Table 1 Example of a plan for the management of hazards in green coffee

Ingredient/step	*Hazard(s)*	*Control measure(s)*	*Acceptable level*	*Monitoring*	*Corrective action(s)*	*Verification*
Green coffee	Pathogenic bacteria, for example, *Salmonella*	No control measure necessary for green coffee with regards to pathogens. High heat treatment (roasting) in subsequent step (see **Table 2**)	No existing norms Not necessary to establish 'acceptable level in end product' due to high heat treatment in subsequent step	Roasting time and temperature	N/A	Quality monitoring system in place (process control)
Green coffee	Foreign bodies (sharp and hard, for example, metallic)	Foreign bodies management procedure at tipping station	$<$2 mm diameter of stainless steel (spherical) or approximately 4.2 mm^3 volume	Foreign body prevention system (magnet) and P2 cleaning	Block lot; Inform QA/production	Metal detector specifications, validation, and online testing
Green coffee	Mycotoxin: Ochratoxin A (OTA)	GAP and goods transportation – Build good relationship with suppliers – Specification addressed to supplier with moisture limit – Release of incoming bulk green coffee based on moisture content result and bean quality attributes	No OTA limits for green coffee established to date (limits have been set for R&G/soluble coffee) Limits set for moisture (12%, reject at 13%) and incoming bean quality (e.g., number of defects)	Moisture content measurement of each lot and quality control (defects, broken beans, moldy, etc.) following incoming inspection	Reject lot, Inform supplier	Sampling and OTA measurement according to sampling procedures
Green coffee	Fumigant Methyl bromide and inorganic bromide	Notification to all exporters/ traders/suppliers that commodities containing residues will be rejected Methyl bromide is being phased out in most countries and fumigation replaced with phosphine	25–50 mg kg^{-1} maximum residue tolerance levels	Material specification	Reject lot	Sampling and analysis
Green coffee	Pesticides	Notification to all exporters/ traders/suppliers that commodities containing residues above legislative levels will be rejected	EU: (EC) No. 149/2008 US: 40 CFR §180.225	Material specification	Reject lot	Sampling and analysis
Decaffeinated green coffee	Caffeine Presence of a high level of caffeine	Decaffeination process: online or at line measurement (e.g., NIR)	Caffeine $<$0.065% - for use in R&G	Material specification	Reject lot	Sampling and analysis

Note: Additional verification measures are discussed in the text.

Abbrevations: CFR, Code of Federal Regulations; EU, European Union; GAP, good agricultural practice; NIR, near infrared; R&G, roast & ground.

cardiovascular risk. The diterpenes cafestol and kahweol are attributed to the hypercholesterolemic effect of boiled coffee, and are extracted during the brewing process. However, the compounds are removed to a large extent by paper filters, and in the case of espresso coffees, the exposure is limited due to the relatively small serving size. As stipulated in Table 2, smaller grinds increase the surface to volume ratio, thereby enabling a better extraction of surface lipids and consequently more diterpenes in the cup. The scientific evidence in the literature in the present day makes it difficult to establish safety limits for total diterpenes, and average coffee consumption is considered unlikely to result in an appreciable increased risk of cardiovascular disease.

Coffee is a rich source of caffeine that provides mild stimulant effects but may be undesired by certain consumers. To cater for this need, manufacturers have developed processes to decaffeinate coffee, applied to the green bean and which remove caffeine to a large degree.

Caffeine, known chemically as 1,3,7-trimethylxanthine, is the major pharmacologically active ingredient in coffee and a central nervous system stimulant. The amount of caffeine consumed through beverages varies considerably and is dependent, for example, on how strong the coffee is prepared, and how much of it is consumed. Robusta is known to contain more caffeine than Arabica coffees.

There are four main methods of decaffeination, according to which substance is used to extract the caffeine: water, ethyl acetate, supercritical or liquid carbon dioxide, and methylene chloride. When organic solvents are employed, residues may be present in the coffee beans. Although the amount of residual solvent in green coffee is very small and no particular legislation has been adopted, there is a need to limit their presence to a minimum. Therefore, the measuring of caffeine as a verification of the decaffeination operation is encouraged. According to legislation within the EC, decaffeinated coffee is a coffee with a caffeine content reduced to 0.1% or less in roasted coffee beans and to 0.3% or less in soluble/instant coffee.

There is ample evidence in the scientific literature that the presence of caffeine above legislative limits in a 'decaffeinated' declared beverage is not a health concern but rather a legislative issue. Therefore, the decaffeination step is identified as a PRP but will nevertheless be carefully controlled to ensure each lot meets regulatory and/or internally set norms.

Control Measures

To effectively manage chemical contaminants such as pesticides and mycotoxins, a clear specification to material suppliers is mandatory. Further, elements that strengthen a control system is the supplier confidence, management at field/crop and transport/storage (warehouse) stages. Generally, the level of pesticide residues found in green coffee is very low. However, analysis of each lot according to the coffee-pesticide profile is simply impossible from a practical as well as economic point of view. It is well documented that if present, their concentration is substantially reduced during storage, roasting, and brewing. The harsh processing conditions (i.e., roasting at temperatures in the range of 180–240 °C) as well as high temperature extraction in the case of soluble coffee manufacture will in most cases lead to a significant reduction of residues should these be present at levels of concern. However, this should not be an incentive for manufacturers to accept lots that contain residues above MRLs. Good agricultural practice (GAP) and existing legislation must be respected at all times. Control measures for pesticides/fumigants (e.g., phosphine, and in some countries methyl bromide) upstream at the supplier level are therefore mandatory and must be firmly embedded in the supplier/grower contract and identified within the HACCP study as a PRP (Table 1). Verification through spot checks or monitoring is highly recommended, the frequency of such checks depending on the supplier confidence level and origins of the green beans. Monitoring should also take into account active substances that are not permitted for use in the commodity (potential abuse), as well as targeted analysis using knowledge of compounds actually applied at farm level to ensure that the commodity complies with National or International Standards and to ensure the safety of the product.

To avoid issues with PAHs, the International Jute Organization has issued specifications for the manufacture of jute bags that limit the amount of unsaponifiable matter to a maximum of 1250 mg kg^{-1}. Some manufacturers have developed a hydrocarbon-free oil, based on vegetable oils, to soften the fibers. Mineral oils in jute sacks should be part of a annual monitoring scheme, to ensure compliance and the safety of the product.

Water, steam, and air circuits are all to be considered within the HACCP study. Potential contamination of quenching water (e.g., microbiological load and absence of pathogens), or air circuits in contact with the roast and ground coffee (e.g., nonfood grade mineral oil from air compressors), may lead to unacceptable amounts of contaminants/residues. The use of food grade mineral hydrocarbons/lubricants at food contact areas is a point that warrants particular attention. In the case of lubricants used in food processing machines, these need to meet the requirements of the European hygiene standard for food processing machines. In the USA, United States Department of Agriculture (USDA)-H1 lubricants can be applied to all friction points where there may be accidental, technically unavoidable contact of lubricant and food product. H2 class lubricants can also be used in food processing but contact with food must be excluded. Manufacturers are encouraged to establish and maintain a lubricant management program, subject to periodical internal review/audit.

Microbiological Hazards

At the typical temperatures of roast, a plethora of thermally driven reactions take place within the coffee bean. The roast temperatures are well above those known to destroy microorganisms and even to large extent decompose mycotoxins. Consequently, the presence of pathogenic bacteria are not considered a hazard for pure roasted coffee as the control of the heat treatment is directed toward delivering sensorial attributes and any deviation will be detected by the sensorial evaluation of the final product (Table 2).

However, a major hazard for coffee is the presence of ochratoxin A (OTA), a nephrotoxic carcinogen produced by the two genera of fungi: *Penicillium* and *Aspergillus*. In fact,

Table 2 Example of a plan for the management of hazards in roasted coffee

Ingredient/step	*Hazard(s)*	*Control measure(s)*	*Acceptable level*	*Monitoring*	*Corrective action(s)*	*Verification/comments*
			No existing norms			Quality monitoring system in place (process control)
Roasting and quenching	Pathogenic bacteria, for example, *Salmonella*	No specific control measure necessary for roasted coffee with regards to pathogens	Not necessary to establish 'acceptable level in end product' due to high heat treatment	Roasting time and temperature	N/A	Control of heat treatment is not necessary as the roasting is driven by quality and any deviation is identified by sensory evaluation of the final product
Roasting and quenching	Acrylamide	None identified to date. Maillard reaction product from asparagine and reducing sugars. Roasting profile not severe enough to degrade acrylamide to a sufficient extent	ALARA. Where valid, 'official' guidance or indicative levels are to be considered	Roasting time and temperature	N/A	Particularly light roasted coffees have relatively high amounts of acrylamide
Roasting and quenching	Furan	None identified to date. Highly volatile compound formed from lipid degradation and sugar fragmentation	ALARA. Where valid, 'official' guidance or signal levels are to be considered	Roasting time and temperature	N/A	Furan has to be considered in changes related to roasting and grinding. Dark roast coffee tends to exhibit relatively higher amounts of furan versus light roasted beans
Grinding	Foreign bodies (sharp and hard, for example, metallic). Hard foreign bodies which have passed through foreign body prevention system may damage the grinder	Foreign bodies management procedure at tipping station	<2 mm diameter of stainless steel (spherical) or approximately 4.2 mm^3 volume	Metal detector specifications, validation, and online testing	Block lot; Inform QA/production	Once a year (depending on factory) the grinder is changed due to wear and tear
Grinding	Presence of high amounts of diterpenes (cafestol and kahweol)	Depending of grinding size diterpenes would be more available for extraction. Control grinding size according to QMS	Coffee oil is more prone to be extracted at finer grind size	Grind size analysis	Block lot; Inform QA/production	Quality monitoring system in place (process control)
Filling and packing	Foreign bodies (sharp and hard, for example, metallic)	Foreign bodies management procedure at filling	<2 mm diameter of stainless steel (spherical) or approximately 4.2 mm^3 volume	Check sensitivity with test pieces and that reject mechanism is working correctly	Hold product; Investigate source; Inform QA	Metal detector specifications, validation, and online testing

Note: Additional verification measures are discussed in the text.

Abbrevations: ALARA, As low as reasonably achievable; N/A, not applicable; QA, quality assurance; QMS, quality monitoring scheme.

coffee has been identified as one of the significant contributors to OTA exposure in the European Community and the Codex Committee on Contaminants in Foods has prepared a draft code of practice for the prevention and reduction of OTA in coffee. Mold contamination of coffee beans can be indirectly controlled through checks that should encompass three main criteria:

1. Dried cherries/pods/husks.
2. Visual damage.
3. Earthy/moldy smell.

The skin of the cherry normally protects the coffee bean itself from contamination with spores. This protection is lost if either cherries or skins and husks are not removed from the green coffee or if the skin is broken, of which broken beans or insect-damaged beans are indicative. An earthy/moldy smell or cup is an indicator for mold damage, and coffees without an earthy/moldy smell showed less than 1% risk to contain more than 10 $\mu g\ kg^{-1}$ of OTA.

Control Measures

In terms of controlling OTA, green bean moisture has been shown to be the main risk factor for OTA formation after the first processing stage. Moisture is regulated within International Coffee Organization (ICO) Resolution 420 and provides for a quality standard for exported green coffee, specifying a maximum moisture level at 12.5%. However, to cater for an additional margin of safety in terms of quality/risk of mold contamination, as well as possible fluctuation due to further storage/transport, manufacturers may choose an internal limit, for example, 12% (Table 1).

Consignments that exceed 13% are rejected, and those between 12% and 13% may lead to compensation claims made to the supplier, dependent on the supplier contract. Within a HACCP study, OTA may be considered a potential hazard. The likelihood of its occurrence depends on the supplier confidence level and the relative risk of exceeding limits due to the coffee origin. GAP and due diligence is the inclusion of verification measures, ideally defined as a surveillance or annual monitoring program of green bean origins (e.g., annual or biannual). Within the European Community, maximum limits have been established for roast and ground coffee and soluble coffees of 5 and 10 ppb, respectively. Notably, some countries have set individual national levels that may be more stringent and/or are based on green coffee beans. Therefore, mold contamination at the green coffee stage must be controlled, i.e., follow defined storage/warehousing and transport procedures and must be part of the manufacturers HACCP system.

Tea and Herbals

Introduction

Tea is the most widely consumed beverage in the world with an estimated daily consumption of 15–20 billions cups. This is due to its refreshing, mild stimulant properties, and also to its medicinal and general health-promoting purposes. In 2008, the world tea production was 3.8 million tons (International Tea Committee). The most commonly found varieties of tea, which are all originating from the same tea plant *Camellia sinensis*, are white, yellow, green, black teas, and oolong. The way in which the fresh leaves of the tea plant are processed and their levels of contact with oxygen determine the different varieties of tea. During the enzymatic oxidation process, tea leaves undergo natural chemical reactions that result in distinctive color and taste characteristics.

Besides traditional teas, there is a growing interest in other plant products termed herbal and fruit infusion (HFI) as they are recognized for their very diverse sensory properties as well as for their potential health beneficial properties. HFI may contain just one or two ingredients or are as minor ingredient blended with teas prepared from *C. sinensis*. Raw materials for herbal infusions are typically parts of plants, fruits, spices, herbs, etc., which do not originate from *C. sinensis*. Plants and parts of plants commonly used in HFI are listed in the European Herbal Infusions Association (EHIA) 'Inventory List of Herbals Considered as Food' in its current version. Other teas can be flavored by the addition of aromas.

Tea and Herbals Manufacture

Although each type of tea has different taste, smell, and visual appearance, tea processing for all traditional teas consists in a similar set of methods with some variations. The most popular teas are green and black. The manufacturing process of the latter involves the following main steps: growing, leaf harvesting, withering (wilting) under hot air stream, sifting, mechanical rolling, fermentation/oxidation for 2–4 h done at high humidity usually at 20–30 °C converting much of the catechins of the leaves into complex tannin, drying to a moisture content below 3% w/w, and finally grading and packing.

In comparison to black tea, the main difference in the processing of green tea is the blocking of the fermentation by heat inactivation of oxidative enzymes either with steam (Japanese method), or by dry cooking in hot pans (traditional Chinese method). Oolong tea falls somewhere between green and black teas as the leaves are only partly oxidized.

The graded tea is packed into containers for shipment from the countries of origin. Different packaging possibilities exist for dried and graded teas, such as wooden tea chests, paper laminate sacks, polythene bags in gunny sacks, polythene bags in cardboard cartons, and vacuum packed cartons/sacks.

In the case of HFI, a plethora of different ingredients originating from various plants or parts of the plants are being used such as roots, tree barks, leaves, flowers, fruits or seeds, and spices. In the EHIA inventory list of herbals, a wide spectrum of different ingredients with more than 400 foodstuffs is listed. Many of these raw materials are not grown in cultivated crops but wild and are then, after harvest, rather sundried than dried industrially at high temperatures.

The manufacturing process of ingredients used for HFI varies a lot due to the broad spectrum of used raw materials but comprises the major steps: growing, harvesting, transport to collection point, cleaning, drying either in the sun or in hot air drying chambers, packing, and storage/transport. HFI may contain just one or two ingredients, for example, ginger,

lemon, or chamomile or are as minor ingredient blended with traditional teas prepared from *C. sinensis*, for example, lemon balm blend and black tea with bergamot (equal to earl gray tea).

When mechanical dryers are being used, both time and temperature of drying should be controlled as well as the moisture of the dried products. A uniform drying of the material should be achieved.

Insects at all stages of their life cycle may be present in teas and HFI. Rigorous inspection and control procedures must be put in place at different levels to detect any sign of infestation in the country of origin, and at the processing plant, the blender/packer level as teas and HFI are submitted to long transportation and are stored for certain periods of time before further processing. Fumigation for the control and elimination of insects should only be applied when necessary with authorized fumigants (note, ethylene oxide is banned in the European Union (EU)) and fumigant residues must be below MRLs.

Hazards and Control Measures

The main food safety risks specific to teas and HFI are foreign bodies, pesticides, and presence of pathogenic microbiological contaminants such as *Salmonella* spp. and mycotoxins.

Physical Hazards and Control Measures

A wide variety of foreign bodies may be introduced in teas and HFI during harvest, postharvest process, transport, and blending/packing and are typically stones, glass, metal, wood, packaging or bag materials, etc.

Various methods such as sieves of different sizes, ferrous material with magnets and air separation to remove efficiently foreign bodies at the country of origin and upstream at the blender and/or packer plants do exist. Presence of foreign bodies represents a low food safety risk in teas and HFI as long as methods to remove them are integral part of the processing operations. Detection and rejection systems (metal detector and magnets) should be installed also downstream at the level of the blender and/or packer as a final verification measure so that all control measures at the countries of origin, during transport, were effective.

Chemical Hazards and Control Measures

At the stage of primary production, pesticide residues usually represent the main chemical hazard. Commonly detected residues in teas and herbals based on the European Tea Committee (ETC) surveillance reports (2001–04) in Indian teas are, for example, dicofol, cypermethrin, deltamethrin, fenvalerate, ethion, and endosulfan. The application of pesticides, if not appropriately controlled, may represent a potential food safety concern, particularly if residues exceed the acute reference dose. The surface area of tea leaves is indeed generally higher than in other types of crops, and thereby the absorption of the pesticides may be quite abundant and depends also on the spraying dose. Not only the tea leaves absorb pesticides but also the soil; some pesticides are known to persist in the soil for many years and may be assimilated by the tea and herbal plants.

Drying at high temperatures of teas and herbal teas will obviously concentrate pesticides present on tea shoots (also called drying factor), but at the same time, it is likely that a reduction of pesticides residues will take place due to the drying process, the heating process specific to green tea and subsequent preparation with hot/boiling water by consumers as reported by some authors.

The use of approved pesticides applied under GAP results in pesticide residues below the stipulated MRLs. This point, however, is not straightforward as MRLs are not aligned, and in some countries such as the USA only very few compounds have designated MRLs for tea ($n=8$). Only 11 MRLs are currently set at the Codex Alimentarius level, whereas Japan has listed >70 compounds and the EU approximately 60 compounds. Such inconsistencies may impact trade, and efforts need to focus on harmonized legislation to avoid trade barriers. In the case of ingredients used for HFI, the multitude of local farmers and the fact that HFI are usually not cultivated make the application of GAP much more challenging mainly for practical reasons, compared with the larger and well-structured traditional tea plantations.

Clear specification to material suppliers is mandatory to manage chemical and also microbiological contaminants. Furthermore, long-term and solid relationships with tea and herbal tea farmers are essential to prevent potential pesticide issues upstream of the supply chain. This is also valid for most of the other food safety concerns (pathogens, mycotoxins, etc.). In addition, monitoring or verification through spot checks is an essential element of the HACCP process and needs to be conducted at a frequency that should be adapted depending on the origin of the teas/HFI and on the supplier confidence level. In this respect, the ETC recommends a list of specific pesticides in teas that should be regularly monitored and sampling plans (2009). In ISO 1839, 'tea-sampling' is specified. The list of pesticides needs to be regularly updated based on the latest food safety information available such as emerging issues, rapid alert system for food and feed (RASFF), regulatory developments, etc.

Most foods contain some aluminum as it occurs ubiquitously in the environment. Beverages (tea, coffee, and soft drinks) and also cereals are the main sources of aluminum from foods. Tea and some spices and herbs contain particularly high levels. Studies showed that high aluminum availability in tea garden soil is the major contributing factors for the enhanced aluminum accumulation in tea leaves and HFI. Teas and herbal materials should not be cultivated in soils contaminated high levels of metals, pesticides, and other environmental contaminants.

In most of different teas and HFI, PAH content is low and does not represent a food safety concern, with perhaps the exception of 'smoked tea,' for example, Lapsang Souchong, which may contain residues of PAH.

Microbiological Hazards and Control Measures

Diverse microorganisms (bacteria/spore formers and fungi) may be present in teas and in HFI. They are indigenous to the

soil and plants in which they are grown. The sources of microbiological contamination may be dust, insects, fecal material from birds and rodents, etc.

The microbial load in teas and HFI is generally high but varies depending on the origin, on the variety of teas (microbial level in green tea is usually low due to the heat treatment), on the plants/part of plants used for HFI, on the process in particular the drying (sun drying versus industrial drying), on the moisture level at different stages, etc. Microbiological guidelines are proposed by the EHIA for herbal infusion raw materials and for finished product (consumer ready dry products), but stricter norms outlined in the specification can be applied if deemed necessary. This would encourage producers to improve hygienic conditions, minimize microbiological contamination, and avoid growth of microorganisms, notably molds and consequently potential mycotoxin formation.

If teas and HFI do not comply with the specification, application of an effective heat treatment (e.g., steam) must be envisaged. Nevertheless, teas and HFIs must not be accepted for heat treatments or other types of treatment if there is evidence of insect damage or/and mold growth because of risk of the presence of mycotoxins. Although reported cases of food outbreak related to tea and HFI are rare, there is evidence of microbial contamination. In 2002, a nationwide outbreak of salmonellosis occurred in Germany, which was traced to contaminated aniseeds. Infants below 13 years of age (42 cases) were principally affected. *Salmonella* agona was isolated from both aniseed containing herbal tea and patients. More recently, in 2007, 14 cases of *Salmonella* senftenberg infection were reported in Serbia and were linked to contaminated fennel seed tea. As teas and HFI (e.g., aniseed, caraway, and fennel seeds) are not only being drunk by healthy consumers but also by sensitive or more vulnerable population groups, such as infants and immune-depressed individuals, it is essential to follow strictly the brewing instructions during preparation. Contamination of tea with other pathogens such as verocytotoxin-producing *Escherichia coli* (VTEC) was also reported in RASFF between 2008 and 2010. It is well documented that *Salmonella* spp. can survive for long period of time in foods with low water activity and some of them are infective at low dose in dry foods. This must be borne in mind as some of these materials are blended as minor ingredient with teas.

During preparation of tea or herbal beverages at home, the brewing with hot/boiling water will ensure the elimination of vegetative pathogens (*Salmonella* spp. and VTEC) potentially present in the product. In this respect, brewing instruction on packages of tea and HFI must be followed by the consumer in order to guarantee the elimination of pathogens potentially present in teas and herbal/fruit infusions. In case an automatic beverage machine is being used, the temperature of the water prepared in the machine has to be high enough to ensure an effective pasteurization of the brewed tea or HFI. Verification that the machine is able to heat water at the desired temperature in the system precisely and over time must be checked by the machine developer through extended life cycle tests. This is particularly essential because tea is generally not perceived as a product at risk for contamination with enteric pathogens.

Inadequate processing and storage conditions, particularly when characterized by high humidity, can provide opportunities for microorganisms to grow, including molds. Growth can take place for the following main reasons: (1) prolonged time between harvest and drying; (2) insufficient drying; (3) insufficient protection from rain, overnight dew during sun drying; and (4) improper dry state during storage and transport contributing to humidity absorption.

One of the important characteristics of teas/HFI are their hygroscopicity, which means that if tea is not adequately protected by suitable packaging materials, they will absorb moisture quickly from the humid and hot ambient air in the countries of origin. Also, condensation under poor storage and transport conditions may occur leading to a localized increase of the water activity and potentially to the growth of microorganisms. The possibility of mycotoxin production by specific molds in high moisture teas and HFI has been reported. Only a minority of molds can generate mycotoxins, many of which are genotoxic carcinogens and may cause cancer. The risk was perceived as nonsignificant by some authors, albeit this subject has not been extensively investigated compared with numerous publications on the mycotoxin contamination of coffee by OTA or of other foods such as nuts or cereals.

In a recent study carried out in the Czech Republic, some 40 samples of green, black, and herbal teas from shops were analyzed. No mycotoxins were found in any of the samples, but some *Aspergillus* species able to synthesize OTA and aflatoxins were identified.

In spite of the long history and wide use of tea and HFI, there are few publications on their contamination with molds and mycotoxins compared with reports on the contamination of other foods such as cereals, nuts, and dried fruits. Liquorice and ginger are used as minor ingredient in some tea blends. Aflatoxins or OTA were identified in liquorice and ginger. Within the European Community, maximum limits for OTA of 20 ppb have been established for liquorice roots and 80 ppb for liquorice extract. Therefore, the tea/herbal tea industry should look at this potential problem in a wider context and possibly greater depth. Once mycotoxins are present, they can persist through processing into the final food product. In addition to the potential food safety issue of mycotoxin synthesis in teas and HFIs, growth of molds on the surface of teas and HFI is unacceptable from a visual and sensory point of view. Sensory properties of the brewed tea and herbal/fruit infusion will be negatively impacted, resulting in a moldy off flavors (musty taste).

To prevent microbiological growth, especially that of toxigenic molds, a safe moisture level must be achieved. The European Union's Scientific Committee on Food in 1997 specifies a maximum moisture level of 10%, which was considered to give an acceptable safety margin for the storage/warehousing of tea considering fluctuation during storage and transport. Generally, moisture content in black tea is largely below 10%. EHIA guidelines recommend a maximum moisture level of 12–13% for herbals, for example, camomile, fennel, lime, peppermint, etc. To determine moisture, ISO 1573 is recommended (103 °C for 6 h).

Cocoa and Derived Products

Introduction

The two distinguishing and pleasurable characteristics of chocolate are the flavor and texture. Chocolate is consumed by almost everyone globally and is considered an enjoyable food; it is generally sold at a price that means it is available to all. The key component of chocolate is cocoa liquor, which is obtained from cocoa. There are four main varieties of cocoa utilized for chocolate, cocoa powder, and cocoa butter production. The most common and widely used cocoa variety is Forastero, which grows largely in West Africa producing an estimated 70% of the world's crop. Criollo, Tinitario, and Nacional are produced in much smaller quantities and when combined make up approximately 10% of the world crop. The different species of cocoa trees (*Theobroma cacao* L.) can be differentiated by the color and shape of their pods, although this can be difficult due to cross-fertilization leading to copious hybrids between the species. Approximately, two-thirds of the cocoa crop is utilized in chocolate products, the remainder goes almost exclusively to beverage and bakery sources.

Manufacture

Cocoa Beans

Storage and Handling

The cacao tree produces pods containing pulp and raw beans. When pods ripen they are harvested from the tree. The pods are opened and the outer pod and placenta are removed, the separated beans are then fermented and dried, which is usually carried out on the farm. The bacteria involved in the fermentation process result in the production of acids and enzymes, which cause death of the beans and breakdown of proteins and sugars to produce cocoa flavor precursors, this ultimately provides the building blocks for the flavor of the cocoa liquor. The drying process then reduces the moisture content of the cocoa beans to between 6% and 8%, which allows the beans to be transported safely as well as removing the acids, which helps to improve the flavor of the cocoa liquor. The cocoa beans are then stored in jute sacks during shipment to manufacturers. On receipt, cocoa bean lots are subject to an intake inspection where checks are made for the presence of molds, damage to bags, signs of infestations, and wet damage. The quality approval of incoming lots is a key input before release of the material into production.

Cleaning and Winnowing

Cocoa bean cleaning is an essential step in the processing of cocoa and often includes air separation, vibration as well as a series of sieves and rotary magnets. The cocoa beans undergo a further processing step known as winnowing. Winnowing involves the predrying or heat treatment of the cocoa beans to allow for separation of the shells from the cocoa nib, the cocoa beans are then broken into large pieces to separate the shells from the nibs. A process known as variable aspiration is often used to remove the maximum quantity of shell without removing cocoa nib.

Roasting

The cocoa nibs are then roasted to bring out the chocolate flavor and color. The temperature and time of the roast depends on the moisture content, type of beans used, and the chocolate or product required from the process.

If the cocoa nibs are to be used to produce cocoa powder, then they must also undergo alkalization, usually with potassium carbonate, in order to develop the flavor and color and increase the solubility of the cocoa powder to be produced.

Milling

The roasted nibs are then milled to create cocoa liquor. Cocoa liquor consists of cocoa particles suspended in the cocoa butter. The temperature and degree of milling varies according to the type of nib used and the product required. Manufacturers commonly use more than one origin of bean in their products, and therefore the different liquors extracted from the different beans must be blended to the required formula.

The processing then takes one of two directions based on the final product required; the cocoa liquor can be used directly in the manufacture of chocolate, or it can undergo further processing involving pressing into two fractions – cocoa butter and cocoa press cake. Cocoa butter is utilized in the manufacture of chocolate whereas the cocoa press cake is further broken down to form cocoa powder, which is mainly utilized in beverages, baking, and desserts.

Chocolate Manufacture

Chocolate manufacture involves the mixing of several ingredients including cocoa liquor, cocoa butter, sugar, milk, emulsifying agents, and cocoa butter equivalents. The proportion of ingredients varies based on the type of chocolate require being manufactured.

Refining

The mixture undergoes a refining process by traveling through a series of rollers until the product becomes a fine flake. The refining process ensures that the particle size is adequately small resulting in the required mouth feel and viscosity of the chocolate.

Conching

Conching further develops the flavor and texture and is a process, which permits the removal of volatiles as well as the even coating of sugar crystals in fat. The conching process also allows flavor transfer into the sugar particles. The speed, duration, and temperature of the conching process influence the flavor of the chocolate.

Tempering

The mixture is then tempered, which involves heating, cooling, and reheating to give the characteristic melting profile of chocolate. The tempering process acts to delay fat bloom in the finished product.

Molding or Enrobing

The chocolate can then be molded or used to enrobe fillings. The molded or enrobed product is then cooled in a cooling chamber.

Distribution

Usually, the final stages of production include packaging and either the metal detection or X-ray of the product before distribution.

Hazards and Control Measures

The food safety hazards specific to cocoa mass production and chocolate manufacture include the potential presence and contamination with foreign bodies and the possible presence and/or contamination of *Salmonella*. These food safety hazards are controlled by a number of prerequisite programs as well as by performing processes that are considered critical to controlling the food safety of the cocoa mass or chocolate. In confectionery, chemical (e.g., presence of mycotoxins due to mold growth) and allergen (e.g., contamination by gluten) hazards are also frequently identified during the HACCP assessment; however, these hazards are not specific to cocoa production and chocolate manufacture and therefore will not be covered in this section. However, it is important to note that hazards will vary between confectionery processing facilities based on raw materials, suppliers, production equipment and processes, to name a few, therefore, in line with the HACCP approach, all hazards should be identified at each process step and suitable control measures effectively implemented.

Physical Hazards

Sources

Cocoa beans are a raw material of agricultural origin and therefore are a major potential source of foreign matter, for example, metal farming implements, pieces of bark, stones, glass, and pests in unprocessed cocoa beans. The fermenting and drying of the beans is nearly always performed directly on the farms, therefore, the likelihood of foreign matter contaminating the raw material is highly likely. In addition, the chocolate manufacturing process is complex in nature and, itself, can also be a major source of foreign body contamination – many pieces of equipment have moving metal parts, which have the capacity to come into direct contact with other parts resulting in metal-to-metal contact. Subsequently, this results in metal entering the process. Examples of such equipment include storage tanks with stirrers, conches, mixers, temperers, and depositors, to name a few. Additionally, polycarbonate molds are commonly utilized in the chocolate confectionery molding process, and these can be prone to breakage in the event of jams.

Control Measures

To control the risk of foreign bodies, an effective foreign body management system must be implemented by a cocoa mass and chocolate confectionery manufacturing establishment. For foreign matter, management in raw materials, auditing of the supplier's food safety practices is crucial as well as the building of a good relationship between supplier and manufacturer. Incoming raw materials must be adequately protected during transport and storage to ensure foreign body contamination is prevented. A check of the condition of raw materials on intake is necessary as well as rejection of damaged goods. Sieves and filters can be installed at the point of raw material intake as well as at other key points in the process as defined by the HACCP study, usually including the end of the chocolate-making process. An essential step in the processing of cocoa beans is cleaning where various methods are used to separate out foreign bodies, including air separation, vibration, sieving, and rotary magnets, although processes can vary greatly. Additionally, further removal of foreign material occurs after the beans have been broken – during winnowing, the shell is separated from the nib and at this point other hard residues such as dried pulp and loose shells are also removed. Ferrous metal contamination is often identified as a risk in the chocolate-making process, and in such a case magnets can be added to the process downstream from ferrous equipment with a possibility of failure. In molded products, a mold breakage procedure should be implemented and involve a thorough check of all potentially affected product. Metal detection is an important step of chocolate production that is purposefully designed into the process. It is usually found at the end of the process to allow for detection of metal hazards coming from all points in the process. It is important to note that such defenses have limitations, and therefore, foreign body prevention should be managed largely through robust prerequisite programs, such as preventive maintenance. This is a key to ensuring a food safe product in preference to a reliance on foreign body detection systems.

Microbiological Hazards

The microbiological hazard of concern during chocolate manufacture and end-product use is *Salmonella*. The low water activity characterized by chocolate (typically approximately 0.4 and 0.5) means that *Salmonella* is unable to grow in chocolate. However, in 1994, Cordier *et al*. elucidated that *Salmonella* has the ability to survive in chocolate products for up to several years. An overview of *Salmonella* food poisoning outbreaks in chocolate have been reported by Werber *et al*., in 2005 (Table 3), and these incidents all involved very low numbers of *Salmonella* cells. It has been hypothesized that the fatty matrix of chocolate actually preserves the *Salmonella* cells from the acidic conditions of the stomach allowing *Salmonella* to survive and ultimately colonize the lower gastrointestinal tract, resulting in the manifestation of clinical symptoms.

Sources

Salmonella can be introduced into chocolate through incoming cocoa beans. Cocoa beans are a known potential source of *Salmonella*, due to poor hygiene conditions during harvesting, fermenting, and drying. The pulp and beans are contaminated during and after breaking of the pod by several sources, some of which include the harvesters, the tools utilized during the harvesting process, soil, leaves, and pests. Following

Table 3 Overview of published chocolate outbreaks due to *Salmonella* contamination

Year	*Country*	*Serovar*	*Vehicle*	*Contamination source*	*Number of affected persons*
1970	Sweden	*Salmonella* Durham	Chocolate products	Cocoa powder	110
1973–74	USA and Canada	*Salmonella* Eastbourne	Chocolate balls: Canada	Cocoa beans	200
1982	England and Wales	*Salmonella* Napoli	Chocolate bars: Italy	Unknown	272
1985–86	Canada	*Salmonella* Nima	Chocolate coins: Belgium	Unknown	–
1987	Norway and Finland	*Salmonella* Typhimurium	Chocolate products: Norway	Avian contamination speculated	349
2001–02	Germany and other European countries	*Salmonella* Oranienburg	Chocolate brands (2): Germany	Unknown	439

Source: Reproduced from Werber D, Dreesman J, Feil F, van Treeck U, Fell G, and Ethelbery S (2005) International outbreak of *Salmonella* Oranienburg due to German chocolate. *BMC Infectious Diseases* 5: 7.

fermentation the beans are dried, often on the floor in the sun, and there is virtually no environmental control, and as a result microbes can contaminate the beans. As a consequence, raw cocoa beans have high microbial levels and the presence of *Salmonella* is a well-recognized hazard. Other raw materials utilized in the chocolate-manufacturing process also need to be controlled because they too could introduce *Salmonella*. Milk powder has been implicated in a large number of salmonellosis outbreaks and heat treatment and cross-contamination controls in the milk powder manufacturing process must be strictly followed. Similarly to cocoa beans, raw nuts may become contaminated with *Salmonella* if they are exposed to poor hygiene conditions. The presence of *Salmonella* has also been linked to other common confectionery raw materials including egg products, flours and starches, lecithin, and coconut.

Inadequate hygiene practices in the cocoa and chocolate manufacturing and processing environment should also be considered as possible sources of microbiological contamination including *Salmonella*. Additionally, another source of *Salmonella* contamination from water includes leaks coming from insufficiently managed equipment (e.g., jacketed water systems) and poorly maintained facilities (e.g., leaking water pipes).

Control Measures

Suppliers of raw materials to be utilized in the manufacture of chocolate should be carefully selected, and their food safety controls audited to ensure that all incoming ingredients are microbiologically safe for use in the chocolate-making process because the microbiological safety of chocolate products relies entirely on the use of safe ingredients and the control of cross contamination during processing and packing.

Roasting of beans or nibs is often a CCP in the destruction of *Salmonella* because temperatures of 70–80 °C achieved during milling, refining, and conching are not sufficient to destroy *Salmonella*. Similarly to cocoa beans, the roasting of raw nuts is a critical step. It is therefore essential that appropriate controls are applied at the roasting step to ensure sufficient temperatures, and roasting times are achieved.

Following the roasting step, there are no further processing stages that will effectively destroy *Salmonella*, therefore, hygienic processes and working practices are essential as preventive measures to avoid *Salmonella* cross contamination and include: Correct hygienic zoning of the cocoa bean process to ensure no possibility of cross contamination from the raw to roasted beans; segregation of raw bean storage and handling areas; dedicated personnel and equipment to potentially contaminated areas; minimization of movement between raw bean and roasted bean areas including hygiene barriers that incorporate a shoe and overall changing area for workers.

Another key element in *Salmonella* contamination prevention in chocolate processes is the control of water. Cleaning procedures used in chocolate manufacturing and processing should always be directed toward dry cleaning as much as possible with a visual inspection on completion. Dry cleaning involves the application of kinetic energy to a surface and often includes manual scraping, brushing, and vacuum cleaning. Additionally, a clear color coding system for cleaning utensils should be implemented ensuring there are dedicated cleaning tools for food contact and nonfood contact surfaces to prevent cross contamination from dirty to clean surfaces. Hygienically, designed equipment is a vital factor in ensuring the ability to effectively clean chocolate processing equipment. Hollow bodies should be avoided, multifaceted surfaces minimized, and it should be possible to easily disassemble and reassemble equipment. Condensates coming from cold water piping, refrigerator coils, and the arches of cooling tunnels, may also act as a potential source of moisture and therefore *Salmonella*. As a result, cool surfaces located in warm areas should be sufficiently insulated and line/environment temperatures carefully monitored. Water leaks coming from closed loop jacketed water systems are common, especially in older equipment. Over time, biofilms may accumulate if water systems are not correctly monitored and treated, and in the event of a microleak, product contamination with *Salmonella* can occur. A key element in controlling this is through a planned preventive maintenance program. Through the formation of a thorough assessment plus historical background of each installation a schedule of planned maintenance should be developed and implemented to control a potential risk before it develops into a hazard. The need to also consider causes of previous *Salmonella* outbreaks as a means of preventing reoccurrences must also be emphasized, for example, since the 2006 Cadbury outbreak a planned preventive facilities maintenance schedule including regular checks on water pipes have prevented further *Salmonella* outbreak recurrences

which, as previously mentioned, should form part of the prerequisite program.

Monitoring programs that consider the environment (e.g., environmental sampling of floors and walls), equipment (e.g., samples taken from various points on a line and closed loop water system sampling), and product (e.g., positive release of part-processed and finished products) are essential to verify the effectiveness of the microbiological control measures as outlined above. A sampling plan should be developed to test, at regularly defined intervals, for *Salmonella* and an appropriate hygiene indicator such as enterobacteriaceae. Sampling points should target susceptible areas. Additionally, investigative sampling will be required. Data should be routinely evaluated and trends identified before the safety of the product becomes compromised. If hygienic processes and practices are insufficient or are not adhered to, then results from the pathogen monitoring program will highlight this. Corrections and corrective actions should then be implemented to manage potential risks. It is important to note that monitoring programs are a means of verifying the effectiveness of the control measures put in place and are not a control measure in their own right.

Conclusion

Pure roast and ground coffee has an inherently low risk of contamination (chemical, microbial, and physical hazards) that may lead to a food safety issue and harm the consumer. In the case of pesticides and fumigants, excursions of chemical residues above the MRL are seldom, and exceptional violation of an MRL is not a health or safety issue as adequate safety margins are incorporated into the MRL to allow a lifetime exposure at or below the given level. Nevertheless, the monitoring of mycotoxins at the green bean stage is important, and the EU Rapid Alert System has identified relatively high OTA levels in some producing countries in Africa, leading to the requirement of stricter controls in these origins. In the case of tea, pesticides remain an ongoing concern, particularly challenging to manage upstream at the growers and farmers level. The risk of microbial contamination in herbals and teas is not to be underestimated, and control is usually managed by the consumer by adding hot or boiling water to the product. Microbial contamination and foreign body hazards are most important to manage in cocoa and chocolate manufacture, requiring the establishment of reliable and robust good manufacturing practices (GMPs) and implementing HACCP at all stages of the supply chain.

The risk of encountering foods in the coffee/tea/cocoa – chocolate categories contaminated with pathogens or other contaminants at an unacceptable level is highly unlikely, provided that manufacturers follow GMP have robust and validated food safety management systems well embedded in their operations.

See also: Bacteria: *Salmonella* Non-Typhi. Mycotoxins: Ochratoxin A. Processing Contaminants: Acrylamide; Chloropropanols and Related Esters; Furan

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SAFETY OF FOOD AND BEVERAGES

Packaging Material and Auxiliary Items

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Glossary

Atactic This term is related to the tacticity of a polymer, which concerns the relative stereochemistry of contiguous chiral repeating units on a macromolecule. In vinyl polymers (as is the case of polystyrene (PS)), tacticity refers to the order in which substituent groups (aromatic ring in the case of PS) are located on one of the two sides of the macromolecular backbone. In the case of an atactic polymer, the substituents are placed randomly on the two sides of the macromolecule. Actually, mostly atactic PS is used although also syndiotactic PS is commercially available.

Bisphenol A The common name for 2,2-(4,4′-dihydroxydiphenyl)propane, 4,4′-isopropylidenediphenol, or 2,2′-bis(4-hydroxyphenyl)propane.

Esterification A chemical reaction that involves an oxygen-containing acid group of a reactant and a hydroxyl group (OH) of another reactant to form a product containing an ester group.

Nanoclay Layered magnesium aluminum silicate platelets.

Shelf life A period of time after which a packaged food, under defined environmental conditions, is no more acceptable in terms of organoleptic properties, nutritional value, and safety.

Standard temperature and pressure Standard temperature and pressure conditions, i.e., a temperature equal to 0 °C and pressure equal to 1 atm.

Aim of Packaging and Required Features

Food packaging serves the following important functions: (1) containing the food, (2) protecting the food against the action of external biological, physical, and chemical factors in order to preserve the food quality, prolong the shelf life of food and insure its safety by slowing down the microbial spoilage, (3) providing convenience for the consumer, and (4) acting as a communicating interface between the producer and consumer.

Selection of a proper packaging material is based on these required functions and also should take into account the cost, weight, transport, esthetic, and processing issues.

Primary packaging, i.e., the one that forms a sales unit for the user or final consumer (e.g., a bottle of water, can of soft drink, and packet of pasta) and directly contains food was mainly focused by the authors.

Packaging Materials and Structures

Relevant Functional and Structural Properties

To protect and preserve the contents, the food packaging material should guarantee adequate barrier to gases, vapors, and aromas, and adequate mechanical, thermal, and electromagnetic properties, depending on the properties of the food and required shelf life.

Barrier Properties

Depending on the material, low molecular weight (m.w.) compounds may or may not solubilize and diffuse through it. Although metallic and glassy materials are virtually impervious to low m.w. compounds, polymeric and cellulosic materials display finite permeability to gases and vapors, spanning a wide range of values. Packaging material should provide adequate 'barrier' to gases, vapors, and aromas, as determined by the preservation purposes. In particular, mass transport of oxygen, carbon dioxide, nitrogen, water vapor, and aromas is of paramount importance. The optimal barrier depends on the type of food and on the desired shelf life. Barrier to external oxygen from the environment to avoid or limit degradative oxidation of fats, barrier to internal carbon dioxide for carbonated beverages, and a proper ratio of the permeability of several gases to keep a desired 'modified atmosphere' in the package headspace are some relevant examples.

The main parameter describing barrier properties of a material is related to permeation. The higher the permeation of a low m.w. compound within a material, the lower is the barrier. It is important to notice that this property is referred to as the steady state condition. Measurements are performed by imposing a different partial pressure of the permeant on the two sides of the film or the sheet to be characterized, or between the external environment and internal part of a container. Either the pure permeant or the permeant present in a

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mixture can be used. Different permeabilities are possibly measured in these two cases: As an example, pure oxygen permeation through a film is generally different (at equal partial pressure) from the case of oxygen permeation in the presence of water vapor.

There are several terms and units to describe permeation, which are as follows:

1. Permeation rate: Is referred to the overall package. It is defined as the permeated quantity per unit time. An example of adopted units is cm^3 (standard temperature and pressure (STP)) day^{-1}.
2. Transmission rate (TR): is defined as

$$TR = \frac{\text{Permeated quantity}}{\text{Area} \times \text{Time}}$$

Unit of TR is mol $(m^2\ s)^{-1}$ or, more commonly in the case of gases, cm^3 (STP) $(m^2\ day)^{-1}$. In this latter case, the term Gas TR is adopted.

3. Permeance (P), defined as:

$$P = \frac{TR}{\Delta p}$$

i.e., TR divided by the difference in the partial pressure (Δp) between the two sides of the film. Examples of units for P are mol $(m^2\ s\ Pa)^{-1}$ and g $(m^2\ s\ Pa)^{-1}$.

4. Permeability ($\overline{P}$) is different from the three previous properties, that can be defined for both heterogeneous and homogeneous films, in that permeability can be defined only for homogeneous films as:

$$\overline{P} = \frac{TR}{(\Delta P/l)}$$

i.e., TR divided by the 'driving force' for permeation (i.e., $\Delta p/l$, where l is the film thickness) between the two sides of the film. Examples of units for $\overline{P}$ are $(cm^3$ (STP) cm) $(cm^2\ s\ cm\ Hg)^{-1}$ or (g cm) $(cm^2\ s\ cm\ Hg)^{-1}$.

Mechanical Properties

Mechanical properties of packaging materials are of importance during packaging manufacture, during the operative life of packaging itself, in the production stages of packaged food, as well as during the distribution stages. Briefly, the most important mechanical properties of the packaging material are mentioned.

1. *Coefficient of friction (COF)*: It is related to the resistance to sliding between the two surfaces of two materials. It is of great importance to evaluate the machinability of a material in processes like printing or lamination. This property can be modified by the addition (mainly in polymeric packaging materials) of proper additives. Both static and dynamic COF are defined and expressed in terms of a ratio of forces.
2. *Mechanical moduli and strength*: A single material property cannot encompass the full complexity of mechanical behavior of a material. Some properties which are technologically relevant in the present context are:
   - Brittleness: It measures the attitude of a material to break under a load without a significant deformation, failing with little or no sign of plastic deformation.
   - Burst strength: It is related to the pressure that is required to bring about the bursting of the package.
   - Ductility: It measures the attitude of a material to deform plastically under a load before breakage.
   - Impact resistance: It is related to the resistance of a packaging material to impact and is measured as the energy (generally expressed in Joule, J) absorbed by a standardized specimen for breaking under a standardized impact.
   - Tear strength: It measures the tearing resistance of a (cellulosic or polymeric) packaging material as measured by the force (generally expressed in N) and absorbed energy (generally expressed in J) needed for the initiation and propagation of a tearing.
   - Tensile modulus: It describes the resistance of a material to deform uniaxially under a tensile load (typical units are MPa or GPa, where Pa stands for Pascal).
   - Tensile strength: It is the maximum tensile strength that a material can sustain before ultimate breakage (typical units are MPa or GPa).
   - Toughness: It is a measure of the energy a material is able to absorb by deforming under a load (typical units are those of energy per unit volume, i.e., MPa or GPa).
   - Creep/relaxation: These are properties related to the viscoelastic nature of a material. In fact, creep is a measure of time-dependent deformation occurring under a stress constant with time, whereas relaxation is a measure of stress reduction with time in the material submitted to a deformation constant with time.

Thermal Properties

The relevant thermal properties of materials for food packaging applications are those related to energy transport and thermal transitions.

1. *Thermal conductivity (k)*: It is defined as the amount of energy transferred in the form of heat per unit time, unit thickness, and unit temperature difference between the two sides of a slab of material of thickness l. Common units are kcal per (m h °C) or W per (m °C). This property is relevant in the processing stages of packaged food (e.g., sterilization) or packaging process of food (e.g., sealing). Thermal conductivity, among packaging materials, is the highest in metals (e.g., approximately 240 W per (m °C) for aluminum), the lowest for cellulosic and foamed polymeric materials (e.g., approximately 0.050 for expanded linear low density polyethylene (LDPE)), and intermediate for inorganic materials (e.g., approximately 1 for common inorganic glasses).
2. *Specific heat at constant pressure (C_p)*: It is defined as the amount of energy in the form of heat to be transferred per unit mass of material to increase its temperature by 1 °C. Common units are kcal $(kg\ °C)^{-1}$ or kJ $(kg\ °C)^{-1}$. This property is relevant in evaluating the degree of protection that the packaging provides against heat. Specific heat, among packaging materials, is the highest in cellulosic and polymeric materials (e.g., approximately 2.3 kJ $(kg\ °C)^{-1}$ for LDPE) and the lowest for metals and inorganic

materials (e.g., approximately 0.9 for aluminum and 0.84 for common inorganic glasses).
3. *Thermal expansion*: Linear (α) and volumetric (β) expansion coefficients are defined, respectively, as the relative change in length or volume per unit temperature change at a fixed pressure. Common units are $^{\circ}C^{-1}$. This property is relevant in evaluating possible breakages of package due to temperature changes. Thermal expansion is the highest in polymeric materials (e.g., approximately 110 $^{\circ}C^{-1}$ for polypropylene (PP)), intermediate for metals (e.g., approximately 25 $^{\circ}C^{-1}$ for aluminum), and the lowest for inorganic materials (e.g., approximately 6 $^{\circ}C^{-1}$ for common inorganic glasses).
4. *Transition temperatures*: The most important transition temperatures are the glass transition temperature (T_G) and melting temperature (T_M). T_G marks a substantial change in the mobility of amorphous regions of a material: below T_G the amorphous regions become much more brittle and rigid. The T_M corresponds to the phase change of the solid crystalline regions of a material into a liquid state. These transition temperatures are extremely important because they are related to the tolerable thermal range for the use of packaging materials, which define the lowest allowable temperature (at which the maximum allowable fragility occurs) and highest allowable temperature (at which unacceptable distortion or chemical modification occur). The highest temperatures among packaging materials are accessible with metals or inorganic glass materials (up to 600 $^{\circ}C$) and the lowest temperature are accessible with some polymeric materials (down to approximately -100 $^{\circ}C$ with fluoropolymers and high density polyethylene (HDPE)).

Electromagnetic Properties

Properties describing the interaction of the packaging materials with electromagnetic radiation are of great importance for protection against the action of light, for the interaction with microwaves, and for esthetic issues of a package.

1. *Refractive index*: It is related to the angles of incidence and refraction at the interfaces of the material surfaces for a radiation passing through the material itself.
2. *Transparency*: It measures the specular transmittance of a radiation through the material.
3. *Transmittance spectrum in ultraviolet (UV), Vis, and IR*: This property quantifies the transparency of the material at specific wavelengths. This is of particular relevance, for example, in quantifying the degree of protection provided by the packaging material against the photooxidation of lipids activated by UV radiation.
4. *Haze*: It measures the degree of opacity of the packaging material.
5. *Gloss*: It measures the shiny appearance of the surface of a packaging material.
6. *Effect of ionizing radiations*: Some radiations like β, γ, and X-radiation, can promote an alteration in some packaging materials. In particular, chain scission, oxidation, and cross-linking can occur in polymeric materials as a consequence of γ-radiation, which is frequently used for sterilizing the material surface to be placed in contact with food.
7. *Interaction with microwaves*: Depending on its structure, a material can interact with microwaves or be transparent to them. Polymeric materials and inorganic glasses are transparent, whereas metals are absorbing/reflective. This interaction behavior is relevant in the application of microwavable packaged food.

Chemical Structure and Morphology of Polymeric, Metallic, Ceramic, and Cellulosic Packaging Materials

Polymeric Materials

Polymers have been used increasingly in the last decades as packaging materials for numerous packaging applications. In fact, these materials can be processed in several forms like films, sheets, trays, bottles, and other types of containers. Polymer molecules, also referred to as macromolecules, have a very high m.w., up to the order of several million daltons. They are obtained by arranging together small repeating units.

Polymers can be classified into two main categories: thermoplastics and thermosets.

Thermoplastics, the most widely used type of polymers in food packaging, are made of independent macromolecules that could be linear or branched at different degrees. The chemical properties of thermoplastics are determined by the functional chemical groups present along the macromolecular chain. Properties of polymers can be finely tuned by changing the types of monomers used to build the polymer (the authors refer to a homopolymer or a copolymer, if, respectively, a single type of monomer or several types of monomers are used) or by blending different polymers. Depending on the chemical structure, the polymers can have a totally amorphous morphology or can be semicrystalline (it is not possible to have a totally crystalline thermoplastic polymer). The T_G involves only the amorphous phase of the material and T_M (higher than T_G) only involves crystalline domains. Properties of thermoplastics can also be tailored by the processing conditions. In fact, polymers can be stretched uni- or biaxially at a temperature between T_G and T_M, thus orienting both the amorphous and crystalline domains in preferential directions and thereby improving the mechanical and barrier properties.

Thermosets consist of a cross-linked network where polymer chains are all linked by chemical bonds. As opposed to thermoplastics, thermosets cannot be reprocessed and are more resistant to chemicals.

In general, polymers for packaging applications always contain additives, such as fillers, antioxidants, plasticizers, and other additives to improve the processing and mechanical properties, and resistance to environmental factors during service life.

The most widely used polymers in food packaging applications are mentioned as follows:

1. *Polyethylene (PE)*: There are actually thousands of types of PEs available commercially. These are rubbery (i.e., T_G is lower than room temperature) semicrystalline polymers. There are three main categories: LDPE, which, due to the branched structure of macromolecules, displays a limited crystallinity and, in turn, a lower density; HDPE, characterized by a more linear structure with few short side-chain branches displaying a higher crystallinity; linear LDPE (LLDPE), characterized by many and very short

side-chain branches. They are used as a heat-sealing layer and barrier to water vapor in the forms, among others, of blow-molded bottles, films, and containers.

2. *PP*: It is a rubbery semicrystalline polymer widely used in the form of nonoriented and oriented films and containers. It has good mechanical properties and high moisture barrier.
3. *PS*: It is the atactic PS used in food packaging applications, which is glassy (i.e., T_G is higher than room temperature) and totally amorphous with a low barrier to gases and water vapor. It is also used in its foamed form, known as Expanded PS, mainly for thermal and mechanical protection purposes, and blended with polybutadiene and grafted polybutadiene–polystyrene copolymer in the form of high impact PS displaying improved impact properties. Barrier properties to gases and moisture are quite low.
4. *Polyvinyl chloride (PVC)*: It is an amorphous glassy and rigid polymer used to produce films and containers. Barrier properties are moderate and it has a limited use due to environmental concerns related to the presence of organochlorine pollutants. Plasticized PVC, obtained by adding plasticizers (phthalates and adipates), is used to produce stretchable film (the so-called 'cling film'), but certain safety issues have been raised related to the migration of plasticizers into the food.
5. *PE terephthalate (PET)*: It is a glassy semicrystalline polymer with a moderate barrier to gases and water vapor. It is used to make films, trays, and bottles.
6. *Polyvinylidene chloride (PVdC)*: Also known as Saran™, it is a copolymer of vinylidene chloride (approximately 90%) and vinyl chloride monomers. It is used to make films and containers. Owing to its very high barrier to gases and water vapor, it is frequently used in the coating of more permeable films (e.g., PP) or as a barrier layer in multilayer packaging structures (films or thermoformable sheets).
7. *Ethylene vinyl alcohol (EVOH)*: It is a semicrystalline copolymer of vinyl alcohol and ethylene monomers (27–48%). It has an exceptional barrier to oxygen that tremendously decreases in the presence of moisture. The barrier to water vapor is low. It is mainly used as an oxygen barrier layer in multilayer laminated or coextruded films and containers.
8. *Ionomers*: They are copolymers made of ethylene and methacrylic acid (up to 15%) monomers. Methacrylic acid groups are partially or totally neutralized by zinc or sodium ions. Surlyn® is the trade name of a widely used class of ionomers. Their use in food packaging applications is related to the very good sealing properties and low melting temperature, which helps with temperature-sensitive foods.
9. *Ethylene vinyl acetate (EVA)*: It is a copolymer of ethylene and vinyl acetate monomers that is mainly used in hot melt formulations, as a sealing layer, and for bags t o package frozen foods.
10. *Polyamides (PAs)*: It is a class of generally semicrystalline polymers encompassing a wide range of glass transition temperatures. They derive their name by the presence of a chain of amide groups formed by the reaction of amine and carboxylic acid moieties of the starting monomers. Nylon 6 and Nylon 6,6 are important examples of PAs. They are used to produce films with high barrier properties.

Table 1 Physical properties of some polymeric materials widely used for food packaging applications

Polymer	*Density (kg m^{-3})*	*Mechanical property (qualitative)*	*Resistance to grease and oil*	*Transparency*	*Temperature range of use (°C)*
LDPE	910–925	Tough and flexible	Good	Poor/fair	−50 to 80
LLDPE	910–940	Tough and extensible	Good	Poor/fair	−30 to 100
HDPE	945–967	Tough and flexible	Good	Poor	−40 to 120
PP	900–915	Moderately stiff and strong	Good	Fair	−40 to 120
PS					
General	1040–1089	Stiff, strong, and brittle	Fair/good	Very good	−20 to 90
HiPS	1030–1100	Tough and strong	Fair/good	Poor	−20 to 90
PVC					
Plasticized	1350–1450	Soft and extensible	Good	Good	−2 to 80
Unplasticized	1160–1350	Stiff and strong	Good	Good	−2 to 80
PET	1380–1410	Stiff and strong	Good	Very good	−60 to 200
PVdC	1600–1700	Stiff and strong	Good	Good	−20 to 130
EVOH	1140–1410	Stiff and strong	Very good	Good	−20 to 150
Ionomers	940–960	Tough and extensible	Good	Very good	−40 to 65
EVA	920–940	Tough and extensible	Good	Very good	−75 to 65
Nylon	1130–1160	Strong and tough	Good	Fair/good	−2 to 120

Abbreviations: EVA, ethylene vinyl acetate; EVOH, ethylene vinyl alcohol; HDPE, high density polyethylene; HiPS, high impact polystyrene; LDPE, low density polyethylene; LLDPE, linear low density polyethylene; PET, polyethylene terephthalate; PP, polypropylene; PS, polystyrene; PVC, polyvinyl chloride; PVdC, polyvinylidene chloride.

Source: Reproduced from Lee DS, Yam KL, and Piergiovanni L (2008) *Food Packaging Science and Technology*. Boca Raton, USA: CRC Press.

Table 2 Barrier properties of some polymeric materials widely used for food packaging applications

Polymer	*Water vapor permeability* $(g\ mil) \cdot (100\ in^2\ day)^{-1}$ *At 38 °C and 90% RH*	*Oxygen permeability* $(cm^3\ (STP)\ mil) \cdot (100\ in^2\ day\ atm)^{-1}$	*Carbon dioxide permeability* $(cm^3\ (STP)\ mil) \cdot (100\ in^2\ day\ atm)^{-1}$
LDPE	1–2	300–600	1200–3000
HDPE	0.3–0.6	100–250	350–600
PP			
Unoriented	0.6–0.7	150–250	500–800
Oriented	0.2–0.5	100–160	300–550
PS	7–10	250–300	900–1050
PET	1–2	3–6	15–25
PVC			
Plasticized	15–40	50–1500	200–8000
Unplasticized	2–5	5–15	20–50
PVdC	0.02–0.6	0.1–2	0.2–0.5
EVOH	1.5–8		
0% RH		0.007–0.1	0.01–0.5
100% RH		0.2–3	4–10
Ionomers	1.5–2	300–450	
Nylon 6	10–20	2–3	10–12

Abbreviations: EVOH, ethylene vinyl alcohol; HDPE, high density polyethylene; LDPE, low density polyethylene; PET, polyethylene terephthalate; PP, polypropylene; PS, polystyrene; PVC, polyvinyl chloride; PVdC, polyvinylidene chloride; RH, relative humidity.

Source: Reproduced from Lee DS, Yam KL and Piergiovanni L (2008) *Food Packaging Science and Technology*. Boca Raton, USA: CRC Press.

Tables 1 and 2 present some important physical properties of polymers used in food packaging.

Inorganic, Nonmetallic Materials

This class of materials, referred to as ceramic materials, has been used for centuries to make food containers. Currently, glasses are in widespread use and are produced from molten inorganic nonmetallic materials. By proper cooling procedures, a noncrystalline solid is obtained. The main component of glasses is silicon oxide, but several other compounds are present, for example, oxides of alkaline earth elements (i.e., calcium, sodium, and magnesium) are added to guarantee a low temperature of melting and to lower the viscosity of the melt. Metal-based compounds are added as coloring ingredients.

Glasses are mainly used to make bottles and containers. These are fragile materials prone to brittle fracture, often initiated by the presence of surface scratches and flaws. For this reason, to strengthen the containers, surface coating is deposited on the surface and chemical or thermal toughening treatments are adopted. These materials are virtually impermeable to gases and vapors and can be used at elevated temperatures, of up to approximately 500 °C.

Glasses are quite inert to most of the substances with the exception of hydrofluoric acid, heated phosphoric acid, and hot alkalies. In acidic medium, a limited leaching of positive ions can occur. Glasses are instead prone to alkaline attack. In hot pressurized distilled water (121 °C), both ion leaching and alkaline attack can occur.

Metallic Materials

The metallic materials mainly used in food packaging applications are aluminum and steel, often coated with tin, chromium oxides, and varnishes. These metals are used to form cans (three- and two-piece cans), collapsible tubes, aerosol containers, and trays.

Aluminum: It is used not only in different forms like cans and foils, but also as a coating (typical thickness of the order of 0.01 μm) to manufacture metallized polymer films with improved barrier to light and low m.w. substances, as compared to the neat plastic substrate. Pure aluminum displays a rather high tensile modulus (approximately 70 GPa), high thermal conductivity, and a relatively low density ($2.71\ g\ cm^{-3}$). It is used in packaging applications either as pure aluminum or in the form of alloys containing manganese, magnesium, iron, silicon, copper, and chrome.

Steel: A steel is an alloy of iron and carbon. In food packaging applications, steel is prevalently used with up to 1% of carbon. Manganese, phosphorus, copper, silicon, and sulfur are also present as alloying elements. Density of steel ($7.85\ g\ cm^{-3}$) is much higher than that of aluminum as well as its tensile modulus (approximately 210 GPa). Because chemical inertness of steel toward foods can be a concern, steel is often coated with inorganic or organic coatings, in food packaging applications. A classic example of coated steel used for canned foods is 'tinplate' that consists of carbon steel coated with a layer of tin. Owing to the cost of tin, steel coated with metallic chromium and chromium oxide has been introduced.

Cellulosic Materials

Cellulosic packaging is based on raw materials containing cellulose fibers and includes corrugated boards and boxes, bags, folding cartons, and cans.

Paper and paperboard: Paper is produced starting from cellulose pulp which is obtained from the raw materials by mechanical/chemical processes. Apart from cellulose fibers, several noncellulosic additives are used in the process of paper

making ranging from fillers and coloring compounds to sizing and plasticizing additives, and many others. The suspension of cellulose fibers and additives is submitted to draining, pressing, and drying, gradually reducing its moisture content to obtain sheets of different densities (increasing the density yields paper, then cards, and finally boards). The amount of moisture determines the final mechanical and barrier properties.

The final properties of paper products to be used in food packaging applications are determined by coating, impregnating, laminating, and finishing steps. Paper is frequently used in multilayer structures with other materials, including aluminum foil and LDPE.

Cellulosic packaging takes the form of flexible films, bags, corrugated board boxes, wrappings, composite cans, and laminated cartons for liquids. They are used, among others, for the packaging of bakery products, biscuits, powders, snacks, fruit juices, milk, and wine. Finally, it is worth mentioning that the oldest commercial transparent flexible film, known under the trade name of cellophane, was produced starting from pure cellulose, originally commercialized by DuPont. The advantages of cellophane include its transparency and gloss, as well as its outstanding barrier properties to gases and, if properly coated (e.g., metallized or coated with PVdC), also to moisture. Owing to its higher cost, starting from the 1970s, it has been replaced in many applications by oriented PP, although its properties, including also stiffness, clarity, and processability, still make cellophane attractive for selected packaging applications (e.g., candies, chocolate, cheese, and snacks).

Biodegradable Packaging Materials

Biodegradation is the chemical process by which organic materials are broken down by enzymes produced by living organisms. Biodegradation of a certain material strongly depends on the conditions in which it is measured (kind of bacteria, temperature, humidity, oxygen concentration, etc.). A polymer could be biodegradable in some conditions (i.e., composting), but not in other conditions (i.e., terrain or marine environment). To reduce the possible ambiguity, some norms that define various characteristics a material must possess in order to be claimed biodegradable have been developed (e.g., EN 13432, ASTM D6400-04, D7081-05, D6868-03, D5511, and D5526).

At the moment, available biodegradable plastics are not able to replace the conventional petroleum-based plastics, both for their costs and limited performance (i.e., low mechanical and barrier properties). However, it is possible to overcome the low performance and process problems by combining several biodegradable materials with different functional characteristics by using lamination in order to make multilayer structures with improved properties. Also by adding to these polymers nanofillers, biodegradable nanocomposites with improved barrier and mechanical properties can be obtained.

The aforementioned cellulosic packaging materials are biodegradable, unless nonbiodegradable coating are used to improve some properties or unless they are laminated with nonbiodegradable materials.

Starch polymers have been in the forefront of the biobased plastics in the plastics market over the last 20 years. Starch is composed of a mixture of two polymers, a linear polysaccharide called amylose and highly branched polysaccharide called amylopectin. Native starch is however too brittle and hydrophilic. To overcome these disadvantages, native starch is chemically, thermally, and/or mechanically processed. Typically, the majority of starch plastics are produced via chemical modification and extrusion/blending of native starch and its derivatives. It is also blended with synthetic aliphatic polyesters to improve the water resistance and processability. The final properties of starch plastics are such that they can be considered potential substitutes for some polyolefins, namely LDPE, HDPE, and PP. European producers have well-established products in the market.

Polylactic acid (PLA) is a thermoplastic, glassy, semicrystalline aliphatic polyester produced via polymerization of a cyclic lactide ester (L,L-lactide). This compound is obtained by the esterification and cyclization of lactic acid, that, in turn, is produced via bacterial fermentation of sugars. The T_G of PLA is approximately 62 °C and the T_M is approximately 175 °C. Many properties of the PLA films are similar to synthetic thermoplastic polymers, i.e., high gloss and transparency, mechanical strength, heat sealability; in addition, PLA is not hygroscopic and has good barrier properties. In fact, PLA can replace PET and PS in several packaging applications in the form of films, sheets, cups, trays, bottles, and containers.

Finally, a class of linear biodegradable rubbery thermoplastic polyesters with good properties for food packaging applications, is that of polyhydroxyalcanoates, obtained via bacterial fermentation of sugars. In particular, polyhydroxybutyrate–valerate copolymer displays properties close to those of PP. The use of this class of polymers in food packaging is still limited due to its performance, processability, and cost as compared to traditional thermoplastics.

Auxiliary Materials

Auxiliary materials are those materials providing additional features to packaging materials or those materials used in the process of manufacturing packaging structures. Examples of the former are lacquers providing improved barrier properties, inks used for printing and labeling, coating used to protect food and package surface in metallic packaging, whereas examples of the latter are adhesives used for laminating polymer films to manufacture multilayer structures.

Regarding the adhesives, after the wetting of the substrates, the adhesive curing takes place by either physical or chemical processes. Examples for the physical curing are the heat-seal coatings and direct labeling adhesives as well as natural latex-based cold seal adhesives. An important example of chemically curing adhesives are solvent-based and solventless polyurethane laminating adhesives. Water-based and UV/electron beam curing systems have also been developed in the last two decades.

Inks for printing on plastic substrates can be divided into four major classes: letterpress and lithographic inks, commonly called paste inks; flexographic and rotogravure inks, which are referred to as solvent inks. Over printing, varnishes

are used in order to cover printing inks with a protective layer and/or to provide functional properties to the packaging such as machinability, gloss or matt appearance, release or sealing properties, scratch resistance, etc. Varnishes called primers are also used to improve the adhesion of inks on some substrates, typically metallized layers.

Several materials are used as coatings for plastic films and containers, with a thickness ranging from few tens of nanometers to a few micrometers, mainly to provide barrier to gases, moisture, and aromas. Examples are micrometric coatings made with polymer nanocomposites and with PVdC (Saran™), nanometric coatings made of SiO_x, AlO_x, and amorphous carbon, nanolayer coatings realized by combustion chemical vapor deposition.

Several types of coatings are used for metal containers to protect the food from direct contact with metal and includes oleoresinous lacquers (enamels), vinyl lacquers (vinyl chloride–vinyl acetate copolymer), phenolic lacquers, epoxy and epoxy-acrylate coatings, and alkyd resins. These materials display different levels of flexibility, adhesion, and retortability. It is worth mentioning that Bisphenol A (BPA), an intermediate in the production of epoxy resins used as an internal coating for a can, has become a major public health issue (see: Bureau of Chemical Safety, Food Directorate, Health Products and Food Branch, Canada. Survey of BPA in Canned Food Products from Canadian Markets (2010)). In fact, BPA can migrate (see Section Migration) from the coating into foods, this phenomenon being particularly relevant for hot-fill or heat-processed canned foods.

Scavenger/absorber materials are frequently used to remove undesired substances from the headspace or to limit the permeation of such substances through the package, by means of physical/chemical mechanisms. Relevant examples are oxygen scavengers, moisture, carbon dioxide, and ethylene absorbers.

Food Packaging Interactions

A packaging material in contact with food can interact with it by releasing undesired substances (migration) to the food and undesired removal of substances from the food (scalping). The amount of migration and scalping should be kept within prescribed limits. Interaction between packaging and food induced on purpose for preservation aiming to prolong the shelf life are discussed in Section Active Packaging.

Migration

Migration can be defined as a food–package interaction in which chemical compounds move from the package into the food. In fact, polymeric packaging materials contain functional additives, residuals from the manufacturing process, and neoforming or decomposition products formed during the processing or aging of plastic materials that can all possibly migrate into the food. The European Union (EU) Regulations EU 1935/2004 and 10/2011 regulate materials and articles intended to come in contact with food and establish the monomers, starting substances, and additives that are authorized in their manufacture as well as the restrictions which they are subjected to, such as overall and specific migration limits (SMLs, see Section International Regulation).

Scalping

Flavor scalping has been reported to be an important factor contributing to alterations of organoleptic quality of packaged food. In particular, it determines the loss of quality related to the absorption of aroma compounds from a food product to the packaging material and may result in a significant alteration of the aromatic profile of the food product. Polymers, in fact, are able to dissolve low m.w. substances present in food and beverages. This phenomenon can be limited to regions at the surface of the materials or may involve mass transport via diffusion through the thickness of the material. Scalping may reduce or alter the flavor of a packed food to such an important and undesirable extent that it should be minimized and possibly avoided.

International Regulation

Important concepts to be defined are those of overall and specific migrations, which are given as follows:

- Overall migration (OM) defines the maximum quantity of substances (nonvolatile) that a packaging structure can release to the foods or food simulants. Substantially this value indicates, in a nonspecific manner, the quantity of substance that migrates into the foods, independently from the kind or nature of the substances.
- Specific migration (SM) defines the maximum quantity of a specific migrable substance that can be released into the foods or food simulants and it must not exceed the fixed specific limitation.

Regulation EU/10/2011 establishes that the OM value is reported in mg dm^{-2} and in particular that "Plastic materials and articles shall not transfer their constituents to food simulants in quantities exceeding 10 mg of total constituents released per dm^2 of food-contact surface (mg/dm^2)." The assumptions are: (1) an intake of 1 kg of food in contact with a particular packaging material per 60-kg person per day and (2) 1 kg of food is exposed to 6 dm^2 surface area of packaging material. As a consequence, the overall level of migration mentioned by the regulation is equivalent to 60 mg kg^{-1} of food mass.

In the USA, the Food and Drug Administration (FDA) regulation assumes that the total daily intake of food and drink is 3 kg per person. An important difference between EU and USA regulation is that in the USA, a consumption factor (CF) and food-type distribution factor (f_T) are used by the FDA to estimate a probable exposure. CF describes the fraction of the daily diet expected to contact specific packaging materials and represents the ratio of the weight of all food contacting a specific packaging material to the weight of all food packaged. CF values for both packaging categories (e.g., metal, glass, polymer, and paper) and specific food-contact polymers

Table 3 Values of consumption factor (CF) for several packaging materials

	Package category	*CF*	*Package category*	*CF*
A. General	Glass	0.1	Adhesives	0.14
	Metal – polymer coated	0.17	Retort pouch	0.0004
	Metal – uncoated	0.03	Microwave susceptor	0.001
	Paper – polymer coated	0.2	All polymers[a]	0.8
	Paper – uncoated and clay-coated	0.1	Polymer	0.4
B. Polymer	Polyolefins	0.35[b]	PVC	0.1
	LDPE	0.12	Rigid/semirigid	0.05
	LLDPE	0.06	Plasticized	0.05
	HDPE	0.13	PET[cd]	0.16
	PP	0.04	Other polyesters	0.05
	Polystyrene	0.14	Nylon	0.02
	EVA	0.02	Acrylics, phenolics, etc.	0.15
	Cellophane	0.01	All others[e]	0.05

[a]Originates from adding CFs for metal – polymer coated, paper – polymer coated, and polymer ($0.17 + 0.2 + 0.4 = 0.8$).
[b]Polyolefin films, 0.17 (HDPE films, 0.006; LDPE films, 0.065; LLDPE films, 0.060; and PP films, 0.037).
[c]PET-coated board, 0.013; thermoformed PET, 0.0071; PET carbonated soft drink bottles, 0.082; custom PET, 0.056; crystalline PET, 0.0023; PET films, 0.03.
[d]A CF of 0.05 is used for recycled PET applications (see the document titled 'Points to Consider for the Use of Recycled Plastics in Food Packaging: Chemistry Considerations').
[e]As discussed in the text, a minimum CF of 0.05 will be used initially for all exposure estimates.
Abbreviations: EVA, ethylene vinyl acetate; HDPE, high density polyethylene; LDPE, low density polyethylene; LLDPE, linear low density polyethylene; PET, polyethylene terephthalate; PP, polypropylene; PVC, polyvinyl chloride.
Source: Reproduced from *Guidance for Industry. Preparation of Premarket Submissions for Food Contact Substances: Chemistry Recommendations* (2002, 2007). College Park, MD: U.S. Department of Health and Human Services - FDA Center for Food Safety and Applied Nutrition. Available at: www.fda.gov/OHRMS/DOCKETS/98fr/001360gd.pdf (accessed on 29 January 2013).

are summarized in Table 3. These values, however, may be modified as new information is received.

The other factor used by the FDA is f_T. Before migration levels can be combined with CF values to derive estimates of probable exposure, the nature of the food that will likely contact the food-contact article containing the food contact substances (FCSs) must be known. To take into account the nature of food contacting each food-contact article, FDA provides f_T for each packaging material to reflect the fraction of all food contacting each material that is aqueous, acidic, alcoholic, and fatty. f_T values for both packaging categories and polymer types are reported in Table 4.

Another important concept adopted by FDA is the threshold of regulation (TOR) in which the attention is focused on the exposure value, that is the amount related to the daily intake. The idea is that if the dietary concentration of FCS, even if no toxicological data are available, does not exceed 0.5 ppb, it does not require any formal regulation. Substances that are demonstrated to be carcinogens and substances that, based on their chemical structure, are suspected to be carcinogens, are excluded from clearance under the TOR process.

Owing to the complexity of food samples, the legislation allows for the migration tests to be carried out using food simulants instead of real samples with the aim of simplifying the analysis. When the aim is to evaluate the amount of OM, the most classical approach is a gravimetric test. After evaporation of the simulant, the dry residue or sample material after the contact is weighted to determine by difference the amount of solid substances migrated.

Regarding the SML, the Regulation EU/10/2011 establishes the SM value in its Annex I, reported in mg per kg. For substances for which no SML are provided, a generic value of 60 mg kg^{-1} shall apply. The evaluation of the SML is usually performed by means of analytical methods. Generally these substances are present in extremely low amounts, measurable at the ppm level in the best case and very often at the ppt level. Food simulants are liquid or solid substances able to emulate the extraction capacity and solubility of the foodstuff. They are generally volatile substances which can be easily removed after contact with the tested material. In Tables 5 and 6 a schematization of food simulants used in the USA and EU is reported.

A further alternative to assess migration from packaging into food is represented by the use of mathematical and semiempirical models, that allow to circumvent possible analytical difficulties and expensive, time-consuming analyses. This approach provides a mean to determine migration levels using limited or no migration data. In fact, these mass transport models are based on the estimation of diffusion coefficients accounting for nature of the migrant and physical properties of the packaging material. The prevailing legislation allows the use of the results of mathematical modeling if it is proven, on the basis of scientific evidence, that migration levels calculated in this way are equal to or higher than those achieved under real storage conditions, i.e., the tests represent a 'worst case' scenario. In fact, migration estimation is accepted in the USA and EU legislation (EU Commission Directive, 2002/72/EC), and valid models based on scientific evidence can be applied to test for compliance with the existing regulations. These models are considered a useful substitute for, or in addition to, experimental data, under limited circumstances. Nevertheless, when the mathematical predictions indicate noncompliance with the legislation, the migration values must be confirmed by laboratory testing.

Table 4 Values of food type distribution factor (f_T) for several packaging materials and types of food

	Package category	*Food-type distribution (f_T)*			
		Aqueous[a]	*Acidic*[a]	*Alcoholic*	*Fatty*
A. General	Glass	0.08	0.36	0.47	0.09
	Metal – polymer coated	0.16	0.35	0.40	0.09
	Metal – uncoated	0.54	0.25	0.01[b]	0.20
	Paper – polymer coated	0.55	0.04	0.01[b]	0.40
	Paper – Uncoated and clay-coated	0.57	0.01[b]	0.01[b]	0.41
	Polymer	0.49	0.16	0.01[b]	0.34
B. Polymer	Polyolefins	0.67	0.01[b]	0.01[b]	0.31
	Polystyrene	0.67	0.01[b]	0.01[b]	0.31
	Impact polystyrene	0.85	0.01[b]	0.04	0.10
	Nonimpact polystyrene	0.51	0.01	0.01	0.47
	Acrylics, phenolics, etc.	0.17	0.40	0.31	0.12
	PVC	0.01[b]	0.23	0.27	0.49
	Polyacrylonitrile, ionomers, and PVdC	0.01[b]	0.01[b]	0.01[b]	0.97
	Polycarbonates	0.97	0.01[b]	0.01[b]	0.01[b]
	Polyesters	0.01[b]	0.97	0.01[b]	0.01[b]
	Polyamides (nylons)	0.10	0.10	0.05	0.75
	EVA	0.30	0.28	0.28	0.14
	Wax	0.47	0.01[b]	0.01[b]	0.51
	Cellophane	0.05	0.01[b]	0.01[b]	0.93

[a]For 10% ethanol as the food simulant for aqueous and acidic foods, the food-type distribution factors should be summed.
[b]1% or less.
Abbreviations: EVA, ethylene vinyl acetate; PVC, polyvinyl chloride; PVdC, polyvinylidene chloride.
Source: Reproduced from *Guidance for Industry: Preparation of Premarket Submissions for Food Contact Substances: Chemistry Recommendations* (2002, 2007). College Park, MD: U.S. Department of Health and Human Services - FDA Center for Food Safety and Applied Nutrition. Available at: www.fda.gov/OHRMS/DOCKETS/98fr/001360gd.pdf (accessed on 29 January 2013).

Table 5 Recommendations for food simulants in USA

Food types as defined in 21 CFR 176.170 (c)	*Food simulants*
Aqueous and acidic foods	10% Ethanol
Low- and high-alcoholic foods	10% or 50% Ethanol
Fatty foods	Food oil (e.g., corn oil), HB 307, miglyol 812, or others

Source: Reproduced from *Guidance for Industry: Preparation of Premarket Submissions for Food Contact Substances: Chemistry Recommendations* (2002, 2007). College Park, MD: U.S. Department of Health and Human Services - FDA Center for Food Safety and Applied Nutrition. Available at: www.fda.gov/OHRMS/DOCKETS/98fr/001360gd.pdf (accessed on 29 January 2013).

Table 6 Recommendations for food simulants in EU

Food types	*Food simulants*
Aqueous foods	10% Ethanol
Foods that have a hydrophilic character and are able to extract hydrophilic substances and which have a pH below 4.5	3% Acetic acid
Foods that have a hydrophilic character and are able to extract hydrophilic substances, alcoholic foods with an alcohol content of up to 20% and those foods which contain a relevant amount of organic ingredients that render the food more lipophilic	20% Ethanol
Alcoholic foods with an alcohol content of above 20% and diary products	50% Ethanol
Fatty food and foods which contain free fats at the surface	All vegetable oils
Dry foods	Tenax (poly(2,6-diphenyl-*p*-phenylene oxide))

Source: Reproduced from Commission Regulation EU n.10/2011.

An important concept in relationship with migration issues, is that of functional barrier. Functional barriers are parts of food-contact materials used in multilayer structure to prevent migration of undesirable substances from layers beyond the barrier. For example, as stated in the Regulation 10/2011, in recent years, plastic food-contact materials do not only consist of one plastic but also combine up to 15 different plastic layers to attain optimum functionality and protection of the food, while reducing packaging waste. In such a plastic multilayer material or article, layers may be separated from the food by a functional barrier. Behind a functional barrier, nonauthorized substances may be used, provided they fulfill certain criteria and their migration remains below a given detection limit. Taking into account foods for infants and other particularly susceptible persons, as well as the large analytical tolerance of the migration analysis, a maximum level of 0.01 mg kg^{-1} in food should be established for the migration of a nonauthorized substance through a functional barrier. However, even when a functional barrier is used, mutagenic, carcinogenic, or toxic to reproduction substances

are not allowed for use in food-contact materials or articles without previous authorization. The same caution should be used when dealing with a functional barrier coupled with materials based on nanoscale engineering, as is the case of nanoparticles. Assessment of the risk should be performed on a case-by-case basis.

An example of allowed use of functional barrier is that of recycled plastics. In fact, recycled plastics may be contaminated by various chemicals and a functional barrier is interposed between the recycled plastic and food to prevent such contaminants from reaching the food.

Finally, some relevant food-contact material issues are specifically addressed in the following:

- *Vinyl chloride monomer*: Polymeric materials and articles which are intended to come into contact with foodstuffs can transfer vinyl chloride monomer to these articles in quantities liable to endanger human health. Directive 78/142/EEC specifies that such materials and articles must not contain vinyl chloride monomer in a quantity exceeding 1 mg kg^{-1} of finished product and must not pass on to foodstuffs more than 0.01 mg kg^{-1} of vinyl chloride monomer.
- *Styrene monomer*: Migration of residual styrene monomer to food from polystyrene in contact with it is another important issue. Food-contact applications of polystyrene include containers for diary products and fruit juice, meat trays, biscuit trays, egg cartons, take-away food and drink cups, and boxes. Undesirable flavor and taint to food are caused by styrene migration. Health concerns derive from reported toxic effect of styrene monomer on the liver and by the fact that it can disrupt normal hormone function; moreover, it also acts as a depressant of the central nervous system, causing neurological impairment and is also considered a possible carcinogen. Nevertheless, it is generally found in such low levels in packaged food that risks for consumers are considered low and styrene monomer migration is mainly considered as an organoleptic/quality problem. In fact, there are no SMLs for styrene in foods. Its content is controlled only by the OM limit of 60 mg kg^{-1}. Content of fat and ethanol in food, temperature, and contact time raise the migrated amount.
- *Phthalates*: Plasticizers are routinely added to polymers to make them more pliable and elastic. Among them, phthalates (also known as phthalic acid diesters) are particularly relevant in relation to migration issues. The five phthalates most commonly used in industry are di-(2-ethylhexyl) phthalate, dibutyl phthalate, di-isononyl phthalate, di-isodecyl phthalate, and benzyl butyl phthalate. Since the early 1980s there have been concerns about the effect of phthalates on human health and there has been particular concern over migration from food packaging to foodstuff. In fact, phthalates can be present in some food packaging materials, including printing inks used on flexible packaging, adhesives used for both paper, board and plastics, regenerated cellulose film (cellophane), aluminum foil–paper laminate, and closure seals in bottles. It should be further noted that many PVC 'cling film' food wraps are no longer made with phthalates, but are now manufactured using other plasticizers. The European Community legislation limits the use of phthalates in food plastics, and, where use is permitted, it limits the migration of these chemicals into foods by setting SMLs.
- *BPA, bisphenol A diglycidyl ether (BADGE), bisphenol F diglycidyl ether (BFDGE), and novolac glycidyl ether (NOGE)*: Endocrine-disrupting compounds like BPA, BADGE, BFDGE, and NOGE, are used in coated materials, plastics, and adhesives as is the case of epoxy-based enamels/lacquers for metal cans. Regulation EC 1895/2005 regulates the epoxy resin derivatives BADGE, BFDGE, and NOGE. In this regulation, BADGE and its hydrolysis product's migration is limited to 9 mg kg^{-1} of food and that of BADGE chlorohydrins to 1 mg kg^{-1} of food. BFDGE and NOGE have been completely banned from food-contact materials.
- *Ink contaminants*: Recently, migration of printing ink and varnish ingredients into foodstuffs has become a focus of interest. This was especially caused by the detection of isopropylthioxanthone (ITX) in baby milk, milk products, and cloudy juices packaged in beverage cartons in 2005. Also the latest findings of 4-methylbenzophenone and benzophenone migrating from printed carton packages into cereals highlight that contamination of foodstuffs through printing inks is still a problem. All these packages were printed with UV inks which contain monomers and oligomers, pigments, additives, and photoinitiators such as ITX. UV printing inks and varnishes have a broad range of applications in food packaging and are used in all kinds of materials like paper and carton board, plastics, and multilayered materials.
- *Mineral oil saturated hydrocarbons and mineral oil aromatic hydrocarbons*: Consumers are exposed to a range of mineral oil hydrocarbons (MOHs) via food, with potential health concerns. In particular, mineral oil saturated hydrocarbons (MOSHs) consist of linear and branched alkanes, and alkyl-substituted cycloalkanes, whereas mineral oil aromatic hydrocarbons (MOAHs) include mainly alkyl-substituted polyaromatic hydrocarbons. MOHs are complex mixtures and little is known of their composition in commercial products. In food-grade MOH, the MOAH content is minimized and is in the typical range of 15–35%. MOHs are present in several food-contact materials, including food packaging materials made from recycled paper and board, and printing inks applied to paper and board. Moreover, MOHs are used as additives in the manufacture of plastics and adhesives used in food packaging, wax paper, and board. Extensive migration of MOSH and MOAH can occur from recycled paper and board packaging in the absence of a proper internal barrier. MOSH may accumulate and cause microgranulomas in several tissues and foodborne MOAH may be mutagenic and carcinogenic.
- *3-Monochloropropane-1,2-diol (3-MCPD)*: The manufacturing process of paperboard food packaging can produce small quantities of 3-chloro-1,2-propanediol (3-MCPD) when wet-strength resins containing epichlorohydrin are used. 3-MCPD is from the same family as 1,3-dichloro-2-propanol, which is known to cause cancer in animals.

- *Diisopropylnaphthalenes (DIPNs)*: DIPNs can migrate from recycled paper and paperboard. In fact they are used for ink-jet printers and as solvents in the preparation of specialty papers. As not all DIPNs are removed during the recycling process, some may be present in the finished board and, under certain circumstances, can migrate into food via direct contact or gas phase transport.
- *Tin and Chromium*: Tin is used for coating steel in tinplate. Tin layer is generally covered by a protective organic layer. Tin migration is only an issue where internal protective coatings are not used. Chromium is used at very low levels as a passivation coating for tinplate and at higher levels for electrolytic chromium coated steel. The process ensures that the only species present are Cr0 and CrIII and not CrVI, which is the toxicologically important species. Chromium in food is not generally regulated (there is a World Health Organization (WHO), limit of 0.025 $m l^{-1}$ for drinking water). The level of migration from uncoated tinplate cans (the only metal foodstuff packaging where migration is likely to occur) is negligible and not considered to be of concern. Future change in environmental legislation is encouraging work on alternative passivation systems.

Special Applications and Perspectives

Packaging structures have evolved in the last two decades, introducing innovative concepts and technologies, which has greatly enhanced the shelf life of packaged food. These new applications rely also on the development of new materials.

Intelligent Packaging

Intelligent packaging is a system able to monitor and give indication on the freshness of the food. Definitions stated in Regulation 1935/2004/EC and in Regulation 450/2009/EC consider intelligent materials and articles as: "materials and articles which monitor the condition of packaged food or environment surrounding the food." In particular, intelligent packaging systems provide functions such as detecting, sensing, recording, tracing, and communicating in order to extend the shelf life of packaged food and enhance its safety. Smart package devices are generally labels or tags that are attached onto primary or secondary packaging and are basically of two types: the first type includes data carriers (such as barcode labels and radio frequency identification (RFID) tags) that are used to store and transmit data. The second type includes package indicators (such as time–temperature indicators, gas indicators, and biosensors) that are used to monitor the external environment and, whenever appropriate, issue warnings.

Active Packaging

Active packaging has been defined as a system in which the product, package, and environment interact in a positive way to extend the shelf life or achieve some characteristics that cannot be obtained otherwise. Definitions stated in Regulation 1935/2004/EC and Regulation 450/2009/EC consider active materials and articles as: "materials and articles that are intended to extend the shelf life or to maintain or improve the condition of packaged food." Active systems usually are obtained by embedding active compounds into the packaging materials that are released into or absorb substances from the packaged food or the environment surrounding the food in order to inhibit or delay food degradation. The relevant example of scavengers has been already mentioned (see Section Auxiliary materials). The European Union Regulation 1935/2004 offered for the first time the opportunity for active packaging to be used in Europe providing general provisions on the safety of active and intelligent packaging and setting the framework for the European Food Safety Agency evaluation process.

Modified Atmosphere Packaging (MAP)

Modification of the internal packaging atmosphere is often used to prolong the shelf life of food. It consists in the modification of the absolute pressure (hypobaric and hyperbaric packaging) and/or of the partial pressure of gases present in the package headspace. In particular, in MAP a proper composition of the internal gas mixture, selected on the basis of the preservation requirements of the specific food, is initially substituted to air. Oxygen, carbon dioxide, nitrogen, and argon are the most used gases for this purpose. The headspace mixture composition evolves with time due to outward and inward permeation of gas molecules and of gas production/consumption exerted by the food. In selecting adequate packaging materials for this application, such as plastic packaging, the ratios of permeability of the gases present in the modified atmosphere should be considered to limit excessive modification of the starting composition of the modified atmosphere.

Nanotechnology in Food Packaging Applications

As in many other branches of science and engineering, nanotechnology is also providing an important impulse for the development of new applications in the field of food packaging, with particular reference to barrier packaging, antimicrobial, and antimycotic packaging, improvement of biobased and biodegradable packaging, active and smart packaging. Important examples include (1) polymer nanocomposites obtained by adding a few percent, by weight, of nanofillers (e.g., nanoclays and silica nanoparticles) to polymers to improve barrier and mechanical properties; (2) biopolymer with nanoadditives for controlling and accelerating compostability and biodegradability; (3) polymers including titanium oxide nanoparticles to improve barrier to UV rays; (4) polymers with zinc oxide and silver nanoparticles to control antimicrobial growth in foodstuffs, and (5) active packaging systems based on nanocapsules designed to release chlorine as an antimicrobial agent.

Processing of Packaging and Packaging Lines

Structure and Manufacturing Procedures of Primary Packaging

Primary packaging can be flexible or rigid/semirigid. The choice depends on the type of food, on preservation and

protection requirements, ease of use, and marketing advantages. A brief overview is reported in the Sections Containers and Flexible Packaging that follow for the different types of primary packaging and their manufacturing procedures.

Containers

Glass bottles and containers are manufactured by blow and blow and press and blow processes, followed by thermal or chemical strengthening treatments and surface coating. Plastic bottles and containers are instead produced by extrusion blow molding and by injection blow molding. Also in this case, coatings may be applied, mainly to improve barrier properties. Frequently, containers can be made with a multilayer structure obtained by the coextrusion of different polymeric materials or lamination of different types of material (aluminum foil, plastic films, and cellulosic layers) followed by folding. Each layer in a multilayer structure is intended for a specific aim (e.g., sealing, barrier to light, gases, and moisture, and mechanical strength). Polymeric containers can also be obtained by thermoforming sheets of plastics. Metallic materials can be shaped in the form of cans, trays, aerosol containers, and collapsible tubes. Cans are produced by rolling of metal sheets, by drawing and wall-ironing and drawing and redrawing. The last two methods are also used for trays. Inner surface of trays and cans, to be put in contact with food, are coated with thermoset lacquers to protect the metal surface and food. Collapsible tubes are produced by impact extrusion.

Flexible Packaging

Polymer films are produced by cast extrusion and blown film extrusion. A tenter frame can be used to orient biaxially cast films. Polymer films can have a multilayer structure obtained by the coextrusion of different polymeric materials, by adhesive lamination of several plastic films, or by extrusion lamination. Typical adhesives used in laminating processes are waterborne, solvent based, hot melt, or reactive 100% solids (solventless). Improvement of barrier properties of plastic films can be obtained by lacquering the surface or depositing a very thin layer of aluminum (metallization) or of silicon or aluminum oxide. To convey information to consumers and for marketing purposes, packaging films are subject to printing processes such as offset, flexography, rotogravure, and digital printing. These films are purchased as reels and then used to prepare bags and pouches, at the site of production of the food product. For example, confectionary machines perform the forming, filling, and sealing of the container. Basically, all the confectionery machines can be grouped into two categories: form-fill-seal and separate packaging machines.

Pasteurization and Sterilization in Food Packaging Applications

Heat pasteurization and sterilization are typically used in food packaging applications. Pasteurization is a treatment that reduces the number of pathogenic microorganisms of the food by heating it at a specific temperature (usually less than 100 °C) for a specific time (from seconds to a few minutes). Heat sterilization is a process of heating the food to a temperature over 100 °C for such time until all microorganisms in the food are killed.

High-pressure pasteurization (HPP) and sterilization are steadily gaining as a food preservation method that also preserves natural sensory and nutritional attributes of food with minimal quality loss. Packaged foods, processed by using this technique, maintain most of their original texture and nutritional qualities, additionally exhibiting an extended shelf life. HPP applies high pressure (i.e., 300–800 MPa) to packaged foodstuffs at a temperature significantly lower than the corresponding heat pasteurization and sterilization temperatures.

Selection of the proper packaging system is crucial for the pasteurization and sterilization treatments performed at atmospheric as well as at high pressures. In fact, packaging must be appropriate for the product to be treated and compatible with the specific method of treatment to be used, i.e., steam, ethylene oxide, ozone, irradiation, and extremely high hydrostatic pressure. Maintenance of sterility of the contents until the package is opened, withstanding physical conditions of the specific process without losing mechanical integrity and resistance to tears and punctures are the main requirements.

For these reasons, multilayer packaging structures with high temperature-resistant materials and good sealing properties are used. In addition, reactive systems with high thermal stability after curing are applied as laminating adhesives for thermal treatments.

See also: Food Additives: Colorants. Food Safety Assurance Systems: Tampering. Food Technologies: Pasteurization; Sterilization. Hazards of Food Contact Material: Bisphenol A and Endocrine Disruption; Food Packaging Contaminants; Nanotechnologies and Nanomaterials; Phthalates

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www.worldpackaging.org
World Packaging Organisation.

SAFETY OF FOOD AND BEVERAGES

Safety of Food in Vending Machines

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Glossary

Ambient temperature foods Foods that are stable at room temperature.

Food safety management plan Also referred to as the HACCP plan – a plan written to show how a company plans to manage and control food safety hazards within its business.

HACCP A system that identifies, evaluates, and controls hazards that are significant for food safety. The acronym stands for hazard analysis, critical control points.

Vending operator The person who cleans and fills a vending machine.

Work instructions Written instructions for vending operators to ensure they carry out their tasks in a manner designed to protect food safety.

Introduction

Vending machines can dispense a very wide range of foodstuffs and drinks including items that are fresh, frozen, long shelf life, or chilled. These include ice cream, plated meals, sandwiches, fresh fruit, baked goods, confectionery, snacks, and hot and cold drinks. In general, products are vended for immediate consumption but some products require heating before consumption. In these cases the vended product is put through a heating process either in the machine or close by. There are no vending machines currently in use in which products are stored at temperatures above ambient for reasons of food safety.

The Vending Market

In most countries with a significant vending presence, North America, USA, Japan, and Australia, the market is dominated by can, bottle, and snack machines whereas in Europe more than 70% of the vending machines serve hot drinks made from powders.

A small proportion of vending machines are owned by individual institutions and cleaned and restocked by them. Some others are operated by catering companies, but the great majority are cleaned, maintained, and filled by an operating company of which there are 10 000 in Europe alone. While a few of these companies are multinational organizations employing thousands of people, most are small- and medium-size enterprises (SMEs) or family businesses operating between a few hundred and a few thousand machines. Vending machines are sited in workplace, leisure, educational, and public sites; in Europe 80% of all machines are sited in the workplace.

Assessing Food Safety Risks in Vending

Hazard analysis, critical control points (HACCP) is a management tool that gives a structured approach to identification and control of hazards. It requires the journey that any foodstuff follows from farm to fork to be divided into steps, the hazards present at each step to be identified and appropriate measures to be put in place to ensure that the food remains safe at all times.

All vending products follow the same general pathway from supplier to consumer. The vending company purchases foodstuffs that are delivered to a warehouse, distributed to locations where machines are sited and used to stock the machines. Drinks machines are received from manufacturers, connected to a source of potable water and filled with ingredients. Companies would break this pathway down into a series of steps and assess the food safety risks at each step in order to derive their HACCP-based food safety management plan. This same technique will be used in this chapter to provide an overall understanding of the safety of food in vending.

Types of Vended Food

From a food safety point of view, food and drink sold from vending machines falls into three categories; food that can be stored at ambient temperature, drinks made from powders or syrups, and food that must be kept chilled or frozen for food safety reasons.

Most of the food and drink sold from vending machines is stable at ambient temperature and humidity. This includes confectionery (chocolate, cereal, and fruit bars), bagged snacks (nuts, crisps, baked, and extruded snacks), and drinks in cans, bottles, and cartons (long-shelf-life milk drinks, fruit drinks,

carbonated drinks, and water). It also includes the powders for hot drinks and the syrups used for postmix still and carbonated drinks vended by machines.

Some foods, however, need to be stored at lower temperatures in order to inhibit microbiological growth. Foods such as sandwiches and salads must be chilled throughout their shelf life in order to be safe.

Other foods are stored frozen and either vended frozen for eating cold, for example, ice cream, or vended to be heated before eating, like ready meals. A further group of machines heat the frozen products within the machine before vending.

The Steps in the Vending Business

All vending businesses have the same basic process for all foodstuffs, whether ambient, chilled, or frozen, which comprises the following steps:

- Purchase of products from manufacturers or wholesalers.
- Transport of the products to the vending company.
- Storage by the vending company.
- Transport by the vending company to the machine.
- Filling and cleaning of the vending machine.
- Storage within the machine and dispensing of the product.

None of these steps provides a means whereby unsafe food can be treated to make it safe, so the safety of food in vending depends on safe food being purchased by the company and that food being kept safe throughout its shelf life.

Each of the steps in the process will be considered separately.

Purchase of Products from Manufacturers

The first step in the process is the purchase of products. The production of foodstuffs should always be carried out under a food safety management plan that minimizes the risks of contamination by bacteria or physical or chemical hazards. Large food companies have quality assurance departments that can audit suppliers and provide confidence that all necessary actions have been taken to minimize risks. Vending companies, with few exceptions, do not have such teams within their organizations and, in consequence, must rely on other means to assure the safety of the products.

If they are buying from one of the major brands then they can rely on the manufacturer having extensive quality assurance procedures in place to maintain the reputation of the brand. However, if they are buying a product with a less well-known brand then they would have to depend on other indicators, for example, whether the supplier had been audited by a large company. It is not sufficient to rely on the supplier having ISO 9001. This is not a measure of the quality or safety of the foodstuffs, but of the paperwork trail within the company. It would be possible to rely on ISO 22000 or the British Retail Consortium (BRC) global standard but unfortunately it is precisely the smaller manufacturing companies who are less likely to have these standards. Less bureaucratic standards for smaller companies, such as Safe and Local Supplier Approval (SALSA) in the UK, have been developed recently but uptake has been slow.

The situation is exacerbated when purchasing chilled foods such as sandwiches and plated salads. Companies who make these products are often small companies who provide products within the local area. They may be audited by the local authority but rarely by a commercial organization with skills in auditing food manufacturing processes. Unfortunately it is the chilled foods that carry most risk of contamination with pathogens such as *Listeria monocytogenes* and, in general, the purchase of novel or local food items requires considerable diligence by the vending operator to ensure that the manufacturer understands their process well enough to guarantee the safety of the food.

Transport to the Company

Products may either be delivered by the supplier or collected by the vending operator company. Many vending companies collect some smaller batches and have larger volumes delivered by the supplier or wholesaler. The only risks to product safety during this step, for foods that are stable at ambient temperature, are those caused by physical or chemical contamination in the vehicle. These may include broken glass or shards of metal or chemicals such as disinfectant or oil from damaged bottles picked up from the vehicle. This is an unlikely event, but it has occurred, and it would be good practice for those checking in the delivery to give a quick look around the inside of the vehicle before unloading. Companies have greater control over their own vehicles and it is important that vending companies have working practices and training in place to ensure that all vehicles operated by them are clean and tidy and pose no threat to food safety.

Chilled or frozen food must be kept chilled throughout its shelf life. It is important that the delivery vehicle has a refrigeration system that is able to keep the food at the appropriate temperature in all weather conditions. The company's food safety management plan would require the temperature to be monitored and there are several ways in which this can be done depending on the technology on the vehicle. Many modern vehicles have systems that allow continuous monitoring of temperature and technology now allows this information to be continuously transmitted to both the supplier and customer in real time. Older vehicles may only have a thermometer and here, as a minimum, the temperature should be recorded on arrival. Because the vehicle thermometer will measure air temperature, the vending operator should also measure the temperature between two packs of product to ensure that the product is within national standards. Further reassurance would be provided by occasional use of a data logger to show the temperature experience of the product throughout the journey.

Storage

Once the product has been received and passed into the vending company warehouse, care has to be taken that the foodstuffs are not contaminated. Vending companies, being

small companies, tend to have limited space within their premises that are often an industrial unit on a trading estate. Within these premises they will store machines, machine parts and foodstuffs, prepare machines for sale, and repair defective machines. Some companies divide the available space with partitions whereas others operate an open plan system. While it is possible to control the risks of physical and chemical contamination in an open plan arrangement it does need careful management. One of the problems with limited space is effective pest control. It is important that the normal practices of pest control, for example, storage off the floor and away from walls, are followed together with securing the services of a reputable pest control organization. Good pest control consists of more than just putting down bait boxes. It involves good hygiene management of the store and regular monitoring and review by the pest control contractor to ensure that the earliest signs of a possible infestation are recognized and acted on.

It is also important that the practices of good warehouse management are followed including good control of stock rotation. This not only ensures that the consumer has quality product but also assists in managing traceability.

The situation with chilled and frozen food requires yet more care. These foods must be kept at safe temperatures at all stages from manufacturer to consumer. Some vending companies receive chilled food into a temperature-controlled store whereas others contract with the supplier of the chilled food to distribute directly to the vending machines. This removes the need for the vending company to have temperature-controlled storage but takes the process out of their control. However, they still need to check that their contractor's procedures are sufficiently robust to ensure that the temperature of the food is properly controlled throughout its shelf life.

When the vending company stores the food themselves they have to manage the temperature carefully to ensure that it complies with national regulations so that there is no risk to food safety. Temperature control is not yet harmonized across the EU and each Member State has its own national regulations with, in some cases, different temperatures for different types of foodstuffs.

To some extent the situation with frozen food is simpler. The product has to be kept frozen, with the maximum temperature being specified by the manufacturer. Generally specific storage and delivery systems have to be established for frozen product in order to maintain product quality.

Transport to the Machine

Products are transported to machine sites by van. These vans will carry both food products and cleaning materials and it is essential that they are kept clean, tidy, and well organized so that there is no possibility of contamination of food products by cleaning materials. In major cities it is frequently impossible for vans to park close to sites and products have to be loaded onto trolleys for transport to the machine. It is important that staff are properly trained in loading and transporting so that fragile products are not damaged or contaminated during this phase of the journey.

Chilled or frozen product should be transported in insulated bags with cold packs to maintain the temperature at safe levels. The company procedure needs to be tested to ensure that product is still at a safe temperature before it is loaded into the vending machine.

Vending companies provide services to a wide range of clients, from large customers with many machines, to small customers with just one machine. The servicing of these sites depends both on the number of machines on a site and on the proximity of the site to a company depot. Sites with many machines will have operators on site all day, whereas smaller sites will be serviced by operators who have a route that may have a number of sites, each with one or more machines. Some of these operators will be based away from the depot and will receive deliveries of stock to their van. Working practices have to be written to ensure that however the stock is delivered, it is always safe from risk of contamination.

Filling and Cleaning

The frequency of cleaning and filling machines is determined to a large extent by the use of the machine. It is not uncommon for very busy machines to be replenished twice a day, but for infrequently used machines to be visited only weekly. The frequency of visiting chilled food machines will be dictated by the shelf life of the product, which is often 3 days.

The only risk to food safety during filling and cleaning a machine selling ambient temperature foods is that of contamination with the cleaning chemicals. This can be avoided by training and work instructions that ensure that chemicals are never near the foodstuffs.

However, there are two additional concerns with chilled food. The first is that the chilled food vending machines are designed only to maintain chilled food at a safe temperature, not to chill the food down from room temperature. The temperature of food does slowly decrease within a vending machine but too slowly to comply with any national temperature control regulations. So, chilled food must be chilled by the supplier before it is delivered to the machine. This creates a problem for some companies who would like to buy their sandwiches from a local shop. The shop must have chilling facilities that will chill the food sufficiently quickly to comply with national temperature control regulations and must devise a method of safely delivering the food to the vending machine without an unacceptable rise in temperature. It is also important that the machine is open for as short a time as possible so that the food inside does not warm up to an unsafe temperature. It is important that work instructions are developed and that staff are trained to ensure that this is the case.

Frozen food also has to be at the correct temperature before loading into the machine. This requires greater thought and planning to ensure that the temperature does not rise during transport through a warm building or while product is being loaded into the machine.

The great majority of chilled and frozen food vending machines display the temperature inside the machine but do not have facilities to provide records of temperature over time. As a check on the performance of the machine it is good

practice to record the displayed temperature when a machine is filled.

Hot drinks machines will need regular cleaning to control bacterial growth in the mixing bowls and dispense area. There are two common ways of cleaning the drink contact parts of the vending machine. Once the parts have been removed they are either cleaned in a bucket on site or bagged up and replaced by a clean set brought from the main site. It has been demonstrated that disinfectant is not needed for cleaning on site, brushing with detergent and hot water followed by rinsing is quite sufficient to keep bacterial populations below a safe level. Provided that the procedure is carried out carefully there is no difference in the effectiveness of these two methods. It is worth noting that there is only one report in the literature of a food poisoning incident being caused by inadequate cleaning of a hot drinks vending machine.

It is important that the person doing the cleaning does not themselves contaminate the machine and that they wash their hands appropriately. As a minimum this should be on arrival at the premises, after using the toilet and before putting on gloves, if these are worn. It is important to train staff to wash their hands correctly. It is also important to be aware that bacteria can be carried on gloves and transferred from place to place, so it would be good practice to change gloves frequently.

It is sometimes forgotten that the cloths and brushes used in cleaning the machine can be a source of contamination and the work instructions should identify the way of keeping them clean between machines.

Storage and Dispensing

Vending machines are placed in a very wide range of locations. Those in office environments are generally free from risk of pest infestation or chemical contamination but these issues should be considered when placing machines in industrial or public sites. For example, machines have been placed in stables, where they have been infested with flies, in print shops and in bus depots where the surfaces of the cups have become contaminated with solvents and diesel fumes. Sites like these should be avoided and machines should be placed in a clean, well lit area, out of direct sunlight and away from doors to the outside that could provide access for ants. In addition they should not be placed under stairways where dust and contaminants could be dropped from above and they should not be sited where they may attract pests such as cockroaches.

Snack or can vending machines are basically merely boxes in which the products are held. However, there are standards for their design and manufacture, which should be followed. In essence the machines should be designed and manufactured to be easy to clean with no dark areas in which pests could hide. The materials of construction should be robust and smooth and corners should be of sufficient radius to be cleaned easily. Food or water contact parts do not have to be made of stainless steel but should be made of materials such as polycarbonate that comply with national or international Regulations. The tubing in hot drinks vending machines is generally of silicone rubber and all parts of the water pathway should be approved for use with drinking water.

Hot drinks machines must be connected to a source of potable water in order to guarantee the safety of the water. Some growth of bacteria can be expected in water as it passes through the pipework in a building and through a vending machine or water dispenser but there is no evidence that this poses any threat to human health.

Machines must be connected through a check valve to ensure that water cannot, under any circumstances, pass back into the mains water system. In the case of machines dispensing carbonated drinks the valve should be a double check valve since carbonated water can dissolve copper. Machines can remain on site for many years and it would be good practice to change the double check valve after 10 years. Because both soft drinks and carbonated water are acidic, it is very important that the pipework in soft drinks dispensing or vending systems is made of a material that will not corrode with acid. Acute illnesses have occurred in the past from copper leaching from piping and tubing downstream of a check valve should be either plastic or stainless steel.

Chilled and frozen food vending machines are programmed so that they will not vend if the temperature inside rises above a preset limit. This makes it very important that such machines are sited out of direct sunlight which could heat the machine beyond the capacity of the chilling system to maintain a safe temperature. Because chilled food items have a short shelf life in the machine it is important that they are removed before their expiry date. Color-coded labels can be useful for highlighting on which day of the week product should be removed. Product removed from the machine must be taken from the site and disposed of correctly.

Traceability

It is good practice for the vending company to keep records of those from who they purchase foodstuffs and this is generally a legal requirement. In Europe, for example, it is part of Regulation 178/2002. It is not necessary to keep records of batch codes or lot numbers of deliveries as, in the unlikely event of a product recall, all product of the relevant brand can be removed from all machines. Once product is back at the company warehouse then it can be checked for date codes or lot numbers as necessary. This procedure will be much less time consuming and bureaucratic than keeping records of every delivery.

See also: Food Safety Assurance Systems: Food Safety and Quality Management Systems; Hazard Analysis and Critical Control Point System (HACCP): Principles and Practice; Personal Hygiene and Employee Health. Food Technologies: Cleaning and Disinfection Technologies (Clean-In-Place, Clean-Out-of-Place). Other Significant Hazards: Physical Hazards in Foods. Safety of Food and Beverages: Water (Bottled Water, Drinking Water) and Ice

Further Reading

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Inglehurst Foods is a research and lobbying organisation for the the vending and HoReCa industry.

SAFETY OF FOOD AND BEVERAGES

Promotional Material

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Glossary

Food contact material Materials and articles intended to come into contact with foodstuffs, including packaging materials, cutlery, dishes, processing machines, containers, etc.

Hazard A microbiological, chemical, or physical property of the promotional item with the potential to cause an adverse health effect.

Nonfood products Articles such as textiles, toys, tableware, electrical devices, etc.

Recall Operation carried out to ensure return of products from consumers to the shop, for example, for quality or safety nonconformities.

Risk evaluation A step in a risk-management process. Risk evaluation is an evaluation of the risk related to a concrete situation and a recognized threat (also called hazard).

Third party Someone other than the principals directly involved in a transaction or agreement.

Withdrawal Removing a product from the warehouses and shelves, for example, quality or safety nonconformities. The operation does not cover goods in the hands of consumers.

Introduction

Promotional items are used to publicize or advertise a product. They are considered as typical marketing tools which, together with public relations and communication activities, are a means to promote awareness and create greater visibility for a brand or a company. They can be printed with a company's name, a logo, or a slogan; they can be distributed at trade shows or conferences, or delivered to consumers packed in/on a food product. Common promotional items are toys, T-shirts, caps, key chains, posters, bumper stickers, pens, booklets, mouse pads, spoons, cups, or mugs.

Promotional items are often used by the food industry. Toys packed inside chocolate eggs or cereal packs, or given away with fast food meals can be cited as concrete examples of promotional items commonly added to the food product. Included into the scope of this article are promotional items foreseen to be in direct contact with food (e.g., cup), packed with food (e.g., toy), or in contact with the mouth or skin of the consumer (e.g., toy, cap, spoon). Cosmetics, as well as tickets for vacations, and samples of food products, are not included into the scope of this article.

Independently from their size or shape, all promotional items must fulfill the following basic requirements: they must be safe, i.e., they must not pose a threat to consumer's health or safety, and they must comply with applicable regulatory requirements.

Although such a statement might seem obvious, the reality appears to be significantly different: based on the number of products recalled by authorities worldwide in 2008–2009, compliance to the above requirements does not seem so straightforward, especially with promotional items targeting children. These can indeed be a source of hazards with serious health consequences varying from death (e.g., suffocation after ingestion of small parts) to potential long-term health effects (e.g., exposure to harmful chemicals as lead paint). Currently, deaths of children due to ingestion of small parts (the small part originated from toy products) are reported in several pediatric and forensic science journals. Given the above, a clear process aimed at ensuring the item's safety needs to be implemented before the product reaches the consumer. This process will guarantee protection of consumer's health and preserve the stability of the manufacturer's business, should the company be (or be perceived as being) incapable of ensuring compliance of its products.

This article will focus on the health and safety risks linked to the promotional items generally used by the food industry, and on the requirements that must be implemented in the product-development phase in order to industrialize a safe product. Examples of the regulatory requirements, international standards, and codes of practice that need to be followed in order to ensure compliance of the promotional material will also be described.

Lessons Learnt

Whenever products bearing a risk are brought to the attention of authorities, the official information is published by most of the governmental bodies on their websites. This publication

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Table 1 Examples of product recalls carried out worldwide in 2009

Type of promotional item	*Health impact for consumer*	*Reason of recall/withdrawal*
Toy	Choking	Small pieces can break from a toy
Doll clothing	Chemical contamination	Surface paints on the pajama pants contain excessive levels of lead
Magnetic dart board	Death	Magnets found by young children can be swallowed or aspirated. If more than one magnet is swallowed, the magnets can attract each other and cause intestinal perforations or blockages, which can be fatal
Battery powered items	Fire and burn	The electric wires in the item or in the battery compartment can short circuit, posing a fire and burn hazard
Decorative candles	Fire and burn	Noncompliance with the European standard EN 15493
Children clothing	Strangulation	Presence of drawstrings in the hood area of the sweatshirt
Children clothing	Chemical contamination	Presence of azo-based dyes; noncompliance with REACH[a] regulation
Inflatable arm bands	Drowning	Holes for the arms which are too large for children; noncompliance with relevant European standard EN 13138
Inflatable swim-ring	Chemical contamination	Presence of phthalates; noncompliance with REACH[a] requirements for toys that can be placed in the mouth by children

[a]The Registration, Evaluation and Authorisation of Chemicals regulation of the European Union.

is easily available to consumers, other authorities, and the media. The number of product recalls notified has risen steeply during the last few years. Examples of promotional items that have been recently recalled by authorities or voluntarily withdrawn due to the high risk they represented for consumers are listed in Table 1.

In Europe, the rapid alert system for nonfood consumer products (RAPEX) establishes the circulation of information among the Commission and member states' authorities on measures taken in relation to products posing a serious health risk to consumers (with the exception of food, pharmaceutical, and medical devices). Every year, RAPEX issues an annual report entitled Keeping European Consumers Safe, on the operation of the rapid alert system for non-food consumer products, which is available on their internet site. The report lists in a very comprehensive manner, among others, the categories of products recalled or withdrawn during the year, together with, for example, information on their country of origin and the risks associated with them. The most frequently notified categories are toys, which represent a significant part of the promotional items used in food industry. Over half of high-risk items notified during the last years originated from China. The main risks linked with toys taken out of the market were choking, associated with presence of small parts. Toys are also taken off the market due to chemical risk, i.e., because of high levels of undesirable chemical substances. This was the reason in 2007 for a massive recall of products by a well-known toy manufacturer.

Similar alert systems also exist in other countries. In the US, for instance, the Consumer Product Safety Commission (CPSC) has the authority to pursue recalls for products that present a substantial product hazard. Companies becoming aware of hazards linked to their products are required by law to immediately inform the CPSC. CPSC works jointly with the company to recall hazardous item from the shelves. All recall notices are published on the CPSC website, publicly available.

Today, international toy manufacturers have also added to their own websites specific pages with data on their recalls, allowing consumers to get appropriate information directly from the source.

Ensuring Quality, Safety, and Compliance of Promotional Items

Promotional items might cause health or safety threats to consumers due to their design, the quality of the raw materials used, and the accuracy of the manufacturing process. These risks need to be duly assessed and corrective actions implemented before reaching the consumers.

The creation process of the promotional item that you recently received at an exhibition or that your child has just found on his cornflakes box can be summarized in three main phases: idea generation/exploration, product development, and product industrialization (Figure 1).

Phase 1: Idea Generation/Exploration

During the first phase (idea generation/exploration), the marketing department evaluates the need in terms of promotional items, explores new concepts, and defines the business strategy. Will it be stickers? Shall we switch to fancy magnets, or innovate with key holders? Will we develop one item per country of sales or shall one item fit all markets? In this phase, the key features of the selected products will be carefully defined, for example, the type of consumer to whom the promotional item is addressed, its intended use (including the potential misuse), the material composition, product shape, and expected shelf life (how many washing cycles will our tennis cap have to last?). Once the idea generation phase is completed and the product definition is clearly set, the development of the promotional item (phase 2) can start.

Phase 2: The Five Key Steps in Product Development

The product development phase, or phase 2, consists of five steps. The first four are carried out practically simultaneously

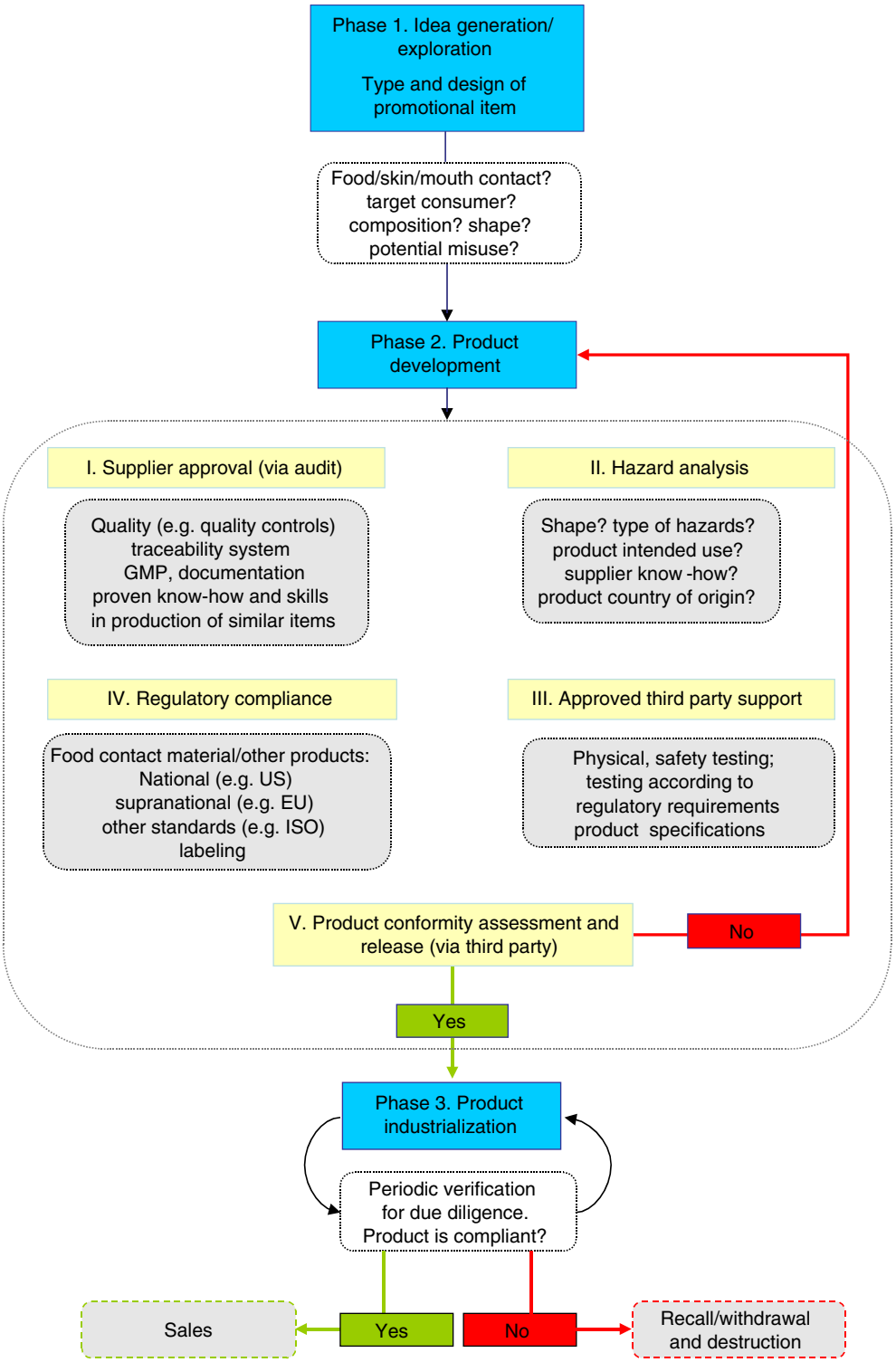

Figure 1 Key phases (in blue) ensuring safety of promotional items, from idea generation to product industrialization. The key phase for ensuring safety of promotional items is clearly the product development (phase 2).

(supplier approval, hazard analysis, third party support, and regulatory compliance). The fifth and last step, consists of the release of the promotional item prototype via a conformity assessment and is generally delegated to an independent third party. It is important to start the product development as early as possible, even before the finalization of the idea-generation phase. By doing so, the threats that can potentially damage the product or the consumer, and the parameters that need to be in place to ensure compliance (e.g., product composition is inappropriate to guarantee consumer safety) can still be

reconsidered and adapted (e.g., change the design or material composition in order to make the product safe).

The five key steps of the product-development phase are as follows:

Supplier Approval

The selection of the supplier is as important as the choice of the promotional item itself. The selected supplier is formally approved by the customer's quality, marketing, and procurement departments based on different criteria. From a quality standpoint, these include the implementation of appropriate quality standards (e.g., a quality control system and a traceability system), a proven know-how in the manufacturing of the product, of typical safety issues potentially linked to it and of applicable regulatory requirements. Customers should make sure that the following aspects are also covered: cleaning of premises and lines, pest control, hazard analysis, product inspection and release, raw material supplier approval, and employee training in quality standards.

Quality control system

A quality control system must be in place at the supplier. The system must be able to identify measures correcting failures in achieving good manufacturing practice (GMP). Quality controls should be documented throughout the production premises, for example, raw material should be controlled at reception to ensure it is in line with agreed requirements. The origins of the raw materials must be known, and their suppliers audited by the product manufacturer. If an audit is not possible (due to logistic or time constraints), appropriate certificates ensuring the quality and regulatory compliance of the raw material must be provided before industrialization (phase 3). A quality control plan should be in place for finished products, possibly in line with the international standard ISO 2859 (sampling procedures for inspection by attributes). The supplier should manage the customer complaints and analyze feedback received from customers in order to improve the parameters of his manufacturing process.

Documentation

Pre-established instructions and working procedures should be made available at the supplier, including product specifications, manufacturing formulae, and processing parameters. Generally, the product specification is a written document containing the detailed description of the product characteristics (size, composition, compliance to regulatory requirements). It is agreed with and signed by the supplier. The specification is also part of the contractual agreement with the client. Certificates of analyses carried out by approved external laboratories (which are relevant to compliance and safety of the promotional material) should also be available on site.

A traceability system

A traceability system, ensuring the supplier can quickly identify in which finished products a given raw material was used, or *vice versa*, should be in place. The system will also be able to localize where a given production of finished items was delivered or is stored. Documents proving traceability implementation need to be available at the production site. A solid traceability system is the best pillar on which recall procedures can be constructed.

Regulatory requirements

The approved suppliers shall be able to demonstrate the required understanding of regulatory compliance matters. This includes knowledge of all components used for manufacturing the promotional item and of the chemicals potentially migrating from it. Suppliers must be aware of the legislation applying to promotional items in their country, as well as to the countries where the product may potentially be sold; they can be asked to share such information with the customer and should be prepared to do so.

The implementation of all the above requirements must be verified through a quality audit, generally carried out by the customer himself or via an accredited third party. Third party groups in charge of such activities will need to be carefully selected and prove to have the appropriate competences. Compliance to regulatory requirements (and to other applicable standards) is also a mandatory point that needs to be carefully evaluated during the audit.

Hazard Analysis

Hazards linked to promotional items

The second key step that has to be carried out to ensure product safety during the development phase is a hazard analysis. A hazard analysis is not only key in ensuring consumer protection, but is also an indispensable tool to protect the manufacturer's business reputation. This could be easily at stake if the media or consumer organizations were to communicate on the injuries or deaths caused by the manufacturer's products.

Clearly, the shape, composition, origin, the intended use of the promotional item, as well as the target consumer, will play a major role in estimating the importance or significance of the hazards associated with the product. It is well known that the shape and structure of the promotional item can be a serious source of safety issues (e.g., choking and wounds), when the product carries strings, or is easily breakable, or bares sharp edges. Lack of GMP at the manufacturer may lead to production parameters out of control, and thus, for example, unexpected presence of toxic components in the final promotional items.

It is the duty of the manufacturer to guarantee safety and compliance of the products delivered. The manufacturer must carry out an assessment that takes into consideration, as a minimum, the health consequences listed in **Table 2**. The hazard analysis of a promotional item follows the same principles of the well-known hazard analysis and critical control point (HACCP) tool, used for ensuring food safety of edible products. In both cases, for food and nonfood products, where a hazard having a significant impact on the consumer's safety is identified, a decision is required as to which control measures to apply in order to reduce the risk to an acceptable level. A modification of the promotional item specification/production process/parameters may also be considered, if needed.

Hazard identification

The following questions can be raised in order to guide the reader in the identification of the hazards that could be linked to promotional materials:

- Composition of the promotional item to define potential contaminants migrating or leaching from the item: Is it

Table 2 Health consequences that need to be considered when developing a promotional item

Type of health consequence	*Origin*
Asthma, cancer, chronic kidney, and liver affection	Specific: Use of noncompliant chemical substances (e.g., lead paints, phthalates in toys) or use of restricted substances above regulatory norms
Intestinal obstruction	Specific: Ingestion of magnets which are easily detachable from the object
Flammability and fire, explosion, electrical shock	Specific: Use of noncompliant raw material/electric devices
Strangulation, entrapment, choking, asphyxiation, septal perforation	Specific: Small parts that can easily fall off/break from the promotional item; presence of strings
Wounds and cuts, laceration, puncture, amputation	Specific: Hazardous shapes of the promotional item
Hot (or cold) burns	Specific: Item not designed for bearing hot or cold temperatures (e.g., nontempered glass cups for hot tea)
Inner ear damage	Specific: Impulsive sound emitted by object (e.g., toy weapons)
Injury by impact of projectiles	Specific: Parts of the premium can act as projectiles

made of plastic (and which one), or of metal? Is the item coated, painted, made with recycled material? Will the item smell due to presence of residual toxic solvents?

- Design of the product to check potential safety threats: Is it made up of several parts? Can these break down easily during manipulation? Small parts that can break off are often responsible for choking issues. Does it contain strings? Strings can cause of accidental strangulation in small children. Are there sharp edges with the potential to cause wounds? Would it pose any other safety risk to the consumer (e.g., burns)?
- Existence of regulatory requirements or other standards for the above mentioned categories of materials or objects.
- Intended use of the promotional item: Is it intended to come in contact with food/mouth (e.g., spoon and glass), or to be packed in direct/indirect contact with food (e.g., toy in a bag-in-box package), or to be placed in contact with the mouth (pacifiers, toys, etc.)? Is it a toy or can it be misused as a toy by children or pets?

Likelihood of occurrence and health consequences

Once the hazards are identified, the risk assessor must consider the likelihood of occurrence of the hazard and the health consequence that this may have on the consumer.

For the evaluation of the likelihood of occurrence of the given hazard, the following elements can be considered:

- Supplier know-how, for example, adequacy of the risk evaluation in place, established know-how in the production of similar promotional items, knowledge of regulatory requirements applying to the product.
- Supplier quality assurance system, for example, existence of on-line quality controls, documentation, application of GMPs.
- Country of origin of the promotional materials, knowing that publications on monitoring and rating a country's political, economical, and financial risk are available, and are updated on a yearly basis.
- Review of the historical evidence of hazards occurring in the categories of products being considered.

The health consequences for consumers will depend essentially on the type of hazard (e.g., small parts breaking off) on the target consumer (i.e., an adult, a child, or a pet), and on the exposure of the consumer to the product (e.g., for direct skin contact or mouth contact). Particular care must be taken when the item is intended for a child, as safety standards will need to be very strict. Indeed children readily put in their mouths a range of different objects; thus, items that are sources of foreign bodies are considered to be high-risk products. Moreover, for pets, the specificity of the animal needs to be considered in the risk evaluation as, for example, the jaws of an adult dog will certainly have a different effect on an object than the jaws of a kitten.

As part of the hazard analysis carried out during the development phase, promotional items packed in/on food products must be considered as an 'ingredient' of the end product, and included in the HACCP study of the food product itself. Clearly, these items should not be in direct contact with food, and should be separated from it by a functional barrier, which prevents the promotional material from contaminating the food product. A functional barrier may be considered to be a barrier consisting of one or more layers. These reduce the migration of authorized monomers and plastics' additives below the specific migration limit (SML), or reduce the potential migration of nonauthorized substances into foods or food simulants to a 'not detectable' level.

Approved Third Party Support

Currently, the manufacturers are held liable for injuries resulting from poor design, from use of inappropriate materials, or from faulty production of articles. Duly selected third parties can help in safeguarding manufacturers (and thus their customers) by providing product safety testing and evaluation. The key element for a third party selection will be the recognition of relevant accreditation bodies, governments, and jurisdictional authorities in most of the countries where the articles manufactured will be launched or marketed.

Best-in-class third parties are able to provide support to product manufacturers on:

- Product design and mock-ups/prototype evaluation.
- Product specification development.
- Various physical testing (mechanical resistance, thermal resistance, life cycle).
- Safety testing.

- Examination and testing according to applicable regulatory requirements.
- Product conformity assessment.

Moreover, they can give very valuable support on a complete range of quality assurance services, including supplier assessments, and laboratory testing.

As a concrete example of support activities supplied by external partners, in the European Union (EU) manufacturers can currently choose between two modules of toy conformity assessment: a self verification or a third party verification. In the case of third party verification, the manufacturer will submit appropriate documentation on the toy to the notified body who will issue an EC type examination certificate. The manufacturer will then need to ensure conformity between his production and the approved model.

Regulatory Compliance

Regulatory requirements applying to promotional items developed for or by the food industry cover items intended;

- to come in contact with food (e.g., spoon and glass);
- to be packed in direct/indirect contact with food (e.g., toy in a bag-in-box package), and;
- to be placed in contact with the mouth (pacifiers, toys, etc.).

Therefore, both product safety regulations and food contact material regulations must be taken into consideration for compliance purposes. These standards are set both at national (e.g., US) and supranational level (e.g., EU). Their main purpose is to protect the consumers and ensure that only safe products are placed on the markets.

Today, regulations do not cover all existing food contact materials, nor all nonfood products. When neither supranational nor national regulations deal with a specific subject/item, the promotional item manufacturer needs to refer to other standards. These include internationally recognized standards, industry-wide standards, community technical specifications, or codes of practice to ensure safety of the material.

Regulatory requirements for promotional items

In terms of product safety, several governments have set a strong legal framework.

In the US, the Code of Federal Regulations (CFR) contains the codification of the general and permanent rules published in the US Federal Register by the executive departments and agencies of the Federal Government. Requirements for nonfood products such as, for example, toys are included in CFR Title 16.

In Europe, the reference document is the General Product Safety Directive (GPSD) 2001/95/EC. One of the objectives of the Directive is to protect consumer safety by setting standards for products that are not covered by sector-specific legislations (e.g., toys, chemicals, cosmetics, and machinery). The Directive provides a generic definition of a safe product: a product which, under normal or reasonably foreseeable conditions of use, does not present any risk or only the minimum risks compatible with the product's use, considered to be consistent with a high level of protection for the safety and health of persons. All products put on the market must comply with this definition.

When there are no specific national rules, the safety of a product is then assessed in accordance with international/supranational standards (see Other standards), and the expectations of consumers.

Regulatory requirements for food contact materials

The legislation on food contact materials has also as an objective the protection of the health of the consumer: the materials in contact with food need to be safe and will not transfer their components into the foodstuff in unacceptable quantities. Food contact materials and articles are regulated in US by the Code of Federal Regulations (CFR), Title 21. In EU they are regulated by the Framework Regulation 1935/2004, by the GMP Regulation (Regulation EC 2023/2006), by specific directives covering groups of materials, and by directives on individual substances used in the manufacture of materials and articles intended for food contact.

Tables 3 and 4 give a list of regulations that need to be applied to ensure product safety. The lists contain references to nonfood products (3) and to food contact materials (4), and are not exhaustive.

Other standards

When this is not dealt by regulatory requirements, several standards, resolutions, or guidelines exist, setting the requirements for manufacturing of safe food contact and nonfood products (e.g., Council of Europe Resolutions (CoE); International Standard Organization (ISO); American Society for Testing and Material Standard (ASTM); European Norms (EN)). Standards help to make sure that products are fit for their purpose, safe, and compatible. Although in principle voluntary and not binding *per se*, some Governments consider them as reference documents which set out criteria of manufactured products to ensure consumer safety, and transpose totally or partially such requirements into their national law.

Tables 5 and 6 list some of the standards that are relevant to promotional items.

Labeling

The labeling of nonfood products is considered by regulatory authorities as another key element to protect consumers.

Generally, the main purpose of a label is to inform consumers on, for example, the origin and composition of the item, the standards according to which it was produced, or its intended use. As an example for Europe, the EU Directive 1935/2004 requires that materials or articles intended to come into contact with foodstuffs, and which are not yet in contact with food when sold, are accompanied by the word 'for food contact' or a specific indication as to their use. This information, however, is not obligatory for articles that, because of their characteristics, are clearly intended to come into contact with food (e.g., a cup or a spoon). If necessary, specific instructions to be observed for safe and appropriate use should accompany the product. The name or trade name and, in either case, the address or registered office of the manufacturer or seller responsible for placing the item on the EU community market, as well as an adequate labeling

Table 3 Examples of regulatory requirements applying to promotional items

Name	*Topic*
EU	
General Product Safety Directive (GPSD) 2001/95/EC	Ensures a high level of product safety throughout the EU for consumer products that are not covered by specific sector legislation (e.g., toys, chemicals, cosmetics, machinery)
Directive on safety of toys 2009/48/EC	Ensures consumers that toys sold in the EU fulfill the highest safety requirements, especially those relating to the use of chemical substances
Decision on phthalates 2005/84/EC	Prohibits the use of certain categories of phthalates in the manufacture of toys and childcare articles intended for children
Decision on magnetic toys 2008/329/EC	Magnetic toys placed on the EU market must carry warning labels. This applies to all toys that contain or consist of loose or detachable magnets, or magnetic components of such size and shape that they can be swallowed by children
Decision on dimethylfumarate (DMF) 2009/251/EC	As of 1 May 2009, all consumer products containing DMF are banned (maximum limit: 0.1 mg DMF per kg of product or part of the product)
Council Directive concerning products which, appearing to be other than they are, endanger the health or safety of consumers 87/357/EEC	Prohibits the marketing, importing, and manufacture of products that look like foodstuffs but that are not in fact edible
REACH, Regulation (EC) 1907/2006	The aim of REACH is to improve the protection of human health and the environment through the better and earlier identification of the intrinsic properties of chemical substances. It concerns the Registration, Evaluation, Authorization, and restriction of Chemicals
Directive 2002/61/EC	Sets restrictions on marketing and use of certain dangerous substances and preparations (azo-dyes)
US	
Code of Federal Regulations, Commercial Practices, Title 16, Chapter II	The regulation covers activities of the Consumer Product Safety Commission (including safety standards and standards for flammability of children's sleepwear and other textiles)
Code of Federal Regulations, Commercial and Foreign Trade, Title 15, Chapter XI – Technology Administration, Department of Commerce, Part 1150 – Marking of Toy, Look-alike and Imitation Firearms	The regulation covers – among others – prohibitions and approved markings relating to toys
US Consumer Product Safety Commission Engineering Test Manual for Rattles	The title is self-explanatory
US Consumer Product Safety Commission Engineering Test Manual for Pacifiers	The title is self-explanatory
US Consumer Product Safety Commission Labeling Requirements for Art Materials Presenting Chronic Hazards (LHAMA)	Labeling hazardous art material is a mandatory rule of the consumer Product Safety Commission
US Child Safety Protection Act, Small Parts Hazard Warning Rule, and Rules for Reporting Choking Incidents	The title is self-explanatory
Age Determination Guidelines: Relating children's ages to toy characteristics and play behavior	The title is self-explanatory
Japan	
The Japan Toy Association Toy Safety Standard	The standard is divided in three parts: Part 1 – Mechanical and physical properties Part 2 – Flammability Part 3 – Chemical properties

or identification to ensure traceability of the material or article, must also be available on the product labeling. In most of the EU countries (and differing from, for example, the US), there is currently no obligation to add the origin of the article on the label of the promotional food-contact material item.

For other promotional items that are not in contact with food (e.g., textiles, pens, toys) several other labeling requirements exist, which cover, for example, the material identification information on the textile sold, age warnings on toys, information that the product meets applicable safety requirements, and sometimes the country of origin. More specifically to the labeling of toys, different legislation exists worldwide (e.g., Japan, Sri Lanka, Taiwan, China, Australia, Fiji, India, south Korea, Malaysia, New Zealand, South American countries, EU, US). The EU toy directive, for example, requires that the 'Conformité Européenne' (CE) marking be affixed visibly, legibly, and indelibly on the toy, to an affixed label or to the packaging before the toy is placed on the market. CE stands for 'European Conformity.' in French. By

Table 4 Nonexhaustive list of food contact material regulations, applying to promotional items in contact with food

Name	*Topic*
EU	
Framework regulation: Regulation on materials and articles intended to come into contact with food, Commission Regulation EC 1935/2004	Sets up general requirements for all food contact materials
Good manufacturing practices : Regulation on good manufacturing practices for materials and articles intended to come into contact with food, Commission Regulation EC 2023/2006	Sets good manufacturing practices that need to be in place at suppliers of materials and articles intended to come into contact with food
Plastics: Regulation on plastic materials and articles intended to come into contact with food, Commission Regulation EU 10/2011	List of authorized substances in plastic materials, multilayer multi-materials; compositional requirements; declaration of compliance; and rules for assessing compliance with migration limits
Active and intelligent materials and articles: Commission Regulation on active and intelligent materials and articles intended to come into contact with food. Commission Regulation EC 450/2009	Ensures safe use of such materials and introduces an authorization scheme for substances used for active and intelligent functions in food contact materials.
Ceramics: Council Directive on the approximation of the laws of the Member States relating to ceramic articles intended to come into contact with foodstuffs. Council Directive 84/500 EC and 2005/31/EC.	Sets migration limits for cadmium and lead which might be released from decoration and glazing. It gives an analytical method for the determination of the migration of these substances
Regenerated cellulose: Commission Directive relating to materials and articles made of regenerated cellulose film intended to come into contact with foodstuffs, Commission Directive 2007/42.	Sets standards for materials made of regenerated cellulose film and intended to come in contact with foodstuffs
Elastomers and rubbers: Commission Directive concerning the release of the *N*-nitrosamines and *N*-nitrosatable substances from rubber teats and soothers Commission Directive 93/11 EEC	Concerns the release of the *N*-nitrosamines and *N*-nitrostable substances from elastomer or rubber teats and soothers
Directives on individual substances or group of substances e.g. Council Directive 78/142 EEC	e.g., Vinyl chloride monomer
USA	
Code of Federal Regulations, Title 21 – Food and Drugs Chapter I – Food and Drug administration Department, Department of health and human services, subchapter B, part 174–178	Detailed requirements for food contact material are listed under the following chapters covering indirect food additives (i.e., additives for food contact materials) 174-General: covers general provisions that apply to indirect food additives (e.g., GMP) 175-Adhesives and components of coatings 176-Paper and paperboard components 177-Polymers 178-Adjuvants, Production aids, and Sanitizers (e.g., colorants for polymers)
Latin America (Mercosur, i.e., Uruguay, Paraguay, Brazil, Argentina)	
GMC RESOLUCION 3/92:	EU regulation is taken as a reference.
GMC RESOLUCION 31/99:	Presently, three general resolutions are in force, covering factory filling equipment in contact with food, a positive list of components allowed to be in contact with food, and analytical methods for verification of food contact materials
GMC RESOLUCION 32/99:	Regarding the plastics food contact materials, there are more than 20 other Mercosur resolutions available
Japan	
Japanese Food Sanitation Law	Chapters IV and V cover, respectively, food contact materials and toys: IV: Apparatus and container packages: general regulatory requirements are set by materials (e.g., metal, glass) V. Toys: test items (e.g., lead), elutions conditions, and regulatory requirements are set

affixing the CE marking to a product, the manufacturer declares that it meets EU safety, health, and environmental requirements, despite its country of origin, and takes sole responsibility for this. Toys that are CE marked can freely circulate in the European Economic Area. Moreover, defined warnings shall specify appropriate user limitations. Where promotional items might be dangerous for children under 36 months of age, for example, they shall bare a warning such as 'Not suitable for children under 36 months' or, 'Not suitable for children under 3 years' or a warning in the form of a graphic (see Annex V, Part B, 1 of the EU Directive 2009/48/EC).

When the labeling cannot be printed, stickered, or engraved on the product itself due to material or size constraints, its content is often added to the leaflet on which are printed the instructions for use.

Table 5 Examples of standards relating to safety of some promotional items

Name	*Topic*
American Society for Testing and Materials (ASTM) Standard Consumer Safety Specification for Toy Safety ASTM F 963	ASTM F963, which includes guidelines and test methods to prevent injuries from choking, sharp edges, and other potential hazards, is part of the Consumer Product Safety Improvement Act of 2008 (CPSIA) The law makes the ASTM F963 standard a mandatory requirement for toys whereas the Consumer Product Safety Commission (CPSC) studies the standard's effectiveness and issues final consumer guidelines for toy safety. ASTM F963 incorporates safety measures already required under federal law
European standard on safety requirements of toys EN 71	Compliance with the standard is legally required for all toys sold in the EU. The standard has been published in 11 parts, containing for example ● Mechanical and physical properties, flammability. ● Specification for migration of certain elements. ● Experimental sets for chemistry and related activities. ● Graphical symbols for age-warning labeling. ● Finger paints. ● Swings, slides, and similar activity toys for indoor and outdoor family domestic use. ● Organic chemical compounds – requirement, sample preparation, and methods of analysis.
International Standard Organization (ISO)	● Safety of toys: safety aspects related to mechanical and physical properties, 8124-1:2009. ● Safety of toys: flammability, 8124-2:2007. ● Safety of toys: migration of certain elements, 8124-3:1997.

Table 6 Examples of standards relating to safety of some food contact material

Name	*Topic*
Council of Europe (CoE)	
Policy statement concerning coatings intended to come into contact with foodstuffs, V1, 10.06.2004	Coatings into contact with foodstuffs
Policy statement concerning cork stoppers and other cork materials and articles intended to come into contact with food, V1, 10.06.2004	Cork materials into direct contact with food
Policy statement concerning lead leaching from glass tableware into foodstuffs, V1, 22.09.2004	Leachable lead from glass
Policy statement concerning packaging inks applied to the nonfood contact surface of food packaging, V1, 21.12.2006, V2 10.10.2007	Packaging inks on the nonfood contact surface of the packaging
Guidelines on metals and alloys used as food contact materials, V1 13.02.2002	Metals and alloys in direct contact with food
Policy statement concerning paper and board materials and articles intended to come into direct contact with foodstuffs, V1 19.12.2002, V2 13.04.2005, V3 11.12.2007, V4 12.02.2009	Paper and board materials intended to come into direct contact with foodstuffs
Policy statement concerning rubber products intended to come into contact with foodstuffs, V1 10.06.2004c	Rubber products intended to come into contact with foodstuffs
Policy statement concerning silicones used for food contact applications, V1 10.06.2004	Silicone used for food contact applications
Policy statement concerning tissue paper kitchen towels and napkins, V1 22.09.2004	Tissue paper kitchen towels

Product Conformity Assessment and Release

The last step of the product-development phase, also called conformity assessment, allows checking that the product (at this stage still in the form of a prototype) is compliant to the quality, safety, and regulatory requirements initially set. Ideally, a third party audit needs to be planned for this. It is strongly recommended to list the key points that are verified by the auditors (e.g., compliance with the defined product specification, regulatory compliance, quality controls, hazard analysis, product design, material compliance, and certificates of analysis) on a template, which is signed off by all parties involved. Once signed, the document will prove that key parameters for product safety and compliance are covered. This signed document will be considered as the official 'release' form and the industrialization will finally start (phase 3).

Phase 3: Product Industrialization

As already mentioned, phase 3 – product industrialization – starts only when the conformity assessment of the product is carried out, the prototype is shown to be compliant with the set requirements, and the product is formally released.

During the industrialization phase, usually lasting from a couple of weeks to several months, samples of promotional items must be taken from the production lines and analyzed according to an agreed control plan (established according to, for example, ISO 2859). The plan includes verification of quality defaults as well as of the potential hazards that were found to be relevant for the product.

Once the items are in the marketplace, the manufacturer will carry out periodic verifications of their compliance for due diligence (often via a third party laboratory). This activity will assure that throughout the production period good manufacturing practices were in place, and were aligned with the defined quality and safety criteria.

Moreover, the manufacturer will monitor the possible consumer complaints. Complaints are a very effective means of measuring the quality and safety of the final promotional item, and the satisfactions of the consumer for the product.

The results of the above-mentioned activities will confirm that the work carried out during the development phase was effective, that the product is compliant both from a quality and regulatory perspective, and that the supplier is reliable. Should a problem occur, then a risk evaluation must be carried out to determine whether the promotional items will need to be withdrawn or recalled from the markets and destroyed. Records of the recall and of the destruction will need to be supplied to authorities, based on request.

Crisis Management and Recall procedures

Noncompliance with the established product safety regulations results in commercialization of unsafe products. The notifications described in Table 1 concern products failing to meet the required standards. Wherever this occurs, commercialization of an unsafe product engenders several corrective actions that can be ordered by authorities (such as withdrawal from markets, recall, or sales ban), or be initiated by manufacturers (withdrawal from the market and recalls).

To perform recalls or withdrawals, the manufacturers need to have in place appropriate procedures ensuring that defective products can be identified, located, and removed from all necessary points of the supply chain. Recall procedures are based on the existence of a traceability system at the manufacturer. These procedures are part of the company's crisis management system, together with a contact list for internal and external stakeholders which is periodically updated in the event of a recall. When products are recalled or withdrawn, the safety of other products manufactured under the same conditions (e.g., with the same raw materials or on the same line) needs to be evaluated and the need for public warning must be also taken into consideration.

Recall procedures need to be tested periodically in order to ensure that the system is working correctly, and records of the verifications need to be duly stored.

Conclusions

Promotional items, if not adequately designed or manufactured, can be a source of hazards for the consumers.

To ensure consumer protection and to avoid a very negative impact on the promoted brand, promotional items must imperatively be safe and comply with relevant regulatory requirements, or other applicable standards (e.g., EN, ASTM, or ISO standard). Product safety and compliance must be fully implemented in the product-development phase, via some key steps, for example:

- promotional item suppliers must be duly selected and approved based on their quality standards and on the knowledge they have on applicable regulatory requirements;
- a hazard analysis must be carried out during the development phase in order to define whether the promotional item can be a threat for the consumer and – if yes – the risk will have to be reduced to an acceptable level (see HACCP approach); and
- where support from third parties is deemed necessary, for example, for safety testing or product conformity assessment, the third party will be duly selected. The selection will be based on recognition of relevant accreditation bodies and governments, in the countries where the promotional items will be launched or manufactured.

A positive assessment of the promotional item conformity ends the product-development phase and is considered as the mandatory release step before the industrialization process. A periodic verification of the promotional item's compliance will be carried out by the supplier for due diligence during the production period.

See also: Food Safety Assurance Systems: Audits of Food Safety Management Systems; Documentation and Record Keeping; Essentials of Crisis Management; Food Safety and Quality Management Systems; Investigation of Incidents in Industry; Labeling and Information for Consumers; Management of Supplier and Raw Material; Recall Systems and Disposal of Food. Hazards of Food Contact Material: Food Packaging Contaminants. Institutions Involved in Food Safety: International Organization for Standardization (ISO). Other Significant Hazards: Food-Related Choking. Public Health Measures: Alerts and Early Warning Systems; Foodborne Disease Outbreak Investigation; Fundamentals of Food Legislation; Modern Approach to Food Safety Management: An Overview. Risk Analysis: Risk Management: Application to Chemical Hazards. Safety of Food and Beverages: Packaging Material and Auxiliary Items. Toxic Metals: Lead

Further Reading

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Relevant Websites

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SAFETY OF FOOD AND BEVERAGES

Risks of Food Adulteration

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Glossary

Economically motivated adulteration (EMA) As defined by the 2009 Food and Drug Administration Open Meeting on EMA, "EMA includes dilution of products with increased quantities of an already-present substance to the extent that such dilution poses a known or possible health risk to consumers, as well as the addition or substitution of substances in order to mask dilution."

Food adulteration As defined by the Food, Drug and Cosmetic Act (FD&C), including if it causes harm, the consumer does not get the benefit of an ingredient (either the product does not include a valuable ingredient or the ingredient is substituted – if it was not valuable it would be considered misbranded, not adulterated), the product includes alcohol, it is filthy or putrid, or could be a banned product (e.g., a banned food additive).

Food defense The protection of the food supply chain from intentional acts that include the intent to harm in the forms of economic, public health, or terror. It includes acts of terrorism that are often referred as bioterrorism.

Food fraud An intentional act of food misbranding for economic gain.

Food misbranding FD&C defines a product to be misbranded if the label is false or misleading, does not include the name and address of the manufacturer, shows inaccurate weight or measure, required text is not included, the container is misleading in some way, a color additive is specifically mislabeled, and it violates the Poison Prevention Act.

Food protection An overarching concept in food protection: food quality, food safety, food fraud, and food defense. A Food Protection Plan was specifically outlined by the US Food and Drug Administration.

Food security The continuous and nutritious supply of food. This is often confused with food defense, that is, the protection of the food supply chain.

Product counterfeiting (broad) The fraudulent representation of a product, not including digital piracy, currency counterfeiting, document forgery, or artwork forgery.

Product counterfeiting (intellectual property rights) A product that violates international intellectual property protection for trademarks, patents, copyright, or trade secrets.

Introduction

The modern era of food regulations in the US began with the Food, Drug, and Cosmetic Act (FD&C) of 1938 which replaced the Food and Drugs Act of 1906. The concepts, and even much of the text, are present in food laws from around the globe. An article by Tousley in 1941 stated "it is doubtful if any piece of recent legislation has been of greater potential significance to the general public than the Food, Drug, and Cosmetic Act which was enacted June 25, 1938." One of the main triggers was a mass adulteration poisoning of medicine that contained diethylene glycol (DEG) – a component of antifreeze. Unfortunately, DEG has continued to be a threat to public health and was recently a lethal adulterant in branded toothpaste in the US in 2007. Both the 1906 and 1938 acts included a major focus on adulteration and misbranding, and the 1938 law greatly expanded the range of products covered. The FD&C eliminated the 'fraud joker' statement which was in an earlier amendment, so it was no longer necessary to prove fraud (intent to deceive) to apply this law.

In the current FD&C, food adulteration is defined and explained in a section on Adulterated Food which is clearly differentiated from Food Misbranding, which is defined in a separate section. For food adulteration, an ingredient or food product is defined as adulterated for factors including if it causes harm, the consumer does not get the benefit of an ingredient (either the product does not include a valuable ingredient or if the ingredient is substituted – if it was not valuable it would be considered misbranded, not adulterated), the product includes alcohol, the product is filthy or putrid, or the product is banned (e.g., a banned food additive). Food adulteration is the result whereas food fraud is the motivation (an intentional act for economic gain).

Specific types of food and drug adulteration have been defined, such as economically motivated adulteration (EMA). EMA does not include misbranding and was defined by the US Food and Drug Administration (FDA) in a Federal Register

notice as "…the fraudulent, intentional substitution or addition of a substance in a product for the purpose of increasing the apparent value of the product or reducing the cost of its production." Also, "EMA includes dilution of products with increased quantities of an already-present substance to the extent that such dilution poses a known or possible health risk to consumers, as well as the addition or substitution of substances in order to mask dilution." The term is not defined in a law, and considering the FD&C, it is argued that if there is EMA there should also be a focus on economically motivated misbranding.

The US Food Safety Modernization Act (FSMA) was enacted in January 2011 and is considered the next major iteration of the laws pertaining to food safety and food defense, including food adulteration and food misbranding. The act includes 11 mentions of 'intentional adulteration' are not explicitly defined. It is intended to include traditional food adulteration as defined in the FD&C, as well as food fraud, smuggling, tampering, and acts of terrorism. Although there are seven mentions of 'misbranding,' each is included in a standard text of "…provide assurances that such food is not adulterated under section 402 or misbranded under section 403(w)."

FSMA is also important because there is a direct focus on prevention not setting specifications or outlining the response. There are 70 mentions of prevent or prevention in the Act. This is a novel challenge for the organization and agencies as the expertise and systems are traditionally more reactive for inspections and testing of products.

Global initiatives are underway to more precisely address food fraud and food defense – root causes of adulteration. Both the Global Food Safety Initiative and the US Pharmacopeia/Food Chemicals Codex specifically address economically motivated food fraud separate from the food defense acts intended to create terror, economic harm, or a public health threat. Also, in June 2012, under the Codex Alimentarius Commission (the 'food book,' which is a set of voluntary standards), the Food and Agriculture Organization of the United Nations produced their first edition of *Prevention and Reduction of Food and Feed Contamination*.

The concept of food adulteration has been a focus of even the very first food laws dating back to Roman times. More recently the concept is being further refined to differentiate between intentional adulteration to create harm (food defense) and those acts for economical gain (food fraud).

Food Protection

The US has been a leader in food protection and food safety regulations. For protecting the food supply, the overarching concept is food protection: food quality, food safety, food fraud, and food defense. In 2007, the FDA created the Food Protection Plan. An example is the FDA Food Protection Plan which states: "The plan focuses FDA's efforts to prevent problems before they start. It employs risk-based interventions to ensure preventive approaches are effective. This was one of the first major US food agency statements that prioritized prevention. It provides for a rapid response when contaminated food or feed are detected, or when there is harm to humans or animals."

Once a product is considered adulterated, there are two standardized responses for common or uncommon incidents. Common incidents fall in the food safety category where routine responses and methods are used. Uncommon incidents are in the food defense category where the contaminant is often something unexpected in food and potentially very dangerous – such as anthrax or an explosive. Tampering falls into the food defense category. The intervention and response steps are calibrated to the perceived level of risk.

The definitions here are from Spink and Moyer (2011).

- Food quality: "Food Quality focuses on the unintentional spoilage or deterioration of food that only results in economic loss, such as an unsalable or down-graded product. This could be due to specific product characteristics deviating from industry reference standards, including expected physical or chemical attributes. Similarly, food fraud can result in economic losses in the form of unsalable product, lower margins, lost tax revenues, or brand equity damages from recalls or consumer concerns. If a food quality incident leads to a product that is harmful, then, although the *cause* is unintentional, the *effect* makes it a food safety incident."
- Food safety: "Food Safety focuses on the unintentional contamination of food by known ingredients, organisms, mishandling, or processing. Food fraud differs since it is an intentional act perpetrated for economic gain. Food fraud also differs from food safety since the types of adulterants are unconventional and could only become known once encountered. Food fraud and food safety are very similar in that both can lead to public health risks."
- Food fraud: "Food Fraud is a collective term used to encompass the deliberate and intentional substitution, addition, tampering, or misrepresentation of food, food ingredients, or food packaging; or false or misleading statements made about a product, for economic gain. Food fraud is a broader term than either the economically motivated adulteration (EMA) defined by the Food and Drug Administration (FDA) or the more specific general concept of food counterfeiting. Food fraud may not include 'adulteration' or 'misbranding,' as defined in the Food, Drug, and Cosmetic Act (FD&C Act), when it involves acts such as tax-avoidance and smuggling. The economic motivation behind food fraud is distinctly different from those for food safety, food defense, and food quality. The *cause* of an event might be food fraud, but if a public health threat becomes involved, the *effect* is an adulterated product and a food safety incident. All of this is under the umbrella of food protection, which encompasses food fraud, food quality, food safety, and food defense."
- Food defense: "Food Defense is a collective term that encompasses preventing and recovering from an intentional and deliberate contamination or tampering of food, motivated by either economic gain or public health harm. Food fraud differs in that the motivation is *only* for the perpetrator's economic gain." For many regulatory activities in the US, Food Defense is defined by Homeland Security Presidential-7 and -9, which define this as terrorism. The

term 'terrorism' is still not clearly defined where one author stated "no single definition of terrorism that commands full international approval."

These concepts are important to clarify when considering prevention rather than intervention and response. The motivation, or cause, is critical to selecting optimal countermeasure for detection and deterrence. Food Fraud poses unique risks due to the unconventional motivation of the perpetrator.

The Food Fraud Risk

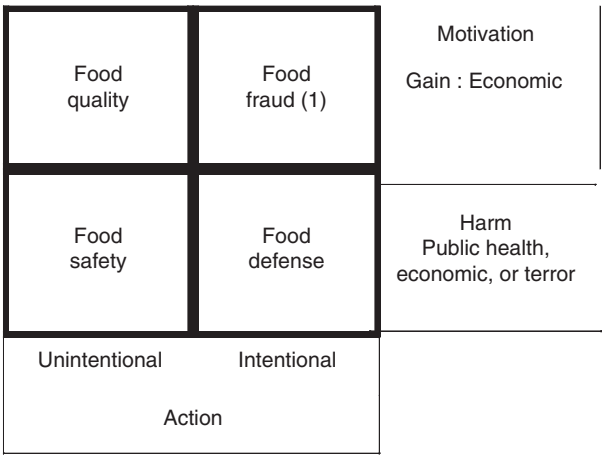
It is important to focus not only on the result of adulteration (contaminated product that is either a food safety or food defense incident) but also on the causes or motivation. To present this, the Food Risk Matrix was developed and published in Spink and Moyer (2011) (Figure 1).

By considering the cause or motivation, countermeasures to detect and deter specific types of risks can be more clearly defined. For example, perpetrator considering food fraud would try to avoid detection where in food defense the intent is to harm the victim – so the food defense attack would be identified quickly due to illnesses.

For food adulteration that is for economic gain, food fraud, there are three types of risks. The food fraud risks are: direct, indirect, and technical. Even though there are public health risks, the motivation is economic gain – the public health risk is an unintended consequence. It is important to consider the worst case scenario where the fraudster is a criminal not concerned with breaking laws, a sociopath not bothered by cheating others, and unaware of the unintended consequences of removing, adding, or modifying food products. The fraudsters who are unaware include operations that are sloppy or filthy. It should be considered that fraudsters do not worry about following standard good manufacturing practices (GMPs), hazard analysis and critical control points, and good hygienic practices which include hand washing and routine sanitation. The following is from Spink and Moyer (2011).

"Direct food fraud risk occurs when the consumer is put at immediate or imminent risk, such as the inclusion of an acutely toxic or lethal contaminant; i.e., one exposure can cause adverse effects in the whole or a smaller at-risk population. *Indirect food fraud risk* occurs when the consumer is put at risk through long-term exposure, such as the build up of a chronically toxic contaminant in the body, through the ingestion of low doses. Indirect risk also includes the omission of beneficial ingredients, such as preservatives or vitamins. *Technical food fraud risk* is non-material in nature. For example, food documentation fraud occurs when product content or country-of-origin information is deliberately misrepresented."

Although these are all risks that apply to all food adulteration, they have been specifically broadened to all fraud. There is a very broad scope of this risk.



(1) Includes the subcategory of economically motivated adulteration and food counterfeiting.

Figure 1 The Food Risk Matrix. Reproduced from Spink J and Moyer DC (2011) Defining the public health threat of food fraud. *Journal of Food Science* 75(9): 57–63.

The Scope of the Food Fraud Risk

As the food supply chains become more global, complex, and consolidated production, new and complex food risks have emerged. Where previous production was on a smaller scale and distributed more locally or regionally, the incidents impacted a smaller number and geographic population. The awareness of food product and safety has increased, including the disruptive acts of terrorism. There is also an emphasis on trusted and engaged supplier network.

It should also be mentioned that the detection methods for fraud are often based on traditional food safety triggers, which include a public health impact. When there is no public health impact – to trigger action – adulterated product may be very prevalent in the marketplace. Another trigger is a quality impact where the product is found not adhering to a production or quality control test. The fraudsters have demonstrated great resilience, intelligence, and ingenuity in circumventing efforts to detect fraud – they adapt to avoid detection including raising the quality of their products.

The technological advancements of the fraudsters, themselves, are amazing. For example, an adulterated product has been found to contain trace amounts of genuine ingredients to deceive authenticity detection tests that only test for the presence of an additive, not the quantity. Some examples of food fraud that could be the root cause of a public health incident include:

- melamine in milk products,
- the carcinogen colorant Sudan Red in foods,
- known *Salmonella*-contaminated peanuts distributed,
- species swapping of fish,
- dilution of fruit juices,
- substandard tomato products up labeled as a higher quality, and
- tax avoidance smuggling of honey.

Although some of these incidents appear to be only technical food fraud risks, the lack of adherence to GMPs lead to constant public health vulnerability. For example, overtreatment of raw fish with too much ice does not seem

Table 1 Top 25 ingredients in scholarly records dataset

Ingredients	*Percentage of total records (%)*
Olive oil	16
Milk	14
Honey	7
Saffron	5
Orange juice	4
Coffee	3
Apple juice	2
Grape wine	2
Maple syrup	2
Vanilla extract	2
Rice	1
Cheese	1
Milk fat	1
Turmeric	1
Vegetable oil	1
Chili powder	1
Sesame oil	1
Cocoa powder	1
Strawberry puree	1
Beeswax	1
Chinese star anise	1
Durum wheat pasta	1
Guar gum	1
Palm oil	1
Paprika	1

like a public health risk, until it is considered that untreated water was probably used.

A review of food ingredient intentional adulteration was conducted by the US Pharmacopeia/Food Chemicals Codex researchers, specifically by Moore *et al.* (2011). From 1980–2010, more than 1000 research reports were identified in scholarly journals and the top 10 targets as broad product groups of olive oil, milk, honey, saffron, orange juice, coffee, apple juice, grape wine, maple syrup, and vanilla extract (Table 1). Broad classes of fraud such as protein adulteration have been researched, as well (Table 1).

The population-wide perspective is important when considering the scope, scale, and threat, but an individual company must take a more macro approach and focus on their own vulnerabilities. Vulnerabilities are only partially defined by the product type or country of origin, and must consider all supply chain and business activities. For example, procuring product from an unscrupulous vendor in a western country may be much more dangerous than buying from a high quality, trusted vendor halfway around the world.

Conclusion

Food adulteration is an evolving concept due to increased detection methods for contaminants ("we're catching more fraud") as well as the growing opportunity for fraudsters to profit from the acts (expanding global markets). The food adulteration risks are being considered from sources across the food protection spectrum including food quality, food safety, food fraud, and food defense. Any food product that is a public health threat is classified as the effect of adulteration though there may be many different types of causes or motivations. Food fraud is a broader term that includes the cause of motivation of the incident. To stay ahead of the growing scope, scale, and threat, new countermeasure approaches are being developed to more efficiently and effectively detect and deter.

See also: Public Health Measures: Health Education, Information, and Risk Communication; Risk Governance. Risk Analysis: Risk Analysis of Hazards in Food: An Overview; Risk Communication

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SAFETY OF FOOD AND BEVERAGES

Safety of Organic Foods

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Glossary

Ecological balance A state of dynamic equilibrium within a community of organisms in which genetic, species, and ecosystem diversity remain relatively stable, subject to gradual changes through natural succession.

Genetically modified organisms The direct manipulation of an organism's hereditary information using biotechnology.

Herbicides Pesticides used to kill unwanted plants (weeds).

Nanotechnology The manipulation of matter with at least one dimension sized from 1 to 100 nm.

Organic certification An approval system run by a third party to convey the practices used in producing the organic food.

Pesticides Substances intended for preventing, destroying, repelling, or mitigating any pest like the damaging influences of weeds, diseases, or insects.

Introduction

Organic food production systems provide clear benefits in relation to the environment and animal health and welfare. The European Commission states as a matter of fact that organic farmers use a range of techniques that help sustain ecosystems and reduce overall pollution. They also explain how organic farming contributes to the protection of natural resources, to biodiversity and animal welfare, and how it helps in the development of rural areas. In simple terms, organic farming is an agricultural system that seeks to provide fresh, tasty, and authentic food while respecting the natural life cycle systems. However, organic foods are often more expensive than their conventionally grown counterparts. This has led to the perception that organic production systems also provide foods that are more nutritious, safer, and have added health benefits, aspects which are very much open for debate.

Looking back at the history of food production, organic farming was the prevailing cultivation method practised for 1000 years as the original type of agriculture. The beginning of the twentieth century saw simultaneous advances in engineering and biochemistry that rapidly and profoundly changed farming. Development of the combustion engine allowed the introduction of tractors and other mechanized farm implements. The chemicals industry developed synthetic fertilizers and a growing range of herbicides and insecticides for weed and insect control. Research in plant breeding led to the commercialization of hybrid seeds requiring more intensive inputs. Fields grew bigger and cropping became more specialized to make more efficient use of machinery. The reduced need for manual labor and work assistance from animals that machinery, herbicides, and fertilizers made possible created an era in which the mechanization of agriculture evolved rapidly.

As a reaction to agriculture's growing reliance on synthetic fertilizers and pesticides, organic farming was revitalized in the 1940s. The term organic farming was coined by Lord Northbourne in a book – Look to the Land – that was published in 1940. From his concept of the farm as an organism, he described a holistic, ecologically balanced approach to farming. He claimed that for this to be attained, the farm itself must have a biological completeness because every branch of work is interlocked with all others. He pointed to vegetables converted through animal digestion into manure that in turn supported the growth of new vegetables in a cycle of great complexity, and highly sensitive to any disturbance of its proper balance (Figure 1).

In the 1970s, global movements concerned with pollution and environmental degradation increased their focus on organic farming. As a result the International Federation of Organic Agriculture Movements (IFOAM) was formed in France in 1972 dedicated to the spread and exchange of information on the principles and practices of organic agriculture across national and linguistic boundaries. In the 1980s, farming and consumer groups around the world began seriously pressuring for government regulation of organic

Figure 1 Organic farming in balance with nature.

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production. This led to legislation and certification standards being enacted starting in the 1990s. Since this time the retail market for organic farming in developed economies has been growing by approximately 20% annually due to increasing consumer demand. A recent set back seen in some countries due to the economic crisis is considered to be temporary.

Defining Organic Food

The rise of organic farming was initially driven by small, independent producers and by consumers. However, the explosive organic market growth has encouraged the participation of larger agribusiness interests threatening the viability of small-scale dedicated organic farms. The meaning of organic farming as an agricultural method can easily be diluted and confused through an increasing commercial emphasis with a multitude of organic certification systems espousing different definitions.

The basic principle of organic farming is to achieve optimum quantities of produce and food of high nutritional quality without the use of artificial fertilizers or synthetic chemicals like pesticides and herbicides. Organic farming does not support the use of genetically modified foods, growth promoters, or hormones. It has been suggested that the application of nanotechnology to food and agriculture is a further technology that needs to be excluded from organic food production. Organic meat, poultry, eggs, and dairy products should come from animals that are given no antibiotics or growth hormones. Organic farmers attempt to rear animals with care and attention to their welfare by letting them grow and develop in the most natural and humane way possible. Organic farming emphasizes the need to maintain appropriate land management and aims to ecologically achieve the balance between animal life, the natural environment, and food crops. The produce that is produced through organic farming is thus supposed to be in its most natural form.

Here are some key differences between conventional farming and organic farming as outlined by the Mayo Clinic scientists:

Conventional	*Organic*
Apply chemical fertilizers to promote plant growth	Apply natural fertilizers, such as manure or compost, to feed soil and plants
Spray synthetic insecticides to reduce pests and disease	Spray pesticides from natural sources; use beneficial insects and birds, mating disruption or traps to reduce pests and disease
Use synthetic herbicides to manage weeds	Use environmentally generated plant-killing compounds; rotate crops, till, hand weed, or mulch to manage weeds
Give animals antibiotics, growth hormones, and medications to prevent disease and spur growth	Give animals organic feed and allow them access to the outdoors. Use preventive measures – such as rotational grazing, a balanced diet, and clean housing – to help minimize disease

However, it must be made clear that the organic movement represents a spectrum of practices, attitudes, and philosophies. On the one hand are those organic practitioners who would not use chemical fertilizers or pesticides under any circumstances. These producers hold rigidly to their purist philosophy. At the other end of the spectrum, organic farmers advocate a more flexible approach. Although striving to avoid the use of chemical fertilizers and pesticides, these practitioners do not rule them out entirely. Instead, when absolutely necessary, some fertilizers and herbicides are very selectively and sparingly used as a second line of defense. Nevertheless, these farmers too consider themselves to be organic farmers.

To clarify the basic understanding of organic farming and to develop a harmonized approach, a number of minimum objectives and principles were introduced. In 2005, the IFOAM approved a set of four principles to inspire the organic movement and to describe the purpose of organic agriculture to the wider world. The principles are intended to apply to agriculture in the broadest sense, including the way people tend soils, water, plants, and animals in order to produce, prepare, and distribute goods. They are concerned about the way people interact with living landscapes, relate to one another, and shape the legacy of future generations.

The four principles of organic agriculture according to IFOAM are as follows:

1. The health principle – organic agriculture should sustain and enhance the health of soil, plant, animal, and human as one and indivisible.
2. The ecology principle – organic agriculture should be based on the living ecological systems and cycles, work with them, emulate them, and help sustain them.
3. The fairness principle – organic agriculture should build on relationships that ensure fairness with regard to the common environment and life opportunities.
4. The care principle – organic agriculture should be managed in a precautionary and responsible manner to protect the health and well-being of current and future generations and the environment.

Following the above principles typical organic farming practices can be defined as including:

1. Wide crop rotation as a prerequisite for an efficient use of on-site resources.
2. Complete avoidance of or very strict limits on chemical synthetic pesticide and synthetic fertilizer use, livestock antibiotics, food additives and processing aids, and other inputs.
3. Absolute prohibition of the use of genetically modified organisms.
4. Use of on-site resources, such as livestock manure for fertilizer or feed produced on the farm.
5. Choosing plant and animal species that are resistant to disease and adapted to local conditions.
6. Raising livestock in free-range, open-air systems and providing them with organic feed.
7. Using animal husbandry practices appropriate to different livestock species.

Implementation of the overall principles and practices will vary according to individual needs and ethical convictions.

Marketing of produce defined as organic will require not only some objective description of parameters adhered to by the particular agricultural production system used, but also during food processing and further handling.

Food processors need organic certification for both, their ingredients and facilities used. This means that buildings, where ingredients are stored, equipment used, product packaging, and storage areas, for final products must all meet set requirements. Cleaning products and solvents need special approval for use in organic food manufacturing or completely rinsed away before organic production. Pest management in organic operations should be dealt with through preventive practices such as exclusion, sanitation, removal of pest habitat, management of environmental factors, mechanical or physical controls, or lures/repellents. In case of mixed processing systems, commingling of organic and nonorganic food must be avoided. Ingredient storage must be dedicated to organic or any containers that have been in contact with nonorganic products or prohibited substances must be thoroughly cleaned so that they pose no risk of contaminating the organic product. Synthetic fungicides, preservatives, and fumigants may not be used in the facility.

Certification of Organic Production Systems

Initially there was a direct communication link between farmers and consumers of organic foods. Early consumers interested in organic food had to buy it directly from growers or farmers markets – know your farmer, know your food was the motto. By talking to farmers and seeing farming activities and conditions, individual acceptance of what constituted organic production was the norm. As demand for organic food continued to increase, high volume sales through supermarkets rapidly replaced the direct farmer connection. A system to convey the practices used in producing the organic food was needed. An organic certification system was the essential solution aimed at regulating and facilitating the sale of organic products to consumers. It was intended to assure quality and prevent fraud, and to promote commerce (Figure 2).

In some countries, certification is overseen by the government, and commercial use of the term organic is legally restricted. Currently, the European Union, the US, Canada, Japan, and many other countries require producers to obtain special certification in order to market food as organic within their borders. In the context of these regulations, organic food is food produced in a way that complies with organic standards set by national governments and international organizations. Some countries, like Australia, have compulsory export standards to assure overseas customers of the authenticity of the organic food whereas adherence to the standard is voluntary for the domestic market. In countries without specific legislation for organic food, consumers must rely on third party certification alone. In any case, certified organic producers are also subject to the same agricultural, food safety, and other government regulations that apply to noncertified producers.

Figure 2 An example of a logo used for certified organic food.

Nowadays, there are more than 385 organizations worldwide that certify organic food, each according to their specific standards. Requirements vary from country to country and between certification bodies, but all involve a set of production standards for growing, storing, processing, packaging, and shipping of the organic food. At a minimum the standards have to comply with government legislation if available, but some certification bodies set standards that exceed government regulations. In general, any business directly involved in food production can be certified, including seed suppliers, farmers, food processors, retailers, and restaurants. For organic businesses, certification identifies suppliers of products approved for use in their own certified operations.

For a farmer to be certified as an organic producer he must know the relevant organic standards and farm facilities and production methods must comply with the specifics in the standards. Extensive documentation is required, detailing farm history and current set-up, and usually including the results of soil and water tests. A written annual production plan must be submitted, detailing everything from seed sources, field and crop locations, fertilization and pest control activities, harvest methods, and storage locations, for the sales of the organic produce. Annual on-farm inspections are required, with a physical tour, examination of records, and an oral interview. Written day-to-day farming and marketing records covering all activities, must be available for random inspection at any time.

Certification for operations other than farms follows a similar process. The focus is on the quality of ingredients and other inputs, and processing and handling conditions. A transport company would be required to detail the use and maintenance of its vehicles, storage facilities, containers, and so forth. A restaurant would have its premises inspected and its suppliers verified as certified organic.

The word organic is central to the certification process. Where organic laws exist, producers cannot use the term legally without certification. However, the organic labeling made possible by certification itself usually requires explanation. In countries without organic laws, government guidelines may or may not exist, although the actual certification is handled by nonprofit organizations and private companies.

Certification is intended to protect consumers from misuse of the term organic, and make buying organic food easier. Internationally, official equivalency negotiations are underway, with some agreements already in place, to harmonize certification between countries and facilitate international

trade. There are also international certification bodies, including members of the IFOAM, working on harmonization efforts. In 2011, IFOAM introduced a new program that attempts to simplify harmonization. The vision is to establish the use of one single global reference defining compulsory input standards to be applied to organic production systems rather than focusing on bilateral agreements.

Quality of Organic Produce

Although input standards for organic production systems can be established, the output quality of the resulting produce is much more difficult to define. There is a common perception that organic foods are of higher quality and provide added health benefits compared to conventionally grown alternatives. However, the weight of available scientific evidence has not shown a consistent and significant difference between organic and more conventionally grown food in terms of safety, nutritional value, or taste. This is not surprising when taking into account marked seasonal and geographical influences that might mask differences due to production methods.

As an example, an extensive recent cross-sectional review of the available literature on nutrient composition and contaminant analysis studies and human trials found that there were no general differences in nutritional value or risk for bacterial contamination between organic and conventional foods. The studies covered unprocessed foods including fruits, vegetables, grains, milk, eggs, chicken, pork, and meat.

Although organic food consumption reduced exposure to any detectable pesticide residues by approximately 30%, pesticide levels were generally within the allowable limits for safety anyway. However, free-range animal production systems might more easily be influenced by environmental contaminants. There are indications that free-range eggs might have slightly higher levels of dioxins, but with little consequence to the overall dioxin burden expected (Figure 3).

Other findings suggested that the consumption of organic fruits and vegetables had no benefit over conventional foods in terms of improved health. No consistent differences were seen in the vitamin content of organic products, and only phosphorus was significantly higher in organic versus conventionally grown produce, but is of little clinical significance.

Figure 3 Synthetic pesticides are not used in organic farming.

There was also no difference in the protein or fat content between organic and conventional milk, but there was some evidence that organic produce contained significantly higher levels of total phenolic compounds, which have antioxidant properties, and that organic milk and chicken contained higher levels of omega-3 fatty acids.

Other studies have found minor differences in ascorbic acid, protein concentration, and several differences in the micronutrient content have been identified between organic and conventional foods, but it does not appear that these have any impact on human health.

Some focus has been placed on the amount of nitrogen content in certain vegetables, especially green leafy vegetables and tubers, when grown organically as compared to conventionally. Although these vegetables, when grown organically, have been found to have lower nitrogen content, there is no consensus as to whether consumption of lower levels of nitrogen translates to improved health.

Most of the research has looked at differences in nutrient availability whereas other health impact parameters have been only scarcely studied. Some results indicate that carrots grown under strict organic conditions improved the immune status by inducing changes in lymphocyte populations, including an increase in regulatory T cells, when fed to laboratory mice. Such findings will still need to be confirmed.

Similarly, most studies that have compared the taste and organoleptic quality of organic and conventional foods report, no consistent or significant differences between organic and conventional produce. However, a few well-designed studies of fruits and vegetables have found minor differences, the majority in favor of organic produce. This might be due to the fact that some organic fruit seems to be drier than conventionally grown fruit. A slightly drier fruit may have a more intense flavor due to the higher concentration of nutrients, and as a result may be preferred by the consumer.

There is also evidence that some organically grown fruits have a higher resistance to deterioration and better keeping quality, attributed to a lower moisture content. On the contrary, because organic fruits and vegetables are not treated with waxes or preservatives, they may spoil faster. Some organic produce may look less than perfect – odd shapes, varying colors, or smaller sizes. However, organic foods should meet the same quality and safety standards as those of conventional foods.

Safety of Organic Produce

There continues to be widespread public belief in arguments that organic food is significantly safer for consumption than food grown conventionally, based mainly on anecdotal evidence and testimonials rather than scientific evidence. However, reviews of the available body of scientific literature have not found any significant differences between the two production systems in relation to safety. Firm conclusions about the relative safety of organic foods have been hampered by the difficulty in proper study design and relatively small number of studies directly comparing organic food to conventional food.

Figure 4 Animal welfare aspects are important when buying organic food.

Claims of improved safety of organic food have largely focused on pesticide residues. Although studies have shown organically grown fruits and vegetables have significantly lower pesticide residue levels, the significance of this finding on actual health risk reduction is debatable as both conventional foods and organic foods generally have pesticide levels well below government established guidelines for what is considered safe.

Reviews have noted that the risks from microbiological sources or natural toxins are likely to be much more significant than short-term or chronic risks from pesticide residues. There is an anticipated increased risk from microbiological contamination due to increased manure use as fertilizer from organisms like *Escherichia coli* O157:H7 during organic produce production, but little evidence of actual outbreaks that can be positively associated with organic food consumption. Other possible sources of increased safety risk from organic food consumption, like use of biological pesticides or the theoretical risk from mycotoxins from fungi grown on products due to the lack of effective organic compliant fungicides, have likewise not been confirmed by rigorous studies in the scientific literature.

When evaluating environmental toxins such as heavy metals, it was noted that organically raised chicken may have lower arsenic levels, although literature reviews found no significant evidence that the levels of arsenic, cadmium, or other heavy metals differed significantly between organic and conventional food products.

Organic regulations ban or severely restrict the use of food additives, processing aids (substances used during processing, but not added directly to food), and fortifying agents commonly used in nonorganic foods, including preservatives, artificial sweeteners, colorings, flavorings, and monosodium glutamate. The American Cancer Society (ACS) has noted that interest in organic food is partly derived from the perceived risk of cancer caused by additives not found in organic foods. The ACS has stated as their official position that whether organic foods carry a lower risk of cancer because they are less likely to include compounds that might cause cancer is largely unknown.

Conclusion

There seems to be little evidence that organic production systems provide healthier and safer food compared to conventionally grown food. However, looking beyond health effects, there are plenty of other reasons to buy organic food. Such reasons include sustainability of agricultural production systems and concerns about animal welfare and the environment. For a sustained growth of organic food, it is crucial to maintain the trust of consumers that organic production adheres to agreed principles through independent certification (Figure 4).

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Relevant Websites

http://www.ifoam.org
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Safety Consideration in Developing Functional Foods

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Glossary

Active phytochemical Compounds that occur naturally in plants that may have biological significance, but are not established as essential nutrients.

Antinutrient Natural or synthetic compounds that interfere with the absorption of nutrients.

Bioactive compound Inherent nonnutrient constituent of food with anticipated health promoting/beneficial and/or toxic effects when ingested.

Biomarkers Indicators of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.

Fortification The addition of an essential micronutrient to a food to improve its nutritional quality and thus provide a public health benefit of nutritionally adequate diets with minimal risk to health.

Nutraceuticals A combination of the words 'nutrition' and 'pharmaceutical' – sometimes used as a synonym for functional foods.

Introduction

The idea of associating certain foods with a beneficial influence on bodily functions emerged in the 1980s in Japan. This was the birth of the modern functional food concept and it has spread from there to become a worldwide dietary and nutritional trend. Functional food development is now a dynamic field in food science due to the increasing popularity of health foods with health conscious consumers and the ability of marketers to promote new and enticing products. It is estimated that the global market of functional foods might reach US$180 billion in 2013 with an annual growth rate of an impressive 7.4%.

In discussing functional foods it is important to first acknowledge that many foods are functional at some physiological level, but some functions might be seen by consumers as more important than others – a fact that the food industry has been keen to explore. With an aging population in many countries, the endeavor to maintain good physical and mental health is a priority topic. What better way then for the industry to capitalize on this need than by being able to provide foods with inbuilt health benefits. And the selling point for functional foods is their link to extra health benefits. Besides the potential problems of bogus health claims, the health concerns regarding functional foods are twofold. One is that they may lead to an unbalanced diet and resulting nutritional deficiencies, but the other is equally important and that is, their safety be assured.

So what constitutes a functional food? Although many definitions exist worldwide, there is unfortunately not yet any official internationally accepted designation of such foods. The difficulty is the fact mentioned above that food products in general are functional as they provide varying amounts of nutrients, energy, or fluids to sustain growth or support vital processes. As a matter of fact a well-balanced and varied diet should be sufficient for normal well-being. However, functional foods are generally considered to offer additional benefits that may reduce the risk of a disease or promote optimal health. Functional foods could thus be seen as foods that have a potentially positive effect on health beyond basic nutrition. But there is still some controversy around applying the concept of functional foods.

The first controversial issue is government prescribed fortification. Common beneficial fortified foods providing particular health benefits include fluoridated water to protect against caries, iodized salt to protect against mental retardation and thyroid dysfunction, and folic acid added to bread, cereals, flour, and other grain products to protect against neural tube birth defects as well as many others. But in an orthodox view of functional foods, government prescribed fortification is not supposed to be part of the concept (Figure 1).

Another difficulty relates to the already existing conventional foods. Some foods now considered to provide positive effects on health are actually natural whole foods where new scientific information about their health qualities can be used to proclaim benefits. Many, if not most, fruits, vegetables, and grains, as well as many fish species contain several natural components that may be argued to deliver health benefits beyond basic nutrition. Examples include lycopene in tomatoes, omega n-3 and n-6 fatty acids in salmon, insoluble fiber in whole grains, and saponins in soy. Many would include such foods in the functional food concept, whereas others think that only fortified, enriched, or enhanced foods with an added component having a health benefit beyond basic nutrition should be considered as functional.

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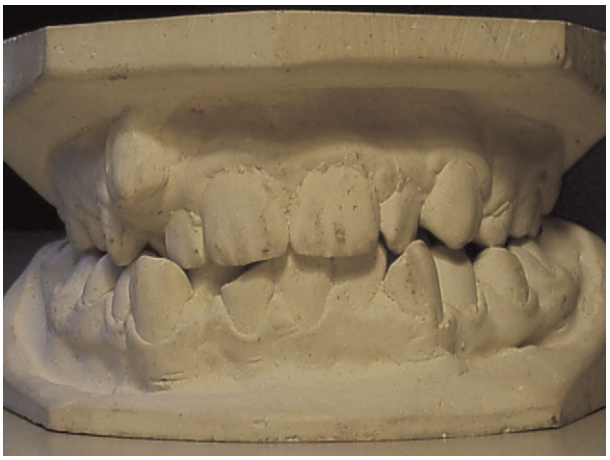

Figure 1 Prevention of tooth decay by fluoridating water is only considered by some as belonging to the functional food concept (photo: Amandabhslater).

Figure 2 Prevention of diseases related to obesity is a claim allowed in Japan (photo: Dennis Sylvester Hurd).

Attempts to Define Functional Foods

There are several attempts to try to better define functional foods from around the world. Although not specifically describing functional foods, the Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) Codex Alimentarius Commission has a definition of foods for special dietary uses. They are foods which are specially processed or formulated to satisfy specific dietary requirements, which exist because of a particular physical or physiological condition, disease or disorder and which are presented as such. The composition of these foodstuffs must differ significantly from the composition of ordinary foods of comparable nature, if such ordinary foods exist. Although foods of this nature are obviously intended to be 'functional,' the wording of the Codex description also implies that newly discovered beneficial effects of traditional foods are not covered, which is a bit of an anomaly.

Clearer rules exist in Japan and China. In Japan, functional foods are legally approved through a government instituted approval process established in 1991. Under their system, the term 'functional' was actually dropped and they are instead called 'foods for specified health use.' The Japanese Ministry of Health and Welfare highlights three conditions that such foods should satisfy. They should be foods, not capsules, tablets, or powders, and should be derived from naturally occurring ingredients. They should be a dietary component to be consumed as part of the normal diet. Finally, they should have a particular function when ingested, serving to regulate a particular body process, such as enhancement of the biological defense mechanisms, prevention of a specific disease (e.g., heart and arterial disease, cancer, hypertension, and obesity), control of physical and mental conditions, or slowing down the aging process (Figure 2).

China has also constructed a unique system for functional foods. Chinese functional foods are legally approved, with a logo issued by the Ministry of Public Health. They can cover one or more of the 24 functional areas defined in the legislation (see Section Validity of Health Claims, below).

In the rest of the world the situation is less transparent. There is no official legal definition of functional foods in the USA. However, some nongovernmental organizations have developed working definitions for functional foods. The American Dietetic Association includes both whole foods and fortified, enriched, or enhanced foods which have a potentially beneficial effect on health in the functional food concept as long as they are consumed as part of a varied diet on a regular basis, at effective levels. The Institute of Food Technologists simply defines functional foods as foods or food components that provide a health benefit beyond basic nutrition.

In developing health claims legislation, Health Canada had a working definition of functional foods as being similar in appearance to, or being, a conventional food that would be consumed as part of a usual diet, and has been demonstrated to have physiological benefits and/or reduce the risk of chronic disease beyond basic nutritional functions.

Equally, the European Union has no official definition of functional foods. However, the European Commission funded a project named the 'Concerted Action on Functional Food Science in Europe.' Participants in this project proposed that a functional food should be defined as beneficially affecting one or more target functions in the body beyond adequate nutritional effects in a way that is relevant to either an improved state of health and well-being and/or reduction of risk of disease. It should be consumed as part of a normal food pattern. It should not be a pill, a capsule, or any form of dietary supplement.

Although there is no consensus around a clear definition of functional foods, a common view seems to be that they should be natural or processed foods constituting a part of a normal diet and containing a bioactive compound with a discrete beneficial health effect beyond normal nutrition. For the purpose of this article, a functional food is a food that is consumed as part of a usual diet, and that is demonstrated to have physiological benefits and/or reduce the risk of chronic disease beyond basic nutritional functions. This definition does not include the so-called 'nutraceuticals,' which are products isolated or purified from foods that are generally sold in medicinal forms not usually associated with food and which have demonstrated health benefits.

Functional Components

To be considered as a functional food it is most important to have proof of the actual claimed beneficial effect of the functional component. Because any functional food must be based on science, its evaluation should principally be contingent on data from biochemistry, physiology, molecular and cell biology, and other relevant biosciences. A key component in the development of a functional food is the identification and validation of relevant biomarkers that can predict potential benefits relating to a target function in the body. This could be a marker directly involved in the biological process or secondary markers of correlated events.

Among many potential candidates of functional components, research into phytochemical, probiotic, or prebiotic substances has shown a variety of possible beneficial effects for a range of foods. Active phytochemicals, such as indoles, thiocyanates, sulfur-containing compounds, allium compounds, isoflavones, and phenols, have all been shown to have some possible beneficial or protective effects. Probiotics such as lactobacilli and bifidus bacteria, which can modify the bacterial flora in the intestine and enhance certain immune functions, possibly promote absorption of certain essential minerals, and protect against some diseases of the intestine and colon. Prebiotics that are oligosaccharides may moderate risks of intestinal disorders, osteoporosis, cancer, and heart disease.

However, to the frustration of many commercial food manufacturing companies, to convincingly establish the beneficial effects of any of these substances has proved to be difficult. Without conclusive scientific evidence it will not be possible to make associated health claims in the regulated markets of industrialized countries.

Validity of Health Claims

The continued growth of the functional food market is very much contingent on the prospects of convincing consumers of their beneficial effects. Thus functional food products typically include health claims on their label touting their benefits. An example would be: 'Cereal is a significant source of fiber. Studies have shown that an increased amount of fiber in the diet can decrease the risk of certain types of cancer in individuals.' However, this could equally be a claim for a drug. In the past there was no middle ground between foods and drugs, and health-related claims were not permitted on foods. Nevertheless, developments at national and international levels have tended to take into account newer research findings into the possible beneficial or protective effects of foods and their ingredients. This has led to the gradual acceptance of well-founded claims for such effects to be associated with specific foods in some parts of the world.

As functional foods are not defined or set aside as a specific class of product in most countries, they are commonly regulated under the existing laws as foods, novel foods, special dietary foods, medical foods, or as drugs, depending on how they are marketed, and the claims that are made for the products in their labeling, or in advertising.

At the international level, the Codex Alimentarius Commission has completed work at a generic level on basic labeling rules, on nutrition claims and nutrition labeling, and claims for foods for special dietary use. In general, these Codex standards and guidelines state that packaged food should not be described or presented on any label or labeling in a manner that is false, misleading, or deceptive, or is likely to create a wrongful impression regarding its character in any respect.

Japan is more specific in relation to health claims on foods. The Japanese rules require that a manufacturer or marketer of such foods present a dossier to the Japanese government with valid information on the ingredients, processing, labeling, quality, safety, and health effects of each product. If the government authorities are satisfied that the dossier supports quality and safety requirements, and substantiates the health effects to be placed on the label or used in advertising, approval of the functional food can be given.

China is explicit in what type of functional claims that can be made. The functions to be considered must fall into one or more of the following exhaustive areas: immune regulation, postponement of senility, memory improvement, promotion of growth and development, antifatigue, body weight reduction, oxygen deficit tolerance, radiation protection, antimutation, antitumor, blood lipid regulation, improvement of sexual potency, blood glucose regulation, gastrointestinal function improvement, sleep improvement, improvement of nutritional anemia, protection of liver from chemical damage, lactation improvement, improvement for beauty, vision improvement, promotion of lead removal, removal of 'intense heat' from the throat and moistening of the throat, blood pressure regulation, or enhancement of bone calcification.

The Food and Drug Administration (FDA) in the USA regulates claims that manufacturers make about the nutrient content of functional foods and their effects on disease, health, or body function. The FDA regulates these types of foods according to whether a food is considered to be a conventional food, a food additive, a dietary supplement, a medical food, or a food for special dietary use. However, some claims will fall outside of the purview of the FDA and be accompanied by the disclaimer: 'These statements have not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure, or prevent any disease.'

The European Commission is responsible for authorizing health claims on foods in the European Union according to legislation on nutrition and health claims applied from mid-2007. Foods covered include natural foods, fortified foods, food supplements, and dietetic foods in the absence of any specific legislation for functional foods. The legislation harmonized the rules governing nutrition and health claims across all Member States of the European Union while providing assurance to consumers that only standardized nutritional claims or specifically authorized health claims may be carried on food. The European Food Safety Authority (EFSA) is responsible for assessing the scientific validity of health claims and advising the European Commission of the results of its evaluations. EFSA's scientific evaluation helps to ensure that claims made on food labeling and advertising regarding nutrition and health are meaningful and accurate, and will thereby help consumers in making healthy dietary choices.

Originally, industry submitted 44 000 claims on functional foods through EU Member States for evaluation by EFSA.

Figure 3 Reduction of blood cholesterol levels is an allowed claim associated with a decrease in the development of coronary heart disease (photo: Gabriella Camerotti).

These claims were consolidated into a list of 4637 differentiated claims. They are differentiated into three areas:

1. general functional claims that relate to the growth, development, and functions of the body, or refer to psychological and behavioral functions or are concerned with slimming or weight control;
2. risk reduction claims that refer to a reduction of a risk factor in the development of a disease, for example, reduction of blood cholesterol levels which is a risk factor in the development of coronary heart disease (Figure 3); and
3. health claims that are linked to children's development.

EFSA completed its evaluations of all general function claims, other than those related to botanicals, in June 2011, having published a total of 341 opinions covering 2758 claims. Using EFSA's scientific advice, the Commission has adopted a list of 222 approved general function claims for use in the European Union. This is less than a 10% approval rate with many functional ingredients considered to be not sufficiently characterized, or with insufficient evidence to establish a cause and effect relationship between the consumption of the food and the claimed effect.

Summarizing the activities needed, it is clear that manufacturers of foods which fit the profile of functional foods must develop or access adequate scientific data to substantiate any claims that they wish to make on products. This will typically involve at least three steps:

1. fundamental research to identify and understand the mechanisms of interaction between the food or ingredient and cellular biochemical functions to be able to demonstrate a potential theoretical effect;
2. elaboration of models and methodologies including possible biomarkers to demonstrate through studies on human nutrition possible functional effects and their impact to justify specific functional or physiological claims; and
3. performance of relevant human nutrition studies to establish the functional effects and benefits to health, including reduction of disease where appropriate, to substantiate the health claim.

Safety Assessment of Functional Foods

Given the differences in regulation of food, special dietary foods, novel foods, foods for special dietary use, and medical foods in different countries and regions, potential manufacturers of these foods must be prepared to fully understand the regulatory requirements which apply in each marketing area to be able to establish their safety.

There are two potential food safety issues related to functional foods. The first one is misdirected or misinterpreted advertising messages in relation to health claims. It is easy to misconstrue a health claim to mean 'the more the better of the food'. If 3 g of plant sterols are good for heart health, what about doubling or tripling the intake of the enhanced food? This could skew the diet away from being balanced and thus be counterproductive. To counter such a response, some countries specify the exact text for any authorized health claim to make sure that it is not overstating the beneficial effect. The second issue is the more traditional food safety evaluation of toxicity, allergenicity, or antinutrient properties.

Many substances in food are considered safe based wholly or in part on the empirical evidence from long periods of consumption, i.e., prior history of safe use. In the absence of a prior history of safe use, potential new functional ingredients must be evaluated for safety before introduction into the market. The safety evaluation of new functional ingredients should adhere to the same safety testing principles used for other substances and should replicate the intended use levels. The safety evaluation should cover both the functional ingredient and the functional food itself. For novel foods that cannot be considered to be equivalent to a traditional food, a more elaborate process will be necessary (see other articles in this encyclopedia).

An objective, science-based evaluation process must establish that the functional components are safe at their estimated use levels. The scope of potential new functional foods is very broad so the safety assessment framework must be effective for many types of functional ingredients over a wide range of consumer intake levels. The nature of the ingredient and the sensitivity of subgroups of the population should be considered. An example is the advice provided by a medical practitioner in Sydney to a pregnant woman to increase her intake of omega n-3 fatty acids by increasing her fish consumption. In response to the advice she exclusively consumed swordfish several times a day for several months resulting in mercury levels well above safe levels.

Typically, a safety assessment of a functional food will include the following steps:

1. documentation of the history of food use;
2. realistic estimates of current and proposed intakes of the functional component for the general population and for high consumers; and
3. a toxicological assessment at predicted intake levels.

Substances without a prior history of safe use will require a comprehensive and critical review of the scientific literature covering their expected biological effects. Based on an initial review, specific studies will generally be required to define:

1. bioavailability and likely modes of action;
2. estimated half-life in the body;
3. estimated dose–response for pertinent potential effects;
4. recognized pharmacologic or toxic effects;
5. evidence of allergenicity; and
6. toxicity testing and safety conclusion.

Any new protein in a functional food should be evaluated for potential allergic effects. Although no single test can absolutely predict the potential allergenicity of a novel protein, the application of a series of tests can provide reasonable assurance that the novel protein is not likely to become an allergen.

Epidemiological studies can confirm relationships between dietary patterns and biomarkers of disease or disease occurrence. Such studies have been widely used to relate diet intakes to the occurrence of heart disease or cancer. However, people can only record food consumed, not isolated ingredients, so food intake studies cannot directly assess the intake of a specific bioactive component. Consequently, epidemiological studies have to combine the food intake data with other compositional data to estimate the actual intake of the substances of interest. However, many bioactive components have not been well characterized and quantitative data for such components may be very limited or nonexistent. In addition, isolating the effect of a specific food or nutrient can be difficult because the substances are consumed as a mixture that may have synergistic effects.

Postmarket Surveillance

To assist in the evaluation of functional foods, postmarket surveillance can be very useful. The term 'postmarket surveillance' refers to the process of acquiring information on the effects of the functional ingredient after it has been introduced into the marketplace. Such evidence can confirm the conclusions reached during premarket evaluations regarding safety and efficacy of the functional component. It will monitor actual consumption patterns and consequential health impact. It can also determine if there are any adverse health effects that were not identified in premarket testing.

A postmarket surveillance program may be active or passive. In an active program, an appropriate professional group is employed to methodically poll consumers regarding intake patterns. It may include additional research to further evaluate tolerability or efficacy or to address scientific questions that arose after marketing. A passive program involves the collection, documentation, and evaluation of complaints about the product and may include reports of adverse health events, often through the inclusion of a free telephone number to the food manufacturer on the label of the product. The information obtained from a passive program cannot establish a causal relationship between the ingredient and the alleged adverse health effect, but remain useful in documenting trends over time and identifying unanticipated effects that may require further evaluation.

See also: Public Health Measures: Assessment of Novel Foods and Ingredients. Risk Analysis: Risk Communication: Novel Foods and Novel Technologies

Further Reading

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Relevant Website

http://www4.agr.gc.ca/AAFC-AAC/display-afficher.doid=1171305207040
Agriculture and Agri-Food Canada website.

Probiotics and Prebiotics

FX Malcata, University of Porto, Porto, Portugal
TS Granja Tavares, New University of Lisbon, Oeiras, Portugal
A Hernández-Mendoza, Center for Food Research and Development, A.C. (CIAD), Hermosillo, Sonora, México

Glossary

Bioavailability Refers to extent and rate at which the active moiety (drug or metabolite) enters systemic circulation, thereby accessing its site of action.
Crohn's disease A chronic inflammatory disease of the intestine that occurs when the immune system mistakenly attacks and destroys healthy body tissue (autoimmune disorder).
Homeostasis The maintenance of metabolic equilibrium within an biological system by a tendency to compensate for disrupting changes.
Xenobiotics Chemical compounds that are foreign to a biological system. They may include naturally occurring compounds, as well as drugs, environmental agents, carcinogens, insecticides, etc.

Current Trends of Functional Foods

In recent years, health has become one of the major reasons of consumers' choice of food. However, the difficulty in having consumers to change their eating habits drastically has prompted the emergence of functional foods that resemble conventional ones in appearance, but contain specific nutraceutical ingredients, i.e., be able to bring health benefits, in addition to responding to nutritional issues. Reduction of incidence of chronic diseases using such dietary supplements will obviously promote one's health, and thus helps reduce the overall costs of public health care.

Several definitions of functional foods exist, which makes it difficult to provide industry with solid information on market trends and potential, or to appropriately protect consumers via legislation. Generally speaking a food maybe considered as 'functional' if, beyond its nutritional effect, it provides benefits on one or more functions of the body, thus improving health or welfare, or reducing the risk of illness; obviously such type of food must be part of a standard diet, consumed on a regular basis and in reasonable amounts. In this definition proposed by Functional Food Science in Europe, one should highlight that: (1) the functional effect is different from the nutritional one; (2) the benefit provided shall be rationalized; and (3) an improvement is to exist in physiological functions, or a reduction in the risk of developing pathological conditions. Because of the growing consumer awareness of the relationship between nutrition and health, the market of functional foods is booming.

To treat or prevent disease, wide ranges of chemotherapeutics are nowadays prescribed to patients; however, exposure to excessive use of antibiotics leads to an imbalance between beneficial and harmful microorganisms, thus turning our body more susceptible to infections brought about by resistant strains. The importance of the digestive tract in this endeavor often owns second relative to heart and brain diseases and conditions, yet it plays an extremely important role in absorbing nutrients and maintaining a state of overall good health. Probiotics are living microorganisms added to food, which remain essentially intact throughout the digestive process so they can reach the colon in viable form where they stimulate metabolic activities. Prebiotics are dietary supplements that selectively stimulate growth of said probiotics during its passage through the gut; and which suppress growth and activity of otherwise deleterious bacteria. Combination of probiotics and prebiotics affect the host beneficially by improving a healthy microbial balance – and adding them together in a single product may bring about synergistic effects.

Among functional food ingredients, prebiotics have been attracting more and more attention, as they offer the potential to change the gut microbial balance toward health benefits in a nonexpensive fashion. Although there are many different types of prebiotics available in the market today (e.g., in bread, cereal bars, spreads, sauces, infant milk formulae, beverages, and drinks), it is important to stress that some are just not so good as advertised; for instance, despite most evidence on prebiotic role against infections being positive, few studies indicate that they may increase the susceptibility to specific gastrointestinal (GI) infections. Hence, the aim of this entry is to describe the probiotics/prebiotics concept, their dietary sources and range of products, and their positive and negative involvement in safety issues.

Concept and Selection Criteria

Probiotics

The word 'probiotics' is derived from the Greek 'pro bios,' which means 'for life'; its origin can be traced back to 1953, when Kollath used it to describe restoration of health of malnourished patients using organic and inorganic supplements. Later, Lilly and Stillwell in 1965 used it to describe compounds secreted by one microorganism that stimulate the growth of another, thus contrasting with the term 'antibiotic.' But it was not until 1974 that the term probiotic acquired its current meaning with Parker – yet an important evolution of this concept has been witnessed; however, there is no consensus about an appropriate definition.

Following recommendations of the Food and Agricultural Organization/World Health Organization working group on evaluation of probiotics in food (2006), the suggested definition describes probiotics as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host. Based on this definition, a significant number of microorganisms have been isolated and labeled as probiotics.

There is a number of minimal criteria to assess the potential of a microorganism to function as probiotic, including: safety criteria (be nonpathogenic, nontoxic, and free of significant adverse side effects); technological criteria (be compatible with the food matrix, as well as the processing and storage conditions associated therewith); functional criteria (be able to survive through the GI tract, and colonize the intestinal mucosa both *in vitro* and *in vivo*); and physiological criteria (be able to demonstrate beneficial effects on the host).

The microorganisms most commonly used as probiotics belong to the (wide) group of lactic acid bacteria (LAB) (e.g., *Lactobacillus* and *Enterococcus*) and the genus *Bifidobacterium* – but *Streptococcus, Escherichia coli, Bacillus*, and *Saccharomyces* strains have also been claimed as probiotics. These bacteria are beneficial to human health by decreasing the presence of intestinal pathogens and/or promoting production of health-related bacterial substances, such us bacteriocins, metabolic products, and short-chain fatty acids (SCFAs), which are generally believed as favorable for colonic health. For instance, SCFAs decrease gut pH to low levels, and enhance mucin production, besides affecting epithelial and immune cells by binding to specific cellular receptors and thus improving the mucosal integrity and defense systems based thereon.

Prebiotics

The definition of prebiotic has been refined over time; in 1995, Gibson and Roberfroid introduced that word by changing 'pro' to 'pre' in the word 'probiotics,' which means 'before' (or 'for') – and defining prebiotics as nondigestible food ingredients that, when consumed, provide beneficial physiological effects on the host by selectively stimulating growth and/or activity of one or a limited number of indigenous bacteria in the colon (Gibson and Roberfroid, 1995). Prebiotics encompass dietary fiber, except for its selectivity toward certain species; and Cummings *et al.* (2001) defended that prebiotics are carbohydrates with relatively short chain length – with oligosaccharide as particularly promising. In 2007, Roberfroid updated the aforementioned definition by stating that prebiotics (inulin-type prebiotics and galactooligosaccharides (GOS)) are ingredients susceptible to colonic enzymatic activity and fermentation, and able to selectively promote specific changes, both in composition and activity, in the desirable types of gut bacteria (e.g., *Lactobacillus* and *Bifidobacterium*) (Roberfroid, 2007). The action of prebiotics is consequently indirect; they indeed alter the GI microbiota composition, by increasing the number of bifidobacteria and lactobacilli (**Figure 1**). However, health benefits of prebiotics go beyond microbiota modulation, and include the impact on immune-associated diseases (e.g., allergies, inflammatory bowel diseases, and cardiovascular diseases), as well as improvement of bowel function, mineral absorption, satiety/obesity control, and certain types of diabetes.

Many prebiotics identified to date are members of the carbohydrate family, but are grouped by their ability to promote growth of specific beneficial (probiotic) gut bacteria rather by structural similarities. According to the International Union of Pure and Applied Chemistry (IUPAC), carbohydrates can be classified based on their molecular size or degree of polymerization (number of monosaccharide units) as monosaccharides, oligosaccharides, and polysaccharides; oligosaccharides are defined as low molecular weight carbohydrates. Based on their biochemical and physiological features, carbohydrates can be classified as digestible or nondigestible (prebiotics as in the case of), and the configuration of the anomeric C atom of the monosaccharide units causes this major difference; nondigestible oligosaccharides (NDOs) have glycosidic bonds resistant to hydrolytic activity, so they are not be broken down into smaller pieces or absorbed in the gut because humans and higher animals do not possess the necessary digestive enzymes. The monosaccharide units usually present in NDOs are fructose, galactose, glucose, or xylose. Dietary fibers encompass both polysaccharides and lignin that are not digested by the endogenous digestive enzymes of the human GI tract; they are classified as water-soluble (e.g., inulin and oligofructose), insoluble (e.g., cellulose), and mixed (e.g., bran).

In his definition, Roberfroid considered only inulin type and GOS as prebiotics, but many other dietary fibers (especially soluble ones) are also to be considered; this is the case of gentiooligosaccharides, glucooligosaccharides, fructooligosaccharides (FOS), isomaltooligosaccharides, mannan oligosaccharides, *N*-acetylchitooligosaccharides, oligosaccharides from melibiose, pectic oligosaccharides, xylooligosaccharides, gums (like arabic gum), hemicellulose-rich substrates, resistant starches (maltodextrin), lactosucrose, oligodextrans, and polydextrose. Furthermore, nonfiber compounds are not precluded from being classified as prebiotics, as long as they abide to such functional criteria; this is the case of germinated barley, gluconic acid, glutamine, lactose, and tagatose. Nevertheless, inulin, GOS, FOS, and its derivatives are the best established and most studied prebiotics; beside inhibiting exogenous pathogens and modulating human gut microbiota, their manufacture is relatively nonexpensive.

Regular dietary sources of prebiotics entail certain fruits and vegetables; for instance, fructans are frequently eaten in

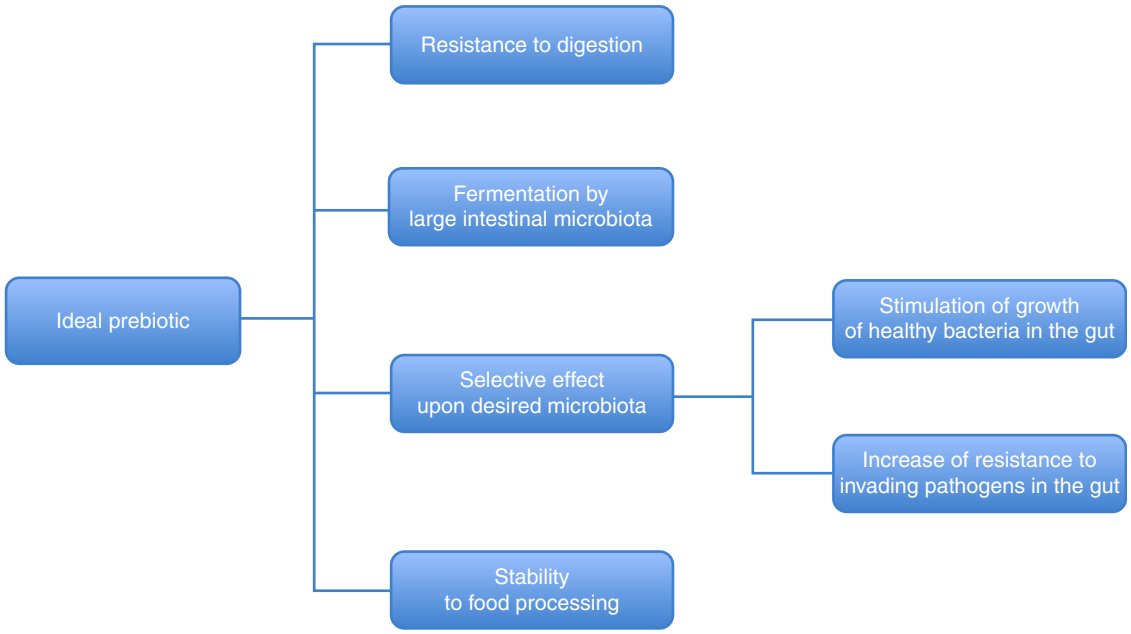

Figure 1 Characteristic features of ideal prebiotics.

classical diets as vegetables – asparagus, wheat, garlic, tomato, leek, artichoke, and onion, its commercial product, inulin, is produced and used as food and feed ingredient. β-Glucans are present in grains and cereals, whereas GOS appear in human breast milk. To bring about a substantial effect, daily intake of prebiotics should be more than 2–3 g; if 5–8 servings of fruits and vegetables were eaten per day, the dietary fiber needs would be fully met. Because the vast majority of the population does not attain that threshold, prebiotics are now added to many common food choices, such as cereals, biscuits, breads, table spreads, drinks, and yogurts.

Health Benefits and Mechanisms of Action

Probiotics

The role of the gut microbiota on host health is increasingly appreciated; the human body, especially the GI tract, is home to a variety of bacteria, which are normally referred as commensal bacteria (meaning that they live in peaceful coexistence with the host); when both, host and bacteria, benefit from each other's presence, then one has symbiotic bacteria. The host provides food and shelter for the bacteria, which digest unused energy sources, thus leading to release of beneficial metabolites. Although in relatively small number in healthy individuals, the human gut also contains (potentially) harmful bacteria. Although the exact mechanisms behind host–microbial interactions are unknown, and also which species of bacteria are involved in maintenance of a healthy gut environment, it is generally accepted that *Bifidobacterium* and *Lactobacillus* genera play a significant role; they indeed play an important role in the maintenance of colonization resistance. These bacteria are consequently pointed as largely responsible to mediate a variety of health effects, such as regulation of intestinal homeostasis; modulation of local and systemic immune responses; repression of procarcinogenic enzymatic activities; promotion of bioconversion of dietary compounds into bioactive healthy molecules; carbohydrate transporters; and interference with the ability of pathogenic bacteria to infect intestinal mucosa, through numerous proposed mechanisms (**Table 1**). Turpin *et al.* (2010) proposed a most comprehensive leistering of benefits, viz.: synthesizing and enhancing bioavailability of nutrients; reduction of symptoms of lactose intolerance; positive influence on urogenital flora following antibiotic and radiation therapies, yeast infections, and vaginitis; prevention and reduction of intestinal tract infections induced by bacteria, virus, *Candida enteritis*, and *Helicobacter pylori*; improved regulation of gut motility (by reducing constipation and irritable bowel syndrome); lower incidence of diarrheal diseases (antibiotic-associated, and caused by *Clostridium difficile* and rotaviruses); promoting maintenance of mucosal integrity; enhancing immune system; reducing risk to certain cancers (e.g., colon cancer); prevention of osteoporosis; regulation of inflammatory conditions, such as inflammatory bowel disease (Crohn's disease and ulcerative colitis); reduction (and even elimination) of small bowel bacterial overgrowth; relieving urinary tract infections; inactivating enterotoxins; preventing dental caries; controlling cardiovascular diseases (by reduction of cholesterol levels and blood pressure); and modulation of insulin resistance and sensitivity.

The mechanisms followed by probiotics when exerting their effects are still largely unknown, but may involve modifying gut pH, antagonizing pathogens via production of antimicrobial compounds, competing for pathogen binding and receptor sites, as well as for available nutrients and growth factors, stimulating immunomodulatory cells, producing lactase, and improving the intestinal barrier.

Table 1 Potential and established health effects of probiotic bacteria

Target health benefit	*Probiotic genus/species*		*Postulated mechanism*
Aid in lactose malabsorption	*Bifidobacterium*	*breve* *infantis* *longum*	Bacterial lactase-mediated hydrolysis of lactose
	Lactobacillus	*acidophilus* *casei* *rhamnosus*	
Prevention of diarrheal diseases (antibiotic-associated, acute, and traveler's diarrhea)	*Lactobacillus*	*rhamnosus* *reuteri* *acidophilus*	Compete with pathogens for nutrients and receptors, induce hydrolysis of toxins and receptors, and regulate intestinal permeability by modulating the epithelial tight junctions
	Saccharomyces	*boulardii*	
	Bifidobacterium	*bifidum*	
Antimicrobial activity	*Lactobacillus*	*rhamnosus acidophilus paracasei* *casei* *plantarum* *reuteri*	Decrease luminal pH, secrete antimicrobial compounds (peptides, organic acids), and block bacterial adhesion to epithelial cells
	Lactococcus	*lactis* L1A	
Anticancer effect	*Bifidobacterium*	*bifidum* *longum* *animalis*	Mutagen binding, carcinogen deactivation, inhibition of carcinogen-producing enzymes (nitroreductase, azoreductase, and β-glucuronidase), enhancement of immune response, influence on secondary bile salt concentration, increase in levels of glutathione S transferase, inhibition of proliferation of damaged cells by increasing apoptosis, and reduction of reactive oxygen species
	Lactobacillus	*rhamnosus* *acidophilus* *casei* *reuteri* *fermentum*	
Immune system modulation	*Lactobacillus*	*acidophilus* LA1 *johnsonii* *rhamnosus* *casei* Shirota	Effect on epithelial, dendritic, monocyte/macrophage, and lymphocyte cells; enhancement of secretory IgA production; and production of modulatory hormones (cytokines)
	Bifidobacterium	*bifidum* Bb12 *lactis*	
Reduction of cardiovascular conditions	*Bifidobacterium*	*animalis* *longum* BL1	Assimilation/binding of cholesterol, deconjugation of bile salts, release of peptides with antioxidative and angiotensin-converting enzyme inhibition activities, and modulation of key genes and proteins involved in hepatic cholesterol metabolism
	Lactobacillus	*rhamnosus* *casei* *acidophilus paracasei plantarum* *helveticus*	
	Enterococcus	*faecium*	
Protection against urogenital infection	*Lactobacillus*	*acidophilus* *fermentum* *casei*	Adhesion to urinary and vaginal tract cells, colonization resistance, and inhibitor production (H_2O_2, biosurfactants)
Reduction of chronic liver damage (cirrhosis, hepatic encephalopathy)	*Bifidobacterium*	*bifidum* *breve* *infantis*	Stimulation of tight junction proteins, increase mucin production, decrease of intestinal bacterium overgrowth, decrease in production of lipopolysaccharides by intestinal microbiota, inhibition of urease-producing gut flora, and balance of production between pro- and anti-inflammatory cytokines
	Lactobacillus	*rhamnosus* GG *plantarum* *casei* *acidophilus*	
	Enterococcus	*faecium*	

(*Continued*)

Table 1 Continued

Target health benefit	*Probiotic genus/species*		*Postulated mechanism*
Prevention of dental caries	*Lactobacillus*	*rhamnosus* GG *reuteri*	Production of antimicrobial substances, binding in oral cavity, stimulation of nonspecific immunity and cellular immune response, and modulation of pH and modification of oxidation reduction potential
Prevention of allergies (rhinitis and asthma) and topic diseases (eczema, atopic dermatitis)	*Bifidobacterium* *Lactobacillus*	*animalis* *lactis* *rhamnosus* GG, *sakei* *acidophilus* *casei* Shirota *paracasei* *johnsonii*	Improvement of gut-associated lymphoid tissue, attenuation of proinflammatory response, induction of release of IFN-γ and TNF-α by monocyte-derived DCs, and modulation of Th1/Th2 balance and mucosal IgA levels as well as allergen-specific B- and T-cell responses
Reduction of urinary stones	*Lactobacillus*	*animalis*	Degradation of oxalate by oxalyl-CoA decarboxylase and formyl-CoA transferase
Prevention of Crohn's disease, ulcerative colitis, and irritable bowel syndrome	*Lactobacillus*	*rhamnosus* GG *casei* Shirota	Enhancement of barrier function (increase in mucus production, enhancement of barrier integrity) and downregulation of proinflammatory agents
Reduction of pain perception	*Lactobacillus*	*paracasei* *reuteri* *acidophilus* *plantarum*	Activation of submucosal immune cell sensitizing sensory terminals, and/or regulation of tight junctions, interaction with ion channel in enteric sensory nerves, and modulation of transcription of genes implicated in pain transmission
Prevention of osteoporosis	*Lactobacillus* *Bifidobacterium*	*helveticus* *salivarius* *brevis* *infantis* *longum* *breve*	Degradation of mineral-complexing phytic acid and stimulation of calcium uptake by enterocytes

Abbreviations: DCs, dendritic cells; IFN-γ, interferon-gamma; IgA, immunoglobulin A, and TNF-α, tumor necrosis factor-alpha.

Regarding regulation of health claims focused on probiotics, the European Union (EU) decided that such claims should be approved (or vetoed) centrally through the European Food Safety Authority (EFSA) (Parma, Italy) to ensure that consumers are not misled by false, ambiguous, or misleading claims of any sort. EFSA, in turn, delivers a scientific opinion based on the dossier submitted with the claim and on the relevant Nutrition and Health Claims Regulation (1924/2006) after resorting to specialized panels. Despite the vast scientific evidence (e.g., peer-reviewed publications, human trials, metaanalyses) describing beneficial effects associated with probiotic consumption in well-designed studies, more than 300 applications pertaining to probiotics submitted under the EU Nutrition and Health Claims Regulation have received negative assessment by EFSA, and consequently no approval by European Commission. Among the most problematic rejections, one may outline refusal of the health claim dossier related to *Lactobacillus casei* Shirota (Yakult strain), *L. casei* DN-114-001 (Danone strain), and *Lactobacillus rhamnosus* GG (LGG) (Valio-licensed bacteria). Other strains rejected by the health panel, on submission to EFSA under digestive and vaginal health claims, were *L. casei* DG CNCM I-1572, *Lactobacillus crispatus* BCCM/ Gent Laboratory of Microbiology (LMG) P-17631 (ID1030, 2950), *Lactobacillus gasseri* BCCM/LMG P-17632 (ID 2956), *L. gasseri* BCCM/ LMG P-18137 (ID 2957, 2958), *Lactobacillus paracasei* CNCM I-1687 (ID 2960, 2961), *L. paracasei* CNCM I-1688 (ID 2962, 2963), *Lactobacillus plantarum* BCCM/ LMG P-17630 (ID 2966, 2967), *Lactobacillus salivarius* CNCM I-1794 (ID 2970, 2971), a blend of *Bifidobacterium animalis* ssp. *lactis* Bf-6, and *Lactobacillus johnsonii* La-1 (ACD-1) (CLbA22) (ID 4231). In addition, EFSA's Panel on Dietetic Products, Nutrition and Allergies rejected *L. casei* DG CNCM I-1572, *Saccharomyces cerevisiae* var. *boulardii*, *Propionibacterium freudenreichii* SI 41, and *P. freudenreichii* SI 26.

Most probiotic health claims have so far been rejected by EFSA on the basis that the strains involved were not adequately characterized, the cause-and-effect links have been poorly substantiated, and ambiguous experimental designs were followed (e.g., inappropriate sample size and daily dose

intake in human trials). EFSA has also criticized the scientific validity of some biomarkers used, e.g., cytokines, chemokines, and antibody titers. The fact that such dossiers were rejected should not be interpreted as lack of substantiated probiotics, nor that products containing rejected strains have not been shown to provide health benefits to humans – nor even that probiotics have failed to satisfy specific safety standards. Moreover, many studies on probiotics proceeded from academic sources with no intention of product testing, and were conducted before EU regulation. Consequently, EFSA has published in 2011 a guidance document on the scientific requirements for health claims related to gut and immune function so as to facilitate submission of applications for authorization of health claims; this guide addresses the beneficial effects and outcome measures that are acceptable for substantiation of claims in these areas. However, such guidance also leaves several important issues unaddressed, such as recognition of biomarkers for probiotic validation, requirements for study protocols and clarification of the definition of 'healthy subjects' – which still leaves a number of hurdles in preparing a dossier that meets EFSA standards. In this regard, an expert panel formed by academic scientists with a documented record in probiotic research recommended that future studies should be designed, executed, and evaluated by multidisciplinary teams consisting of microbiologists, nutritionists, statisticians, etc.; should always formulate a precise and concrete hypothesis, and appropriate goals and parameters before starting a trial; should ensure that trials have a sufficient sample size, so that they are adequately powered to reach statistically significant conclusions, either supporting or rejecting any *a priori* hypothesis – and taking into account adjustment for multiple testing (this might necessitate more than one recruitment site), appropriate duration, focus on single, primary objective, and evaluation of multiple parameters only when they are hypothesis driven.

Probiotics as Intestinal Barrier against Ingested Toxic Compounds

It is well established that diet is an important etiological factor in human carcinogenesis, as mutagenic/carcinogenic substances have been found in food – e.g., heterocyclic amines, acrylamide, and mycotoxins. However, the ability of probiotic bacteria to adhere to mucus and/or intestinal epithelial cells is one of the mechanisms aimed at protection of the host organism from pathogen invasion and noxious molecules. In this respect, it has been reported that intestinal bacteria can bind mutagenic compounds *in vitro*, and the ability of such bacteria to bind those toxic compounds correlates well with *in vivo* assays – as noted by reduced rates of intestinal toxin absorption after exposure to the bacterial strains. Some LAB may indeed act as biological barrier under normal intestinal conditions, thereby reducing the bioavailability and toxic effects of these dietary xenobiotics.

Biological Control of Intestinal Absorption of Aflatoxin (AF)

AFs are a group of toxic secondary metabolites produced in nature during growth of some fungi (*Aspergillus flavus, Aspergillus parasiticus*, and *Aspergillus nomius*) on a variety of substrates, such as grains, cereals, and dried fruits in their pre- and/or postharvest period, storage, and/or transport. As many as 20 forms of AFs and their metabolites exist; of these, AFB_1 is considered one of the most potent mycotoxins in nature. In humans, its ingestion through contaminated food has been associated with liver and kidney necrosis, and mutagenic, carcinogenic, teratogenic, immunosuppressive, and cytotoxic effects, as well as Reye's syndrome. Moreover, presence of AFB_1 goes along with the very high incidence of primary liver cancer in some countries. Various pre- and postharvest methods have been implemented to detoxify and/or inactivate AFs, which include removal of AFs when they are present in identifiable portions, or degradation by physical and/or chemical methods. However, these strategies are far from being effective in detoxifying food because they generate adverse effects on nutritional and sensory quality, exhibit poor performance and/or its implementation is expensive.

Therefore, biological methods have attracted considerable interest as alternative to reduce or minimize the toxic effects of food contaminated with AFs. Such biological methods include biodegradation of mycotoxins in food, or diet modification through improved nutritional content – either by addition of phytochemicals or by addition of agents able to taken up a degrade AFs. In this respect, recent studies have shown that select strains from *Lactobacillus, Bifidobacterium*, and *Lactoccocus* genera can remove AFs in the *in vitro*, *ex vivo*, and even *in vivo*; it appears that their antiaflatoxigenic mechanisms entail binding of AFs by some of its cell wall components, but the binding mechanisms are not well-understood yet. Lahtinen *et al.* (2004) have suggested that AF binds predominantly to polysaccharides and peptidoglycans of the bacterial cell wall (BCW), including a relevant role of teichoic acid.

AFs and probiotic bacteria

Although direct experimental evidence on the suppression of cancer in humans mediated by probiotic bacteria is still scarce, many indirect evidence is available: for instance, the *in vitro* assay conducted at University of Perugia, Perugia, Italy, demonstrated the ability of LAB obtained from various commercial dairy products to inhibit the genotoxic effect of a potent carcinogen (4-quinoline-1-oxide), but such protective effect is strain and genus dependent. Additionally, studies involving animal models have established beyond reasonable doubt an indirect relationship between probiotic microorganism consumption and cancer development. Among the underlying mechanisms, the following appears likely: (1) modulation of immune system (improved resistance to chemicals, inflammation, and other factors); (2) alteration of metabolic activities of the intestinal microflora (production of antitumorigenic and antimutagenic compounds); (3) alteration of physicochemical conditions in the colon (improved intestinal permeability, delayed or denied absorption of toxins, and improved renewal of colonocytes); (4) improvement (quantitatively and qualitatively) of the intestinal microflora, reducing the putative producers of carcinogens and cancer promoters (by improvement of the intestinal microecology as more bile acid-degrading bacteria, and fewer bacteria-producing azoreductase, nitroreductase, β-glucuronidase, and β-glucosidase); and (5) binding and/or degradation of carcinogens themselves.

The latter hypothesis is of particular relevance with regard to probiotic bacteria: the cell wall of Gram-positive bacteria

chiefly comprises a single homogeneous layer of peptidoglycan or murein (20–80 nm), composed of identical subunits of sugars and amino acids that form a mesh-like layer. Its sugar component consists of alternating residues of β-(1,4) linked, *N*-acetylglucosamine (NAG), and *N*-acetylmuramic (NAM) residues (Figure 2(a)); attached to the NAM acid is a tetrapeptide chain, which can be cross-linked to the peptide chain of another strand forming the 3D mesh-like layer (Figure 2(b)). Teichoic acids are associated with peptidoglycan through a covalent bond with the NAM acid, to the cell membrane lipids (lipoteichoic acids), or to a terminal D-alanine in the tetrapeptide cross-links between molecules of NAM (Figure 3); the said acids extend to the surface of the peptidoglycan – and contribute to provide a negative charge to the cell wall. Recall that the basic structure of teichoic acid is a linear chain of repeated units of glycerol or ribitol linked via phosphodiester bonds (Figure 3); this basic structure allows for minor changes, for example, inclusion of sugar residues or replacement of the side chain, as well as frequency of substitution in linear chain or polymer backbone. However, several bacteria produce exopolysaccharides (EPS) that play an important protective role against conditions of desiccation, toxic agents, bacteriophages, and osmotic stress, while also allowing adherence to surfaces and formation of biofilms. These EPS are present in two different forms, depending on their location: as surface polymers closely related to the cell surface,

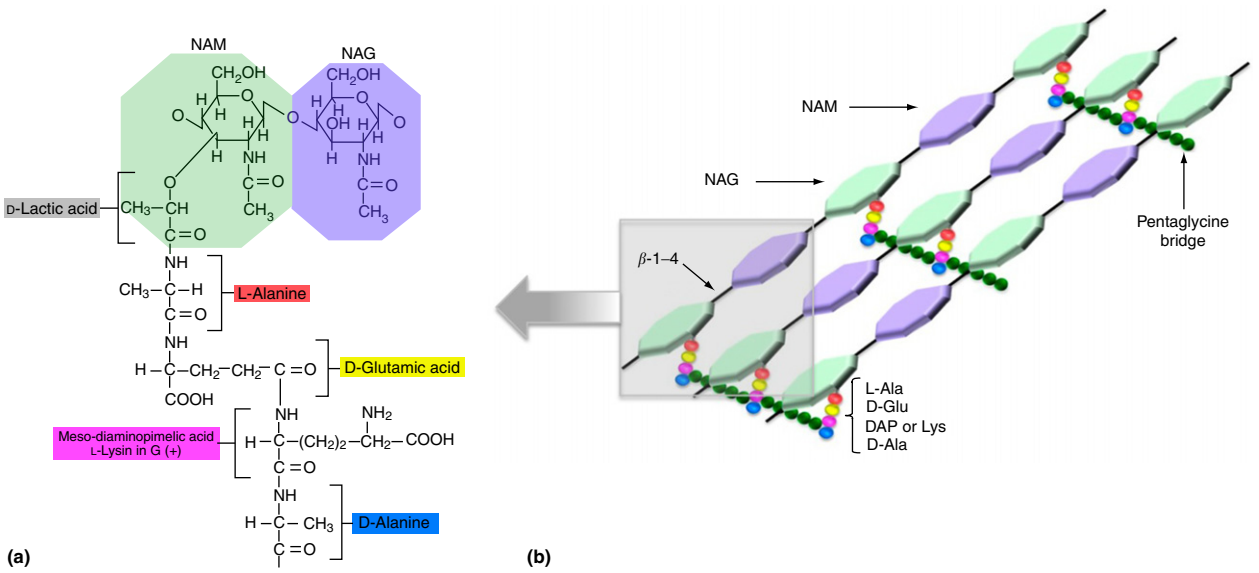

Figure 2 Peptidoglycan subunit (a) and structure (b).

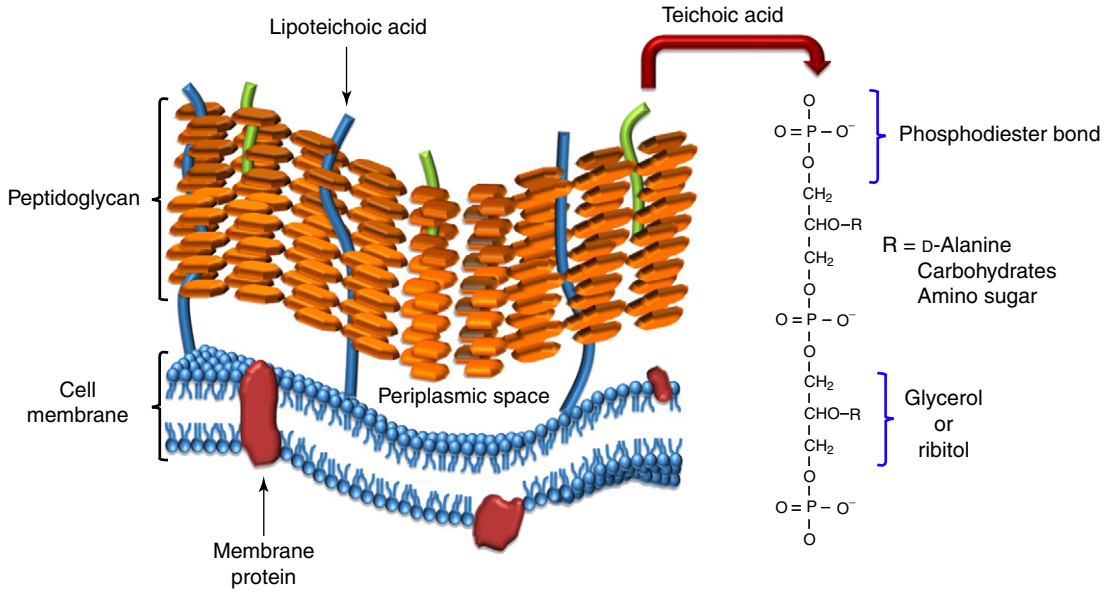

Figure 3 Typical cell wall of Gram-positive bacteria and basic structure of teichoic acids.

or viscous polymers loosely associated therewith. Information concerning the presence of polysaccharides, teichoic and lipoteichoic acids, as well as surface proteins apparently relate to the binding capacity of AFB_1 by probiotic bacteria.

Zhang and Ohta (1991) have accordingly reported that some intestinal bacteria are capable of inactivating pyrolyzed mutagens by absorption; both intact cell and cell wall fragments bound the mutagen at different extents, thus suggesting that the main components involved in binding are polysaccharides and peptidoglycan. It was also reported that purified cell walls of *Bifidobacterium longum* (SBT 2928) and four different strains of *Lactobacillus acidophilus* have a higher binding capacity of 3-amino-1,4 dimethyl-5H-pyrido [4,3-b] indole compared with the crude extracts, peptidoglycans, or cell extracts. Additionally, the mutagen-binding capacity was reduced when the cell walls were treated with compounds that degrade carbohydrates or release polymers therefrom (metaperiodate or trichloroacetic acid, respectively); conversely there was no effect when they were treated with proteolytic enzymes (trypsin or proteinase K). Two viable strains of LAB were also tested for their ability to bind such carcinogenic compounds in human diet as heterocyclic amines, benzo(a)-pirene, and AFB_1, which was influenced by pH. The results of recent studies on the ability of viable and nonviable probiotic bacteria (*Lactobacillus* and *Bifidobacterium*) to remove AFs in aqueous media, probably via the BCW or some of its components such as peptidoglycans and polysaccharides, support the hypothesis that some probiotic bacteria are able to bind specific dietary contaminants, in a process dependent on the strain used, the nature of the bacterial population and its viability, its physiological state, and pH of the medium.

The efficiency of nonviable bacteria (inactivated by heat treatment or acid) to bind AFs, indicate that toxin removal is not dependent on active metabolism; the inhibitory effects of periodate and pronase E (compound and nonspecific enzyme able to degrade carbohydrates and proteins, respectively) support that carbohydrates and proteins in the cell wall are crucial in the process of AFB_1 binding, with a major role of cell surface hydrophobicity in the case of *Bifidobacterium bifidum* BGN4. Additionally, Lahtinen *et al.* (2004) carried out experiments to establish which components of the cell envelope maybe involved in the AFB_1 binding process by LGG and evidence was found for AFB_1 binding to cell wall peptidoglycan or compounds tightly associated with it (e.g., proteins not covalently bound). Although cell wall peptidoglycans and polysaccharides appear as the two most important compounds in binding of AFB_1 by probiotic bacteria, recent evidence has shown that teichoic acids might be highly related in this phenomenon (Figure 4). The actual extent of binding of AFB_1 by LGG in the presence of divalent ions (Ca^{2+}) is low, so it suggests that the strongly cationic character of the polycation (Polymyxin B) may reduce AFB_1 binding by blocking possible interactions of AFB_1 and teichoic acids on the surface of BCWs.

However, there are variations of structure among wall teichoic acids (such as inclusion of sugar residues in the polymer backbone, or side chain substitution of the polyol residues) – and it is likely that all these structural variations convey different AFB_1 binding properties: they (partially) explain the differences in AFB_1 binding ability among bacteria. Additionally, changes

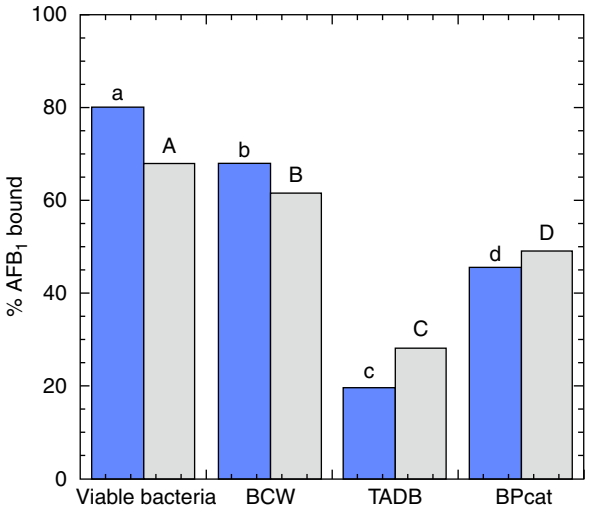

Figure 4 Percentage of AFB_1 bound by viable bacteria (*Lactobacillus reuteri* (*n*), *L. casei* Shirota (*n*)), BCW, teichoic acid-deficient bacteria (TADB), and bacteria treated with polycation (BPcat). Reaction conditions: pH 7.2, initial level of 6 μg AFB_1, exposure time of 4 h, inoculum of 1–3 × 10^{10} CFU per ml, and temperature of 37 °C. Treatments with different letters for each column are statistically different from one another for each strain ($p \leq .05$); capital letters represent statistically significant differences for *L. casei* Shirota, and small letters represent statistically significant differences for *L. reuteri*.

in surface morphology of the bacteria induced by the AFB_1 binding were determined using atom force microscopy (Figure 5). The interaction of AFB_1 with the cell wall causes conformational changes that alter the bacterial cell surface, probably via attachment to the primary polymer layer.

Prebiotics

Probiotic bacteria are highly specialized in metabolizing complex carbohydrates, being primarily carbohydrate-fermenting bacteria; the products of carbohydrate fermentation are beneficial to host health, in opposition to products released by proteolytic activity and amino acid fermentation. However, prebiotic carbohydrate may directly interact with pathogens, thus also protecting the host. The first step in pathogenesis is the interaction between pathogens and epithelial cell surface receptors – which is likely affected by the presence of prebiotics that mimic host cell receptors; some prebiotics have indeed been shown to structurally resemble the saccharide-containing glycoproteins of intestinal cells. Inhibition of bacterial adherence is strain dependent, so the effects shown by prebiotics may be due to modulation of virulence genes of the pathogen; expression of virulence genes could in turn be related to prebiotics acting as signaling molecules, or to a more global control as catabolite repression.

Prebiotics and Immune Function

Several pieces of evidence suggest that prebiotics do influence some aspects of host immunity. Supplements containing

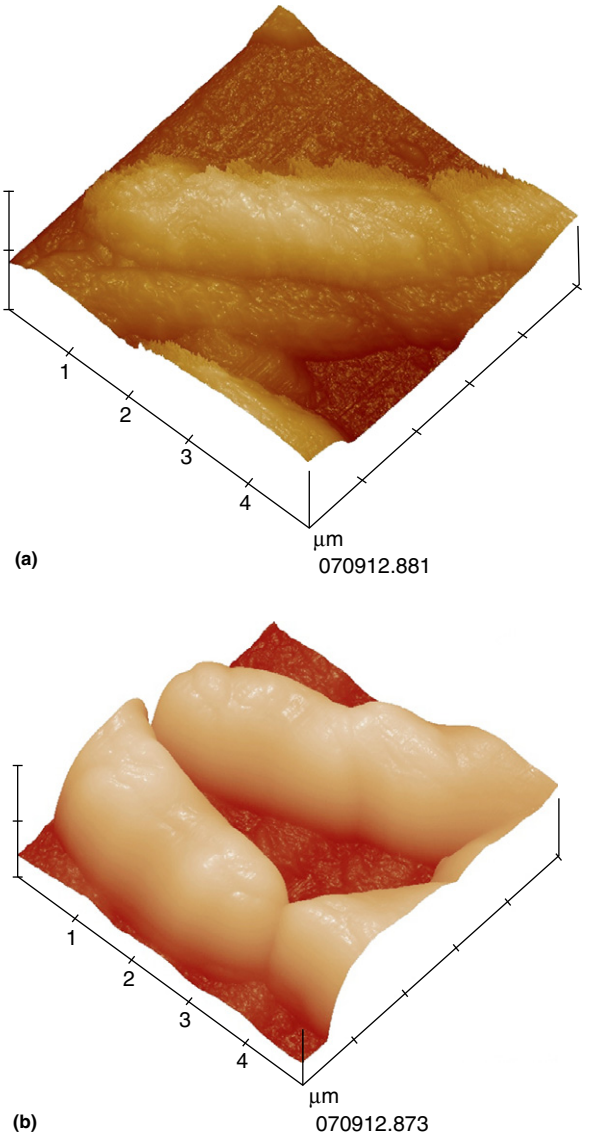

Figure 5 Atomic force micrographs, taken in taping mode (scan size 5.0 µm, scan rate 0.15 Hz), of *Lactobacillus casei* Shirota: (a) with, and (b) without, AFB_1 bound to their cell surface.

prebiotics, especially β-fructans, either alone or in combination with other components (e.g., other prebiotics, antioxidants, vitamins, minerals, and fats, as well as probiotics) were studied via ingestion by animals and humans – and resolved the effects on gut-associated lymphoid tissues (GALT), made up of the mucosa-associated lymphoid tissues of the gut and located underneath an epithelial layer and mucus layer, and the systemic immune system itself – via measurement of blood immune markers and immune cell responses. Recall that specific epithelial layer cells are responsible for antigen transport from the gut into the GALT, where antigen is processed and exposed to lymphocytes (that play an important role in the antitumor immunity and destruction of virus-infected cells, which are in turn responsible for activation and transportation to mesenteric lymph nodes (MLNs) and blood (where they are relocalized in the intestine).

Innate immune system

Studies related to GALT in innate immune system showed enhancement of cecal macrophage and granulocyte numbers and respiratory burst, as well as increase of peritoneal macrophage phagocytic activity in response to antibiotic treatment following ingestion of prebiotics. Major histocompatability complex (MHC) II molecule expression was also shown to increase in antigen-presenting cells in the MLN of rats. It appears that intake of prebiotics can improve the innate immune system of the gut, thus leading to a beneficial effect on the host's primary response to infection; however, lymphocyte natural killer cell activity was not affected by said prebiotics.

The systemic immune system has been more widely studied regarding prebiotic supplementation than GALT; however, all animal studies pointed at minimal (or none) effect of various prebiotics and prebiotic mixtures. No effect on monocyte, eosinophil, and neutrophil numbers in the blood, nor on phagocytic activity in the blood or spleen were found; as observed in GALT, MHCII expression was increased in antigen-presenting cells in the spleen and thymus of rats; and likewise prebiotic supplementation did not affect natural killer cell activity in peripheral blood, though increased natural killer activity of splenocytes, and natural killer cell-like cytotoxic function was unfolded in the spleen. Conversely, a decrease in monocyte and granulocyte phagocytosis was observed in human studies.

Adaptive immune system

As immunoglobulin A (IgA) antibodies prevent adherence of pathogens to the gut mucosa surface, IgA is used as a marker, to measure the potential effect of supplementation with prebiotics. In GALT animal studies, the animal used and its age seems determinant to enhance the prebiotic-mediated immune function: the effect is higher in younger animals, perhaps due to their gut immune system being under development, and thus more susceptible to modulation. Other studies using T cells as markers show that the effects of prebiotic supplementation on cell-mediated immunity in GALT are dependent on site of origin of cells and animal model used. In what concerns the systemic immune system, prebiotic supplementation tends to be associated with a decrease in serum antibody concentrations, a decrease in proportion of B cells in the peripheral blood, and a decrease in serum IgG_1 – yet no effect on total serum Ig exists. Once again, prebiotic supplementation causes little effect on systemic immunity, and – if present, it is a suppressive effect. As with GALT, prebiotics may alter T-cell subpopulations, but appear not to have any effect on lymphocyte proliferation in the spleen.

Unlike results reported in human studies regarding the innate immune system, experimental results regarding the adaptive immune system suggest modification by prebiotics: An increase in blood B cell numbers was indeed recorded, but no effect on serum Ig concentrations (IgA, IgG, and IgM) was achieved; an increase in fecal secretory IgA levels (correlating with enhancement of antibody response in GALT by prebiotics) was observed, but no effect on salivary secretory IgA levels. Prebiotics may also increase the response to some vaccines (or vaccine components), but different results on blood lymphocyte subsets are found depending on the site of origin of the cells.

Table 2 Selected effects of prebiotics on infections and inflammation in animal models and in humans

Type	*Effects on infections*	*Effects on inflammation*
Inulin	*Animal studies*	
	In *Salmonella typhimurium*-infected host, decrease in severity of enterocyte sloughing and epithelial damage, via decrease in ileal Na^+	Decrease in mucosal damage in several parts of the gut and decrease in myeloperoxidase activity in induced colitis
	Decrease in mortality by *S. typhimurium*	Plus probiotics, decrease in colonic inflammation after induced colitis
	Decrease in *S. typhimurium* translocation to liver and levels of serum aminotransferase	
	With calcium deficiency, increase in *Salmonella enteritidis* colonization and translocation and increase in myeloperoxidase activity in cecum and colon	
	Decrease in densities of *Candida albicans* in small intestine	
	Vanishment of mortality from *Listeria monocytogenes*	
	Decrease in *Oesophagostomum dentatum* fecal egg count, as well as total colonic intestinal worm recovery	
	Plus fiber, decrease in *O. dentatum* fecal egg count and numbers in large intestine, as well as female worm fecundity	
	Decrease in fecal egg count, number of worms recovered in large intestine, and fecundity of female *Trichuris suis*	
	Plus fiber, decrease in fecal egg count and worms of *T. suis*	
	Human studies	
	Plus probiotic *Saccharomyces boulardii*, eradication of *Helicobacter pylori* in children	Reduction in total histological and endoscopic scores, mucous exudates, and total pouchitis disease activity index in patients with ileal pouch–anal anastomosis
	Plus fiber and probiotics, decrease in duration of antibiotic therapy, and incidence of postoperative upper and lower urinary tract infections in adult patients undergoing liver transplantation	Reduction in disease activity scores and increased expression of toll-like receptor 4 on dendritic cells in lamina propria in patients with ileocolonic Crohn's disease
	Plus fibers and probiotics; decrease in duration of antibiotic therapy; and the bacterial infections in wounds, peritonitis, and pneumonia in adult patients undergoing duodenal surgery	Plus probiotics, amino acids, and vitamins, decrease in abdominal pain, distension, and constipation in irritable bowel syndrome in adults
	Plus fibers and probiotics, decrease in systemic infection rate, septic complications and inflammatory response syndrome, severe sepsis, and duration of stay in intensive care unit and under mechanical ventilation	
	Increase in calcium and magnesium absorption	
Fructooligosaccharides (FOS)	*Animal studies*	
	In *S. typhimurium*-infected host, decrease in severity of enterocyte sloughing and decrease in epithelial damage	Decrease in gut mucosa inflammation, myeloperoxidase activity, and macroscopic damage in induced colitis
	In *S. typhimurium*-infected host, increase in ileal glutamine transporters and prevention of diarrhea	Decreased disease activity index and damage to distal colon in colitis-induced animal, faster recovery from damage
	Decreased shedding of *S. typhimurium* in feces and decreased mortality thereby	Decrease in occurrence and severity of intestinal lesions, dependent on clostridial species used to induce colitis
	Decreased cecal counts of *S. enteritidis*	
	Decreased mortality by *Candida difficile*	
	Decreased densities of *C. albicans* in small intestine	
	Decreased mortality by *L. monocytogenes*	

	Decreased mortality by *Escherichia coli,* and of symptoms of diarrhea, anorexia, pyrexia, and dehydration Plus probiotics, decrease in duration of diarrhea induced by *Rhesus rotavirus*	
	Human studies Increase in lactic acid bacteria concentration, fecal content of bifidobacteria, and mucin excretion Decrease in duration of diarrhea in children aged 1–14 years with acute diarrhea Plus vitamins and minerals, decrease in median days of upper respiratory tract infections in adults aged >65 years Decrease in relapse of diarrhea, in consecutive patients suffering from *Clostridium difficile*-associated diarrhea Increase in feeling of well-being, in healthy adults traveling to countries with a high risk of travelers, diarrhea Reduction in incidence of diarrhea-associated symptoms (fever, uncomfortable bowel movements, antibiotic use, and daycare absenteeism) in nonbreastfed infants Decrease in pathogenic Clostridia and lower incidence of diarrhea, vomiting, and fever during supplementation, and decrease in number of infectious diseases requiring antibiotic treatment in healthy infants Plus probiotics, reduction in incidence of pathogenic bacteria and multiple organisms in nasogastric aspirates	Reduction in disease activity scores and increased expression of toll-like receptor 4 on dendritic cells in lamina propria
Galactooligosaccharides (GOS)	*Animal studies* Reduction in numbers of *S. typhimurium* in organs (chiefly spleen), prevention of colonization and associated pathology of *S. typhimurium* in ileal loops Decrease in prevalence of *L. monocytogenes* in intestinal samples and in liver, spleen, and mesenteric lymph nodes (MLNs)	
	Human studies Decrease in incidence and duration of travelers' diarrhea, less abdominal pain, and better assessment of overall life quality in healthy adults traveling to countries with high risk of travelers' diarrhea Plus probiotics, decrease in respiratory infection frequency in infants	
Xylooligosaccharides	*Animal studies* Increase in prevalence of *S. typhimurium* in liver, spleen, and MLNs, and higher levels of serum haptoglobin Decrease in prevalence of *L. monocytogenes* in intestinal samples, and in liver, spleen, and MLNs	
Soyaoligosaccharides (SOS)	*Animal studies* In *S. typhimurium*-infected host, increase in ileal glutamine transports and prevention of diarrhea	
Transglucosylated oligosaccharides	*Animal studies* Plus probiotics, prevention of *S. typhimurium* growth in intestine, and subsequent translocation of some bifidobacteria	

(*Continued*)

Table 2 Continued

Type	*Effects on infections*	*Effects on inflammation*
Lactulose	*Animal studies* Reduced excretion in feces of *S. enteritidis*, and calcium-decrease translocation to the systemic circulation	
FOS/inulin	*Animal studies* Increased IgG in blood and fecal IgA against *S. typhimurium* (virulent strain) and macrophage phagocytic activity	Decrease in development of colitis (in terms of gross cecal scores, inflammatory histological scores in cecum and colon, and inflammation of gut mucosa) in induced colitis Plus probiotics, decrease in bacterial translocation to MLN, colonic myeloperoxidase activity as indicator of inflammatory granulocyte infiltration, and disease activity index in induced colitis
	Human studies Plus protein, fat, carbohydrate, vitamins and *Lactobacillus paracasei*, reduction in infections of respiratory, skin, gastrointestinal, and genitourinary nature in elderly free-living adults immunized with influenza and pneumococcal vaccines Increase in intestinal permeability to infants	Decrease in fecal calprotectin as marker of intestinal inflammation and perception of abdominal pain Plus probiotic, decreases in TNF-α and interleukin 1 alpha messenger ribonucleic acid (mRNA) levels in mucosal tissue and C-reactive protein levels in blood, and in mucosal tissue mRNA levels of β-defensins upregulated in ulcerative colitis
GOS/inulin	*Human studies* Increase in intestinal permeability to infants	
SOS/inulin	*Human studies* Plus *Lactobacillus rhamnosus*, zinc, and iron, decrease in duration of diarrhea in infants with acute diarrhea, and moderate dehydration	
FOS/GOS	*Human studies* Reduction in number of infectious episodes and incidence of recurring infections in infants with parental history of atopy Reduction in episodes of diagnosed infections, respiratory tract infections, and antibiotic prescriptions Reduction in intestinal infections and respiratory infections during the first year of life in healthy infants Decrease in incidence of acute diarrhea and number of children with more than three episodes of upper respiratory tract infections per year, as well as number of children receiving more than two antibiotic courses per year	Reduction in development of atopic dermatitis

Prebiotics in Infection and Inflammation

To improve host immune defenses, prebiotics would be expected to decrease susceptibility to and/or severity of infection. Available studies provide a consistent picture of prebiotics improving host resistance, thus preventing or reducing microbial gut infections (Table 2). It should be emphasized that a prebiotic effect does not necessarily imply that unwanted microorganisms are unable to ferment the prebiotic, as they may be stimulated thereby in the presence of surrounding microbiota.

A portfolio of distinct results of prebiotics have been reported in human studies (Table 2), including effects on frequency and duration of diarrhea; incidence of upper respiratory tract infections; intestinal permeability; and skin, GI, and genitourinary infections. Mostly studies show absence of or a beneficial effect, and, in general, suggest a reduction in incidence or duration of infections; nevertheless, with regard to advantages studies of prebiotics in adults are less convincing than studies in infants and children. Synbiotics (i.e., foods containing probiotics and prebiotics) appear to exert some beneficial effects on infections of intensive care patients, who are particularly susceptible to modifications of their immune system; the prebiotic effects in what concern the bacterial gut infections depend chiefly on the prebiotic site of action and the infective site of the pathogen.

In animal models of inflammation, prebiotics (e.g., β-fructans) convey a fairly strong protective effect in models of colitis and necrotizing entercolitis, unlike allergic airway eosinophilia. Findings appear be related to the proximity of the prebiotics site of action, as it happens with infection. Inflammatory bowel conditions in human adults are also improved following prebiotic supplementation, and a synbiotic supplement increases the protection against acute pancreatitis. Maybe due to the nature of the irritable bowel syndrome regarding relapse and remission pattern, most trials have failed to report beneficial effects of prebiotics administration.

Risks and Safety Assessment

Functional foods have been increasingly supplemented with probiotics and prebiotics despite uncertainties regarding their efficacy and safety. To be accepted as probiotic- and prebiotic-supplemented food, such ingredients should undergo preliminary comprehensive studies in order to determine their potential short- and long-term benefits, and their nutritional adequacy and toxicity – so as to eventually lead to a safe product.

The minimum effective prebiotic dose of a functional food ingredient to justify a health claims is 5–10 g day^{-1}, leading to a consumer exposure of 10–20 g day^{-1} (or even more) – thus becoming a nonnegligible fraction ($>$2–4%) of the daily diet. In this context, it is interesting to analyze the approach adopted to evaluate the safety of these products, since safety of most foods relies only on assessment of its macrocomponents – instead of undergoing evaluation as done with a food additives or drugs.

A food ingredient of natural biological origin, which has been widely consumed for its nutrient properties, without known detrimental effects, which is subject only to conventional processing, and for which unknown safety hazards exist, should be regarded as generally recognized as safe (GRAS). Hence, prebiotics (e.g., inulin-type fructans and GOS) have been recognized with their GRAS status, as their history of safe use is sufficient to guarantee safety to public authorities, users, and consumers. In the case of most prebiotics, however, the data of toxicological trials are insufficient (or not fully appropriate) for a complete safety assessment; although a great number of beneficial effects (e.g., prevention of lifestyle-related diseases) and toxic studies suggest that the administration of probiotics and/or prebiotics does not raise safety concerns of any kind some evidence points at adverse effects (or less beneficial side effects) of prebiotics regarding pathogen infections – e.g., increased susceptibility to pathogen translocation; and safety and clinical effects of a given product cannot be extrapolated to others. Furthermore, published evidence show that prebiotics increase the total number of beneficial bacteria, yet their clinical benefits may be subject to debate.

A wide range of probiotics and prebiotics (alone or incorporated in food) has been made available in recent years and this calls for caution in overinterpretation of some results; there is indeed too much uncertainty to draw reliable (and general) conclusions at present. Accreditation organizations and related food committees are very careful when they recommend routine ingestion of prebiotics, owing to the lack of data on long-term effects. In general, no dangerous risks to consumer health by prebiotics have been unfolded. The GI symptoms reported by some consumers are similar to those that follow an excessive consumption of fibers – and such reactions are largely individual in nature, so each consumer may experience a personal set of tolerance limits to these products, so they can adjust consumption to their own sensitivity.

See also: Safety of Food and Beverages: Safety of Probiotics and Prebiotics

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Safety of Probiotics and Prebiotics

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Glossary

Denaturing gradient gel electrophoresis A method for separating deoxyribonucleic acid (DNA) fragments according to their mobility under increasingly denaturing conditions.
Necrotizing enterocolitis Bacterial infection in the intestine, primarily of sick or premature newborn infants.
Pancreatitis Inflammation of the pancreas.
Prebiotics A selectively fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon the host health.
Probiotics Live microorganisms, which when administered in adequate amounts, confer a health benefit on the host.
Pulsed field gel electrophoresis An electrophoretic technique in which the gel is subjected to electrical fields alternating between different angles, allowing very large DNA fragments to snake through the gel, and hence permitting efficient separation of mixtures of such large fragments.
Sepsis A systemic inflammatory response to a infection.

Introduction

Probiotics are defined by Food and Agricultural Organization of the United Nations (FAO)/World Health Organization (WHO) as "live microorganisms, which when administered in adequate amounts, confer a health benefit on the host." One of the most recent definitions of prebiotics is by Gibson *et al.* (2010) saying that prebiotics are "a selectively fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon the host health." This definition is still limited to the gastrointestinal tract; however, a possibility to widen it to also include the oral cavity, skin, and urogenital tract is discussed.

Fermentation by lactic acid bacteria (LAB), molds, and yeasts is one of the oldest methods for a natural safe preservation of foods. The genus *Lactobacillus* has played a major role in food preservation. Today most of the probiotic bacterial strains that are used belong to the genus *Bifidobacterium* and the LAB group, e.g. lactobacilli. These are bacteria that have been safely used in foods by humans for a long time and have acquired a status as 'generally recognized as safe' (GRAS). Many prebiotic carbohydrates have their origin in plants and vegetables and occur naturally in chicory root, asparagus, onions, different vegetable roots, wheat, and oats that have been consumed as part of our daily food for thousands of years without any adverse effects.

During the past three decades, the number of food products with added pro- and prebiotics has increased considerably. Also new genera, species, and strains are introduced as probiotics. A few cases of adverse effects have been documented in severely ill patients or immune compromised individuals. This puts the focus on the necessity to handle the safety aspects of pro- and prebiotics in an appropriate way, and especially when probiotics are given to potentially vulnerable persons, the safety issues should be considered with great care.

This overview article deals with two kinds of safety issues for probiotics, namely, the potential risk of adverse effects from probiotics and the possibility to improve safety in a product by the use of food-grade bacteria or probiotic bacteria. The article also addresses the risk of adverse effects of prebiotics.

Safety Concerns Linked to the Probiotic Microorganisms

General Safety of Probiotics

Today both different bacteria and yeasts are used as probiotics (Table 1). Because the most commonly used probiotic bacteria belong to the *Lactobacillus* and *Bifidobacterium* genera, most of our knowledge on the general safety of probiotics is based on the experience gained from their use. Despite the common use of probiotics, reported correlations between consumption of probiotics and adverse effects are very rare. Adverse effects have only been reported in seriously ill patients or immune compromised persons, with a very few exceptions. Bifidobacteria and lactobacilli have been isolated from persons with bacteremia; however, no case is known where commercially used probiotic strains have been identified to cause an infection in healthy humans. Furthermore, in Finland, a country where the consumption of probiotics has increased significantly during the

Table 1 Examples of microorganisms used as probiotics in food, food supplements, and feed

Microorganism used as probiotics
Genera *Lactobacillus*
Species
● *Lactobacillus acidophilus*
● *Lactobacillus bulgaricus*
● *Lactobacillus casei*
● *Lactobacillus plantarum*
● *Lactobacillus rhamnosus*
● *Lactobacillus reuteri*
Genera *Bifidobacterium*
Species
● *Bifidobacterium bifidum*
● *Bifidobacterium infantis*
● *Bifidobacterium lactis*
● *Bifidobacterium longum*
Streptococcus
Genera *Enterococcus*
● *Enterococcus faecalis*
● *Enterococcus faecium*
Saccharomyces cerevisiae *biovar.* boulardii
Propionibacterium
Genera *Bacillus*
● *Bacillus subtilis*
● *Bacillus licheniformis*
Escherichia coli

past decades, no correlation between the increased consumption of probiotics and increase in bacteremia caused by lactobacilli has been identified. The risk of *Lactobacillus* infection in healthy persons has been calculated by Bernardeau *et al.* (2006), based on the ingestion and infection frequency of lactobacilli in France, to be approximately one in 10 million individuals during a century of probiotic consumption. This means that lactobacilli are a nearly negligible risk. In a large study supported by National Institutes of Health, 12 databases were searched for studies and reviews on safety of probiotics. It resulted in 622 studies being included in the analyses. The conclusion from this study is that 'the available evidence in randomized controlled trials does not indicate an increased risk; however, rare adverse events are difficult to assess.'

Safety of Probiotics When Used in Vulnerable Human Populations

The cases identified when probiotics have caused illness have been nearly exclusively when they have been given to seriously ill persons, for example, severely immune compromised individuals, people in hospital care, and neonates. Probiotics have been shown to have a potential to significantly lower the risks of infections and the need for antibiotics and thus positively influence the chance to become healthy. However, when used for those purposes in severely ill or hospitalized individuals, a few cases where the probiotic organisms have given an infection have been identified. When probiotics are used under those circumstances, they cannot be considered as food but are used as drugs and therefore a risk–benefit calculation needs to be done. However today, no probiotics are documented or sold as drugs, but as foods or food supplements, meaning that the risk–benefit calculations are missing. If probiotics are going to be used in the future for these groups at risk, careful assessments of risks must be done.

Areas Where Safety Issues Linked to Probiotics Are Especially Important

Transmissible Antibiotic Resistance

Humans or animals exposed to probiotic strains carrying antibiotic resistance genes that might be transferred to the commensal microbiota *in vivo* are at a risk. This issue has been addressed in the European Union (EU) founded project 'Biosafety Evaluation of Probiotic Lactic Acid Bacteria for Human Consumption' and in the 'Assessment and Critical Evaluation of Antibiotic Resistance Transferability in the Food Chain' project where the antibiotic resistance character of a large number of strains was analyzed and recommendations on the determination of safety was given. The cut-off values for different antibiotics in different bacterial species were set. Antibiotic susceptibility of other strains belonging to the same species can be compared with those cut-off values to judge the possible acquired antibiotic susceptibility in a specific strain. Special focus on the transfer of antibiotic resistance genes and the presence of virulence genes has been given to the genus *Enterococcus*. The antibiotic resistance questions have also been in focus in the International Organization for Standardization (ISO) and International Dairy Federation joint Action Team on Probiotics.

Probiotics to Severely Ill Individuals

In severely ill patients, the gut function is critical and the risk of a breakdown of the gut barrier is increased. This increases the risk of sepsis, systematic inflammation, and multiorgan failure. The potential for probiotics to act beneficially in this group is large; however, the risk of adverse effect is also increased compared with healthy individuals.

A number of studies on the incidence of infection and the need for antibiotics in patients undergoing abdominal surgery have shown significantly fewer infections in patients who were given probiotics. In one study, the probiotic prophylaxis in predicted severe acute pancreatitis (PROPATRIA) study, a combination of six strains, belonging to the *Lactobacillus* and *Bifidobacterium* genera were given to patients severely ill with pancreatitis. The incidence of infection was similar in the placebo and the probiotic groups; however, the mortality rate was significantly higher in the probiotic group, 16% compared with 6% in the placebo group. The discussions on demands for better risk assessment methods when probiotics are given to the seriously ill patients raised by this study were summarized by Morrow *et al.* (2012).

A large review study by **Whelan** and **Myers (2010)** was done, according to Cochrane and Preferred Reporting Item for Systematic Reviews and Meta-Analyses (PRISMA) recommendations, to investigate the possible adverse effects of probiotics given to patients receiving nutritional support. The participants were hospitalized patients – undergoing surgery or transplantation or pancreatitis patients. A total of 53 trials

were included where 4131 patients received probiotics and 3643 placebo. According to the outcome of mortality or infection, most trials showed less mortality and less infections or no effect in the probiotic group compared with placebo. There were case reports of adverse effects for 32 patients due to infections with *Lactobacillus rhamnosus* or *Saccharomyces boulardii*. The main risk factor was the use of a central venous catheter and increased translocation.

Immune Compromised Persons

The gut microbiota is essential for the development and maintenance of the immune function, which also involves maintenance of the gut barrier function. Probiotics might give a great benefit to immune compromised persons; however, this is also a group of individuals where the risk of adverse effects, such as sepsis is large. The mechanisms behind probiotic effects on the immune system are not very well known. Some strains have been seen to stimulate the immune system and enhance the response to pathogens, whereas some other strains can decrease inflammation and allergy. Thus to achieve the desired effects on the immune system, the choice of strains is very important.

Infants

The sterile gut of a newborn baby is quickly colonized with different microorganisms, including bifidobacteria, lactobacilli, enterococci, and *Escherichia coli*, and at 1 year of age the gut microbiota is similar to the adult one. The colonization of the gut is crucial for its maturation and for the development and maintenance of the immune system. Many studies have been conducted on probiotics in healthy full-term infants with positive effects on health and no adverse effects. Still, long-term effects are not well studied and methodologies to identify infants at possible risk for adverse effects are missing. Thus there is a need for more studies on long-term effects of probiotics in term infants.

Preterm infants are often separated from their mothers and raised during their first period of life under strict hygienic conditions, with impaired enteral feeding and exposure to antibiotics. This means that the colonization of the gut is very different from that of term infants, for example, fewer species are colonizing the gut and the colonization with bifidobacteria is delayed. A risk in preterm infants is the development of necrotizing enterocolitis (NEC), which is a rare but severe consequence of an immature gut and impaired mucosal barrier. Intervention studies with probiotic strains of lactobacilli, bifidobacteria, or streptococci have been seen to reduce the incidence and severity of NEC. Neither in this study by Dani *et al.* (2002) nor in a study by Lin *et al.* (2008), altogether including 729 infants, was sepsis identified. Thus in preterm infants, the use of probiotics to diminish the risk of NEC is very promising but risk–benefit estimation needs to be done. As in term infants the long-term effects of probiotics is not known and need to be investigated.

Humans produce L(−)-lactate only and all the D(+)-lactate present in humans is produced by microorganisms or through microbial conversion of L(−)-lactate to D(+)-lactate. If large amounts of D-lactate is built up in children, it may create acidosis. This has been detected in children with short bowel syndrome and with a gut microbiota dominated by lactobacilli. Acidosis has not been detected in healthy children, but it cannot be ruled out that giving large doses of LAB producing the D(+)- isomer might be a risk. To avoid this, probiotics given to children shall produce only L(−)-lactate.

Weight Gain and Probiotics

The gut microbiota has been shown to be important for carbohydrate and fat metabolism. The microbiota differs between obese and lean persons and the major difference identified so far is that the diversity is less in obese subjects. These results together with the fact that probiotics have long been used to avoid the need for antibiotics to keep animals raised for meat production healthy, and for quick gain of weight initiated a question on whether the use of probiotics induces obesity. However, it is important to keep in mind that when young animals get probiotics they get fewer infections and grow faster because they are healthy. From this no conclusion on cause of obesity can be drawn. There are studies that have shown that probiotics might influence fat metabolism in a favorable way and reduce fat deposition. Thus today there are no data supporting the view that probiotics contributes to obesity in humans.

Safety Regulations and Probiotics

In Europe, the European Food Safety Authority (EFSA) has introduced an approach for safety assessment of bacterial strains including probiotics called 'qualified presumption of safety' (QPS). The approach was introduced in 2007 and is updated every year for new microorganisms. The safety assessment of specific taxonomic groups of microorganisms is done according to taxonomic identity of the strain, body of knowledge, possible safety concern (pathogenicity), and intended end use. If the taxonomic identity does not raise safety concerns, the taxonomic unit can be put on the QPS list and the only further safety studies needed are on antibiotic resistance. If a microorganism does not belong on the QPS list, considerable assessments of food safety are needed before it can be used in foods. The genera *Lactobacillus* and *Bifidobacterium* are all on the QPS list.

FAO/WHO introduced guidelines for the use of probiotics in foods. This includes establishing the safe use in traditional products, absence of transmissible antibiotic resistance, absence of risks with virulence properties, no production of D-lactate, no toxin production, no hemolytic properties, no side effects in human interventions, and epidemiologically known to be safe (the latter is done postlaunching). No proper instructions on how the documentation shall be done are available. Lactobacilli, bifidobacteria, and *Saccharomyces cerevisiae* belong to the organisms stated as GRAS.

Canada has recently developed guidelines for the industry called 'recommendations for the evidence requirements for efficacy, safety, and quality of natural health products containing probiotics.' In Norway, the Norwegian Scientific Committee for Food Safety has evaluated the safety of a few probiotic strains. Guidelines for the regulation of probiotics in food are to be introduced.

In China, guidelines are available since 2005 and all foods with a health claim require an application to and approval by

the State Food and Drug Administration concerning efficacy, safety, and quality.

Basic Safety Documentation for Probiotics

Based on the regulations in different countries and from the available literature, the following issues are important for a proper safety assessment of a probiotic organism:

- Proper taxonomic identification at strain level.
- The nature of the microbe (linked to taxonomy).
- Absence of transmissible antibiotic resistance genes.
- Genetic stability.
- Absence of infectivity and translocation properties.
- Absence of toxin production.
- The population for which the product is intended for must be considered and if it is intended for any vulnerable population, extended risk assessments need to be done.
- Method of administration.
- For production of the probiotic products, good manufacturing procedures need to be used to ensure the hygiene and safety standards needed.

Sequencing of the genome of a probiotic organism is a useful tool to analyze the presence of possible genes that are linked to unwanted characteristics, such as antibiotic resistance and toxin production.

Modern molecular biological methods should be used for proper taxonomic identification at species and strain levels. These include the analysis of the ribosomal ribonucleic acid subunit 16S, the use of strain-specific primers, and deoxyribonucleic acid finger printing techniques, such as ribotyping, pulsed field gel electrophoresis, and denaturing gradient gel electrophoresis.

Safety Issues Linked to Prebiotics

The target microbiota of prebiotics is dominated by the genera *Lactobacillus* and *Bifidobacterium*. It is the bifidobacteria that have been in the main focus and the possibility of these influencing the gut microbiota and increasing the number of bifidobacteria has gained much attention. The requirement for a prebiotic was more precisely described by Gibson *et al*. as a substance resistant to gastric acidity and hydrolyzes by mammalian enzymes and adsorption. It can be fermented by intestinal microflora and it selectively stimulates the growth and/or activity of intestinal bacteria associated with health and wellbeing.

Many prebiotic carbohydrates such as inulin and fructooligosaccharides (FOS) are natural constituents of foods. In the US, inulin and FOS have a status as GRAS since 1999. No severe side effects have been seen with prebiotics and the major side effect attributed to prebiotics is cause of osmotic diarrhea, rumbling, and abdominal pain due to bloating, cramping, and/or flatulence. This is because the polysaccharides and oligosaccharides are not broken down by intestinal enzymes but transported to the colon where they are fermented. Daily high doses, 40–50 g, have been seen to cause osmotic effect, whereas low doses 2.5–10 g may cause fermentation and possible flatulence. It appears that the chain length of inulin-type prebiotics influence the severity of side effect, that is, shorter chain length increases the side effect. The theory behind this is that shorter inulin molecules are metabolized primarily in the proximal colon and thus are more rapidly fermented, whereas longer chains are fermented in the distal colon.

The daily dose required to achieve a positive effect on the gut microbiota or on other health effects from prebiotics is in most studies 2.5–10 g and most products on the market today have doses of 1.5–5 g per portion. This means that the dose for a positive effect touches the dose for a mild side effect. Most probably the large majority of humans can eat this amount without side effects but if more and more products are being fortified with prebiotics, this needs additional attention (Table 2).

Table 2 Examples of carbohydrate used as prebiotics in food and feed supplements

Carbohydrates with documented prebiotic effect
Fructans
● Short chain fructooligosaccharides
● Fructooligosaccharides
● Inulin
Galactooligosaccharides
● Transgalactooligosaccharides
● Lactulose
Carbohydrates suggested to be prebiotics
Polydextrose
Glucans
Resistant starch
Pectins

Discrimination between the EFSA Health Claim Regulation and Safety Issues

When EU launched the Regulation (EC) No 1924/2006 in 2007 for health claims, the basis for the regulation was a concept of consumer protection, that is, the labeling of foods should be appropriate according to nutrients and health claims. So far no health claim for pro- or prebiotics has been approved. Van Loveren *et al.* (2012) have summarized the reason for health claim rejection and link to safety. The reason for health claim rejection is not a matter of safety of probiotic or prebiotics. The reason is instead linked to that in some cases the probiotic bacteria has not been taxonomically identified to the appropriate detailed level and before the regulations, studies on pro- and prebiotics were done more in an explorative way and not according to the current claim regulations. In 2011 and 2012 EFSA published guidelines for human intervention studies in different areas, and the application of those guidelines will facilitate studies and dossiers for approval of health claims. If a probiotic bacterium is included on the EFSA QPS list, which also most probiotics are, safety is not an issue for getting health claims. Thus the common demands for health claims and for safety judgment is that the probiotic or prebiotic used is appropriately

identified. However, if the probiotic strain is not on the QPS list, safety considerations are recommended according to the novel food regulations.

Use of Probiotic Bacteria/LAB to Increase the Safety of a Product

Use of Microorganisms for Natural Biopreservation of Foods

LAB, fungi, and molds have been used for the preservation of foods for thousands of years. The most common method of biopreservation is fermentation. The preservation effect is due to the production of organic acid, diacetyl, and hydrogen peroxide but also to specific antimicrobial compounds such as bacteriocins and antifungal peptides. Mathematical models and predictive microbiology have been used to address the importance of process factors together with acid and bacteriocin production. When the food industry now is looking for natural preservation methods, the use of LAB with its GRAS status is attractive. Bacteriocin-producing LAB have been applied to improve food quality and safety in sourdough and sausages production and in cheese production for competition, antilisteria, and anticlostridia purposes. The most studied bacteriocin is nisin for its preservative effects in foods, especially cheese. Also the potential of application within the malting and brewing process to diminish the growth of naturally occurring unwanted microorganisms has been suggested. The possibility to use bacteriocin-producing bacteria as probiotics for human and animal health to lower the risk of infection and for therapeutic purposes is an interesting possibility with a potential to lower the wide use of antibiotics. Bacteriocin production might also strengthen the competiveness of a probiotic bacteria in complex microbial communities such as those in the gut.

Binding of Toxic Compounds (Mycotoxins, Heterocyclic Aromatic Amines, and Heavy Metals)

Production of mycotoxins in foods and feeds create severe spoilage and is a threat to human health. The mycotoxins might be carcinogenic, immunotoxic, neurotoxic, nephrotoxic, or hepatotoxic. The use of LAB as natural preservatives to produce organic acids and bacteriocins to diminish the growth of the unwanted microflora as well as the capacity of LAB to bind mycotoxins is an interesting natural preservation method. One of the most common mycotoxin is aflatoxin, which is produced by *Aspergillus* strains of fungi. The ability to bind aflatoxin is strain dependent, and binding of toxic substances has been seen both in *in vitro* and *in vivo* situations. Different examples on the potential for probiotics to bind and detoxify mycotoxins have been described by Amalaradjou and Buhnia (2012) and Salminen *et al.* (2010), for example, different probiotic strains have been seen to bind and reduce the bioavailability of different forms of aflatoxin, including the most potent one aflatoxin B1, ochratoxin A, fusarium toxins, and patulin. Studies have shown that the mechanism behind removing of mycotoxins most probably is binding of the toxins and not a metabolic process since, for example, both dead and live probiotics have the ability to remove the toxins. Similarly, strains of *L. rhamnosus, Propionibacterium*, and *S. cerevisiae* have been shown to bind aflatoxin B1 also in the chicken or ruminant gastrointestinal tract and thus have the potential to be used as a feed additive to reduce the bioavailability of aflatoxin. Only a few studies have been performed in humans and a study in China showed that feeding a dietary supplement with specific strains of probiotics reduces the urinary excretion of aflatoxin B1. It should be noted that the binding capacity seems to be strain specific.

The production of toxins by cyanobacteria in aquatic and marine environments and specifically in drinking water is a risk of poisoning. The possibility to remove microcystin toxins produced by cyanobacteria in drinking water has been investigated using various lactobacilli and bifidobacteria separately or in combination with promising results.

When meat is cooked at 150–300 °C heterocyclic aromatic amines (HAA) are formed. These compounds have a high mutagenic potential and might contribute to gastrointestinal cancer. The possibility to utilize probiotic bacteria to eliminate theses mutagenic substances is interesting and investigations by Nowak and Libudzisz have shown *in vitro* that the probiotic bacteria *Lactobacillus casei* DN-114001 reduces the amount of three different HAA compounds by 27–99%, indicating that the toxins are metabolized.

The use of different microorganisms to bind metal cations has been studied for waste management. This could be expanded to foods by the use of food-grade microorganisms. The potential to use probiotic bacteria in this area for the decontamination of foods and the intestine from heavy metals have been investigated and the ability to bind, for example, lead and cadmium, has been shown to be dependent on the strain and pH.

See also: Public Health Measures: Assessment of Novel Foods and Ingredients. Safety of Food and Beverages: Probiotics and Prebiotics; Safety Consideration in Developing Functional Foods

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SAFETY OF FOOD AND BEVERAGES

Safety of Irradiated Foods

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Glossary

Codex Alimentarius International Standards for food (codes, practices, guidelines, etc.) as adopted by the Codex Alimentarius Commission in which more than 160 states are represented; this Commission works under the Joint FAO/WHO Food Standards Program.

Dose (radiation absorbed dose) The amount of energy at a point of interest in a volume of matter containing a given amount of mass, expressed as the quotient of energy imparted divided by the mass contained; SI units are Joule per kilogram (J/kg^{-1}) or Gray (Gy). Note: dose is usually expressed in kGy (kilogray).

Free radical An atom or molecule with an unpaired electron; usually highly reactive and lasting – in particular in humid media as food – only for extremely short periods of time.

***G*-value** The yield of chemical changes in an irradiated substance, expressed as the number of specified chemical changes per amount of energy imparted; traditionally given as 1/100 eV, in SI units as ne per Joule (1/Jo).

Polyploidy Occurrence of cells containing more than the normal number of chromosomes; for humans the normal is 46. Occasionally, double, triple, or multiple sets are found.

Radiolysis Chemical change caused by ionizing radiation, typically the cleavage of molecules.

Radiolytic product A substance produced in the process of radiolysis, i.e., any substance submitted to ionizing irradiation, including food.

Wholesomeness The safety of food for consumption. This has to be considered for food processed by ionizing radiation, and covers aspects such as radiological safety, toxicological safety, microbiological safety, as well as nutritional adequacy.

Introduction

Food irradiation is the best studied food technology ever. More than 60 years of research is well documented, and during this period the sensitivity of analytical methods has increased tremendously. Meanwhile, new and more sensitive analytical methods have evolved, which have added new insight. Experiences gained while researching into food irradiation have added to the fundamental knowledge about food, food processing, nutrition, food chemistry, and engineering.

A number of national expert panels have confirmed this conclusion. On top of all, a Joint FAO/IAEA/WHO Expert Committee on Food Irradiation (JECFI) and a Joint FAO/IAEA/WHO Study Group on High-Dose Irradiation (JSGHDI) have validated this technology and confirmed that it is wholesome. Furthermore, the World Health Organization (WHO) has recommended its use and complained that unfounded resistance to introduce this technology negatively affects those countries and regions where the benefits are most needed. The arguments against food irradiation and their scientific background have been evaluated and refuted by many expert groups; however, opponents and activists against food irradiation still rely on these long refuted considerations. Of course, it is well documented that in political disputes about new technologies the negative has more success.

It is fair to say that nearly all the arguments against food irradiation voiced by advocacy groups have a sound scientific background. However, all these considerations and arguments have been carefully checked and finally refuted based on sound science.

It should be understood that this short article on the safety of irradiated food cannot substitute a full scientific review of this issue; the entries below may be taken as headlines or query terms for further interest in this topic.

Definition of Wholesomeness

The terminology of 'wholesomeness' was first introduced as a legal conception by the US FDA in 1957:

> "The term wholesome means sound, healthful, clean, and otherwise fit for human food."

Another terminology which is also easier to translate into foreign languages is 'safety for consumption.' With regard to food processed by ionizing radiation, the JECFI in 1980 listed a number of further aspects to be specifically discussed: radiological safety, toxicological safety, microbiological safety, and nutritional adequacy.

Role of Overall Average Dose

The terminology of 'overall average dose' was introduced by JECFI in 1980 to provide a tool to estimate the total chemical turnover induced by a given radiation absorbed dose (short dose) in order to allow a more generalized estimate of chemical changes even in situations where a full and detailed analytical verification would not be possible. As the main chemical changes are well-known and characterized by the respective *G*-values (number of molecules changed per amount of energy imparted, usually measured in eV) of the individual chemical compounds making up the food item under study and as the full spectrum of the chemical composition of such food is also known, a calculation of the final changes can be made and the results can be confirmed by chemical analysis. There are a number of hypothetical chemical changes induced by ionizing radiation, but as the main transformations are well known, this amount can be subtracted and the remaining unknown amount can be estimated. Thus, using overall average dose can help to estimate which risk might originate from this small amount of compounds assuming the highest possible specific toxicological effect of these compounds. And, conversely, assuming the maximum tolerable concentration of these hypothetical compounds, the standard approach of toxicology can also be used to extrapolate the tolerable intake by a consumer for any substance under scrutiny. Having arrived at such value, the maximum tolerable overall average dose can be derived with the help of the summarized *G*-values for the food under consideration. This concept also allows for the estimation of expected chemical changes when large quantities of food are irradiated, not all receiving the identical dose or being irradiated under varying situations or at different times. It is very important to understand this approach by JECFI in order to make a reasonable use of their findings and conclusions.

The above considerations are valid for the reason that chemical transition in the dose range applicable for the food treatment is linearly proportional to the dose. Consequently, summing-up overall components is allowable, and a summary *G*-value can be derived and multiplied by the grand average of the dose in order to estimate the total turnover. More detailed considerations are not needed here, and the interested reader can find such information in the respective text books.

General Aspects of Chemical Changes

Processing food by ionizing radiation is a physical process imparting energy; but the final purpose is to induce chemical changes by many other processing methods like cooking, salting, pickling, etc. The advantage of ionizing radiation is that it does not heat the food and, therefore, does nost change the identity of the food; a dose of 10 kGy will heat water only by 2.5 °C! In other words, a raw meat will remain raw, a fresh fish will remain fresh, a fresh fruit will remain fresh, and a deep-frozen product will remain deep-frozen. In most – nearly all – applications of radiation processing of food, the target chemical is a rupture of the DNA molecules thus disabling the biological activities in a living plant, microorganism, or animal cell. This is achieved by direct hits cleaving the molecule or by indirect action of free radicals mainly formed in water. Of course, the components of the DNA molecules remain and their overall composition is not affected. However, any molecule hit by ionizing radiation will undergo changes characterized by their respective *G*-value; and there are molecules, for example, unsaturated fatty acids with their double bonds, which are particularly sensitive, possibly resulting in rancidity. For such reasons it is essential in several applications to design the irradiation process and treatment in a way as to minimize such undesirable changes, for example, by irradiation in the deep-frozen state.

Particular Chemical Changes

In judging the toxicological implications of the chemical changes to be expected, great interest has been paid to the possible existence of unique radiolytic products (URPs). The idea had been that there must be cleavage molecules which cannot be produced by other techniques of food processing. Their detection would also help to screen for irradiated foods in the marketplace that are not labeled appropriately. With one single exception, such URPs have not been found; the exception is the class of 2-alkylcyclobutanones (2-ACBs), a group of compounds derived from the fatty acids making up the fat moiety. Because of their particular chemical structure, some toxic properties had to be expected and a number of studies in model systems have been conducted. Finally, the conclusion was that these compounds have some toxicological potential but no consequence for the human consumption of irradiated food containing fat. However, with an enormous increase in the sensitivity of analytical methods, the existence of these URPs meanwhile has become a standard method for the detection and verification of irradiated food in the marketplace.

Biological Changes

As most applications of food irradiation aim at some biological effect, it was quite natural to question whether such changes would have any effect on the consumer. The changes in the DNA are not relevant to the consumer as the DNA does not contribute to nutrition. Another effect is the change of cell permeability, which might be used to increase the yield in juice production; but again, this has no nutritional consequence. Microorganisms are killed, but the remaining chemical compounds have no implication on nutrition. Ripening of fruits can be delayed or accelerated but this again has no nutritional consequence; however, the treated fruits might become less palatable, for example, by softening of the tissue. There are a number of microorganisms less sensitive to ionizing radiation, and it might be quite difficult to eliminate their spores. This latter effect is not different from other processes; for example, some microorganisms are quite tolerant to heat treatment, and the process must be designed appropriately to achieve the desired effect and the microbiological safety of the final product. Furthermore, it is virtually impossible to eliminate viruses by irradiation; the necessary dose will be much higher than needed for sterilization. Parasites

and insects that occur in food are quite sensitive to irradiation; they however, are not contributing to nutrition. There is a vast literature available on the biological effects of ionizing radiation, and reviews and textbooks give a lot of information for the practical exploitation of the irradiation process.

Radiological Safety

The question whether irradiated food may become radioactive is a very sensitive concern of the general public and laypersons. First of all, it must be understood that any object, including food, is already radioactive: it is the primordial radioactivity that originates from the creation of our world. For example, if we consider potassium, there is always the isotope K-40, which is naturally radioactive. If we consider carbon – a main chemical constituent of our food – it always contains some C-14, which is created by cosmic radiation in the world's atmosphere. Hence, the question of radioactivity induced by the radiation processing of food must be considered in this context. To avoid any measurable induced radioactivity, the number of permitted radiation sources has been limited to gamma-rays from cobalt-60 or cesium-137, to electrons up to 10 MeV particle energy, and X-rays generated from converting electrons with up to 5 MeV (USA up to 7.5 MeV) from machine sources. In other words, any kind of radiation known to induce radioactivity – for example, neutrons – are not permissible. However, there are also several isotopes of the elements contained in food that may be activated theoretically, even by the energies of the permitted radiation types. However, such isotopes as indium-119 are very rare in food and are not activated to any measurable extent, and, therefore, will not contribute to any measurable increase in the natural radioactivity of our food. In the early days, national authorities were required to measure the radioactivity of all irradiated food, but this requirement is no longer maintained according to the scientific evidence that no radioactivity is induced.

Toxicological Safety

No doubt, there are radiolytic changes in any irradiated food, which are partially responsible for the beneficial effects in food irradiation. Most of the radiolytic effects only change the relative composition of the chemical spectrum contained in food. This was confirmed by a number of experiments including microbial test systems, feeding tests on a wide variety of animals, and even studies with human volunteers. None of these studies revealed any negative effect. This is also true for the 2-ACBs (see Particular Chemical Changes). These particular compounds have a toxicological potential. However, because of the extremely low concentration of those components in irradiated fat-containing food, it has not been found to be relevant. From the wealth of experimental results and the conclusions reached by national and international expert bodies, it must be accepted that irradiated food is safe to consume. This is valid without any restriction on the maximum tolerable dose, without any discussion of overall average dose, as long as the irradiated food remains palatable and maintains its technological properties. This conclusion has already been incorporated by Codex Alimentarius in its International Standard on Irradiated Food, but has not yet been implemented worldwide for national regulations.

Microbiological Safety

From the beginning of the research into food irradiation, there have been concerns that irradiation of food could cause genetic changes in microorganisms and by repeated irradiation microorganisms might become resistant to ionizing radiation as already proven for chemical treatments. However, none of the many experimental studies rendered any evidence for such suspicion. It is also true that all species of microorganisms have a different sensitivity to irradiation; the same is true for heating and other treatments. Consequently, radiation processing will inevitably change the relative spectrum of microorganisms present, except in the case of total sterilization, which can also be achieved by irradiation. The relevant question, consequently, is whether such relative change can favor the outgrowth of hazardous microorganisms, thus creating a risk for the consumer. No experiment hitherto has confirmed such suspicion. Furthermore, it has been confirmed that any irradiated food when undergoing spoilage will still have sensory warning signals for the consumer, particularly smell and appearance. There is a rule that irradiated food needs the same care for handling in the kitchen as food processed by any other technology. Another question that has been asked this whether some mutation of irradiated microorganisms would not only create microorganisms more resistant to ionizing radiation but also create more hazardous microorganisms producing more toxic substances. There have been scientific publications on such effects; however, the irradiated microorganisms needed to be cultivated in a protected laboratory environment and shielded from the competition of the normal microbial populations in our food. In such laboratory studies, it has been shown that the mutated microorganisms did produce more toxins per surviving microorganism; however, such mutated microorganisms would never survive under conditions of the real food environment.

Nutritional Adequacy

In the initial JECFI conclusions of 1980 it was already, pointed out for consideration that certain groups or populations living on a diet restricted in variation and composition would encounter nutritional problems if all their food would be irradiated without exception. However, it is quite unlikely that, for example, the boat people in Thailand would receive all their food irradiated. It is also quite unlikely that any other special group would encounter nutritional deficiencies if some of their typical food has been processed by ionizing radiation. It has further been shown that any permission of radiation processing would not have any consequence on the supply of nutritionally appropriate food to the consumers. It is true that some nutrients are affected by ionizing radiation and may be even totally depleted at extremely high doses; in such cases other food consumed will compensate for such deficiencies.

In extreme situations, as for astronauts, their radiation-sterilized food will be carefully controlled for the sufficient supply of all indispensable nutrients, and occasionally even supplements are added to compensate for possible deficiencies.

Expert Evaluations of Wholesomeness

All these concerns and suspicions about the wholesomeness of irradiated food have been considered by national and international expert committees. Without validating the personal expertise of the members of the various committees, it must be convincing that all the committees came to the conclusion that irradiated food is safe to consume. Of course, there have been studies reporting negative effects; however, where put in context, it has been proven that these results could not be substantiated in subsequent experiments by independent expert and research groups. Other reported negative effects have been shown to have no relevance in the real situation of human nutrition. In any field of science, there will always be some dissent by researchers in minority. However, for judging the situation and arriving at legal consequences for regulations or bans, it is indispensable to hear the results from the mainstream of science. This vote is clear: there is no argument based on sound science against the general accepting of radiation processing for irradiated food. Based on this argument, in 2003, Codex Alimentarius has accepted food irradiation without any limitation to the kind of food or any restriction to maximum permissible dose.

Concerns about Food Irradiation

Opponents to food irradiation and advocacy groups have voiced a range of arguments, which are not related to the wholesomeness of irradiated food: the nuclear industry wants to get rid of their waste; interest the process is mainly from the military; the necessity of huge transportation vehicles carrying radioactive materials and the risk of a spill on the roads; workers in irradiation facilities are not sufficiently protected; and radioactive emissions from irradiation facilities are much higher than those of nuclear power plants. This variety of arguments illustrates the emotional and ideological basis of this reasoning. In fact, such technically based arguments can best be refuted by the truth: food irradiation is not related to nuclear waste, the interest is not mainly from the military (and what would be the argument?), there are no huge transports of radioactive materials for the large number of irradiation facilities world wide (irradiating huge volumes of nonfood items), workers in such facilities are sufficiently protected, and there are no radioactive emissions from irradiation plants. Having looked into such concerns and the arguments to resolve them it becomes evident how difficult it would be to pass arguments against the wide-spread skepticism into the wholesomeness of irradiated food.

There are a number of concerns that have, at least, some scientific foundation and justification: foods may become devitalized, denatured, and dead; spoiled food may be cosmetically converted to make it marketable, thus convincing the consumer; natural goodness of food is destroyed; food may be over-irradiated and become harmful; a dose high enough to kill microorganisms, insects, plants and animals, and even humans cannot be healthy when applied to food intended for human consumption; irradiated food can become re-contaminated undermining the purpose of the treatment; bacterial toxins already formed will not be eliminated; and consumers might place too much trust too the effect of the treatment to limit spoilage. The arguments needed to answer the concerns need some basic understanding of human nutrition. There is no evidence that certain vital forces may be ingested with our food; nutrition relies exclusively on the components of which food is made up, i.e., vitamins, minerals, amino acids, proteins, starch, and so on. Consumption of food such as a raw potato is not nutritious as the raw starch is indigestible and only after cooking and consequent denaturation does it becomes digestible. There is no chance to revert spoilage with the help of radiation processing: a spoiled fish will remain stinky, a rotten potato will remain rotten, and so on; the consumer will be warned by the symptoms of spoilage not to consume such items the same way as with the unirradiated food. Natural goodness of food is assumed, particularly for products of organic farming, and most regulations do not permit radiation processing for such products; however, there is no argument based on sound science for such conclusion. On the contrary, processing by ionizing radiation would be the best method to be combined with organic and sustainable agricultural production. It does not change the identity, quality, and nature of the product, and it could be used to avoid the application of any chemical treatment.

It must also be considered that a large number of experimental studies have been published that report a detrimental or even noxious effect of radiation processing of food. All these publications have been considered and validated by national and international expert panels. All these experiments have been repeated by other groups of scientists in order to verify the published findings. In many of these follow-up studies, the experimental parameters also have been varied in order to render a better insight into the underlying principles. All this wealth of accumulated scientific knowledge has led to the final conclusion that irradiated food is safe to consume. However, for the layperson it would remain very difficult to verify these conclusions without knowing and understanding the wealth of the available publications.

The hypothesis of the existence of radiotoxins has been postulated, in order to explain the specific chemical effects induced by ionizing radiation. However, all experiments to verify such theory failed. The other common argument is that free radicals are introduced, which is true. However, in a system containing water in abundance, i.e., in food, such free radicals have a very short lifespan and disappear immediately by recombination or by reaction with other compounds of the food. These latter effects have been extensively analyzed in the studies reported above. The evidence is, even from feeding studies with rather dry substances as irradiated milk powder, that no harmful effects could be found. As expected, the free radicals contained (and proven by electron spin resonance (ESR) analysis) immediately decay on contact with the saliva

of the test animals; and if not, they are quenched away by the aqueous chemistry of the digestive system.

An Example: The Polyploidy Issue

A frequently cited study to prove that irradiated food may be unsafe to consume is an Indian experiment on malnourished children in a hospital; the diet to foster recovery contained freshly irradiated wheat. First of all, it is not known what the increased-rate of polyploidy could mean; in other words, the biological significance of polyploidy is not known; what is known for sure is that a certain spontaneous rate of polyploidy is a part of life. Polyploidy means the occurrence of cells that do not have the normal number of chromosomes (for humans 46), but the duplicate or even multiple sets (i.e., 92, 136, 184, or even more). To do a valid evaluation on polyploidy, a sufficiently large number of cells must be examined and counted under the microscope. There are a number of technical deficiencies in the Indian study even thought it is published and peer-reviewed. It is not only the low number of slides counted, but also the lack of the full information about material and methods applied. For example: how was the freshly irradiated wheat integrated in the diet? Whole wheat, baked, or cooked? It is evident that the children did not consume raw grains. There are sufficient reports that other parameters may influence the occurrence of polyploidy cells as even the circadian rhythm of food intake may have an effect. National and international experts have evaluated the technical details of this study and concluded that the experimental design was essentially unsuitable to draw any conclusion and that there were even founded doubts on the statistical validity of the results. A basic observation, easy to understand even by a layperson: a group of 15 malnourished children in custody of the hospital had been chosen; three subgroups with five children each were formed, (1) normal hospital diet for recovery, (2) this diet with inclusion of unirradiated wheat, and (3) a diet containing the irradiated wheat (0.75 kGy). An experimental plan with such small treatment groups can never be sufficient to draw any statistical conclusion. And even more puzzling, the authors appear to be unable to produce in their reference group of children the expected standard incidence of polyploidy of approximately 0.8% (normal) or 1.8% (slightly above normal), but instead report an absolute zero. What was the underlying fundamental error in these experiments? Furthermore, the number of cells counted for each child was rather low (500), and data for all the five children of a group were pooled in the report. Pooling of data is only acceptable after a check of the statistical quality, i.e., answering the question whether the observations belong to the same group of data. Consequently it was found that a single child within one group having an increased polyploidy would contaminate the data and no statistical verification is possible. There was no health evaluation reported and one child might have been affected by inflammation of an inner organ which is also known to increase polyploidy.

With the poor information given by the authors, nobody would be able to repeat and confirm their experiments and results. Furthermore, there would be ethical limitations to execute such experiments on children (volunteers?) in a critical state of malnutrition. For such reasons, model systems, in particular test animals under malnutrition conditions, have been used in a number of experiments. None of these studies could reveal an incidence of polyploidy caused by the irradiated feed.

Reference to this issue of polyploidy is made here, as the respective publications have been used aggressively at many instances by opponents to food irradiation and advocacy groups. Never an answer was given to the scientific doubts into these studies. Several follow-up publications on the initial Indian study have been published by opponents, still insisting on the enhancement of polyploidy, but none giving any answers to the basic doubts in these studies.

In summary, the scientist/expert must pay respect to the concerns voiced by laypersons; however, there is still a need to translate the results from the mainstream of science into information understandable by ordinary people. This is also important with regard to food irradiation as the regulators essentially are laypersons.

Regulations and Wholesomeness

By common consent it is agreed that governments are obliged to protect their people. Particularly in relation to food, it is generally assumed that consumers are unable to judge the quality of their food. This is also a consequence of the industrial production of food, where the applied treatments and the used ingredients and additives are not obvious from the appearance of the product. For such reasons, also, the food sector is governed by a wide range of regulations. At the beginning of the twentieth century, no regulations of food irradiation existed, which for some countries implied "what is not regulated is not permitted," and for other countries "not regulated means not forbidden". During the first five decades of scientific work, some concerns were raised that food processed by ionizing radiation might impose some hazards with its consumptions; scientific research commenced into the wholesomeness of irradiated food. In 1957, the world's first commercial irradiation facility for food (spices) was put in operation in Germany (the situation: not regulated means not forbidden); however, already in 1958 a general ban of food irradiation was introduced in Germany as a precautionary measure with the reasoning that not enough was known about this new technology. However, the USA, for example, took the other approach, and beginning in the early 1960s permitted certain commodities for certain treatments at specified doses. But even here, the uncertainty about the wholesomeness of irradiated food led to the cancellation of a number of the early clearances. This situation only changed with the emergence of JECFI, which initially recommended even conditional and which in 1980 declared any kind of food to be wholesome up to a treatment of 10 kGy overall average dose. This finding was adopted in 1983 by Codex Alimentarius in its General Standard on Irradiated Food.

Despite the fact that after this decision irradiated food had to be considered safe for consumption and, consequently, no further protection of the consumer needed to be implemented, very few governments implemented the full Standard

into national regulation. Instead, a number of countries began to permit radiation processing of clearly defined food categories or even subgroups from a category and introduced dose limits far below the set value of 10 kGy. Of course, such restrictions were never based on considerations of wholesomeness. However, the US approach to issue clearances on the basis of received petitions allowed the industry to introduce food irradiation on a commercial scale and to bring such products on the market. This approach allowed the US regulators to re-evaluate the wholesomeness aspect any time a new petition was submitted. Petitioned by the industry, most dose values were set below the 10 kGy as no higher dose was needed for the respective application. And again in the USA, the 30 kGy maximum dose for spices was derived from wholesomeness considerations: as spice is a minute component of food ingested and considering the original definition of overall average dose. The conclusion was that in this particular case the ingestion of an extremely low concentration of hypothetically harmful radiolytic products would not pose any health risk. The exact value of 30 kGy was derived the following way: the minimum dose needed to eliminate harmful microorganisms from this dry product was assumed at 8 kGy; as common irradiation facilities would be able to provide a dose range between maximum and minimum of less than 3, the maximum dose for the intended treatment would be calculated to be 24 kGy; and rounding this value ended at a regulated 30 kGy.

The European Union (EU) in 1999 regulated food irradiation compulsory for all the member states using the terminology of overall average dose; only a single commodity was listed at a dose value of 10 kGy. The member states were permitted to maintain any clearance that was in place before this general regulation. In other words, the EU-regulation is not governed by consideration of wholesomeness, otherwise there would be no justification for any differentiation by country.

The 10 kGy overall average dose of JECFI and Codex Alimentarius did not provide for radiation sterilization of food, which requires doses up to or above 50 kGy. Such applications were used in South Africa for rations of out door enthusiasts and in USA for their astronauts. This created the desire to arrive at a verdict on the general wholesomeness of radiation sterilized food, and both countries – through their membership to, the International Consultative Group on Food Irradiation (ICGFI), were among the sponsors of the JSGHI, in 1997. Both governments now have the confirmation that their decisions are based on the assured wholesomeness of the radiation-sterilized products.

Conclusion

Irradiated food is wholesome, this means safe to consume regardless of kind of food and dose applied. This is even valid for the highest doses as long as the food remains palatable i.e., of high quality to enjoy its consumption, and as long as the technological properties, for example, the thickening effect of starch ingredients is maintained. There is a wealth of scientific publications on this topic, and mainstream science confirms again and again these conclusions. Any adverse or negative finding has been refuted by substantiated arguments and scientific evidence.

See also: Food Technologies: Food Irradiation

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SAFETY OF FOOD AND BEVERAGES

Safety of Genetically Modified Foods

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Glossary

Arthropods Any invertebrate of the phylum Arthropoda, having a segmented body, jointed limbs, and usually a chitinous shell that undergoes moltings, including the insects, spiders and other arachnids, crustaceans, and myriapods.
Biofuel Fuel derived from organic matter (obtained directly from plants, or indirectly from agricultural, commercial, domestic, and/or industrial wastes) instead of from fossil products.
Bioremediation Degradation of organic contaminants (such as chemicals, heavy metals, and oil) in the soil or water, by the action of cultured microorganisms selected for their ability to metabolize the specific contaminants.
Endotoxin A toxin, usually heat resistant, present in the intact bacterial cell but not in cell-free filtrates of cultures of intact bacteria.
Eutrophication The process by which a body of water becomes enriched in dissolved nutrients (as phosphates) that stimulate the growth of aquatic plant life usually resulting in the depletion of dissolved oxygen.
Lignocelluloses A combination of lignin and cellulose that strengthens woody plant cells (biomass).
Monoculture The cultivation of a single crop on a farm or in a region or country; monoculture crops do not provide a rich habitat for other flora and fauna and inhibit diversity.
Nucleator An ice nucleus is a particle, which acts as the nucleus for the formation of an ice crystal.
Prion Protein particle similar to a virus but lacking nucleic acid, thought to be the infectious agent responsible for scrapie, variant Creutzfeldt-Jakob disease (vCJD), and certain other degenerative diseases of the nervous system.
Sporulation Formation of spores, usually very resistant to heat and dessication.
Supercooling Supercooling is the process of chilling a liquid below its freezing point, without it becoming solid.
Superweeds A wild plant that has been accidentally pollinated by a genetically modified plant and contains that plant's abilities to resist herbicides and insects.
Transgenic Organism containing genes altered by insertion of DNA from an unrelated organism, and to have that trait expressed in the offspring.
vCJD A variant of Creutzfeldt–Jakob disease caused by the prion associated with bovine spongiform encephalopathy and contracted by consuming infected beef or beef products, which are invariably fatal.

Introduction

Genetic modification of plant and animals can occur spontaneously as part of the evolutionary process. However, selective breeding of plants and animals in agriculture allowed small human nomadic group to settle down and eventual form larger urban societies with more opportunities for diversification of employment activities and the improvement of life. In other words, human control of plant and animal genes gave rise to civilization as we know it in the present day. In the past few centuries, agriculture has benefitted from improvements in crop and animal varieties, which was able to keep up with the food demands of an ever-increasing human population. These benefits were most apparent when climate was suitable, infestation was minimal, and crop spoilage lose was controlled; however, the cycle of feast and famine continued to occur well into the nineteenth and twentieth centuries, particularly in developing regions. As the world faced limitations of arable land and fresh water and other challenges, there was a motivation for research to improve productivity by modifying plants by introducing changes in the DNA. This became feasible once the genome was better understood and tools to manipulate the DNA structure were developed in the latter half of the twentieth century.

The commercial use of genetically modified crops in agriculture began in1990s by a few multinational corporations based in the United States (US) that possessed this new technology. Genetically modified plants (GM plants) were generated in a laboratory by altering their genetic makeup to introduce desired qualities. This was usually done by either inserting or deleting genes from a plant's genome by genetic engineering. These new organisms with recombinant DNA are generally referred to as genetically modified organisms (GMOs). In 1994, the first commercial GM product was introduced in the US. The *Flavr Savr* tomato had a deleted gene, which caused postharvest fruit softening, and thus the genetically modified (GM) tomato had a longer shelf life. However, it was taken off the market shortly afterwards because it could not compete with conventionally bred tomatoes with long shelf lives and better flavor. In 1996, GM maize

(corn) and GM soybeans were approved and planted in the US, and thereafter processed foods containing GMOs started to appear in retail stores in the US and Canada. The genetically derived advantage of most GM crops, such as soybean, corn, rapeseed, and cotton, is the ability to be resistant to specific herbicides or certain plant pests and pathogens.

The Use of GM Technology in Agriculture and Foods

Canola (Canadian oil, low acid) for human consumption was developed in the early 1970s in Manitoba through conventional plant breeding from rapeseed to distinguish it from natural rapeseed oil, which has much higher erucic acid content. By 1998, a more disease- and drought-resistant variety was developed through genetic engineering. In the present day, Canola is produced widely in Canada, the US, and other countries, and it is generally recognized as safe by the United States Food and Drug Administration (USFDA), and in 2013 was permitted in infant formulas with Canola oil at levels up to 31% of the total fat blend.

Other widely consumed GM products are corn and soybeans from GM crops. The herbicide glyphosate inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase, which is present in plants, fungi, and bacteria but not in animals. This enzyme is a key step in the formation of hormones through production of aromatic amino acids. The use of the broad-spectrum herbicide became much more widespread with the development of Roundup® ready (GM) soybeans and maize, which contained the glyphosate-*N*-acetyltransferase gene. With the application of the herbicide, these GM crops would not be affected, but certain weeds would be killed. In the present day, different commercial glyphosate products are able to control more than 100 broadleaf and grass varieties of weeds. Toxicological studies showed that even though new metabolites are formed in the edible parts of the GM crops that were not observed in conventional crops, the Joint FAO/WHO Meeting on Pesticide Residues concluded there was no human health concerns for the short- or long-term consumption of these commodities or their products. The number of approved GM crops worldwide is expected to increase from 30 in 2009 to 110 by 2015. Even in Europe where concern by the population to GM foods is greatest, approximately 30 million tons of GM crops are imported each year and many varieties of herbicide-resistant maize are now allowed to be grown in the European Union (EU). However, glyphosate is only slowly degraded by soil microorganisms and may pose a risk of water contamination. In addition, resistance of some species of weeds to the herbicide is a growing concern.

Another successful application of GM technology is the insertion of a gene for the biological pesticide produced by *Bacillus thuringiensis* (Bt), a close relative of the very common soil and dust bacterium *Bacillus cereus.* In 1901, Bt was first observed in a colony of sick or dying silkworms in Japan. The main difference between Bt and *B. cereus* is that Bt produces an endotoxin that kills lepidoptera. This is accomplished by the protein toxin, which occurs as a parasporal body ('crystal') in the bacterium during sporulation. The insect gut proteases activates the toxin proteins, allowing them to bind to receptors, and affect the midgut cells by forming pores in the larval digestive tract (hemocoel). These pores allow naturally occurring enteric bacteria to enter the hemocoel, where they multiply and cause sepsis The Bt toxin in the form of spray-dried wettable powder of the Bt culture became commercially available in the 1950s and was used extensively in Canada in a spray over wide areas of forests infested by spruce budworm and gypsy moth. In forestry, however, by the mid-1980s, Bt strains had virtually replaced the major chemical pesticides for spruce budworm and gypsy moth control in Ontario, Quebec, and the Atlantic provinces. Since then, various modifications have been made to target certain insects, mainly destructive caterpillars. However, for food and forage crops, its use has been more limited, mainly targeted against cabbage worms, tomato hornworm, European corn borer, alfalfa caterpillar, and alfalfa webworm. Bt can be applied through overhead irrigation systems or as granules. Available data suggest that spores may remain in soil from months to years under field conditions, but little is known about the longevity of the toxin in soil or water.

Two isolates of this genus are highly active against insects of great economic importance; Bt subsp. *kurstaki* attacks lepidopterous insects and Bt subsp. *israelensis* kills mosquitoes and black flies. The Bt *kurstaki* strain is the one used most frequently as a spray to control caterpillars on vegetables. Bt insecticides are the only bacterial insecticides in widespread use, and one advantage they have is that they neither target pollinators, like bees, nor predators or parasites of the pests of concern. In 2012, the European Food Safety Authority conducted a risk assessment on the Bt *kurstaki* strain and concluded that the health risk to mammals, reptiles, amphibians, birds, algae, and nonlepidoptera terrestrial arthropods, and probably soil microorganisms is low. From a GMO perspective, Bt maize is a variant of maize, genetically altered by inserting the gene for Bt toxin into the maize genome to kill European maize borer and more recently the maize ear worm and root worm. Unlike Bt, transgenic plants like corn do not release the Bt toxin. Instead, the cell must be digested by the insect in order to release the active ingredient in the gut. This is an improvement on the sprayed Bt because it is not susceptible to degradation by sunlight or washed away by rain. Most sprayed formulations are less effective over time, perhaps a few days or weeks after application, unlike the GM version, which is effective for the life of the plant. One risk however, is that continual exposure of insects to the GM derived Bt may confer resistance to insect predation.

Although insects are capable of developing high levels of resistance under laboratory experiments, this has not been observed to any great extent where crops have been sprayed. Now it is generally agreed that 'high dose/refuge strategy' is the most promising and practical approach to prolong the effectiveness of Bt toxins. This requires toxin free host plants as refuges near insecticidal crops, and toxin doses intended to be sufficiently high to kill insects. After more than a decade because of initial commercialization of Bt crops, most target pest populations remain susceptible, but field-evolved resistance has been documented in some populations of three noctuid moth species feeding on Bt maize in Puerto Rico and South Africa and in Bt cotton in the southeastern US. Field outcomes are consistent with predictions from theory, suggesting that

factors delaying resistance include recessive inheritance of resistance, abundant refuges of nonBt host plants, and two-toxin Bt crops deployed separately from one-toxin Bt crops. The use of Bt crops is popular worldwide with more than 32 million hectares in cultivation, including Bt cotton and Bt potatoes. Even some countries with concerns about GM foods in general, such as in the EU, permit the use of Bt transgenic crops, and it is likely their use will expand in the future. Other GMOs permitted in the US and some other countries include cotton resistant to the herbicide bromoxynil; delayed ripening tomatoes; squash, zucchini, and papaya modified to resist viruses (80% of Hawaiian papaya is genetically engineered because there is still no conventional or organic method to control ringspot virus). Sugar beets that are glyphosate resistant have been approved in Australia, Canada, Colombia, EU, Japan, Korea, Mexico, New Zealand, Philippines, Russian Federation, Singapore, and the US.

The potential of this technology can also be used to enhance nutrition such as vitamin production; a good example of this is 'golden rice', a GM variety of *Oryza sativa* rice, which produces beta-carotene, a precursor of vitamin A, in the edible parts of rice, produced in 2000. Golden rice was created by transforming rice with two beta-carotene biosynthesis genes: Phytoene synthase from a daffodil and *crtI* from an *Erwinia* species, and actually is golden in color, quite distinct from nonGM rice. The reason for the research was to plant this variety in regions, such as in Africa and India, where thousands of children die each year from a lack of vitamin A. In 2005, a newer variety producing much more beta-carotene was developed, but unfortunately neither the original nor the newer version is yet grown for human consumption. The GM crop approach for vitamin A fortification is seen by many as a less expensive and more practical alternative to vitamin supplements or a change in diet to greater consumption of vegetables and animal products.

The usual concerns expressed about GM crops have also been raised in regard to golden rice: spread of GM genes into the environment; loss of local varieties and biodiversity; opening the door to more controversial GMOs; obscene profits made by multinational companies from those who can least afford the cost of the seed; and vitamin A could be derived from other food sources. Other opponents have argued that adults and children would have to eat inordinate amounts of golden rice to see any benefit. However, recent trials showed that golden rice delivered dietary vitamin A as good as supplements and better than the natural beta-carotene in spinach. To permit widespread use, GM companies have now agreed that farmers could obtain the seed and replant it free of charge, unless they made more than USD$10 000 a year from the crop. Field trials have been conducted, and it is hoped that golden rice will meet the regulatory conditions for its production and be on the market in 2015.

Another beneficial application of GM technology is vaccine production and delivery through GM plants. Selected DNA from hepatitis B and cholera viruses injected into banana saplings might allow the plant to produce antigenic proteins without any infectivity component. Consumption of these bananas (and some other modified vegetables like potatoes and carrots) would build up antibodies in the consumer to fight these diseases in a similar way to injecting or ingesting traditional vaccine. This may be a more efficient and less costly way of vaccinating large populations against specific diseases.

GM research with plants will accelerate in the future, and some of the outcomes may prove to be both economically and environmentally acceptable to governments and the public. Some plants and trees could be engineered for capturing large amounts of carbon, which would be sequestered in roots and stems. Perennial grasses like switchgrass and *Miscanthus* may have the best immediate potential because of their extensive root systems. Other examples are GM trees to grow faster; yield better wood, say for construction and for biofuel; resist pest invasion and extreme climatic conditions; and even detect biological attacks by developing trees that change color when exposed to biological or chemical contamination. However, environmental concerns will prevent any large scale adoption of these, particularly as pollen released from trees is uncontrollable over large areas.

GM Microorganisms

One of the first scientists to select bacteria for desired attributes was Louis Pasteur who helped to develop pure cultures for wine and beer production that are still used in the present day. Where the desired product is only produced naturally by microorganisms, difficulty in growing the bacteria on an industrial scale have resulted in low yields of the product. With today's technology the specific gene for the product can be inserted into another microorganism (a bacterium, yeast, or fungus) that is easier to culture. Thus, these GM microorganisms, sometimes called genetically engineered microorganisms, have been used for many years to produce vitamins, enzymes, food additives, and processing agents for the food industry. These include vitamins B_2 and C, xanthan thickeners, regulators of citric acid acidity, amino acids, such as glutamate and enzymes, used in the production of cheeses, baked goods, fermented beverages, and glucose. Because the final product is highly purified, like a specific enzyme, there is no altered DNA or other substances to be concerned about in the product. Because the GM organisms are contained in the fermentation vessel, there is also no release into the environment, and thus, regulatory oversight is less stringent.

However, there are relatively few examples where a GM microorganism has been approved for use in the environment including agriculture. In the agriculture area, some viruses have been genetically modified to increase their toxicity to the host, such as baculovirus containing a gene for scorpion toxin against caterpillars. *Lactococcus lactis* is a bacterium widely used for years safely in the dairy industry and is a potential vector for delivering vaccine antigens through the human mucosa. However, despite much work in this area no commercial human vaccine has yet been produced from this organism. Before their release into the environment, GM microorganisms have to be shown to be both effective and safe. These essential attributes include:

1. ability to survive and multiply in the ecosystem into which they are introduced;
2. stability of the new genetic material and low potential for this material to transfer horizontally to indigenous organisms;

3. ability of the GM microorganisms to function as designed under the different environmental conditions expected; and,
4. lack of adverse impact on the broader ecosystem where they are introduced.

The Gram-negative bacterium *Clavibacter xyli* ssp. *cynodontis* (Cxc) is a common endophyte living in the xylem of Bermudagrass (*Cynodon dactylon*) and also in a few weed species and can be made to colonize maize, rice, sorghum, oats, and white millet through artificial inoculation. The gene coding for the CrylA(c) protein from Bt ssp *kurstaki* is inserted into the chromosome of a wild-type Cxc. Maize plants inoculated with recombinant Cxc; and artificially infested with larvae sustained up to 80% less damage than plants inoculated with either wild-type Cxc or uninoculated plants. In agricultural research experiments, such bacteria have been incorporated into soil to facilitate crop growth by fixing nitrogen and applied directly onto crops to kill pests. The release of transgenic resistant to pests and pathogens could increase selection pressure on those organisms and increase the likelihood of them becoming resistant, thus rendering the GM microbial pesticides ineffective. One possible example could be Bt toxin genes introduced into a *P. fluorescens,* a common colonizer of maize plants and into several species of nitrogen-fixing *Rhizobium*. The Bt genes produce toxins against various insect pests including caterpillars, mosquitoes, and beetles.

Another application for GM microorganisms is prevention of loss of crops. Water does not necessarily freeze at 0 °C and may be supercooled in a liquid state unless there is an ice nucleator to form the ice. Ice-nucleating bacteria perform this function, which is beneficial to avoid their own intracellular freezing damage. Because these bacteria are present on crops, they can result in plant cellular frost damage. Therefore, research has been done on the development of GM microbial ice-minus bacteria (a modified *Pseudomonas syringae,* commonly found in vegetation) that can delay ice formation and death of the plants during freezing temperatures. Ice-minus technology is designed to depress the critical temperature at which frost damage begins by displacing the natural population of ice-nucleating organisms with the modified *P. syringae,* which lacks the genes for the ice-nucleating protein. These bacteria are sprayed across the leaves and other plant parts so that ice will not form unless the frost is more severe. However, after some small trials on strawberries and tree fruits, there has been no government approved initiative to apply this technology to regions that can be affected by frost. Because climate change is creating extreme weather events, seasonal damage by cold may become more frequent in areas where this has been a rare phenomenon in the past. This may have benefitted tart cherry and apple farmers in Michigan in 2012 if it had been used because an unseasonably warm March brought blossoms out early to be hit by a late frost in April. The cherry crop was almost nonexistent, and the apple crop loss was the largest since the 1940s.

In recent years, more GM microorganisms have been developed for biotechnological processes that require introduction of the modified organisms into the environment in substantial numbers. For instance, GM microorganisms have the potential for bioremediation applications in soil, groundwater, and activated sludge environments and degrading organophosphate and carbamate pesticides. A modified *Pseudomonas fluorescens* has been used in field trials, where its bacterial hemoglobin can impact polluted sites, where oxygen availability limits the growth of aerobic bioremediating bacteria. If these are effective and safe, the impact could be more land being turned over to agricultural use. Another example of a GM microorganism with gene deletions beneficial to agriculture is that used to control crown gall disease occurring in several plants caused by the soil-borne bacterium *Agrobacterium tumefaciens*. *Agrobacterium radiobacter* produces an antibiotic against the disease and is often used as a control agent. However, prolonged use of this control agent results in transfer of the plasmid that contains both the antibiotic and resistance genes. The GM microorganism *A. radiobacter* was unable to transfer this plasmid to *A. tumefaciens*is and thus became the first GM microorganism to be used in a commercially available pesticide in the 1990s.

GM microorganisms need to be tested against a range of possible targets and released only after their specificity has been established. Many of the early examples of GM microorganisms released into the environment had genes deleted from their genome, such as the ice-minus and *A. radiobacter* examples discussed above. This makes them apparently less risky than those with added genes and possibly safer than the naturally occurring microorganisms they replace. Nevertheless, each GM microorganism should be assessed according to its own particular properties. Appropriate risk assessments should be carried out before agreeing to the widespread release of these DNA-modified microorganisms. International input should be sought because even if these have been shown to be safe in one country or region, they may have a deleterious effect in a different ecosystem in another country. Unfortunately, it is almost impossible to predict long-term effects of introducing a new species into the environment. At the same time, there are many examples of introduced plants, insects, and animals, some accidentally and some deliberately, where these have gone very wrong and whole ecosystems have deteriorated. Thus, there can be both promotion of reckless introduction of GM microorganisms by researchers and industry and also overt rejection of any GMOs by consumers groups, usually with the government in the middle trying to balance the need for new technologies guided by the precautionary principle in the recognition that any release may be irreversible.

Another issue is the intentional misuse of GM microorganisms for criminal purposes, such as creating synthetic pathogens, or enhancing their virulence. Although most governments have eschewed research into 'germ warfare', the technology in the present day is available to small laboratories to generate bioterrorism weapons. Fortunately, the delivery system for mass infection is more difficult to establish, but small targeted attacks could be achievable by one or a few scientists, as seems to have been the case with the *Bacillus anthracis* attacks in 2001 where the agent was delivered by mail. In the agricultural area, a modified pathogen affecting large areas of crops could be economically devastating.

GM Animals

GM animals for food have also been developed, although none is currently in the market. In September 2010, the USFDA stated it was willing to consider approval of GM salmon, which grow twice as fast as native species because of the insertion of a gene that produces a continuously released growth hormone. GM Atlantic salmon have an added gene from the ocean pout that acts as an 'on switch' to produce the growth hormone year-round. This, therefore, shortens the time it takes to grow a mature salmon, which has the same flavor, texture, color, and odor as a regular salmon. This rapid growth should make salmon more plentiful and cheaper for the public. There are concerns, however, that GM fish might escape from growing pens and then breed with native fish, possibly causing catastrophic effects on the life cycle of wild fish, especially as GM salmon may out-compete the wild salmon population for limited food sources. If the USFDA approves the sale of the salmon, it will be the first time the government has allowed modified animals to be marketed for human consumption, and under existing guidelines, the fish would not have to be labeled as genetically modified. However, the environmental concerns would still need to be addressed.

There are other applications of GM technology to animals that are in development or close to approval. A GM pig, called enviropig, has been genetically modified in Ontario to be able to metabolize plant phosphorus more efficiently and consequently excrete less polluting phosphorus (up to 70% less) in its feces. Pig manure is high in phytate, a form of phosphorus, so when farmers use the manure as fertilizer, phosphates enter the watershed and cause eutrophication of the water by algae blooms that deplete oxygen and kill the aquatic life. The enviropig has an enzyme that allows breakdown of phytate without release of phosphorus. A transgene construct composed of the promoter segment of the murine parotid secretory protein gene and the *Escherichia coli* phytase gene is introduced into a pig embryo. Although the approval process in Canada was going ahead, funding ceased and the project is currently halted.

Another environmentally positive GM approach is to reduce flatulence from cows. During digestion of grasses, bacteria in the cow produce methane, which is a major greenhouse gas. Various research approaches in Australia, Canada, and the US are being taken to reduce release of methane in cattle by removing microorganisms that produce methane in the gut or rumen or that produce metabolic products other than methane, or modifying the grass the cattle feed on. Other applications being developed include GM cattle that produce more fat-free milk and increased amounts of casein for better cheese production.

Products Derived from GMOs

Products derived by GM microorganisms have been in use for many years. Because the final products are often highly purified, no residual DNA is present. Thus, the main issue is the specifications for the GM product. In the case of hepatitis B vaccine, the GM derived product was purer, safer, and cheaper than the original vaccine derived from human blood. As mentioned earlier, many food ingredients and processing aids, like enzymes, are produced by GM technology and have been evaluated for safety by national food safety authorities as well as by the Joint FAO/WHO Expert Committee on Food Additives. Because they are produced in closed systems, environmental concerns are minimal.

Perhaps the most infamous GM product is bovine somatotropin hormone (BST), which is a protein hormone naturally found in the pituitary gland of cattle and which is produced in cows after giving birth to initiate lactation. If BST is injected into nonlactating cow, milk production is induced without the need for a long gestation period. It can also be administered to lactating cows to significantly increasing milk production (10–25%). In the last few decades, a genetically modified source of BST has been developed in the US. Through recombinant DNA technology, a genetically modified *E. coli* bacterium is used to produce large amounts of the hormone, which is called rBST (recombinant bovine somatotropin hormone). The US is the only developed nation to permit cows to be given rBST and approximately 20% of herds use the hormone. Although the EU does not permit the use of rBST, milk produced using the technology may be imported and sold without labeling. There are various arguments against rBST use including uncertainty about its safety to consumers over a lifetime, economic reasons (too much milk may lower the price, and consumer reluctance to purchase such rBST milk or products made with the milk), and animal health reasons (increased risk of clinical mastitis, reduced fertility, and increased risk of lameness). The Codex Alimentarius Commission has not been able to achieve a consensus on the approval of rBST, especially as Canada and the EU have not approved its use for animal health reasons.

There has been recent interest in the use of GM microorganisms for the ethanol industry. Lignocellulosic producing plants such as switchgrass, straw, and various wood chips are cheap ingredients to make ethanol and reduce the biofuel industry's reliance on maize and other crops. Much work has been expended on developing GM microorganisms that convert the pentosans derived from the celluloses to ethanol. GM microorganisms that can convert xylose and other pentose sugars along with cellulosics-derived sugar constituents to ethanol would greatly improve the efficiency of such production and lower costs. Modified *Saccharomyces cerevisiae*, *Zymomonas mobilis*, *E. coli*, and *Klebsiella oxytoca* have been the most successful GM microorganisms so far, but much work has yet to be done before these lignocellulosic products can be cheaply and efficiently converted to fuel.

Medical advances may be made possible in the near future through the use of GM animals. For instance, GM hens lay eggs that have miR24, a molecule with potential for treating malignant melanoma and arthritis, and also human interferon b-1a, an antiviral drug. GM goats have been raised to produce a constituent of human milk as well as antithrombin for people whose blood needs thinning. Antithrombin, normally harvested from human plasma, is used in patients preceding surgery to stop blood clotting but carries a risk for blood-borne diseases. Other GM goats are being engineered with a gene from a spider for dragline silk to produce silk protein in their milk; this silk milk would be transformed in web-like

'biosteel' material. Another example is GM cows that produce large amounts of albumin in their milk, which can be used to treat burn patients and aid those with liver and kidney diseases.

Positions of Proponents and Critics

In the present day, GM crops and foods are a multibillion dollar industry that is controlled by the companies that produce them. Farmers who wish to plant GM seeds must pay a premium so that those who invested in the research and develop can recoup their costs and make a profit in the same way as drug companies or any research intensive business. Some potential customers feel this is a monopoly by large multinational corporations and that prices of seed and other GM products will always remain artificially high. This is particularly a concern in developing countries where the value of GM crops may be the greatest, especially if there is drought, flooding and extremes of temperatures that are exacerbated by climate change. However, many of these countries, such as Zambia in Africa, refused shipments of whole kernel GM maize because of concerns that farmers would grow the seed and jeopardize future trade with the EU.

GMOs in agriculture became a contentious issue when first shipments of GM maize and soybeans were shipped from North America to Europe. The advantages claimed by GM use proponents are: (1) more effective control of destructive insects through targeted pest control and less resistance by pests to sprayed insecticides or those delivered through irrigation; (2) much less use of actual chemical pesticides with less chance of indiscriminate dispersion in the environment including wind drift and water runoff, and destruction of beneficial insects; and (3) reduced tillage with subsequent soil erosion required to remove weeds. However, these are advantages that accrue to the farmer and not to the consumer. Consequently, the distribution of the risks and benefits is an issue in risk communication.

One of the most used GM products is the glyphosate-based herbicide (e.g., Roundup®) against weeds where farmers do not need to hoe or remove weeds mechanically or use traditional herbicides. However, there is a fear that superweeds resistant to this herbicide will eventually emerge, making crop yields less in the future. There are also concerns that pollen from GM crops will fertilize nonGM species, or seeds from GM plants will spread uncontrolled GM genes into the environment, sometimes called 'genetic contamination' or 'genetic pollution' and also lessen diversity in crops. GM crops are meant to be isolated from crops grown in nonGM areas, and if pollen transmission is limited as with maize, this is feasible. But this would be impossible if GM trees were developed, for example, to be resistant to spruce budworm and withstand drought as airborne pollen transmission by tree is often measured in kilometers.

The development and sale of GM foods has been challenged on safety, economic, and environmental grounds. Evaluation of GM foods for human food safety requires a demonstration of substantive equivalence in nutrition compared to its unmodified counterpart. To decide if a modified product is substantially equivalent, the product is tested by the manufacturer for unexpected changes in a limited set of components, such as toxins, nutrients, or allergens, that are present in the unmodified food. The manufacturer's data are then assessed by the appropriate regulatory agency. That data, along with data on the genetic modification itself and resulting proteins (or lack of protein), are submitted to regulators. If regulators determine that the submitted data show no significant difference between the modified and unmodified products, then the regulators will generally not require further food safety testing. However, if the product has no natural equivalent, or shows significant differences from the unmodified food, or for other reasons that regulators may have (for instance, if a gene produces a protein that had not been a food component before), the regulators may require that further safety testing to be carried out. In addition, special studies are required to demonstrate that the GM food contains no new proteins (or increased level of a known protein) capable of causing allergenic reactions. A standard safety test may consist of the following steps:

1. Analysis of the chemical composition of the relevant plant parts, measuring nutrients, antinutrients (those that interfere with the absorption of nutrients) as well as any natural toxins or known allergens;
2. Assess the risk of gene transfer from the food to microorganisms in the human gastrointestinal system;
3. Study the possibility that any new components in the food might be allergens;
4. Estimate how much of a normal diet the GM food would comprise;
5. Estimate any toxicological or nutritional problems revealed by this data in light of data on equivalent foods; and
6. Additional animal toxicity tests if there is the possibility that the food might pose a risk.

In this regard, two GM products failed initial safety testing because of possible allergic reactions and were discontinued. To date, no adverse health effects caused by GM products approved for sale have been documented. However, environmental concerns and labeling remain major issues for marketing GM foods.

Opponents of GM foods are demanding more rigorous environmental testing before any approvals are granted. Although public knowledge of GMO technology and GM foods is generally low, consumer acceptance of the technology is highly variable. In Canada, China, and the US, many GM foods are on the market and no special labeling is required. However, the EU, Japan, Malaysia, Australia, and other countries have taken a cautious approach to GM food and have established labeling and traceability requirements for GMOs and their derived food products. These requirements dictate a physical separation of GMOs and nonGMOs throughout the whole supply chain. At the production and raw commodity level, this requires a certification scheme; this becomes more complicated for GM ingredients, especially those which are otherwise indistinguishable from their nonGM counterparts, such a vegetable oils. In some cases, conventionally grown crops and food made from them may contain a small amount of GMOs due to cross-pollination. Current analytical methods are capable of detecting approximately 1% of GM food in a mixture, and this is used as the *de facto*

limit in most legislation. Therefore, if a product is found to contain more than 1% GMO, it is considered to be mislabeled, and subject to regulatory action, even if it poses no safety concern. Another potential problem of labeling GM food is that it could, in principle, be applied at the foodservice level. As with the term 'organic', some businesses may try to use 'nonGM' as a marketing advantage and thus further contribute to so far scientifically unfounded fears of consumers to GM foods.

The US follows a scientific, risk-based assessment appealing to the concept of substantial equivalence, and the notion that zero risk in food safety regulation is not practical, given that conventional foods are already presumed to be safe. In contrast, the EU follows a more precautionary approach to risk management of GMOs and has abandoned the concept of substantial equivalence. In 1999, the European Council formalized a moratorium on GMO approval by recommending to the European Commission an amendment to the existing regulations. The provisions of the recommendation were for the EU to take a thoroughly precautionary approach to future approval of GM crops, and that GM crops should not be placed on the market until it could be demonstrated that there is no adverse impact on human health and the environment, and that principles regarding traceability and labeling be applied. A revised regulatory framework was subsequently approved by the European Parliament and European Council in 2003. Critics of the EU's adoption of the precautionary principle argue that it has become less of an approach for risk management and more of a tool for lobby groups to influence the regulatory process, undermining the role of science in that process. The EU has already proven its willingness to reject World Trade Organization (WTO) decisions where there is scientific uncertainty regarding the potential health effects of a product and strong public support for the continued moratorium (as occurred in the case of bovine growth hormones (BGHs), where the EU has accepted the imposition of countervailing sanctions against some of its exports to maintain the moratorium on BGHs).

It has been argued that in drought-prone developing countries, especially in areas of Africa, the use of GM crops with a tolerance to limited rainfall could be the only means to stave off famine and malnutrition for millions of people. However, even there GM crops and products have become a contentious issue. In 2002, many countries in eastern and southern subSaharan Africa experienced their worst food crisis in a decade affecting 15 million persons. The governments of Malawi, Mozambique, Zambia, and Zimbabwe rejected United State food aid because of concerns over the inclusion of GM maize. GMO advocates in the US, Canada, Australia, and Argentina (all major GM food exporters), publically stated that these governments were allowing millions of their countrymen to starve because of unfounded fears of health or other concerns. GMO opponents indicated that offering GM maize to these countries was a means of encouraging African countries to adopt GM technology because the exporters were having a hard time having their products accepted in Europe. The US government stated that it did not separate GM and nonGM grains and had insufficient nonGM grains to supply these countries. The grain was not milled before being sent, the extra cost claimed to be a factor, but opponents of the aid argued this was not done so that farmers could replant some of the grain as seed and make these countries potential GM crop growers in the future.

This had been previously demonstrated in Mexico where farmers planted GM varieties received as food. This has become a contentious issue in that country where seeds are smuggled across the border from the US, the world's largest maize producer, with more than 70% of crops being from GM varieties. Some Mexican farmers in the arid northern flatlands are planting banned GM maize to reduce crop loss. In contrast, farmers in the south are concerned that pollen from these GM crops will fertilize native maize species and reduce the pool of local maize varieties, which may be as many as 10 000. However, some officials in Mexico indicate that Mexican farmers will not be able to compete without GMO crops. In 2008, Mexico, the US, and Canada lifted all maize tariffs under the 1994 North American Free Trade Agreement, and Mexico imported nearly 35% of maize for its residents. Because maize costs have risen because of the biofuel industry, there is an argument that growing and harvesting of GM crops would reduce Mexico's dependency on the US for its maize.

Eventually, South Africa milled the US GM maize and Malawi, Mozambique, and Zimbabwe accepted the milled food to be distributed as famine relief. Because Zambia continued to refuse the milled product, it became a rallying point for advocates both for and against biotechnology, using Zambia's stand as an example. The refusal by the EU to state that this GM maize were safe for African governments led the US to file a case against the EU through the WTO. As a result of the extensive publicity in Europe, nonGM producers gained much more of the EU market, and the US ended up with a surplus of GM maize, which it was unable to sell. In August 2003, the US, Canada, and Argentina took the EU to the WTO for suspending approvals for biotech products, and for six member states' national bans on EU-approved GMOs. The US claims to have suffered $200 million in lost sales for maize products alone and $100 million for soy products. European governments voted with a clear majority in 2005 to retain existing national bans on GMOs.

In 2006, the WTO Dispute Panel ruled on three aspects of the EU's regulation of GM crops: first, the EU had acted inconsistently with its obligations under the SPS Agreement (Agreement on the Application of Sanitary and Phytosanitary Measures) by applying a *de facto* moratorium on approvals on new GM crops between June 1999 and August 2003; second, in the case of specific measures delaying the approval of 24 new GM crops, the EU had breached its obligations under the SPS Agreement; and third, safeguard measures implemented by six EU member states against the import or marketing of specific GM crops were not based on any risk assessment as required by the SPS Agreement, and hence the EU had acted inconsistently with its obligations under that Agreement. However, the ruling did not consider the source of the contentious issues: (1) the safety of GM foods; (2) substantial equivalence that states that GM foods are like conventional foods; and (3) whether the GMO approval process by the EU is consistent with its obligations under the WTO. Some commentators feel that the US may have won a battle for marketing GM foods but lost the war in Europe where there is still much opposition to GMOs on the market.

However, with the WTO ruling, antiGM governments, such as Zambia's, have lost one of their arguments for not adopting the technology. Drought struck Zambia again in 2005 and some farmers would like to have had permission to plant GM crops. Among the proponents is the Biotechnology Outreach Society of Zambia, which accepted the 2003 findings of a team of Southern African scientists that GM crops pose no immediate risk to humans and animals and advised the southern African nations to embrace the technology because of its potential to increase agricultural yields. In contrast the Jesuit Center for Theological Reflections and the Kasisi Agricultural Training Center thought that GM crops would bring negligible benefits to Zambian farmers and could threaten the sustainability of small-scale farming in Zambia. The government suggested that farmers should consider alternatives to maize, such as winter maize, which grows well in dry conditions, and cassava, and to irrigate their fields with water from wetlands. Although the US leads the world in GM crops (>16% of the total planted), the recent drought in 2012, which cut the maize production by approximately 20%, may increase the desirability of GM crops. In theory, some GM varieties are able to withstand extreme climate change. However, it is interesting that the varieties planted for the 2012 maize and soybean crops in the midwest did not fare well against the recent drought.

However, another issue is the use of ingredients from GM products, such as lecithin. Maize oil and soy oil are sources of lecithin, which is widely used in processed food as an emulsifier. This is a highly purified product with no protein or DNA remaining from the GM crop source. Nevertheless, consumer concerns about genetically modified food have extended to highly purified derivatives from GM food. In 2000, the EU required labeling of food containing additives derived from GMOs, including lecithin, even though it is not possible to distinguish a GM product from a conventional one through any laboratory tests (i.e., total equivalence). Thus, industries wanting to sell lecithin in Europe have to maintain a written tracking record of all their sources if they wish to avoid any labeling infractions. Labeling may eventually be an issue in the US and Canada where national governments do not require labeling of GM foods or ingredients. However, in 2013, a proposed new law in New Mexico defines any product containing more than 1% of a genetically modified material as a product that would require a disclosure label on the 'immediate container' or wrapper and would require the label to be 'easily legible.' A referendum for a similar measure in California was defeated in November 2012.

Conclusion

As Ramjoué (2008) states: The most fundamental question to be answered is whether and to what extent a given society believes that a certain technology, in this case the use of genetic engineering in agriculture, is a useful and appropriate way forward. It seems that some countries in the world agree that GM products, like drugs, are a benefit overall, and other countries are either ambivalent or totally opposed. These past recognize that once GM crops become a part of agriculture, they cannot be eliminated, and future trade could be adversely affected. Actually, even in regions like the EU, which has many Member States opposed to GM foods, a certain proportion of GM ingredients may be permitted because of inadvertent exposure in the food chain to some GM components, and it would appear in the very long run, GM products are going to be present in all countries, coming from large farms with mass-produced monocultured crops. This will be accelerated because of more extreme weather events making growing, harvesting, and storing of crops more difficult and with fewer proportions of a society willing to work on the farm. May be this will result in cheaper food for the masses and probably no long-term health effects, but with a probable loss of the small farmer and local varieties unless the public sees value in the foods produced in the same way as wines, cheeses, and organic foods have developed their uniqueness and remained thriving industries.

See also: Foodborne Diseases: Overview of Chemical, Physical, and Other Significant Hazards; Overview of Emerging Food Technologies. Institutions Involved in Food Safety: Consumer Organizations; FAO/WHO Codex Alimentarius Commission (CAC); Food and Agriculture Organization of the United Nations (FAO). Mycotoxins: Aflatoxins; Mycotoxins – General. Other Significant Hazards: Food Allergies and Intolerances. Public Health Measures: Challenges of Industrialized Countries in Food Safety Management. Risk Analysis: Risk Assessment: Chemical Hazards; Risk Assessment: Principles, Methods, and Applications; Risk Communication: Chemical Hazards

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Safety of Regional Specialities – Korean Fermented Foods

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Glossary

Critical control point A processing factor of which a loss of control would result in an unacceptable food safety or quality risk.

Fermentation A kind of food processing that is the conversion of carbohydrates to alcohols and carbon dioxide or organic acids using yeasts, bacteria, or a combination thereof, under anaerobic conditions.

Food safety A scientific discipline describing the handling, preparation, and storage of food in ways that prevent foodborne illness.

Hazard A biological, chemical, or physical agent in, or condition of, food with the potential to cause an adverse health effect.

Hazard Analysis Critical Control Point A systematic preventive approach to food safety and pharmaceutical safety that addresses physical, chemical, and biological hazards as a means of prevention rather than finished product inspection.

Soybean Sauce and Soybean Paste

Korean-style soybean sauce (*Kanjang*) and soybean paste (*Doenjang*) are produced in a single process using *Meju*, a fermentation starter. The *Meju* is prepared from cooked soybeans. Soybeans are soaked overnight in water, cooked for 2–3 h, and mashed by pounding. The substance is shaped like a brick or a ball, dried in the sun, and stored in a stack covered during the night for several days. Through this process, molds (especially *Aspergillus oryzae*) are grown on the surface, and bacteria (typically *Bacillus subtilis*) are generated inside the *Meju*. The enzymes from molds and bacteria hydrolyze the soybean proteins into amino acids, and the carbohydrates into sugars and organic acids. The amino acids and sugars interact with each other in a browning reaction, resulting in the characteristic dark brown color and meaty flavor. Well-fermented *Meju* is immersed in an earthen jar filled with brine and ripened for several months. The brown color and meaty flavor leach into the brine. During this period, salt-tolerant yeasts grow in the mash, particularly *Saccharomyces rouxii*, which produces the aroma of soybean sauce. The liquid portion becomes soybean sauce (*Kanjang*) and the precipitates become soybean paste (*Doenjang*). Soybean sauce produced thusly is boiled once and stored in an earthen jar for years.

Gochujang, a unique hot bean paste, is one of Korea's most common and popular seasonings. It is made with *Meju* and malt made from barley. Malt powder is mixed with cereal porridge made from rice, glutinous rice, or barley. The enzymes in malt hydrolyze the starch into sugars and reduce the consistency of the mixture. *Meju* powder, red pepper powder, and salt are added to the partly saccharified porridge, with thorough mixing, to form a paste that is transferred to an earthen jar. The top is covered with salt to prevent mold growth. The jar is placed in the sun for further fermentation. The proteins in soybean and cereals are degraded into amino acids to produce a meaty flavor. During fermentation, a wonderful harmony of the meaty flavor from hydrolyzed proteins, the sweet taste of hydrolyzed starches, the pungent taste of red pepper, and the saltiness is achieved, and a new characteristic flavor stimulating the appetite of Koreans is formed.

Safety concerns of these fermented soybean products include possible contamination with mycotoxin-forming molds during *Meju* fermentation, biogenic amine formation during the aging process in brine, and contamination with *Bacillus cereus*. Much research has been conducted in Korea on these safety concerns. It has been proven that aflatoxins are degraded to 80–90% after 2 months of fermentation and degraded to 100% after 3 months of fermentation.

In addition, samples of soybean sauce and soybean paste were collected from various markets in Korea to detect aflatoxin levels. According to a report supported by the Korea Food and Drug Administration (KFDA) in 2006, aflatoxin levels were not detected in a total of 14 samples of *Doenjang*, *Kanjang*, and *Gochujang*. Another report performed in 2004–2005 showed that aflatoxin B_1 was detected in one of the seven soybean sauce samples and in 2 of the 56 soybean paste samples by enzyme-linked immunosorbent assay/high-performance liquid chromatography (ELISA/HPLC). Because the detected levels were 1.81 and 0.05–0.17 ppb, respectively,

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they were under the permitted level (10 ppb) of Korea Foods Standards (Table 1). Although they are within safe levels, right manufacturing processes and management practices (including cultivating and storage technology) are important to minimize the contamination with aflatoxin, ochratoxin, biogenic amines, and *B. cereus* at every stage of production.

Several factors can be considered in degrading aflatoxins during fermentation. Ammonia produced during the fermentation, light, microbial competition with *Bacillus* spp., and addition of charcoal or vitamin C were reported to help reduce aflatoxins in soybean products. In addition, increasing concentrations of CO_2 or N_2 suppressed the formation and growth of aflatoxin because aflatoxin producing molds are aerobic organisms requiring O_2. The most important factor is the prevention of any mold growth, which results in aflatoxin production because any subsequent cooking will not destroy it.

Table 1 Regulatory standards for traditional Korean fermented foods

	Fermented soybean products	*Kimchi*	*Fermented fish products*
Aflatoxin ($\mu g\ kg^{-1}$)	<10 ppb (vitamin B_1) <15 ppb (total aflatoxin)	– (<10 for red pepper powder)	–
B. cereus	<10 000 per 1 g (excluding *Meju* and soy sauce)	–	–
Coliform group	Negative (limited to sterilized mixed paste)	Negative (limited to sterilized packaged products)	Negative (limited to *Jeot* and spiced/seasoned *Jeot*)
Escherichia coli	–	–	–
Harmful metals ($mg\ kg^{-1}$)	–	Lead <0.3 Cadmium <0.2	–

'–' Indicates no regulations.

Source: Reproduced from Korea Food & Drug Administration (KFDA) (2008) *Food Code*. Seoul: Korea Food Industry Association.

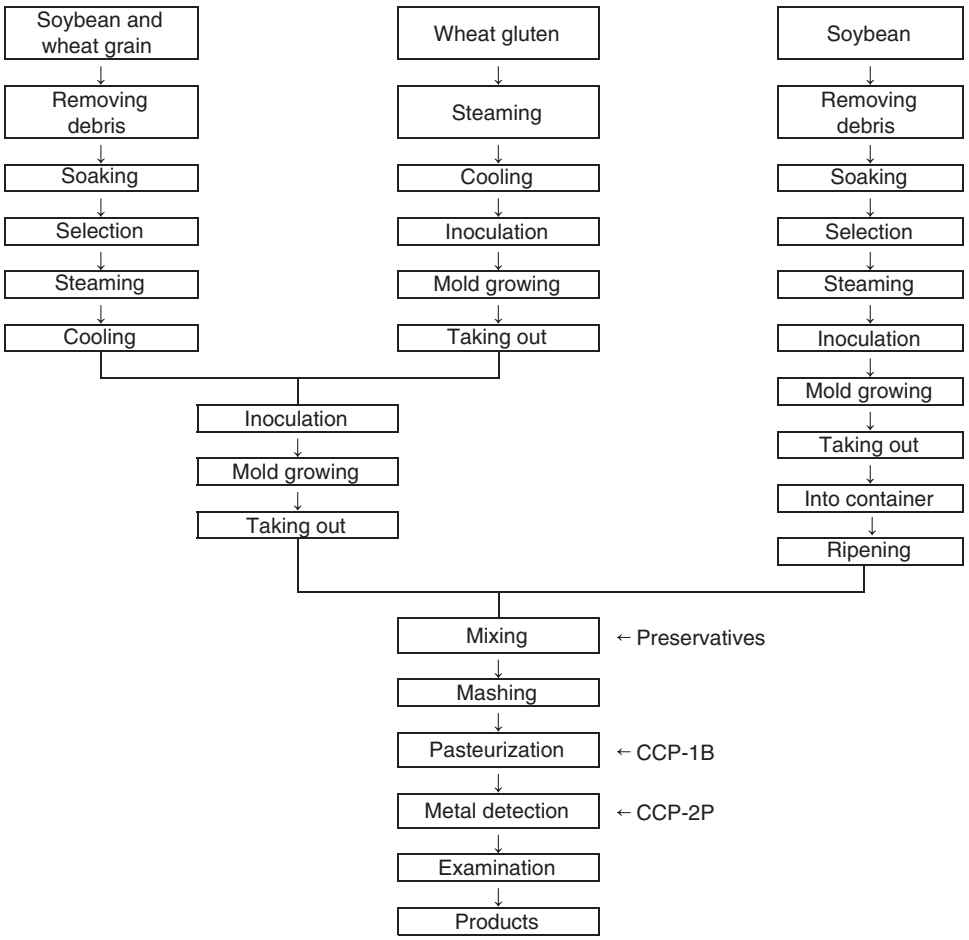

Figure 1 Flow chart of the procedure for *Doenjang*, marked with CCPs.

It was suggested that storage temperatures should be maintained at 0–7.5 °C because the optimal temperature for aflatoxin production is 25–30 °C. The food industries in Korea are therefore launching refrigerated soybean products for enhanced safety. Current refrigerated soybean paste accounts for 16% of the sales. This method was also reported to remove off-flavor and improve taste.

Adopting controlled fermentation techniques using *Koji*, a starter of Japanese soybean products, is a safe alternative to replace the traditional method. Most of the industrial production of soybean sauce and soybean paste in Korea utilizes *Koji* instead of *Meju* because *Koji* contains *A. oryzae* rather than *Aspergillus flavus*, which produces aflatoxin. However, the taste and flavor of soybean products fermented with *Koji* are not as rich as those made in traditional ways. It is thus worthwhile to introduce both these methods in the food market to meet consumer preference.

As an approach to eliminate, minimize, or prevent hazards from farm to fork, the hazard analysis critical control point (HACCP) system is highly regarded. This control system includes seven principles designed to prevent problems before they occur and to correct deviations as soon as they are found. The critical control points (CCPs) are placed in the flow chart of operational steps, where practices can be applied. The KFDA has put efforts in developing HACCP models for improving safety and reducing hazards in Korean fermented foods. The following models for soybean products are the examples. The potential hazard lists of fermented soybean products, *Doenjang*, *Kanjang*, and *Gochujang*, can be summarized as follows: biological hazards (pathogens, such as *E. coli* and *B. cereus*, yeast, and molds), chemical hazards (aflatoxin and pesticide residues), and physical hazards (foreign substances, such as grass, seeds, sticks, and wood waste). According to the HACCP model suggested by the KFDA, the CCPs of *Doenjang* and *Gochujang* are identical (Figures 1 and 2). Two CCPs (CCP-1B and CCP-2P) were noticed in their procedures. It mentions that pathogens, such as *E. coli* and *B. cereus*, should be controlled in the pasteurization step (CCP-1B) with critical limits, appropriate time, and temperature (60–80 °C). The metal detection step (CCP-2P), a procedure to remove iron and stainless steel using a metal detector just before packaging, is another critical point. The quality control for the raw material (soybean) is relatively easy in Korea because most companies purchasing it in bulk from the Korea Jang Cooperative.

Kanjang has only one CCP (CCP-1B) for a pasteurization step at 80–85 °C to manage pathogenic bacteria (Figure 3). Bacterial count of *B. cereus* should be less than 10^4 cells per gram in these soybean products after the thermal treatment.

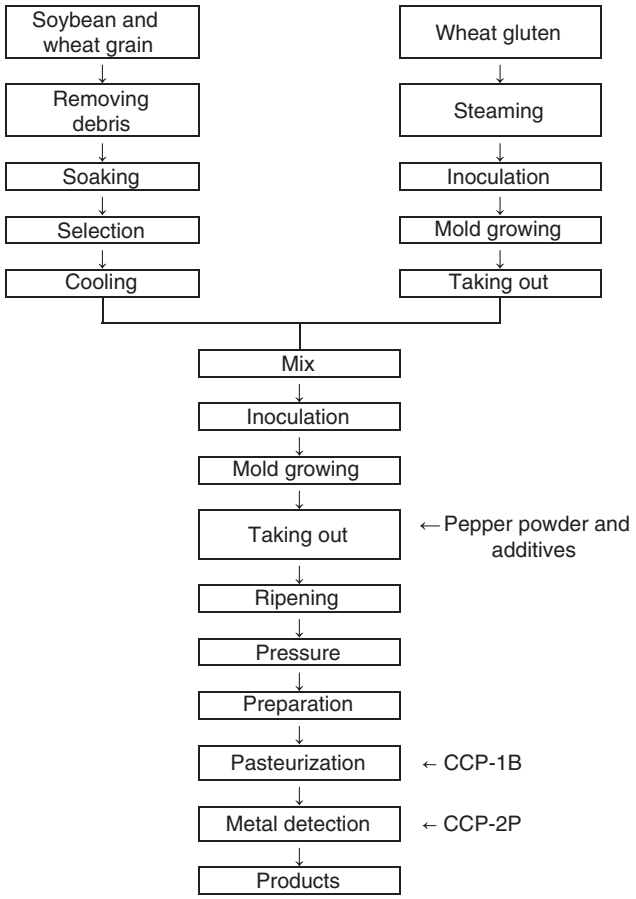

Figure 2 Flow chart of the procedure for *Gochujang*, marked with CCPs.

Fermented Fish Products

Fermented fish products in Asia are generally salt-fermented products: fish sauce, fish paste, and cured fish. When the salt concentration is higher than 20% of the total weight, growth of pathogenic and putrefactive microorganisms can be prevented. In this case, the products do not need other preservative means. The first criterion for classification in this group is the degree of hydrolysis, which is influenced by fermentation time and temperature, added enzyme sources, and the water content. The fully hydrolyzed liquid is defined as fish sauce. The cured fish is confined to represent the partially hydrolyzed fish products that retain the original shape of fish in the exuded liquid, and this form is frequently used as a side dish for rice meals. Fish paste is characterized by partially dried salted fish, which restricts the degree of hydrolysis and produces a homogeneous and solid condiment. Each type can be further subdivided by the type of raw materials, such as fish species, portion of fish, etc.; accordingly, numerous products can be named. In Korea, more than 30 products are included in the category of cured fish.

When the salt concentration is lower than 20%, the salted fish undergoes rapid spoilage, and other means of preservation is needed. Lactic fermentation by the addition of carbohydrate is an old method for fish preservation in low-salt processes. Rice, millet, flour, and even syrup (or sugar) are used as the carbohydrate source. The amount of added carbohydrate and the salt concentration primarily control the extent of acid fermentation and maintain quality. An alternative method keeps the low-salt fermented fish with vinegar at low temperatures. This method is practiced widely in the Scandinavian countries. Many Asian countries produce salt-cured and dried-fish products, for example, *Plakem* in Thailand, *Jambalroti* in Indonesia,

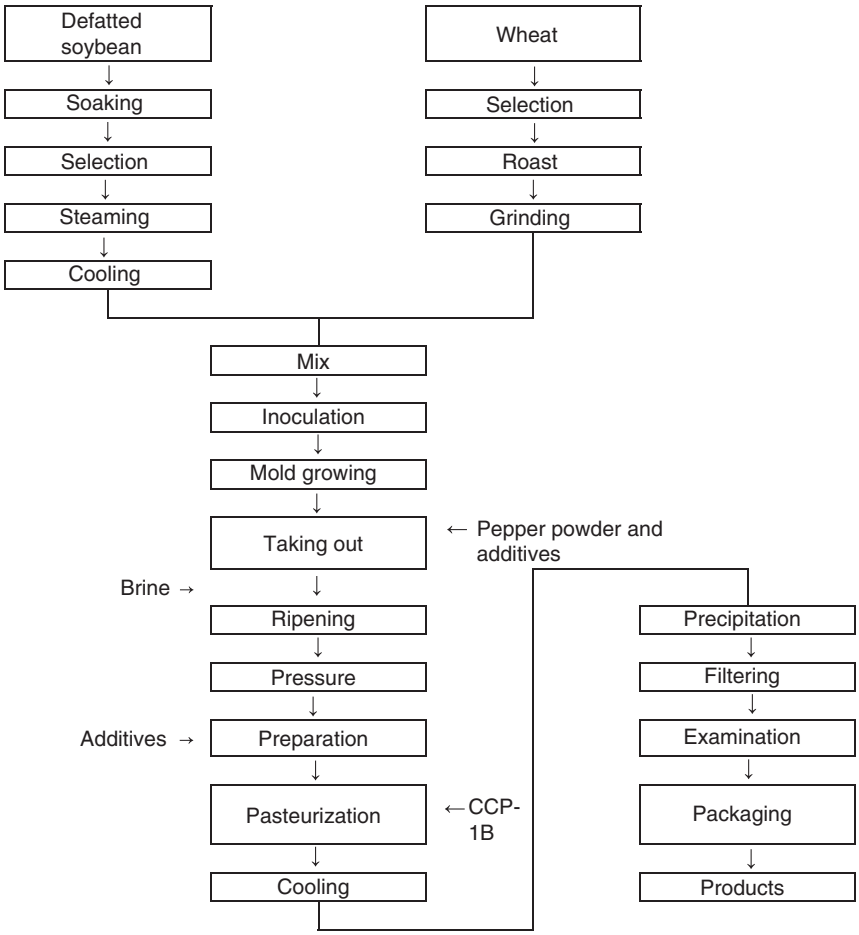

Figure 3 Flow chart of the procedure for *Kanjang*, marked with CCPs.

Maldive fish in Sri Lanka, and *Gulbi* in Korea, but the role of fermentation in these products is not fully understood.

Fermented fish products can be divided on the basis of the enzyme hydrolyzed versus the microbial fermented. The products are subdivided into four groups depending on the enzymatic hydrolysis: (1) hydrolysis in >20% salt, (2) hydrolysis in salt + drying, (3) hydrolysis at low temperature, and (4) hydrolysis at low pH. The products preserved by microbial fermentation are subdivided into two groups: (1) fermented with added carbohydrate and (2) fermented without added carbohydrate.

The major potential hazard associated with proteinaceous foods, such as fermented fish, is from the growth of pathogenic bacteria such as *Vibrio* spp., presence of parasitic worms, and the production of physiologically active amines. Of particular concern for unheated foods in anaerobic conditions is the possible growth of *Clostridium botulinum* and its toxin production.

It is evident that neither the high-salt nor the low-salt lactic fermented fish products will cause the growth of any pathogenic bacteria once they are prepared with the appropriate salt content and/or low pH. However, the improper storage of raw fish before salting and insufficient acid production in a very low-salt fermentation can cause an outbreak of botulism. The botulinum toxin is destroyed relatively easily by cooking, but it is very stable in salty and acidic environments. The fermented fish products most frequently incriminated in *C. botulinum* type E poisonings are *Sushi* (a type of *Narezushi*) and *Kirikomi* (a type of Shiokara) in Japan, and salmon egg cheese (fermented crushed salmon roe) among British Columbia First Nation Peoples and Alaska Indians.

The physiologically active amines, such as histamine formed by the bacterial decarboxylation of histidine, may be produced in amounts sufficient to cause poisoning in certain fishes. *Jeot-gal* is the generic name of high-salt fermented fish products, which are used not only for side dishes but also for additives in making *Kimchi*. *Jeot-gal* contains large amounts of precursor amino acids of biogenic amines because it is made from the muscles and viscera of seafood and salts. It is therefore important to reduce the biogenic amine content. Several studies suggested that the addition of garlic and glycine can inhibit amino acid decarboxylase activity in *Myoelchi-jeot* (made of anchovies). In fact, the cadaverine and tyramine contents were reduced by up to 18.4% and 30.9%, respectively, in the culture treated with garlic extract. Glycine has the greatest inhibitory activities on biogenic amine production. The contents of putrescine, cadaverine, histamine, tyramine, and spermidine were reduced by 32.6%, 78.4%, 93.2%, 100%, and 100%, respectively, compared with the control. Therefore, there is no doubt that these findings can help improve safety. Nowadays, many people are trying to reduce sodium intake because they

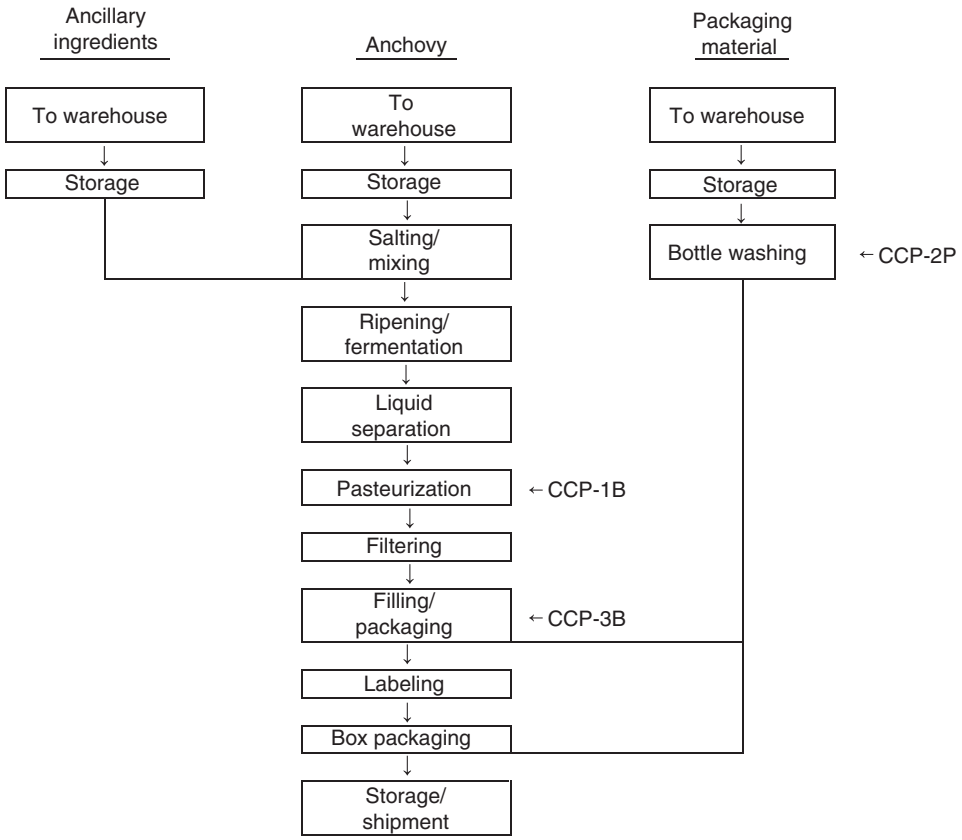

Figure 4 Flow chart of the procedure for liquid fermented anchovy, marked with CCPs.

are paying attention to their health and well-being. Keeping this in mind, companies are investing in cooling systems to produce refrigerated products with less salt. Following the HACCP, plan for liquid fermented anchovy is based on products that are produced not in a cooling system but in a traditional way (Figure 4). The possibility of pathogen growth results in a CCP at the pasteurization step (CCP-1B). Sufficient bottle washing is required to eliminate debris inside the bottle (CCP-2P). Proper filling and a packaging system (CCP-3B) are essential to prevent decomposition and microbial contamination due to poor sealing.

Fermented Vegetable Products

Kimchi is a unique fermented vegetable product with a long tradition in Korea. It is a common side dish served with cooked rice and other dishes. There are more than 50 varieties of *Kimchi* classified by the use of raw materials, processing methods, and the season and locality of preparation. Korean cabbage and Korean radish are the most popular vegetables for making *Kimchi*; but cucumber, carrot, onion, and even eggplant can be used as the primary vegetable. Fermented fish sauce is an important subingredient providing enzymes and flavor substances for fermentation. Salt, garlic, and red pepper play an important role in controlling the type of microflora in *Kimchi*. Production of organic acids at the cost of carbohydrates and resultant pH reduction contribute to maintaining freshness of the vegetables during the storage period.

A recipe for the simplest *Kimchi* may include Korean cabbage 100 g, garlic 2 g, green onion 2 g, red pepper powder 2 g, and ginger 0.5 g with an optimal salt content of 3.0%. Whole (or cut) cabbage is salted with 15% brine for 3–7 h, washed twice with fresh water, and drained. Other minor ingredients are chopped, combined, mixed with the treated cabbage, placed in containers, and tightly sealed. The length of time for completion of the fermentation depends on the salt content and temperature, 3 or 4 days at 20 °C and 2–3 months at 5 °C.

The content of reducing sugar decreases rapidly at the beginning of *Kimchi* fermentation, whereas the total acidity increases. The optimal pH and acidity for the best taste is 4.2 and 0.6% (as lactic acid), respectively. The number of aerobic bacteria decreases rapidly at the beginning of *Kimchi* fermentation, when anaerobic bacteria dominate. However, at the later stages of fermentation, surface film-forming aerobic bacteria start to grow, and texture softening and taste deterioration take place. During *Kimchi* fermentation, the type of microflora changes. The number of *Leuconostoc mesenteroides* decreases after 10 days of fermentation in a 3.5% salt content at 14 °C, whereas *Lactobacillus plantarum*, which is considered to be a key to the *Sauerkraut* production process, reduces the quality of *Kimchi*. It

is also worthwhile to note that there is a considerable increase in the vitamin B group during winter *Kimchi* fermentation. That is, the amounts of vitamin B_1, B_2, B_{12}, and niacin may reach as high as twice the initial amounts at the optimal maturation of *Kimchi* and then decrease as the taste of *Kimchi* deteriorates due to overfermentation. Vitamins A and C are reduced slightly during the fermentation, but it is an excellent way of preserving these vitamins during the winter season.

There have been several controversial debates on the safety of *Kimchi* in terms of nitrate, nitrite, secondary amines, and biogenic amines. It was reported that the levels of nitrate in the vegetable decrease rapidly over 4 days of fermentation at 20 °C, whereas the contents of nitrite and secondary amines increase slightly and before decreasing. The change in nitrate reductase activity during *Kimchi* fermentation follows the same pattern as the change in nitrate concentration. This indicates that *Kimchi* fermentation reduces the nitrate level in vegetables through the action of microorganisms without increasing the concentrations of nitrite or secondary amines to any significant level.

The formation of nitrite and secondary amines during *Kimchi* fermentation was of great concern to many researchers in Korea in the 1970s. However, it was found that the amounts of nitrite and secondary amines in *Kimchi* were very low compared with those in sausages and fish. The possibility of nitrosamine formation during fermentation was investigated because fresh cabbages contain large amounts of nitrate varying from 55 to 2500 ppm. However, the levels of nitrate are reduced rapidly from 135 to 70 ppm after 4 days of *Kimchi* fermentation and further down to 50 ppm after 10 days of fermentation at 20 °C. Thus, the subject is of less concern.

To examine the biogenic amine contents in commercial *Kimchi*, eight types of *Kimchi* products were analyzed by HPLC. The biogenic amine contents were between 0 and 150 mg kg^{-1}, which is an acceptable level for human health. The putrescine and cadaverine levels seem to be slightly high in the *Baechu Kimchi* sample compared with those of other samples. To reduce these amines, addition of *Jeot-gal* can be adjusted because the biogenic amines in *Jeot-gal* are transferred to *Kimchi*.

It is well known that *Kimchi* has strong antipathogenic and antimicrobial activities. In fact, *Clostridium perfringens* disappeared after 2 days of *Kimchi* fermentation; *Staphylococcus aureus* and *Salmonella typhimurium* after 4 days; and *Listeria monocytogenes*, *Vibrio parahaemolyticus*, and *E. coli* after 5 days, whereas the number of lactic acid bacteria increased from 10^5 to 10^8. This antimicrobial effect of *Kimchi* appears to result from the combined effects of the organic acids and bacteriocin produced during fermentation, and the *Kimchi* ingredients (garlic, onion, ginger, etc).

Contamination with parasite eggs in *Kimchi* imported from China was a big scandal in Korea in 2005. The types of parasite

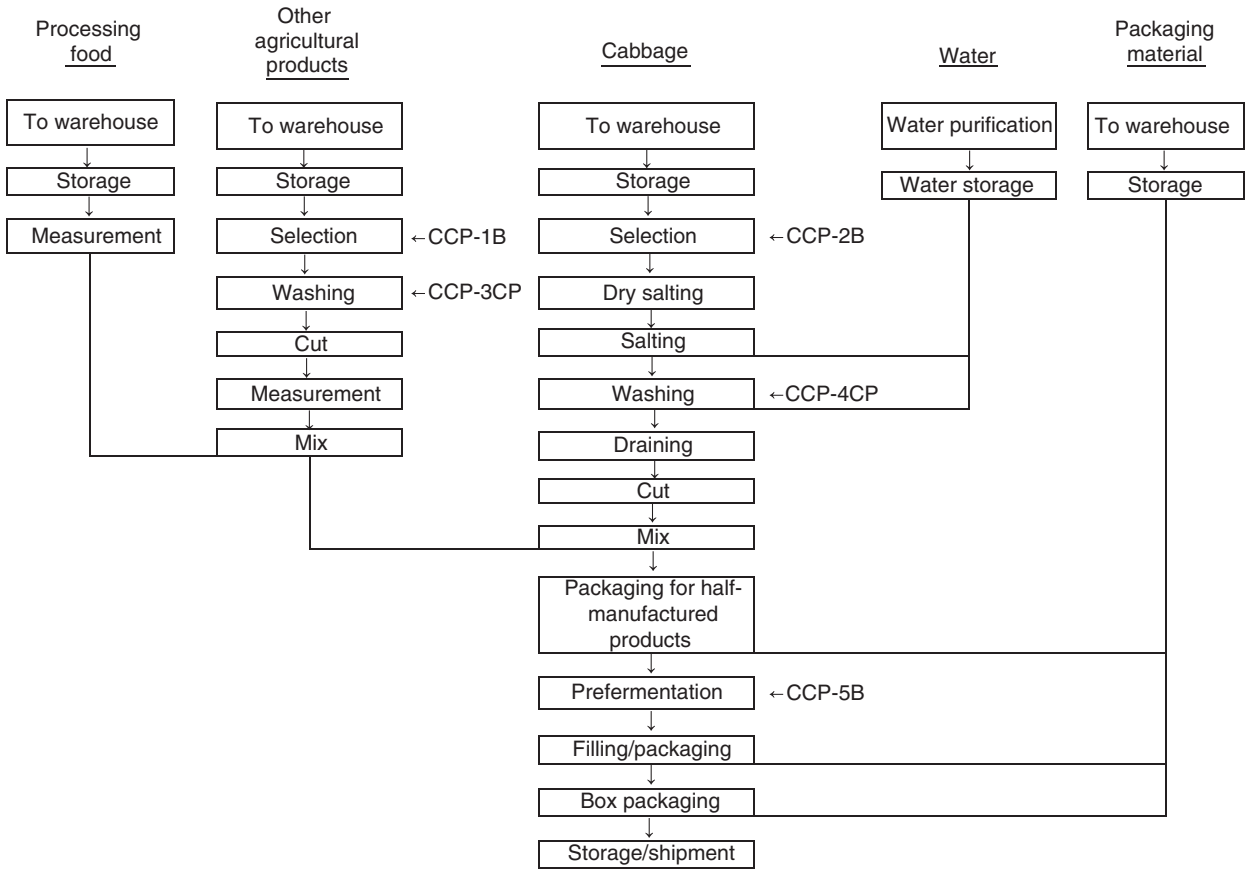

Figure 5 Flow chart of the procedure for *Baechu Kimchi*, marked with CCPs.

eggs found in *Kimchi* were *Ascaris lumbricoides* (roundworm), *Ancylostoma duodenale* (hookworm), *Trichostrongylus orientalis*, and *Isospora belli*. Although parasite eggs are mostly inactivated in *Kimchi* juice, which contains 3–5% salt, 0.8% organic acids (mainly lactic acid and acetic acid), and CO_2, the KFDA considered this issue as a safety incident at that time. It created a severe trade conflict with China, and the import and export of *Kimchi* were severely damaged.

To identify potential problems and possible corrective actions in the production of *Kimchi*, the KFDA has suggested the HACCP model, as shown below (Figure 5). Several CCPs (CCP-1B, CCP-2B, CCP-3CP, CCP-4P, and CCP-5B) have been identified by the analysis of biological, chemical, and physical hazards. Because *Kimchi* is a ready-to-eat product, a hazard analysis must be conducted to determine whether there are food safety hazards that are reasonably likely to occur. Among these items of hazard analysis, both microbiological evaluation and detection of pathogens of cabbages, ingredients, instruments, utensils, employees, and working area are essential. Therefore, the selection steps CCP-1B and CCP-2B (choosing good raw materials and removing decomposition and spoilage parts from materials) in the beginning of the HACCP plan is very significant for preventing pathogens, molds, parasites, and other contaminations from the raw materials to the final products.

The next step to consider is the washing procedure for other agricultural products and cabbages (CCP-3CP and CCP-4CP). Sufficient washing in accordance with the standard operating procedure (SOP) can help to remove the pesticide residues and foreign substances. Unlike other HACCP plans, the prefermentation step (CCP-5B) can be a critical point in making *Kimchi* because inappropriate temperature and time can result in abnormal fermentation.

Another example of pickled vegetable products, *Danmooji* (also known as *Takuan* in Japan), can be introduced. It is made from radish and used widely in Korea and Japan. In addition to being served alongside other types of Korean dishes, it is also used as a filling for *Kimbab* (rice roll in laver sheet). *Danmooji* is made by first salting radish, desalting it, and seasoning it with vinegar and sugar. The finished *Danmooji* is usually yellowish because food industries use coloring agents for this effect. The HACCP plan for *Danmooji* suggested by KFDA indicates five CCPs (CCP-1C, CCP-2CP, CCP-3B, CCP-4B, and CCP-5B) (Figure 6). They include proper seasoning preparation (CCP-1C) not to exceed the limits of food additives, proper washing (CCP-2CP) to remove the pesticide residues and foreign substances, proper filling/packaging (CCP-3B) to prevent decomposition and microbial contamination from poor sealing, and proper pasteurization (CCP-4B) and proper cooling (CCP-5B) to ensure microbial contamination and growth at the final stages. The amount of free chloride (more than 1 ppm) is required to maintain microbiological control in cooling water.

HACCP systems introduced in this article are one of the models that verify the safety of traditional foods. To ensure the validity and the good performance of a HACCP system, the system must be applied in combination with other food safety

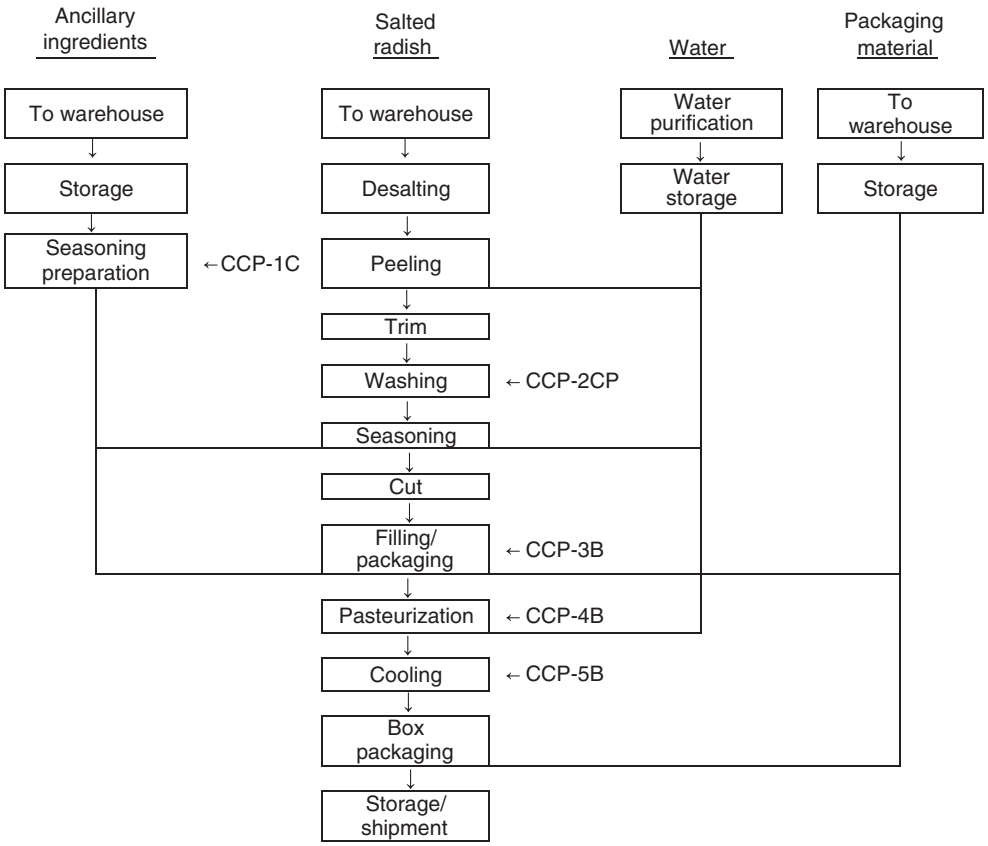

Figure 6 Flow chart of the procedure for salted radish (*Danmooji*), marked with CCPs.

assurance systems, such as good manufacturing practice (GMP), good handling practice (GHP), as well as personnel training. In fact, quite a number of industries have already obtained certification schemes, such as good agricultural practice (GAP), GMP, GHP, and International Organization for Standardization (ISO) standards. In addition, international standards, such as Codex standards, can facilitate the global trade of traditional foods as free trade agreement is increasing between countries. *Kimchi* was first registered in Codex in 2001, followed by *Gochujang*, *Doenjang*, and *Ginseng* in regional Codex 2009. Having Codex standards for more traditional foods will facilitate exports.

See also: History of Food Safety and Related Sciences: History of Foodborne Disease in Asia – Examples from China, India, and Japan

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Safety of Human Milk: Microbiological Aspects

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Glossary

Bioactive factors These factors are constituents of human milk that have specific functions for the infant in addition to nutrition. Example: Lactoferrin binds iron and suppresses the growth of *Escherichia coli*.

Breast milk Milk produced by the human breast stimulated either by infant suckling or mechanical pumping by hand or machine.

Breastfeeding To feed human infant at his/her mother's breast. To suckle.

Colostrum First milk produced after delivery.

Donor milk Milk pumped by a mother to be given to an infant not her own

Risk/benefit ratio The risk of a contamination versus the tremendous benefit of breastfeeding or receiving human milk.

Species specificity With respect to mammary secretion each species provides a milk specific for the offspring of that mammalian species and its nutritional and growth needs.

Introduction

Lactation is the physiologic completion of the reproductive cycle and occurs in all mammalian species including the human. Of more than 4000 mammalian species, each one produces a milk that is carefully engineered to meet the nutritional needs of that specific species as determined by the offspring's maturity at birth, the rate of physical growth and especially the rate of brain and neurological growth. The process of lactation is designed so that the offspring receives the feeding directly from the gland that produces the nutrient thus eliminating any intervention by the mother or utilization of equipment. There is no opportunity for invasion of infection. This discussion of human feeding will, however, also include situations where milk is removed from the breast and fed to the infant using man-made devices with and without any processing of the liquid. It will also include a description of human milk banking.

The Value of Human Milk

Any discussion of human lactation and breastfeeding requires first an understanding of the uniqueness of human milk and the importance of breastfeeding to both mother and infant. When specific issues are discussed such as maternal infection, medications, or complications of the infant it is the risk/benefit ratio that is the issue. What is the risk of a maternal infection to the nursling for example compared to the tremendous value of being breastfed. Thus, the answers are complex and multifunctional.

The most compelling reason to promote breastfeeding is species specificity. Human milk is designed for the nutritional needs of the human infant who will be exclusively nourished by it for 6 months and then continue to receive mother's milk for months to years while weaning foods are added to the diet. Human milk supports growth of brain and body as the sole nutrient. The human infant is the most immature of all mammals at birth except for the marsupials thus brain growth is the most significant need of the human newborn. The biochemistry of human milk includes nutrients not found in other species' milk especially the cow and the goat. Cholesterol, docosahexaenoic acid (DHA), and taurine are essential to human brain growth for instance but do not occur in cow milk. The micronutrients in human milk are easily absorbed by the human infant. The milk also contains enzymes that contribute to the digestion and absorption of all macro- and micronutrients.

There are a number of infection protection constituents in human milk (Table 1). Lactoferrin is an excellent example of a constituent with multiple roles. Lactoferrin binds iron to make iron unavailable for *Escherichia coli* that depends on iron for growth. When lactoferrin binds to iron, *E. coli* cannot flourish and the physiologic flora of the newborn gut thrives. The normal flora of the breastfed infant is *lactobacillus bifidus* (*Bifidobacterium bifidum*). The small amount of iron in human milk is totally absorbed by the infant. Only 10% of the iron in formula is absorbed in contrast.

Bioactive Factors

The proteins in human milk are not only a vital nutrient but also prevent infection and inflammation as well as promoting growth.

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Table 1 Component functions in human milk

Function	*Component*	*Process*
Biosynthesis of milk components in mammary gland	Phosphoglucomutase	Synthesis of lactose
	Lactose synthetase	Synthesis of lactose
	Fatty acid synthetase	Synthesis of medium-chain fatty acids
	Thioesterase	Uptake of circulating triglyceride fatty acids
	Lipoprotein lipase	Uptake of circulating triglyceride fatty acids
Digestive function in infant	Amylase	Hydrolysis of polysaccharides
	Lipase (bile salt-dependent)	Hydrolysis of triglycerides
	Proteases	Proteolysis (not verified)
	Xanthine oxidase	Carrier of iron, molybdenum
	Glutathione peroxidase	Carrier of selenium
	Alkaline phosphatase	Carrier of zinc, magnesium
Preservation of milk components	Antiprotease	Protection of bioactive proteins (i.e., enzymes and immunoglobulins)
	Sulfhydryl oxidase	Maintenance of structure and function of proteins containing disulfide bonds
Anti-infective agents	Lysozyme	Bactericidal
	Peroxidase	Bactericidal
	Lipases (lipoprotein lipase, bile salt-dependent lipase)	Release of free fatty acids that have antibacterial, antiviral, and antiprotozoan actions
Anti-inflammatory agents	Vitamins A, C, and E	Scavenges oxygen radicals
	Catalase	Degrades hydrogen peroxide
	Glutathione peroxidase	Prevents lipid peroxidation
	Platelet-activating factor acetylhydrolase	Degrades platelet-activating factor
	α1-antitrypsin	Inhibits inflammatory proteases
	α1-antichymotrypsin	Inhibits inflammatory proteases
	Prostaglandin 1	Cytoprotective
	Prostaglandin 2	Cytoprotective
	Epidermal growth factor	Promotes gut growth and function
	Transforming growth factor-α	Promotes epithelial cell growth
	Transforming growth factor-β	Suppresses lymphocyte function
	Interleukin 10	Suppresses function of macrophages and natural killer and T cells
	Transforming growth factor-α receptors I and II	Binds to and inhibits transforming growth factor-α

Source: Reproduced from Hamosh M (1989) Enzymes in human milk: their role in nutrient digestion, gastrointestinal function and nutrient delivery to the newborn infant. In: Lebenthal E (ed.) *Textbook of Gastroenterology and Nutrition in Infancy*, 2nd edn. New York: Raven, and Hamosh M (2001) Bioactive factors in human milk. Breastfeeding 2001, Part I: The evidence for breastfeeding. *Pediatric Clinic of North America* 48: 69.

Leukocytes are present in milk in very high levels in colostrum and in lesser amounts continually throughout lactation. Specific antibodies and other antimicrobial factors protect the infant from infections. The immunologic benefits of human milk are numerous covering soluble factors, cellular components and those that are hormone like (Table 2). These bioactive properties of human milk range from proteins (i.e., lacotoferrin and lysozyme) hormones (such as erythropoietin, prolactin, and insulin) growth factors, neuropeptides (TNF, alpha, Il-6) anti-inflammatory agents (enzymes, antioxidants), and nucleotides (see Table 2). It is not only the presence of these factors that explains the immunologic properties of human milk but also their dynamic actions that affect infection protection in the normal development of the infants immune system. Although the fetus receives some maternal antibodies across the placenta, the newborn infant depends on the transport of secretory IgA (sIgA) immunoglobulins in colostrums and mature milk. sIgA provides local protection to the mucous membranes of the GI tract from birth throughout lactation.

Although the protective properties of human milk can be divided into cellular facts and humoral factors, it is important to recognize that the constituents of human milk are multifunctional, interactive, and complementary and have not been duplicated in artificial infant feedings. The nonimmunoglobulin antipathogen factors in human milk are shown in Table 3. This is a long list of specific antipathogens that have been demonstrated to protect against specific bacteria, viruses, and toxins.

The Mucous Membranes as Barriers

The mucosal immune system in the infant is immature but stands ready to respond to the stimulating factors in human milk. The primary function of the mucosal surfaces is immunologic even though it may perform other functions. The mucosal surface of the gut also serves to absorb nutrients. These barrier mucosal surfaces with their large surface areas

Table 2 Immunologically and pharmacologically active components and hormones observed in human colostrum and milk

Soluble	*Cellular*	*Hormones and hormone-like substances*
Immunologically specific	Immunologically specific	Epidermal growth factor
Immunoglobulin	T lymphocytes	Prostaglandins
sIgA (11S), 7S IgA, IgG, IgM IgE, IgD, secretory component	B lymphocytes	Relaxin
		Neurotensin
	Accessory cells	Somatostatin
	Neutrophils	Bombesin
T-cell products	Macrophages	Gonadotropins
Histocompatibility antigens	Epithelial cells	Ovarian steroids
		Thyroid-releasing hormone
	Additional cells	Thyroid-stimulating hormone
Nonspecific factors	Stem cells	Thyroxine and triiodothyronine
Complement		Adrenocorticotropin
Chemotactic factors		Corticosteroids
Properdin (factor P)		Prolactin
Interferon		Erythropoietin
α-Fetoprotein		Insulin
Bifidus factor		Cytokines
Antistaphylococcal factor(s)		Interleukins
Antiadherence substances		
Epidermal growth factor		
Folate uptake enhancer		
Antiviral factor(s)		
Migration inhibition factor		
Gangliosides		
Nucleotides		
Antisecretory factor		
Spermine		
Soluble CD14		
Carrier proteins		
Lactoferrin		
Transferrin		
Vitamin B_{12}-binding protein		
Corticoid-binding protein		
Enzymes		
Lysozyme		
Lipoprotein lipase		
Leukocyte enzymes		

Source: Modified from Ogra PL and Fishaut M (1995) Human breast milk. In: Remington JS and Klein JO (eds.) *Infectious Diseases of the Fetus and Newborn Infant*, 4th edn. Philadelphia: Saunders.

and constant exposure to microorganisms, foreign proteins, and chemicals predisposes the membrane to damage and infection. The infant's immune system is developing the ability to respond to and protect against invasive pathogens as well as learn to tolerate other commensal organisms that exist on these surfaces. The bioactive factors in human milk supplement this immune protection of the mucosa while limiting inflammation. This functional barrier also includes the actions of enzymes, chemicals, acidity or pH, mucus, immune globulins, and indigenous flora. Secretary immune globulins sIgA and IgM behave at the epithelial surface without inflammation but by limiting adherence and transmigration yet facilitating phagocytosis of potential pathogens.

Study of the microbial colonization of the intestinal tract has resulted in the demonstration of the effects of probiotics that are found amply in human milk. A number of probiotic or healthy bacteria that exist in the human body to protect against the growth of pathogens are defined as microorganisms that can exist within a host while affording benefits for the organism and the host. The best known of the probiotics are *Lactobacillus rhamnosus* GG, *Bifidobacteria infantis*, and *Bifidobacteria bifidus*. Many others are considered to be on this list and are even available commercially. Human milk promotes their growth and is an important protective mechanism associated with breastfeeding.

It is well established that human milk is effective in controlling bacterial infection. Numerous articles have been published demonstrating this protection. The Agency for Healthcare Research and Quality (AHRQ) investigated the values of human milk and published two reports of their findings. A review of the literature reveals a long list of antibodies present in human milk against bacteria including *E. coli*, *Bacteroides fragilio*, *Colostridium tetani*, *Haemophilus pertussis*, *Diplococcus pneumonia*, *Corynebacterium diptheriae*,

Table 3 Nonimmunoglobulin antipathogen factors in human milk

Antipathogen	*Pathogen*
Ganglioside GM_1	Cholera toxin Labile toxin of *Escherichia coli* Toxin of *Campylobacter jejuni*
Globotriaosylceramide	*Shigella* toxin I Shigalike toxin of *E. coli*
GM3	Enteropathogenic E. coli
Fatty acids	Enveloped viruses *Giardia lamblia*
Chondroitin sulfate	HIV
Sulfatide	HIV
Glycoprotein (mucin)	Inhibition: rotavirus *in vitro* and *in vivo*
Glycoprotein (mucin, glycosaminoglycan)	HIV
Lactadherin	Rotavirus
Mucin	Adherence: S-fimbriated *E. coli*
MUC 1	Poxviruses, HIV
Glycoprotein (mannosylated)	*E. coli* intestinal adherence
Large macromolecule	Respiratory syncytial virus
Macromolecule-associated glycans	Norovirus, *P. aeruginosa*
Oligosaccharides	Adherence: *Streptococcus pneumoniae* and *Haemophilus influenzae*, enteropathogenic *E. coli* *Listeria monocytogenes*
Fucosylated oligosaccharide	Adherence, invasion, *C. jejuni*, stabie toxin of *E. coli* stable toxin *in vivo*, *Vibrio cholera*
Sialyllactose	Cholera toxin, *E. coli*, *Pls. aeruginosa*, Influenza virus *Aspergillus fumigates*, Polyomavirus, *Helicobacter pylori*

Abbreviations: GM, Granulocyte-macrophage; HIV, human immunodeficiency virus.

Source: Modified from Newburg DS, Ruiz-Palacios GM, and Morrow AL (2005) Milk glycans protect infants against enteric pathogens. *Annual Review of Nutrition* 25: 37–58.

Table 4 Nonantibody, antibacterial protective factors in human milk

Factors	*Proposed mechanisms of action*	*Organisms affected*	*Effect of heat*
Bifidus factor	Inhibits replication of certain bacteria in GI tract by causing proliferation of lactobacilli	Enterobacteriaceae, including shigellae, salmonellae, and some *E. coli*	Stable to boiling
Complement components	Opsonic, chemotactic, and bacteriolytic activity	*E. coli*	Destroyed by heating at 56° C for 30 min
Lysozyme	With IgA, peroxide, or ascorbate, causes lysis of bacteria	*E. coli* Salmonellae	Some loss (0%–23%) at 62.5° C for 30 min; essentially destroyed by boiling for 15 min
Lactoferrin (nutrient binders)	Binds ferric iron	*E. coli* *Candida albicans*	Two thirds destroyed at 62.5° C for 30 min
Lactoperoxidase	Oxidizes bacteria	*E. coli* *Salmonella* Typhimurium	Presumably destroyed by boiling
Nonantibody proteins: receptor-like glycolipid or glycoprotein	Inhibit bacterial adherence	*Vibrio cholerae*	Stable to boiling for 15 min
Gangliosides (GM1-like)	Interfere with attachment of enterotoxin to GM1 cell membrane ganglioside receptors	*E. coli* and *V. cholerae* enterotoxins	Stable to boiling
Nonlactose carbohydrate factors	Prevent action of stable toxin	*E. coli* stable toxin	Stable at 85° C for 30 min
Milk cells (macrophages, polymorphonuclear leukocytes, B- and T-lymphocytes)	By phagocytosis and killing: *E. coli*, *S. aureus*, *S. enteritidis* By sensitized lymphocytes: *E. coli* By phagocytosis: *C. albicans* lymphocyte stimulation by *E. coli* K antigen		Destroyed at 62.5° C for 30 min

Source: Modified from May JT (1984) Antimicrobial properties and microbial contaminants of breast milk: an update. *Australian Paediatric Journal* 20: 265, and Pickering LK and Kohl S (1986) Human milk humoral immunity and infant defense mechanisms. In: Howell RR, Morriss Jr., RH, and Pickering LK (eds.) *Human Milk and Infant Nutrition and Health.* Springfield, IL: Thomas.

Table 5 Nonantibody, antiviral, and antiprotozoan factors in human milk

Factors	*Proposed mechanisms of action*	*Organisms affected*	*Effect of heat*
Lipids (unsaturated fatty acids and monoglycerides)	Inactivate lipid-enveloped virus	Herpes simplex	Stable to boiling for 30 min
		Semliki Forest virus	
		Influenza	
		Ross River virus	
Macromolecules	Inhibit attachment and penetration	Herpes simplex	Most stable at 56 °C for 30 min
		Coxsackievirus B_4	Destroyed by boiling for 30 min
		CMV	
		Rotavirus	
α_2-Macroglobulin protein	Inhibits hemagglutinin activity	Influenza	Stable to boiling for 15 min
		Parainfluenza	
α_1-Antitrypsin	Trypsin-dependent inhibition	Rotavirus	Stable to boiling for 10 min
Bile salt-stimulated lipase	May generate fatty acids and monoglycerides that inactivate organisms	*Giardia lamblia*	
		Entamoeba histolytica	
Nonlipase macromolecule	Unknown	*G. lamblia*	
Milk cells	Induced interferon by virus or phytohemagglutinin; induced lymphokine by phytohemagglutinin; induced cytokine by herpes simplex virus; lymphocyte stimulation by rubella, CMV, herpevirus, measles, mumps		Destroyed at 62.5° C for 30 min

Source: Modified from May JT (1984) Antimicrobial properties and microbial contaminants of breast milk: an update. *Australian Paediatric Journal* 20: 265, and Pickering LK and Kohl S (1986). Human milk humoral immunity and infant defense mechanisms. In: Howell RR, Morriss Jr., RH, and Pickering LK (eds.) *Human Milk and Infant Nutrition and Health*., Springfield, IL: Thomas.

Salmonella shigella, *Chlamydia trachomatis*, *V. cholera*, *S. Aureus*, and several strains of Streptococcus. Human milk also contains other protective nonantibody properties (Table 4).

Protection against viruses in human milk has also been vigorously investigated. Antibodies have been demonstrated against polio virus, coxsachievirus, echovirus, enterovirus, influenza virus, Reovirus, RSV, Rotavirus, and rhinovirus. In tissue culture, human milk will inhibit the growth of these viruses. The various factors associated with the protection against viruses and protozoa in human milk are demonstrated in Table 5.

In summary, it is well established that human milk has many properties that protect against bacteria, viruses, and protozoa. These factors act in many ways including the stimulation of the mucous membranes to develop ongoing protection.

In addition to the infection protection and immune properties of human milk, there are many other unique constituents that enhance brain growth, mature the nervous system, provide enzymes that facilitate the digestion and absorption of nutrients, and provide hormones that enhance physiologic processes. Of particular interest are the hormones associated with appetite control, Leptin adiponectin, Grelin, Insulin-like growth receptor (IGF), Resistin, and obstatin; these hormones are associated with the fact that breastfed infants cannot be overfed. Breastfeeding is associated with a normal growth curve and not obesity. The growth curves developed by the World Health Organization using only healthy breastfed children demonstrate a more physiologic Body Mass Index (BMI). The BMI is calculated as follows: weight (kg)/height $(m)^2$ = BMI (metric).

The Process of Human Milk Feeding

When an infant receives his mother's milk directly by suckling at the breast, the milk he receives is clean. Mid-stream cultures of human milk contain no bacteria. The flora on the mother's skin includes benign staph and diptheroids thus the infant does receive some skin bacteria while suckling. The infant is sterile at birth but is quickly colonized by his environment. It is ideal if the infant is colonized with his mother's bacteria that serve to protect the infant from being colonized by pathogens in the environment. The mother should always wash her hands before breastfeeding. There is no need to wash her breast, however, because human milk contains many antibacterial components as noted in Tables 3–5. If milk is pumped by the mother and left in a clean container for 8 h at room temperature, there are fewer bacteria per milliliter than the number retrievable at the time of expression because the milk destroys these bacteria. Unaltered human milk is safe for the infant suckling at the breast.

Extracting the milk from the breast and feeding the milk to infant by the bottle and nipple introduces some possibilities for contamination as any time an infant receives bottled milk. Extracting the milk by hand, as all mothers should be taught to

Table 6 Storage of human milk for home use

Breast milk	*Room temperature*	*Refrigerator*	*Freezer*
Freshly expressed into closed container	6–8 h (78 °F or lower)	3–5 days (39 °F or lower)	2 weeks in freezer compartment inside refrigerator 3–6 months in freezer section of refrigerator with separate door 6–12 months in deep freeze (0 °F or lower)
Previously frozen Thawed in refrigerator but not warmed or used	4 h or less (i.e., next feeding)	Store in refrigerator 24 h	Do not refreeze
Thawed outside refrigerator in warm water	For completion of feeding	Hold for 4 h or until next feeding	Do not refreeze
Infant has begun feeding	Only for completion of feeding; then discard	Discard	Discard

Developed from Recommendations of the Milk Banking Association of North America, Inc. and current literature. *2011 Best Practice for Expressing, Storing and Handling Human Milk in Hospitals, Homes and Child Care Settings.* © Human Milk Banking Association of North America, 3rd Edition, 2011.

Table 7 Suggestions for milk storage for infant at home

Wash hands thoroughly
Polyethylene bags are acceptable for home use
Refrigerate or freeze milk after expressing
Use fresh milk whenever possible
Freeze milk that will not be used within 2 days
Use milk stored in a self-defrosting freezer within 3 months (top of refrigerator)
Use milk stored in a deep freezer within 12 months
Use the oldest milk first. Date container at time of collection

do, provide clean milk. She should also wash her hands before handling her lactating breast and before manually expressing milk. If she chooses to use a mechanical pump to express the milk, all of the pump parts that touch the breast or the milk should be chemically cleaned between pumpings and should be sterile when she first uses the equipment. No one else should use these disposable parts although the mechanical parts of the pump that do not touch the breast or the milk may be shared if cleaned with soap and water between individuals.

The containers where the milk is placed in for storage should be sterile if the infant is sick or hospitalized. The milk should be refrigerated as soon as possible unless it is immediately fed to the infant. If it is going to be stored for more than 4 days it should be frozen as soon as it is cooled, although it has been shown that it can be used if stored up to 8 days. These guidelines apply to milk saved for a healthy baby by a healthy mother (Tables 6 and 7).

Guidelines for saving milk for mother's sick and/or premature infant are much more rigid. Sharing stored, unpasteurized milk with other infants is not recommended. One concern in refrigerating milk for days is not bacterial growth but the possibility that the lipase normally present in human milk will begin to break down the lipids. An occasional mother produces large amounts of lipase that digests her milk if stored rendering it rancid and rejected by the infant. Brief scalding before storing has prevented this action by reducing the amount of lipase for these women.

Human Milk Banking

Human milk banks have been operating for more than 100 years. During this time the 'rules and regulations' have been developed. The Human Milk Banking Association of North America (HMBANA) has been the organization in North America that has standardized milk banking as it is not controlled by any agency, state, or federal. The guidelines start with the selection of donors who must be healthy, their infants must be healthy, their obstetrician and pediatrician must approve of their patient being a donor. Screening tests are also required. The donor must follow instructions for milk collection, storage, and transport. The milk bank pools the samples, pasteurizes the fluid, stores in sterile individual containers and freezes it. Its use is limited to infants whose physicians prescribe it. There is a fee to cover the processing. Donors are not paid for milk. The supplies are limited to sick infants in Neonatal Intensive Care Units and their graduates, i.e., medical need. Pasteurization carried out by heating at 62.5 °C for 30 min (Holder method) is commonly used. High temperature, short-term (HTST method) requires 72 °C for 15 s. It has been thoroughly studied and is considered more effective with less impact on the milk constituents. This method is used by commercial milk banks. This donor milk has proved to be not only safe but also life-saving for infants who receive it.

Careful study including both before and after cultures have shown that some are not removed by pasteurization so that the commercial milk banks utilize filtration. Of particular concern is *Bacillus cereus* that has been reported in one neonatal intensive care unit from contaminated ventilator equipment in one outbreak. Twenty two other cases are reported in the literature of infants in NICUs. This bacillus is anaerobic, spore-forming, gram positive, or negative bacterium found in dust, air, and water. In adults it has been associated with foodborne gastroenteritis. The magnitude of this problem or risk of cereus being a problem for banked human milk is not known. It is known to be a problem in powdered, artificial cow-milk-based formula.

Storage of human milk at home for one's own infant should follow the guidelines developed by the Academy of Breastfeeding Medicine who has developed a specific protocol

for choosing the container, collection, and storage. The details of storing and warming are included in the protocol which can be accessed on the Academy of Breastfeeding Medicine website at: http://www.bfmed.org/Resources/Protocols.aspx. One is cautioned not to refreeze thawed breast milk, not to warm it in a microwave, milk left in the feeding container after the feeding is completed should be discarded.

The use of banked donor human milk has been shown to be safe if processed by HMBANA guidelines. Many sick infants have benefited by its use. See table of Definition 7.

Prematures fed human milk (either mother's milk or donor milk) have a lower incidence of infection especially necrotizing enterocolitis. They also have shorter hospital stays compared to infants with comparable problems who do not receive human milk.

Summary

Breastfeeding by a healthy woman for her own baby is safe when suckled directly from the breast. Milk extracted from the breast for later feeding is safe if simple guidelines are followed the infection protective constituents of human milk magnify that safety. Donor human milk is safe if the simple guidelines are followed the infection protective constituents of human milk magnify that safety. Donor human milk is safe if the simple guidelines for donor selection, collection, pasteurizing, storing, and dispensing the milk are followed. Human milk has such tremendous benefits for the human infant that issues of maternal problems rarely outweigh the benefits.

There is a theoretic concern if the breastfeeding mother is ill, infected or takes certain medications. The magnitude of the concern is determined on an individual basis often described as the risk/benefit ratio. What is the risk of the problem compared to the tremendous benefit of human milk and being breastfed. Most local infections of the breast or a case of mastitis are usually not an indication to interrupt breastfeeding. However, it is recommended that a mother with HIV should not breastfeed in developed countries. In developing countries such as Africa it is safer to breastfeed in this case because the risk of infection and diarrhea that breastfeeding protects against is so high. Most maternal medications are safe or a safe alternative drug can be utilized. Radioactive medications used therapeutically would be contraindicated if they have a long half life such as Iodine 118. Here again a specific case should be discussed with the physician who can consult the Library of Medicine database, LactMed at http://toxnet.nlm.nih.gove/cgi_bin/sis/htmlgen?LACT. Consultation is also available at the Lactation Study Center at the University of Rochester School of Medicine (585) 275-0088 daytime, weekdays.

See also: Foodborne Diseases: Foodborne Diseases and Vulnerable Groups. Safety of Food and Beverages: Safety of Human Milk: Chemical Aspects

Further Reading

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Relevant Websites

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Safety of Human Milk: Chemical Aspects

GG Moy, Food Safety Consultants International, Geneva, Switzerland

Glossary

Endocrine disruptor Any exogenous chemical, or mixture of chemicals, that can interfere with any aspect of hormone action.

Toxic equivalency factor (TEF) Relative toxicity of an individual congener of TCDD, TCDF or dioxin-like PCB to the most toxic congener, 2,3,7,8-TCDD (which is assigned a TEF value of 1.0).

Toxic equivalent (TEQ) TEQs are used to report the toxicity-weighted mass of mixtures of dioxin-like chemicals based on their toxic equivalency factors (TEFs).

Introduction

Human milk is the natural and best food for infants because it contains the optimal composition to meet their nutritional needs in early life and provides significant immunological and psychological benefits. Evidence for the health advantages of breastfeeding has continued to increase. Recent studies confirm that the singular intestinal flora of the newborn is supported by the oligosaccharides in human milk so as to exclude potential pathogens. It is well established that breastfeeding reduces child mortality and has health benefits that extend into adulthood. The World Health Organization (WHO) recommends exclusive breastfeeding for 6 months, followed by continued breastfeeding with appropriate complementary foods.

Unfortunately the wholesomeness and safety of human milk has been compromised by the presence of exogenous chemicals, which may pose a health risk for the infant. To some extent, the contamination of human milk is a reflection of living in the modern world where chemicals play a large role. For example, pharmaceuticals are essential health technologies, but they may also be contraindicated during the lactation period because they can be excreted in human milk. Similarly, inhalation or dermal absorption can also result in the contamination of human milk. For example, dermal absorption of personal care products has resulted in the presence of triclosan, ultraviolet filters, and musk fragrances in human milk. However, the most widespread route of exposure to potentially toxic chemicals that appear in human milk is through food. Although an exogenous chemical may be present in human milk, this does not automatically mean that the chemical represents a health risk for the breastfed infant. For this purpose, the risk analysis framework is used to assess the potential impact of these chemicals on health. Yet, even this scientific approach has proven to be challenging because of uncertainty in assessing long-term health effects on the infant. At present, only in very rare situations involving high levels of contamination has it been prudent to advise mothers to limit or terminate breastfeeding.

Human milk is a unique biological matrix because it can provide exposure information not only about the breastfed infant, but also about the mother and the highly vulnerable fetus. The noninvasive method for the collection of human milk is simple and relatively free of risk, in contrast to the collection of blood or adipose tissue. Human milk is considered to be one of the most important biota for the monitoring of so-called persistent organic pollutants (POPs), such as dichlorodiphenyltrichloroethane (DDT) and dioxins, which are known to accumulate in the food chain. Consequently, human milk monitoring can also yield information about the levels and trends of POPs in the environment as well as the food supply. Better understanding of sources of harmful environmental chemicals can aid in the management of such chemicals by eliminating or reducing emissions or by establishing limits for their presence in specific foods. Although this article mainly addresses POPs, many of the principles and practices can be applied to other chemicals that may be found in human milk. Note that this article is the counterpart of another article in this encyclopedia.

Hazard Identification and Characterization of POPs in Human Milk

POPs are a group of chemicals which have been intentionally or inadvertently produced and introduced into the environment. Owing to their stability and transport properties, they are now widely distributed around the world, and are even found in places where they had never been used, such as the Arctic regions. Given their long half-lives and high fat solubility, POPs tend to biomagnify in the food chain and are particularly high in long-lived predatory species. POPs appear

at higher concentrations in fat-containing foods, especially meat, eggs, milk, and certain fish. POPs also bioaccumulate in the human body and are found in human milk. The original concerns about POPs were related to the organochlorine pesticides, such as DDT, but more recently, the focus of attention has been on POPs of industrial origin, most notably polychlorinated biphenyls (PCBs), and industrial by-products, especially polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). However, not all POPs are transmitted through food. For example, in the case of polybrominated diphenyl ethers, which are flame-retardant chemicals sprayed on cloth and used in plastics, the main exposure is through dust. As a group, POPs are of concern because many of these chemicals have been identified as endocrine disruptors.

Since 1963, the WHO Panel of the Joint Food and Agriculture Organization of the United Nations (FAO)/WHO Meetings on Pesticide Residues evaluated the safety data for a series of POP pesticides and established corresponding acceptable daily intakes (ADIs) for their use on food crops. The ADIs were deemed to present no appreciable risk when consumed over a lifetime. These included DDT, aldrin, dieldrin, endrin, heptachlor, and lindane. At the same time, the FAO Panel recommended a large number of Maximum Residues Limits (MRLs) for residues of these pesticides on food and animal fodder, which were subsequently adopted by the Codex Alimentarius Commission. Later when many countries withdrew the agricultural uses for these pesticides because of environmental and safety concerns, the WHO Panel reevaluated these pesticides as environmental contaminants and converted the ADIs to tolerable daily intakes, which were sometimes lower than the original ADI. Similarly, the FAO Panel converted the MRLs into Extraneous Maximum Residue Limits, setting many of these at the limit of prevailing methods of analysis.

Other pesticides that fall within the POPs category include chlordane, mirex, and toxaphene, but these were not approved for use on food crops. Hexachlorobenzene was approved as a fungicide on seeds, but proved to be extremely toxic and persistent in the environment, with a half-live of over 20 years. It is still one of the most commonly detected POPs in food in many countries that used this pesticide. In addition to these pesticides, alpha- and beta-hexachlorocyclohexane which occur as contaminants in the production of technical-grade gamma-hexachlorocyclohexane, i.e., lindane, are also POPs of concern. However, as all uses of lindane have been prohibited (except for medical treatment of head lice and scabies), these contaminants are no longer entering the environment.

POPs that are industrial chemicals or by-products have been evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) as contaminants in food. For example, JECFA has established tolerable intakes for PCDDs, PCDFs, and dioxin-like PCBs. In its most recent evaluation in 2001, JECFA established a provisional tolerable monthly intake for dioxins, dibenzofurans, and dioxin-like PCBs of 70 picograms (pg) (expressed in WHO toxic equivalents (WHO-TEQ)) per kg body weight per month, which is one of the lowest tolerable intakes ever set. In doing so, JECFA noted that mean exposure estimates for many populations approached or exceed this value.

Numerous environmental studies in wildlife and in laboratory animals have provided evidence that POPs may be involved with altered endocrine function, reproductive and immune dysfunction, neurobehavioral and developmental disorders, and cancer. More recently, some authors have implicated exposures to POPs in reduced immunity and increased infections, developmental abnormalities, and neurobehavioral impairments in infants and children as well as reproductive impairments, aspects of metabolic syndrome, and cancer in adults. Some POPs are being considered as a potentially important risk factor in the etiology of human breast cancer. More detailed discussions of these issues are provided elsewhere in this Encyclopedia.

Exposure Assessment of POPs in Human Milk

Since its establishment in 1976, the WHO Global Environment Monitoring System/Food Contamination Monitoring and Assessment Programme (or simply, GEMS/Food) has collected information on the levels and time-trends chemicals in food and human milk. In 1998, GEMS/Food published a literature search and health assessment of certain organochlorine pesticides and PCBs in human milk, which raised a number of health concerns. However, generally little information was available on the donors and frequently, basic information was not provided.

In regard to PCDDs, PCDFs and dioxin-like PCBs in human milk, WHO conducted a series of three surveys covering the periods 1987–88, 1992–93, and 2000–03, which included a number of countries in Europe. To ensure reliability and to improve comparability, WHO GEMS/Food carried out interlaboratory analytical quality assurance studies with a mixture of organochlorine pesticides. Only a few laboratories consistently performed well. Some laboratories failed to detect the presence of a chemical, whereas others reported chemicals that were not present. For the third round of WHO-coordinated exposure studies, the State Laboratory for Chemical and Veterinary Analysis of Food (CVUA) in Freiburg, Germany was the only laboratory to qualify as a WHO reference laboratory for these POPs in human milk based on stringent preagreed criteria. Since that time CVUA has also been designated as the European Union reference laboratory for dioxins. In the intervening years, the quality of analysis for PCDD/Fs has generally improved in terms of both capability and capacity and other quality parameters, possibly driven by demanding limits set for regulating foods by the EU following food scares, in particular, the dioxin incident in Belgium in 1999. Cost of analysis has also decreased significantly. Food is the most important source of exposure to POPs for those who are not occupationally exposed.

Stockholm Convention on POPs

In 2004, the Stockholm Convention on POPs was ratified by over 150 governments to decrease environmental and human exposure to initially 12 priority chemicals in this class. Subsequently in 2009, nine additional POPs were included under the convention and in 2011, endosulfan was added.

Table 1 Persistent organic pollutants included in the Stockholm Convention

Chemical name	*Pesticide*	*Industrial chemical*	*By-product*
Aldrin	X		
Chlordane	X		
DDT	X		
Dieldrin	X		
Heptachlor	X		
Mirex	X		
Toxaphene (over 200 isomers)	X		
Hexachlorobenzene	X		
Polychlorinated biphenyls (209 isomers)		X	
Polychlorinated dibenzodioxins (75 isomers)			X
Polychlorinated dibenzofurans (135 isomers)			X
Included as of 2009			
Chlordecone	X		
Alpha-hexachlorocyclohexane	X		X
Beta-hexachlorocyclohexane	X		X
Lindane (gamma-hexachlorocyclohexane)	X		
Hexabromodiphenyl		X	
Hexabromodiphenyl ether and heptabromodiphenyl ether		X	
Pentachlorobenzene	X	X	X
Perfluorooctane sulfonic acid, its salts, and perfluorooctane sulfonyl fluoride		X	
Tetrabromodiphenyl ether, and pentabromodiphenyl ether		X	
Included as of 2011			
Endosulfan (technical grade)	X		

A complete list of Stockholm Convention POPs along with their original sources (pesticide, industrial chemical, or by-product) is given in Table 1. Of particular relevance to human milk monitoring was Article 16 which requires periodic effectiveness evaluations of the convention. In this regard, experts convened by the United Nations Environment Programme (UNEP) considered how to implement these evaluations and recommended that a global survey of human milk be carried out in close collaboration with the WHO. This was later approved by the Stockholm Convention's Conference of Parties and in 2005, a memorandum of agreement was signed by the WHO and UNEP to formalize the arrangement.

WHO-Coordinated Global Survey of Persistent Organic Pollutants in Human Milk

In responding to the needs of the Stockholm Convention on POPs, WHO GEMS/Food developed a new protocol for the 4th WHO-coordinated global survey of POPs in human milk that would meet the combined health, food safety, and environmental objectives of WHO, UNEP, and their member counties. The protocol for survey included all 12 POPs initially covered by the Stockholm Convention and was designed based on the advice of international experts in the field and on extensive experience gained in previous surveys. To promote reliability of the data and comparability across time and among countries, participants are encouraged to adhere as closely to the protocol as possible. Given that breastfeeding reduces child mortality and has health benefits that extend into adulthood, every effort was made to protect, promote, and support breastfeeding in the context of these studies.

Analysis of Pooled and Individual Samples

Because of the high cost and analytical difficulty, the protocol uses pooled samples to monitor levels of PCDDs, PCDFs, and dioxin-like PCBs in human milk. This also avoids some ethical issues that may arise if the concentrations are known in the milk from a particular individual. These analytically complex POPs require ultrapure solvents and sophisticated equipment (high resolution gas chromatograph with two high resolution mass spectrometers coupled in tandem) and therefore have been analyzed at the WHO reference laboratory at CVUA. However, basic pesticide POPs and certain marker PCBs (analytically simple POPs) are analyzed in individual samples because of the lower cost and ease of analysis, which uses a gas chromatograph with an electron capture detector. This type of equipment is available in many developing countries. The analysis of the analytically simple POPs in developing countries was supported by periodic analytical proficiency testing. Table 2 provides a list of analytically complex and simple POPs and, where appropriate, their residue definitions, included in the 4th WHO-coordinated survey.

Results for Analytically Complex POPs

This was the 4th WHO-coordinated human milk survey of these chemicals undertaken since 1987. The current levels of PCDD/Fs were in the range of 4–10 WHO-TEQ (pg g^{-1} fat). However, perhaps the more important issue for human health and the environment is that levels have continued to show a decreasing trend over the past 20 years. Data for those countries that participated in three or more of the surveys is given in Figure 1.

The current levels of dioxin-like PCBs were in the range of 5–11 WHO-TEQ (pg g^{-1} fat). As with PCDDs and PCDFs,

Table 2 Analytically complex and simple pops and their residue definitions included in the 4th WHO-coordinated survey of persistent organic pollutants (POPs) in human milk

Analytically complex POPs
Polychlorinated dibenzodioxins (PCDDs) (total to be expressed in WHO-TEQs)
2,3,7,8-tetrachlorodibenzodioxin (TCDD)
1,2,3,7,8-pentachlorodibenzodioxin (PeCDD)
1,2,3,4,7,8-hexachlorodibenzodioxin (HxCDD)
1,2,3,6,7,8-HxCDD
1,2,3,7,8,9-HxCDD
1,2,3,4,6,7,8-heptachlorodibenodioxin (HpCDD)
1,2,3,4,6,7,8,9-octachlorodibendioxin (OCDD)
Polychlorinated dibenzofurans (PCDFs) (total to be expressed in WHO-TEQs)
2,3,7,8-TCDF
1,2,3,7,8-PeCDF
2,3,4,7,8-PeCDF
1,2,3,4,7,8-HxCDF
1,2,3,6,7,8-HxCDF
1,2,3,7,8,9-HxCDF
2,3,4,6,7,8-HxCDF
1,2,3,4,6,7,8-HpCDF
1,2,3,4,7,8,9-HpCDF
1,2,3,4,6,7,8,9-OCDF
Dioxin-like polychlorinated biphenyls (PCBs) (total to be expressed in WHO-TEQs)
Mono-ortho PCBs
IUPAC No. 105
IUPAC No. 114
IUPAC No. 118
IUPAC No. 123
IUPAC No. 156
IUPAC No. 157
IUPAC No. 167
IUPAC No. 189
Nonortho PCBs
IUPAC No. 77
IUPAC No. 81
IUPAC No. 126
IUPAC No. 169
Analytically simple POPs
Aldrin
Chlordane (total)
Alpha-chlordane
Gamma-chlordane
Oxy-chlordane
trans-Nonachlor
Dieldrin
Dichlorodiphenytrichloroethane (DDT) (total)
o,p′-dichlorodiphenyldichloroethane (DDD)
p,p′-DDD
o,p′-dichlorodiphenylethylene (DDE)
p,p′-DDE
o,p′-DDT
p,p′-DDT
Endrin (total)
Endrin
Endrin ketone
Heptachlor (total)
Heptachlor
Heptachlor epoxide
Hexachlorobenzene
Hexachlorocyclohexane (HCH) (total)
Alpha-HCH
Beta-HCH
Lindane (gamma-HCH)
Mirex
Toxaphene (total)
Parlar 26
Parlar 50
Parlar 62
Polychlorinated biphenyls (PCBs)
Marker PCBs
IUPAC No. 28
IUPAC No. 52
IUPAC No. 101
IUPAC No. 138
IUPAC No. 153
IUPAC No. 180

Abbreviations: IUPAC, International Union of Pure and Applied Chemistry; WHO-TEQs, World Health Organization toxic equivalents.

levels of dioxin-like PCBs have fallen over time as shown in Figure 2.

Results for Analytical Simple POPs

The new WHO protocol included analytically simple POPs, which basically are comprised of organochlorine pesticides. However, data on such POPs using the new protocol were available from only Belgium, Finland, Norway, and Sweden (see Table 3). Additional data collected with the WHO protocol will become available when the results of other countries are tabulated.

Although the first studies of DDT in human milk go back to the 1950s, the levels reported in such studies are only considered indicative because some reports were highly variable and few details on the sampling and methods of analysis were provided. The WHO conducted a literature search and prepared a risk assessment of the exposure of infants to some of these analytically simple POPs, which can be a useful point of reference. It should be noted that some of these POPs are extremely stable in soil and consequently, levels in food and in human milk may continue for many years. In developing countries where many of these POPs have been banned more recently and where some of these POPs, for example, DDT and lindane, are still used, levels in human milk are expected to be higher.

Risk Characterization of POPs in Human Milk

The risk characterization of POPs in human milk is hampered by the lack of consensus on health-based reference values that would protect nursing infants. JECFA has stated that the ADIs it establishes do not apply to infants under the age of 12 weeks. Although epidemiological studies suggest that exposure of the developing fetus to POPs can lead to significant long-term health effects, the additional exposure of the infant through contaminated human milk needs further study. However, measuring the levels of chemicals in human milk can provide the needed information, which is essential for understanding the risk posed by certain chemicals at critical

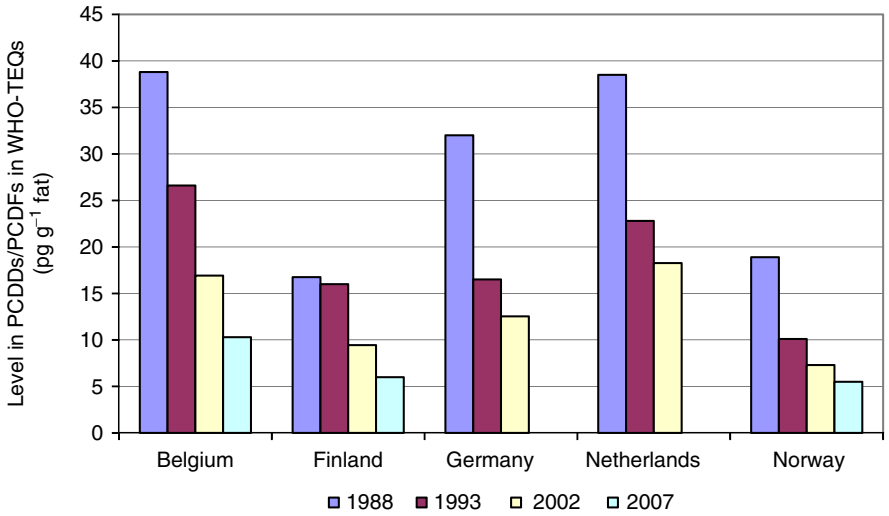

Figure 1 Trends in levels of PCDDs and PCDFs in human milk in selected countries.

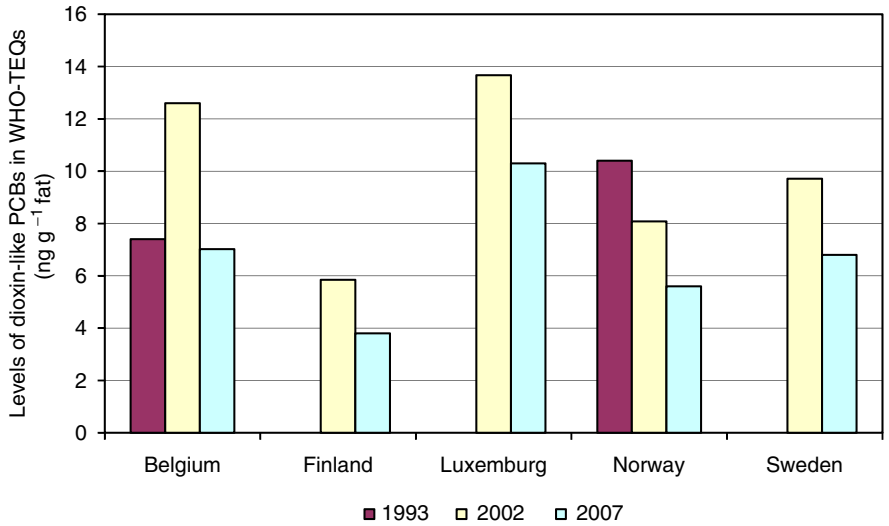

Figure 2 Trends in levels of dioxin-like PCBs in human milk in selected countries.

periods of development and for generally reducing exposure from food and the environment.

Risk Management of POPs in Human Milk

Because of health concerns raised by exposure to POPs *in utero* and through human milk, the risk management of POPs should be considered by both governments and individual mothers to reduce the risk of possible harm to the infant.

Primary preventive measures to eliminate and reduce the introduction of POPs in the environment are the most effective long-term way to control exposure to these chemicals. When possible, the use and emissions of POPs should be further reduced, including assuring the safe disposal of existing stocks.

Because food is the main source of exposure for the vast majority of individuals, responsible authorities should examine their food monitoring and control programs to assess whether greater attention should be paid to foodstuffs potentially high in POPs. It is also important to geographical areas with potential for increased infant exposure levels resulting from highly contaminated foodstuffs or other sources.

Maximum limits in foods and animal feed ingredients that are high in POPs should be considered. Limits established by the European Union for PCDD/Fs include milk and milk products, including butter fat – 3 pg g^{-1} fat; hen eggs and egg products – 3 pg g^{-1} fat; liver and derived products – 6 pg g^{-1} fat; fish oil – 2 pg g^{-1} fat; and, fish (flesh) 4 pg g^{-1} fresh weight.

Girls and young women should limit their intake of foods known to contain high levels of POPs in order to reduce their body the burden of POPs. Dietary advice for girls and women of child-bearing age should be promoted including consumption of less contaminated foods, for example, certain organic foods.

Epidemiological studies linked to POPs in food and human milk are urgently needed to assess the possible

Table 3 Levels of analytically simple POPs in human milk in selected countries

Basic POPs (ng g^{-1} fat)	*Belgium*	*Finland*	*Norway*	*Sweden*
Aldrin	ND	ND	ND	ND
Chlordane group	7.8	1	3.6	2.2
Dieldrin	6.7	1.5	2.5	1.8
DDT group	156.3	33.1	69.6	81.9
Endrin group	ND	ND	ND	ND
Heptachlor group	5.3	0.55	0.6	0.8
Hexachlorobenzene	15.0	2.7	16.9	7.1
Hexachlorocyclohexane (HCH) group				
Alpha-HCH	ND	ND	ND	ND
Beta-HCH	12.0	4	5.8	6.6
Gamma-HCH	0.7	1.3	0.5	0.9
Toxaphene (Parlar) group	2.3	1.9	3.7	2.4
Mirex	ND	ND	ND	ND

Abbreviations: DDT, Dichlorodiphenyltrichloroethane; ND, not detected; POPs, persistent organic pollutants.

long-term health risks to the newborn and growing infant from the intake of POP-contaminated breast milk. This will enable the provision of adequate advice on the best breastfeeding practice for mothers at risk, such as the maintenance of body weight during the lactation period. Of course, it also must be acknowledged that there is a large body of literature on endocrine disruptors that indicates that early life exposures can induce diseases that do not manifest until puberty or adulthood. Thus, epidemiology studies must consider this long timeframe implicated in many POP-associated human diseases.

Responsible authorities should consider incorporating mechanisms to assess potential health risks posed by contaminants in human milk into their national risk assessment procedures, as is already done for pharmaceuticals. In this regard, health-based reference values for exposure to POPs in human milk contamination will be required.

Decision-making for any contemplated intervention should take into account the well-established benefits of breastfeeding as well as socioeconomic factors. Except in the most extreme cases, mothers can and should be reassured that breast milk is by far the best food to give their babies.

Risks Posed by Lead in Human Milk

Whereas lead is toxic to essentially all organs of the body, it is particularly dangerous for infants and young children as it can cause blood and brain disorders. Lead has been demonstrated to irreversibly reduce the intelligence quotient of children at extremely low levels of exposure. It is also associated with learning, attention, and more recently, to violent behavior. Lead will accumulate in the bones of girls and young women through exposures from food, water, soil, and other environmental sources. During lactation, maternal bone lead stores from these past exposures may be mobilized along with calcium as part of the normal changes during pregnancy.

As with POPs, the early studies of lead in human milk lack sufficient details on the sampling and analytical quality assurance and methods to understand if the reported levels are accurate and representative. Very large ranges of values have been reported, but the elimination of lead in paint and fuel for motor vehicles in many countries has contributed to the decline of lead levels in human milk. Levels of lead in human milk reported from a number of countries are given in Table 4.

Table 4 Lead levels in human milk in selected countries

Country	*Year*	*Number of samples*	*Lead in milk (μg l^{-1})*
Australia	1998	9	0.7 $\pm$ 0.7
Canada	2003	25	2.08 $\pm$ 1.67
China	2008	1600	5.11
Brazil	2009	92	2.90 $\pm$ 1.7
Greece	2005	95	0.15 $\pm$ 0.25
Iran	2009	44	10.4 $\pm$ 4.7
Nigeria	2002	89	1.77 $\pm$ 0.24
Mexico	2004	310	1.4 $\pm$ 1.1
UK	2004	255	1.5 $\pm$ 1.2
Spain	2011	100	15.56
Sweden	1995	75	0.7 $\pm$ 0.4
Turkey	2005	143	2.34 $\pm$ 1.0
USA	2002	15	6.1 $\pm$ 1

As with POPs, the exposure to lead by girls and young women should be avoided. However, exposure to lead is possible from food, water, soil, or air. In some cases, traditional medicines and cosmetics can contain lead. It is also important to prevent the mother from immediate exposure to lead. Because there is a linear relationship between lead in human milk and maternal blood, monitoring maternal blood during pregnancy can be used as a screening tool. For example, maternal blood lead levels of 100 μg l^{-1} generally translate to approximately 5 μg l^{-1} in human milk, concentrations that are considered of little or no concern. In general, levels below 10 μg l^{-1} are considered acceptable for human milk. However, if levels are excessive, interventions may be considered. For example, a study in Mexico in 2004 indicated that lead mobilization can be minimized if the mother has a diet that is adequate in calcium. In 1994, The US National Institute of Health Consensus Conference on Optimal Calcium Intake recommended that for pregnant and lactating women the optimal daily intake of calcium should be 1200 mg day^{-1}.

Further details on lead are provided elsewhere in this encyclopedia.

Risks Posed by Mercury in Human Milk

Both organic and inorganic mercury can be present in human breast milk. Organic mercury in the form of methyl mercury is more hazardous for children, although inorganic mercury is also of potential concern. Methyl mercury, like lead, is a neurotoxin and can cause deficits in learning and memory. Methyl mercury levels in human milk are relatively low because it is tightly bound to protein and is not subject to mobilization. However, if the maternal diet is high in methyl mercury, human milk can become contaminated as the compound is metabolized. Because methyl mercury is readily absorbed in the intestine of a nursing infant, exposure through human milk may place the infant at risk. Serious adverse health effects have been documented in cases where the mothers of breastfed infants were exposed to high levels of methyl mercury via consumption of fish.

The second form, inorganic mercury, is more easily transmitted through human milk but less than 15% is absorbed by the gastrointestinal tract of the infant. Mercury levels in milk are generally three times lower than the levels in the mother's blood. Therefore, *in utero* exposure is probably more important than lactational exposure to mercury.

The average levels found in human milk are far below those that cause acute poisonings. Most mothers are exposed to methyl mercury through fish consumption, particularly consumption of predator fish such as swordfish, shark, and tilefish that are known to be high in methyl mercury. Other sources of mercury include coal burning, incineration, chlorine manufacturing, and mining, as well as some natural sources. Inorganic mercury exposure primarily comes from cereal grains, which are consumed in large amounts.

In most studies, total mercury is analyzed because of the difficulty in differentiating between organic and inorganic species. Data on the levels of total mercury in human milk are limited because hair and maternal blood are preferred matrices for biomonitoring. Table 5 provides reports on levels of total mercury in human milk in a number of countries.

Because of the established adverse neurological effects of mercury, both pregnant and nursing women should avoid the consumption of fish that are high in methyl mercury. Various education programs for women of child-bearing age have been developed as countries attempt to address other environmental emissions. As noted earlier, the biomonitoring of maternal blood and hair can also provide useful markers of exposure that can be used to assess the safety of human milk. Further details on mercury are provided elsewhere in this Encyclopedia. In addition, a recent opinion of the European Food Safety Authority (EFSA) on mercury and methyl mercury in food includes consideration of human milk.

Table 5 Levels of total mercury in human milk in selected countries

Country	*Year*	*Number of samples*	*Total mercury in milk ($\mu g\ l^{-1}$)*
Austria	2002	116	1.59 ± 1.21
Iran	2012	80	0.86 (high fish consumers) 0.13 (low fish consumers)
Slovakia	2005	158	0.94
Spain	2011	100	0.53
Turkey	2010	44	3.42 ± 1.66

Special Considerations When Undertaking Studies Involving Human Milk

Studies involving human milk have a number of special considerations that need to be taken into account. Because human subjects and tissues are involved, the following areas should be addressed when planning to conduct any survey of human milk.

Ethics

Any study that involves human subjects must be carefully planned and approved by an authorized ethics review committee to be certain that the rights of the individuals participating in the study are protected. Mothers donating samples of their milk should be informed of the nature and purpose of the survey and asked to sign an informed consent form for this purpose. It is the responsibility of the lead researcher to ensure that the protocol for the study meets all national ethical and informed consent requirements. This includes adequate safeguards to ensure the confidentiality of any information regarding the donors and especially the results, which should only be identified by code. Based on national requirements, donors may be provided with the results of their samples. If such information is provided, considerable judgment must be used in characterizing various levels of POPs. The provision of individual results should always be accompanied by an explanation giving the range of other results and a short interpretation of the health significance of the values.

General Principles

Because studies will often involve primiparae mothers, the practicality, feasibility, and sustainability of sample collection from the donor's perspective should be given the highest priority. In addition, the following principles should be considered during development of the protocol and implementation of the study:

- Breastfeeding should be protected, promoted, and supported.
- The health benefits of breastfeeding for both mother and baby should be clearly and consistently communicated.
- Sampling of milk should not be an undue burden on the mother nor should it compromise the nutritional status of the infant.
- Ethical review, including prior informed consent, should be respected.
- Safeguarding of confidential information should be assured.

- Analytical quality assurance should be independently confirmed.

Criteria for the Selection of Donors

The criteria for selection of donors need to be designed to reduce the variability among the individual samples due to factors that are known to influence the levels of environmental chemicals in human milk. When the intention is to assess trends in levels over time, the reduction in variability is particularly important. However, overly stringent criteria in the selection of donors may give rise to an insufficient number of qualified donors. Consideration of available statistics on primiparae mothers and experiences from other studies involving mothers may be of assistance when developing selection criteria for donors. The following criteria were developed for a study of POPs and may be appropriate for other studies of chemicals that bioaccumulate in humans:

- Mother should be primiparae.
- Mother should be under 30 years of age.
- Both mother and child should be apparently healthy, including normal pregnancy.
- Mother should be breastfeeding one child only (i.e., no twins).
- Mother should have resided in the area for at least the previous 10 years.
- Mother should not reside in local areas where emissions of POPs are known to occur.
- Mothers should be available for sample collection within 3–8 weeks of delivery.

The location of residence of the donor, usually urban or rural, may also be associated with different exposure levels. Living in highly polluted areas, such as in the vicinity of incinerators, pulp and paper industries, and metal industries, is known to influence exposure to some chemicals. Persons with markedly different exposures should not be included in the survey to avoid skewing the results. Factors known to have such effects should be included in the protocol as exclusion criteria.

Collection of Samples

Once breastfeeding is well established, sampling can be carried out between 3 and 8 weeks (21 days to 2 months) after delivery. Donors should receive verbal and written information concerning the survey. The procedures of the survey should be explained, particularly the rights of the donor to withdraw from the survey without prejudice. Following this, the donor should be requested to give their written consent on a standard informed consent form. The sample can then be collected.

Preferably mothers should provide the sample at a local place of contact where collection can be supervised. At least 50 ml of milk in total should be collected by hand expression after a feeding or while the infant is nursing on the other breast, to take advantage of the let-down reflex of the mother. Depending on the wishes of the mother and local customs, a human milk pump to facilitate expression can be provided. Alternatively, the mother may collect the sample at home, in which case manual expression is preferred. If so, she should be given detailed instructions for taking, storing, and transporting of milk samples. Mothers should also be given a clean glass jar with a protected screw cap to collect and store the milk sample. Sample collection jars should be labeled with the donor's individual identification code and not the name of the mother. Milk samples may be stored in the refrigerator at approximately 4 °C for a maximum of 72 h, or for longer times in the freezer at −20 °C.

Biosafety

One of the criteria for selecting women as potential donors is that both the mother and infant should be apparently healthy following a normal pregnancy. The reasons for this criterion are to avoid extra demands on a mother who is already experiencing difficulties and to minimize results that may be caused by medical conditions. For POPs, a sudden loss of weight may mobilize fat stores and increase the levels of POPs in the milk. Consequently, donors with previously diagnosed clinical hepatitis, malaria, acquired immunodeficiency syndrome, and other such diseases should be excluded from the study. In many countries, pregnant women are screened for a number of infectious diseases so that their health status can be evaluated.

In countries which have established human immunodeficiency virus (HIV) screening of pregnant women, the National Coordinator should decide whether HIV-positive donors should be excluded from the study. In this regard, potential weight loss of donors could be an issue as well as the biosafety of the samples. In some countries, discrimination based on HIV status is not allowed legally and in certain countries, a person's HIV status is considered confidential. Although the infectivity of human milk from HIV-positive mothers is considered low when ingested by infants, for the purpose of this study, such milk should be considered infectious unless it is decontaminated. Therefore, any milk sample known or suspected to be contaminated with HIV should be decontaminated by heating at 62.5 °C for 30 min. Such treatment does not alter the levels of POPs in the sample. Similarly for countries with HIV morbidity and no HIV screening, human milk samples should be considered contaminated and heat-treated as above. Staff working with such samples should be suitably trained and provided with personal protective equipment.

Number of Samples

To get statistically reliable data, a minimum of 50 individual samples was recommended for each country. To determine the feasibility of this number of samples, information on the number of infants born to primiparae mothers should be available from the health statistics databases and other records. However, some flexibility may be necessary for countries with small populations and/or low birth rates. If this is a problem, extending the sample collection period should be considered as the first option to increase the number of available donors. In some cases, reducing the number of

donors may be unavoidable, but the impact on the statistical power of the survey to detect differences between time periods should be carefully considered. However, the power of the survey can be increased by the inclusion of more than 50 individual samples and is encouraged. In particular, countries with populations greater than 50 million should include at least one additional participant per one million population over 50 million. Countries with populations well over 50 million (or with sufficient resources) are encouraged to prepare a second pooled sample (or more) if feasible.

Pooled versus Individual Samples

Because some analytical methods involve ultrapure solvents and sophisticated instrumentation, the cost of analysis may be prohibitive. For example, the cost of analyzing PCDDs, PCDFs, and dioxin-like PCBs was estimated to be US$2000 per sample when some of the earlier studies took place, although this has reduced considerably in recent years. Obviously, the analysis of pooled human milk samples is also far less expensive than the analysis of individual samples. In addition, it is easier for each donor to provide the lower volume of milk required for pooled analyses. If the purpose of the study is to estimate an average level for a population, this approach can save time and money because combining equal amounts of each sample represents a physical averaging of all the samples. However, the analysis of individual samples can provide information on the distribution of exposures and on factors possibly contributing to exposure. In addition, such data are also essential to statistically validate that two average values are indeed different based on the distributions of their individual results.

Analytical Considerations

Once a chemical of interest has been identified, the next task is to define for analytical purposes the actual residue(s) to be measured. In most cases, this will include the parent compound, but for many chemicals, the number of possible isomers may be quite large. For example, PCBs exist as 209 possible isomers; PCDDs have 75 isomers; and, PCDFs have 135 isomers. In other cases, such as DDT, degradation products are also included in the residue definition. Another complication in relation to POPs is that the potential to bind to endocrine receptors varies significantly among different POPs as well as among the isomers of the same POP. Therefore, the concentrations of various POPs must be corrected with its corresponding WHO toxic equivalence factor. Consequently, the residue definitions for PCBs, PCDDs, and PCDFs are based on a combination of not only their expected distributions, but also their relative toxic potencies.

See also: Environmental Contaminants: Dioxins, Furans and Dioxin-like Polychlorinated Biphenyls; Environmental Estrogens – Hazard Characterization. Hazards of Food Contact Material: Bisphenol A and Endocrine Disruption. Pesticide Residues: Organochlorines. Safety of Food and Beverages: Safety of Human Milk: Microbiological Aspects. Toxic Metals: Lead; Mercury

Further Reading

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World Health Organization.

SAFETY OF FOOD AND BEVERAGES

Halal Food Requirements

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Glossary

Hadith Sayings of Prophet Muhammad to denote examples for one to follow.
Halal An Arabic word meaning lawful or permitted to consume, variantly spelled as hallal, halaal, halalan, or helal.
Shari'ah Comprehensive Islamic laws governing a Muslim's life, including those of halal food laws and practices.
Sunnah Traditions of Prophet Muhammad including his sayings and practical examples of actions to teach Muslims about the observance of their faith.
Zabiha An Arabic term, literally meaning slaughtered but generally perceived as manually slaughtered by a Muslim, as opposed to mechanical slaughtering of some animals especially poultry.

Introduction

The objective of this article is to describe halal laws as they apply in the food industry, and to elaborate on ensuring the integrity of the process of making halal food including halal certification. It also highlights and emphasizes the importance of understanding how halal foods are produced, and how important its compliance is to all stakeholders, producers, marketers, regulators, and consumers. Special emphasis is on the religious significance of dietary laws for Muslims, as they are understood by the food industry in order to serve this segment of the global population exceeding 1.57 billion halal consumers.

The halal dietary laws determine which foods are lawful or permitted for Muslims. These laws are found in the Quran, the holy book and *Sunnah*, the practice of Prophet Muhammad, as recorded in the books of the traditions, called *Hadith*. Islamic law is referred to as *Shari'ah* and has been interpreted by Muslim scholars over the years. The basic principles of the Islamic laws remain definite and unaltered. However, their interpretation and application may change according to the time, place, and circumstances. Besides the two basic sources of Islamic law, the Quran and *Sunnah*, two other sources of jurisprudence are used in determining the permissibility of food, when a contemporary situation is not explicitly covered by the first two basic sources; the first is *Ijma* meaning a consensus of legal opinion, and second is *Qiyas*, meaning reasoning by analogy. In the latter case the process of *Ijtihad*, or exerting oneself fully to arrive at an answer to the problem, is used.

Current issues of genetically modified organisms (GMOs), animal feed, hormones, etc., are discussed in the light of these two concepts and several other lesser sources of Islamic jurisprudence. Unconventional sources of ingredients, synthetic materials, and innovations in animal slaughter and meat processing are some of the issues Muslim scholars are dealing with in helping the consumers make informed choices.

It is important to note that, the health aspects of eating are an important consideration with halal food laws. These laws are viewed by the community as divine guidance without a need for explanations. Only in recent times some people have felt a need to try to justify them as health laws.

Basis of Halal Food Laws

Why do Muslims follow the halal dietary laws? The main reason for the observance of halal food laws in the Islamic faith is to follow the Divine Orders.

> "O ye who believe! Eat of the good things wherewith We have provided you, and render thanks to God if it is He whom ye worship." (Quran II: 172)

God reminds the believers time and again in the Holy Scripture to eat what is 'Halalan Tayyiban,' meaning 'permitted and good or wholesome.'

> "O, Mankind! Eat of that which is Lawful and Wholesome in the earth..." (Quran II: 168)

> "Eat of the good things. We have provided for your sustenance, but commit no excess therein." (Quran XX: 81)

Again in the Quran, Chapter VI, titled the Cattle, Muslims are instructed to eat the meat of animals on which God's name

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has been invoked. This is generally interpreted as meaning that an invocation has to be made at the time of slaughtering an animal.

> "Eat of that over which the name of God hath been mentioned, if ye are believers in His revelations." (Quran VI: 119)

Although Muslims eat what is permitted specifically or by implication, albeit without comment, they avoid eating what is specifically disallowed, such as:

> "And eat not of that whereupon God's name hath not been mentioned, for lo, it is abomination. Lo! The devils do inspire their minions to dispute with you. But if ye obey them, ye will be in truth idolaters." (Quran VI: 121)

The majority of Islamic scholars are of the opinion that this verse deals with proper slaughtering of allowed animals.

As Muslim dietary laws relate to divine permissions and prohibitions, if anyone observes these laws, he/she is rewarded in the hereafter and if anyone violates these laws, he/she may receive punishment accordingly. The rules for those foods that are not specifically prohibited may be interpreted differently by various scholars. The things that are specifically prohibited are just a few in numbers, as summarized in the following verse:

> "Forbidden unto you are: carrion and blood and swine flesh, and that which hath been dedicated unto any other than God, and the strangled, and the dead through beating, and the dead through falling from a height, and that which hath been killed by the goring of horns, and the devoured of wild beasts save that which ye make lawful, and that which hath been immolated to idols. And that ye swear by the divining arrows. This is abomination." (Quran V: 3)

Although these permissions and prohibitions as a divine injunction are enough for a Muslim to observe the laws, it is believed that the dietary laws are based on health reasons that suggest impurity or harmfulness of prohibited foods.

The Halal Market

Why are we concerned about halal in the secular world? Because halal is an important component of the food business. Most people, even in the food industry, are not aware of the breadth of foods that are under religious supervision. This section provides a background on the economic aspects that make it important for the food industry to have a better understanding of halal.

The Muslim population in the US is developing a stronger marketplace presence each year. Over the past 30 years many halal markets and ethnic stores have sprung up, mainly in the major metropolitan areas. Most of the eight million Muslims in North America observe halal laws, particularly the avoidance of pork, but the food industry has, for the most part, ignored this consumer group. Although there are excellent opportunities to be realized in the North American halal market, even more compelling opportunities exist on a worldwide basis as the food industry moves to a more global business model. The number of Muslims in the world is more than 1.57 billion people and trade in halal food products is approximately $661.6 billion. Several recent reports estimate the Muslim population to be higher than 1.60 billion globally and estimate the global halal trade at $2.3 trillion total and $661.6 billion for food alone. Many countries of South Asia, Southeast Asia, the Middle East, and Northern Africa have predominantly Muslim populations. Although only approximately 15% of India's population is Muslim, it is the second largest Muslim country in the world after Indonesia. In many countries halal certification has become necessary for products to be imported.

Although some Muslims purchase kosher food in the US, these foods, do not always meet the needs of the Muslim consumer. The most common areas of concern for the Muslim consumer when considering the purchase of kosher products are the use of various questionable gelatins in products produced by more lenient kosher supervisions and the use of alcohol as a carrier for flavors, as well as a food ingredient. The details of both ideas will be developed later in this article.

In recent years, many other universities are also exploring kosher/halal and multicultural food options.

Discussion on Halal Food Laws

The halal dietary laws define food products as halal or permitted and haram or prohibited, whereas a few items may fall into *Makrooh* or detestable category. The Islamic food laws deal with the following five major issues; all but the last one are in the animal kingdom.

1. Prohibited animals.
2. Prohibition of blood.
3. Method of slaughtering/blessing.
4. Prohibition of carrion.
5. Prohibition of intoxicants.

The Islamic dietary laws are derived from the Quran, a revealed book; the *Hadith*, the traditions of Prophet Muhammad; and through the extrapolation and deduction from the Quran and *Hadith* by Muslim jurists.

There are 11 generally accepted principles pertaining to halal and haram in Islam for providing guidance to Muslims in their customary practices.

The basic principle is that all things created by God are permitted, with a few exceptions that are prohibited. Those exceptions include pork, blood, and meat of animals that have died of causes other than proper slaughtering, food that has been dedicated or immolated to someone other than God, alcohol, intoxicants, and inappropriately used drugs.

1. To make lawful and unlawful is the right of God alone. No human being, no matter how pious or powerful, may take it into his hands to change it.
2. Prohibiting what is permitted and permitting what is prohibited is similar to ascribing partners to God. This is a sin of the highest degree that makes one fall out of the sphere of Islam.

3. The basic reasons for the prohibition of things are due to impurity and harmfulness.
4. A Muslim is not supposed to question exactly why or how something is unclean or harmful in what God has prohibited. There might be obvious reasons and there might be obscure reasons. The underlying principle behind the prohibitions remains the Divine order: "FORBIDDEN UNTO YOU ARE....".
5. What is permitted is sufficient and what is prohibited is then superfluous. God prohibited only things that are unnecessary or dispensable while providing better alternatives. People can survive and live better without consuming unhealthy carrion, pork, blood and the root of many vices, and alcohol.
6. Whatever is conducive to the prohibited is in itself prohibited. If something is prohibited, anything leading to it is also prohibited.
7. Falsely representing unlawful as lawful is prohibited. It is unlawful to make flimsy excuses, to consume something that is prohibited, such as drinking alcohol for supposedly medical reasons.
8. Good intentions do not make the unlawful acceptable. Whenever any permissible action of the believer is accompanied by a good intention, his action becomes an act of worship. In the case of haram, it remains haram, no matter how good the intention or how honorable the purpose may be. Islam does not endorse employing a haram means to achieve a praiseworthy end. The religion indeed insists not only that the goal be honorable but also means chosen to achieve it be lawful and proper. Islamic laws demand that the right should be secured solely through just means.
9. Doubtful things should be avoided. There is a gray area between clearly lawful and unlawful. This is the area which falls under doubtful. Islam considers it an act of piety for the Muslims to avoid doubtful things, for them to stay clear of the unlawful. Prophet Muhammad said:

> "The halal is clear and the haram is clear. Between the two there are doubtful matters concerning which people do not know whether they are halal or haram. One who avoids them in order to safeguard his religion and his honor is safe, while if someone engages in a part of them, he may be doing something haram."

10. Unlawful things are prohibited to everyone alike. Islamic laws are universally applicable to all races, creeds, and sexes. There is no favored treatment of a privileged class. Actually, in Islam, there are no privileged classes; hence, the question of preferential treatment does not arise. This principle applies not only among Muslims but also between Muslims and non-Muslims.
11. Necessity dictates exceptions. The range of prohibited things in Islam is quite limited, but emphasis on observing the prohibitions is very strong. At the same time, Islam is not oblivious to the exigencies of life, to their magnitude, or to human weakness and capacity to face them. A Muslim is permitted, under the compulsion of necessity, to eat a prohibited food to ensure survival – but only in quantities sufficient to remove the necessity and avoid starvation.

Prohibited and Permitted Animals

Meat of pigs, boars, and swine is strictly prohibited, as are the carnivorous animals such as lions, tigers, cheetahs, cats, dogs, and wolves. Also prohibited are birds of prey such as eagles, falcons, osprey, kites, and vultures.

Meat of domesticated animals like ruminants with split hooves, for example, cattle, sheep, goat, and lamb, is allowed for food, as are camels and buffaloes. Also permitted are the birds that do not use their claws to hold down food, such as chickens, turkeys, ducks, geese, pigeons, doves, partridges, quails, sparrows, emus, and ostriches. Some of the animal and birds are permitted only under special circumstances or with certain conditions. Horsemeat may be permitted for consumption under some distressing conditions, discussion of which is beyond the scope of this article. The animals fed unclean or filthy feed, for example, formulated with biosolids (sewage) or protein from tankage, must be quarantined and placed on clean feed for a period varying from 3 to 40 days to cleanse their systems before slaughter.

Food from the sea, namely, fish and seafood, are the most controversial among various denominations of Muslims. Certain groups, particularly Shi'a, only accept fish with scales as halal, whereas others consider as halal everything that lives in the water all the time. Consequently, prawns, lobsters, crabs, and clams are halal but may be detested (*Makrooh*) by some and hence, not consumed. Animals that live both in water and on land, amphibians, such as frogs, turtles, crocodiles, and seals are also not consumed by the majority of observant Muslims.

There is no clear status of insects established in Islam except that the locust is specifically mentioned as halal. Insects are generally considered neutral. However, from deduction of the laws, it seems that both helpful insects like bees, ants, and spiders, and harmful or dirty creatures like lice, flies, and mosquitoes are all prohibited as food. Among the by-products from insects, the use of honey was very highly recommended by Prophet Muhammad. Other products like royal jelly, wax, shellac, and carmine are acceptable to be used without restrictions by most; however, some may consider shellac and carmine to be questionable.

Eggs and milk from permitted animals are also permitted for Muslim consumption. Eggs from chicken, ducks, geese, etc. and milk from cows, goats, sheep, and buffaloes are halal.

Prohibition of Blood

According to the Quranic verses, blood that pours forth is prohibited for consumption. It includes blood of permitted and nonpermitted animals alike. Liquid blood is generally not offered for sale or consumed by Muslims or non-Muslims, except in certain tribal areas, but products made from blood are available. There is general agreement among Muslim scholars that anything made from blood is unacceptable. Products like blood sausage and ingredients like blood albumin are either haram or questionable at best, and should be avoided for product formulations.

Proper Slaughtering of Permitted Animals

There are special requirements for slaughtering the animal:

- An animal must be of a halal species.
- It must be slaughtered by a competent Muslim of sound mind.
- God's name must be invoked on each animal at the time of slaughter.
- Slaughter must be done by cutting the throat in a manner that induces rapid and complete bleeding, resulting in the quickest death. Generally accepted method is to cut at least three of the four passages, i.e., carotids, jugulars, trachea, and esophagus. Some Islamic scholars do accept mechanical slaughter, particularly of poultry. However, in recent years the trend has reverted toward manual slaughter of these animals.

The meat of animals thus slaughtered is called zabiha or dhabiha meat. "Verily God has prescribed proficiency in all things. Thus, if you kill, kill well; and if you perform dhabiha, perform it well. Let each one of you sharpen his blade and let him spare suffering to the animal he slays."

Islam places great emphasis on the gentle and humane treatment of animals, especially before and during slaughter. Some of the conditions include giving the animal proper rest and water; avoiding conditions that create stress; not sharpening the knife in front of the animals; and using a very sharp knife to slit the throat. Only after the blood is allowed to drain completely from the animal and the animal has become lifeless can the dismemberment, cutting off horns, ears, legs, etc. commence. Animal-derived food ingredients like emulsifiers, tallow, and enzymes must be made from animals slaughtered by a Muslim, for them to be halal. There are some differences between Sunni and Shi'a Muslims as well as some cultural differences regarding slaughtering animals.

Hunting of permitted wild animals, like deer, and birds like doves, pheasants, and quail is permitted for the purpose of eating but not merely for deriving pleasure out of killing an animal. Hunting during the pilgrimage to Mecca and within the defined boundaries for pilgrimage is prohibited. Hunting is permitted with any tools, for example, guns, arrows, spears, or trapping. Trained dogs may also be used for catching or retrieving the hunt. The name of God must be invoked at the time of shooting a bullet or releasing the tool rather than after catching the hunt. The animal has to be bled by slitting the throat as soon as it is caught. If the blessing is made at the time of pulling the trigger or shooting an arrow and the hunted animal dies before the hunter reaches it, it would still be halal as long as slaughter is performed and some blood comes out. Fish and seafood may be hunted or caught by any reasonable means available as long as it is done humanely and invoking God's name is not required in such conditions.

The requirements of proper slaughtering and bleeding are applicable to land animals and birds. Fish and other creatures that live in water need not be ritually slaughtered. Similarly there is no special method of killing the locust.

The meat of the animals that die of natural causes; for example, diseases, being gored by other animals, being strangled, falling from a height, through beating, or killed by wild beasts; is unlawful to be eaten, unless one saves such animals by slaughtering before they actually become lifeless. Fish that dies naturally and is floating on water or lying out of the water is still halal as long as it does not show any signs of decay or deterioration.

Meat of Animals Killed by Christians or Jews

There has been considerable discussion and controversy among Muslim consumers as well as Islamic Scholars about the permissibility of consuming meat of animals killed by the Christians and Jews, known in the Quran as People of the Book. The issue focuses on whether meat prepared in the manner practiced by either faith would be permitted for Muslims.

In the Holy Quran, this issue is presented only once in Chapter V, verse 5, in the following words:

> "This day all good things are made lawful for you. The food of those who have received the Scripture is lawful for you, and your food is lawful for them."

This verse addresses the Muslims and seems to establish a social context where Muslims, Jews, and Christians could interact with each other. It addresses Muslims saying that food of the People of the Book is lawful for them and Muslim food is lawful for Jews and Christians. In most discussions, scholars try to deal with the first part, food of the People of the Book, and avoid the second part, food of Muslims, altogether, leaving that decision to the People of the Book.

The majority of Islamic scholars are of the opinion that the food of the People of the Book must meet the criteria established for halal and wholesome food including proper slaughter of animals. They believe that the following verse establishes a strict requirement for Muslims.

> "And eat not of that whereupon God's name hath not been mentioned, for lo! It is abomination." (Quran VI: 121)

However, some Islamic scholars are of the opinion that the above verse does not apply to the food of the People of the Book and there is no need to mention the name of God at the time of slaughtering. It is up to the regulatory agencies in the halal food importing countries, halal certifiers for export or domestic consumption, or the individual Muslim consumers to decide how to interpret this verse.

Prohibition of Alcohol and Intoxicants

Consumption of alcoholic drinks as intoxicants is prohibited according to the Quran (V: 90–91), as follows:

> "O you who believe! Fermented drinks and games of chance, and idols and divining arrows are only an infamy of Satan's handiwork. Leave it aside in order that you may prosper. Only would Satan sow hatred and strife among you, by alcohol, and games of chance, and turn you aside from the remembrance of God, and from prayer: Will you not, therefore, abstain from them?"

The Arabic term used for alcohol in the Quran is *khamr*, which means, that which has been fermented, and implies not

only to alcoholic beverages like wine, beer, whiskey, and brandy, but also to imply all things that intoxicate or affect one's thought process. Although there is no allowance for added alcohol in any beverage like soft drinks, small amounts of alcohol contributed from food ingredients may be considered an impurity and hence ignored. Synthetic or grain alcohol may be used in food processing for extraction, precipitation, dissolving, and other reasons, as long as the amount of alcohol remaining in the final product is very small, generally below 0.1%. Each importing country may have its own guidelines, which must be understood by the exporters and strictly adhered to.

In the West, food may be cooked in alcohol to enhance the flavor or impart distinctive flavor notes. Wine is the most common form of alcohol used in cooking. Although one may think that all the added alcohol evaporates or burns off during cooking, the fact is that it does not. The alcohol retained in the food products varies depending on the cooking method.

Even after cooking for 2.5 h, up to 5% alcohol remains in the food. Although there is little chance of intoxication by eating such food, the use of alcoholic drinks in cooking is categorically discouraged.

Food Preparation, Processing, and Sanitation

There are no restrictions about cooking in Islam, as long as the kitchen is free from haram foods and ingredients. In food companies, haram materials should be kept segregated from halal materials. The equipment used for nonhalal products has to be thoroughly cleansed using proper techniques of acids, bases, detergents, and hot water. It is recommended that food processors use dedicated equipment and utensils for producing halal food.

Gelatin

Important in many food products, gelatin is probably the most controversial of all modern halal ingredients. Gelatin can be derived from pig skin, cattle bones, and cattle hides. In recent years, some gelatins from fish skins have also entered the market. Fish gelatins can be produced halal with proper supervision, and acceptable to almost all of the mainstream religious supervision organizations. Most currently available gelatins – even if called kosher – are not acceptable to the mainstream US kosher supervision organizations and Islamic scholars. Many are, in fact, totally unacceptable to halal consumers because they may be pork-based gelatin. All major manufacturers and some smaller ones are currently producing certified halal gelatin from cattle bones and hides of animals that have been slaughtered by Muslims.

Biotechnology

Ingredients made through biotechnology and genetic engineering of organisms, GMO ingredients have gained acceptance by Islamic scholars since the production of vegetable chymosin or synthetic rennet. The basis for this acceptance includes the fact that the genetic material is isolated from permitted animal and copied onto microbial systems to produce nature identical ingredients. Thus, the original source of the gene is essentially lost by the time it enters the food products. The production conditions and media for the microbial growth meet halal requirements. Many food ingredients and crops are now produced through genetic modifications and very few questions are raised about their acceptance.

Dealing with Halal Supervision Agencies

In practical terms, the food industry works with halal supervision agencies to obtain permission to use the supervision agency's trademark symbol on their products. In this way, the industry can make claims in the marketplace that are credible to those intentionally purchasing these products. Halal supervision is taken on by a company to expand its market opportunities, domestically as well as internationally. What criteria should a company use to select a supervision agency? Supervision fees must be taken into account, and the agency's name recognition is a consideration. Out of hundreds of halal certifications around the globe, only a handful are accepted by importing countries like Malaysia, Indonesia, Singapore, United Arab Emirates, and other Middle Eastern countries. Besides credibility and acceptance, other important considerations include:

1. Responsiveness in handling paperwork; providing Muslim inspectors at the plants on a timely basis; and in doing routine inspections at a defined frequency during the year.
2. Willingness to work with the company on problem solving.
3. Ability to clearly explain their halal standards and their fee structure.
4. Whether or not an agency's halal standards meet the company's needs in the marketplace; meaning the acceptability among the consumers and importers.

Many halal certification bodies have sprung up throughout the world; some of them are nonprofit organizations, whereas other government agencies, private for profit businesses, multinational quality assurance corporations, and even some private individuals. Some groups and individuals have resorted to certifying their own products. If one has any interest in exporting to Muslim countries or countries with a significant Muslim population, it is extremely important to know which countries will accept the supervision of which agencies.

In recent years we have started to see products that have dual halal and kosher certification. The first of such products were the ready-to-eat meals for the US Military (meals ready-to-eat, MREs), but the market has expanded to other industrial and consumer products. Some of the civilian versions of the MREs are now available in long-term shelf-stable (nonrefrigerated) form that makes them convenient for transport, store, and use. Only vegetarian and dairy products have received dual certification.

Ingredient companies should be particularly careful in selecting a supervision agency. They should try to use a mainstream halal supervision agency because most halal food manufacturing companies will require such supervision. The

ability to sell to as many customers as possible requires a broadly acceptable agency.

The halal symbol of the certifying agency or individual doing the certification may appear on the packaging. In some industrial situations, where halal and nonhalal products are similar, some sort of color coding of product labels and packages may also be used. Most of these religious supervision symbols are trademarks that are duly registered. In many countries a generic halal symbol, i.e., the word halal in Arabic in a circle has been used indiscriminately. Muslim consumers do not have much faith in such a symbol. In North America some small companies have used similar generic markings or just the word halal or letter H to signify that food is halal, but such symbols are not widely accepted. The Islamic Food and Nutrition Council of America uses a registered trademark logo of the letter 'M' inside a closed crescent. Many other halal logos have started to appear on packages in North America, United Kingdom, Pakistan, and many other countries. Some countries like Indonesia, Malaysia, Brunei, Singapore, and Thailand have central halal control bodies, each with their unique logo. As the volume of halal products offered in local and international markets grows, it is expected that determining the standards for a halal certification will become more complex.

Animal Welfare Issues

Lately, animal welfare has been in the news a great deal, especially in Europe, where the European Union has formulated a set of guidelines especially for religious slaughter of animals. The World Organisation for Animal Health (OIE), an international organization comprising 167 member countries has also developed guidelines on animal welfare that include humane treatment of animals during transport and slaughter. According to OIE guidelines both kosher and halal conventional slaughter are considered as humane, meeting animal welfare considerations. Some of the largest fast food chains have adopted a set of animal welfare standards that determine the purchasing requirements of products they use. In the USA, the most widely recognized animal welfare standard was developed by the American Meat Institute; however, other industry organizations and even individual food chains claim to have their own standards. In Islam, animal welfare is not limited to slaughter only; it starts with raising the animals on the farm and is carried through transport and slaughter. The concept of proper treatment of all types of animals is very comprehensive and deep rooted in the faith of Islam.

See also: Safety of Food and Beverages: Kosher Food Requirements

Further Reading

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MIHAS and Halfest Expos.

SAFETY OF FOOD AND BEVERAGES

Kosher Food Requirements

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Glossary

B'de-eved After the fact.
Beit Yosef No lung adhesions.
Chai Life or the number 18.
Chalef Jewish religious slaughter knife.
Challah Special bread offering.
Chodesh New flower.
Cholev Yisroel Jewish milk.
Chometz Prohibited grains for Passover (wheat, rye, oats, barley and spelt).
Gevinas Yisroel Jewish cheese.
Glatt No more than two lung adhesions on large animals.
Halacha Jewish religious law.
Halal Muslim dietary laws.
Kitnyos Rabbinically prohibited materials for Passover.
Kosher Jewish dietary laws.
L'chatchilla Before the fact.
Mashgiach Jewish religious supervisor.
Matzos Unleavened bread for Passover.
Mevushal Heated grape juice and wine.
Mishnah The oral law as written down in the Talmud.
Pareve Neither meat nor dairy.
Pas Yisroel Jewish baking.
Schmura Watched wheat for Passover.
Shochet Jewish religious slaughterman.
Talmud The codification and commentary on the oral law.
Torah The written law, the first five books of the Hebrew scriptures.
Tovel To immerse vessels in water.
Treife Not kosher.
Yashon Old flour.

Introduction

The objective of this paper is to describe the kosher laws as they apply in the food industry, particularly the US with a special emphasis on how these laws might affect food safety and related issues. To understand their potential impact on food safety, one must have some understanding of how kosher foods are produced, and how important kosher compliance is to consumers, i.e., its impact on spiritual health. According to the World Health Organization, health is defined as a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity. Thus, a violation of the kosher laws by those companies making and marketing kosher foods represents a breach of faith that can negatively impact the consumer, i.e., dependent on these products for their continued spiritual health and social well-being.

[☆] Preliminary note: The information in this article is as accurate as possible at the time of submission (August 2011). However, the final decision on any application rests with the religious authorities providing supervision. The ruling of the religious authorities may differ from the information presented here.

The Kosher Laws

The kosher (kashrus) dietary laws determine which foods are 'fit or proper' for consumption by Jewish consumers who observe these laws. The laws are Biblical in origin, coming mainly from the original five books of the Holy Scriptures, the Torah, which has remained unchanged since then but responds to modern needs. At the same time that Moses received the Ten Commandments on Mount Sinai, Jewish tradition teaches that he also received the oral law, which was eventually written down many years later as a text known as the Mishnah, which was then incorporated into the Talmud, which is the major Jewish law book. This oral law is as much a part of Biblical law as the written text. Over the next 3500 years, the meaning of the Biblical kosher laws have been interpreted and extended by the rabbis to protect the Jewish people from violating any of the fundamental laws, and to address new issues and technologies. The system of Jewish law is referred to as 'halacha.'

Why do Jews follow the kosher dietary laws? Many explanations have been given. The following explanation by Rabbi I. Grunfeld summarizes the most widely held ideas about the subject.

These food laws are viewed by the Jewish community as given to the community by G-d without a need for explanation. Only in modern times have some people felt a need

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to try to justify them as health laws. For example, the claim that it was necessary to protect Jews from trichinosis in pork was a reason for these laws. There are only two problems with this idea – apparently mummified pork obtained from the Egyptian pyramids did not have any signs of the trichinosis cyst and the incubation period for trichinosis is 10–14 days – making it very unlikely that the cause and effect needed for such a rule would have been figured out at that time.

> "And ye shall be men of a holy calling unto Me, and ye shall not eat any meat that is torn in the field" (Exodus XXII:30). Holiness or selfsanctification is a moral term; it is identical with...moral freedom or moral autonomy. Its aim is the complete selfmastery of man."

To the superficial observer it seems that men who do not obey the law are freer than law-abiding men, because they can follow their own inclinations. In reality, however, such men are subject to the most cruel bondage; they are slaves of their own instincts, impulses, and desires. The first step towards emancipation from the tyranny of animal inclinations in man is, therefore, a voluntary submission to the moral law. The constraint of law is the beginning of human freedom.... Thus the fundamental idea of Jewish ethics, holiness, is inseparably connected with the idea of Law; and the dietary laws occupy a central position in that system of moral discipline which is the basis of all Jewish laws.

> "The three strongest natural instincts in man are the impulses of food, sex, and acquisition. Judaism does not aim at the destruction of these impulses, but at their control and indeed their sanctification. It is the law which spiritualizes these instincts and transfigures them into legitimate joys of life."

The Kosher Market

Why are we concerned about kosher in the secular world? Because kosher is an important component of the food business (e.g., in the US approximately 40% of packaged goods are kosher certified) and impacts how a company does its food processing, with potential positive and negative benefits to food safety. Most people, even in the food industry, are not aware of the breadth of foods that are under religious supervision. This section provides a short background on the economic aspects that make it important for the food industry to have a better understanding of kosher.

The kosher market according to Integrated Marketing, an advertising agency specializing in the kosher food industry, comprises greater than 100 000 products in the US, approximately 250 billion dollars worth of products are estimated to have a kosher marking on them. The deliberate consumers of kosher food, i.e., those who specifically look for the kosher mark before purchasing, are estimated to be greater than 10 million Americans. They are purchasing almost 15 billion dollars worth of kosher product. Annually greater than 10 000 companies produce kosher products and the average US supermarket has 13 000 kosher products. Less than 1/3 (possibly as low as 20%) of the kosher consumers are Jewish (900 000 year-round consumers). Other consumers who at times find kosher products helpful in meeting their dietary needs include Muslims, along with others such as Seventh Day Adventists, vegetarians, vegans, people with various types of allergies – particularly to dairy, grains, and legumes (the latter two are addressed with Passover products) – and general consumers who value the quality of kosher products, even though there is rarely a one-to-one correlation between kosher and these consumers' needs.

Kosher

The Kosher Dietary Laws

The all year around kosher dietary laws predominantly deal with three issues, all focused on the animal kingdom (but because of these laws, vegetarian and vegan products are carefully checked to be sure no slaughtered animal products have come in contact with these foods):

A. The Allowed Animals,
B. The Prohibition of Blood, and
C. The Prohibition of Mixing of Milk and Meat.

Additionally, for the week of Passover (in late March or April) restrictions on 'chometz,' the prohibited grains (wheat, rye, oats, barley, and spelt) in other than unleavened form – and the rabbinical extensions of this prohibition – lead to a whole new set of regulations, focused in this case on the plant kingdom.

In this paper we will also discuss additional laws dealing with special issues such as grape juice, wine, and alcohol derived from grape products; Jewish supervision of milk; Jewish cooking, cheese making, and baking; equipment kosherization; purchasing new equipment from nonJews; and old and new flour.

The kosher laws are an internally consistent logic system and have an implied 'science' behind them – which may or may not agree with modern science. This system is the basis upon which rabbis work through problems and come up with a solution for new issues brought to their attention through a process of asking questions.

Allowed Animals

Ruminants with split hoofs that chew their cud, the traditional domestic birds, and fish with fins and removable scales are generally permitted. Pigs, wild birds, ostriches, and fish without removable scales are prohibited as are all crustacean and molluscan shellfish. Almost all insects are prohibited such that carmine and cochineal, which are used as natural red pigments, are not permitted in kosher products by most rabbinical supervisors. However, honey and shellac (lac resin) are permitted as will be discussed later in this section.

Four classes of prohibited animals are specifically described in the Torah. These are simply those animals that have one kosher sign but not both. These animals are no more or less nonkosher than other nonkosher animals; none are kosher. They are listed specifically only to clarify the text. In modern times the prohibition of pork has often been the focus with respect to both the kosher and halal laws, because pork is such a major item of commerce in many parts of the world.

With respect to poultry, the traditional domestic birds are kosher but birds in the rattrie category (ostrich, emu, and

rhea) are not kosher as the ostrich is specifically prohibited in the Bible (Lev. XI:16).

The only animals from the sea that are permitted are those with fins and scales. All fish with scales have fins, so the focus is on the scales. These must be visible to the human eye and must be removable from the fish skin. A few fish remain controversial, especially swordfish, whose scales do not seem to belong to any of the biologists standard scale types. The Conservative Movement also permits sturgeon, which most Orthodox authorities consider nonkosher.

Most visible insects are not kosher. The exception includes a few types of grasshoppers, which are acceptable in the parts of the world where the tradition of eating them has not been lost. The recent development of exhaustive cleaning methods to prepare prepackaged salad vegetables, which was driven in part by the desire to receive kosher certification for such products, eliminates a lot of visible insects so products are available without requiring further extensive special inspection procedures. Presumably all of this produce washing and insect removal also should make these products cleaner and safer for consumers, especially with some of the concerns for hand washing by workers in the fields. Kosher consumers have appreciated the use of pesticides to keep products insect free. Modern integrated pest management (IPM) programs that permit an increase in the level of insect infestation in the edible portions of fruits and vegetables can cause problems for the kosher consumer. Honey and other products from honey bees are covered by a unique set of laws that permits honey and beeswax. Most rabbis extend this permission to the use of lac resin or shellac, which is used in candy and fruit coatings to provide a 'shine.'

Prohibition of Blood

Ruminants and fowl must be slaughtered according to Jewish law by a specially trained religious slaughterman (shochet) using a special knife designed for the purpose (chalef). Thus, any wild animal or bird would have to be captured alive and then slaughtered.

The knife must be extremely sharp and have a very straight blade, i.e., at least twice the diameter of the neck of the animal to be slaughtered. It is the process itself, and the strict following of the law, that makes a product kosher, and not the presence or absence of a blessing over the food. However, before a slaughter session the shochet does make a blessing. The animal is not stunned before slaughter. If the slaughter is done in accordance with Jewish law and with the highest standards of modern animal handling practices, the animal will die without showing any signs of stress. In 1958, the US Congress declared kosher slaughter and similar systems, (e.g., such as halal) to be humane, but included an exemption for preslaughter handling of the animal before kosher and halal slaughter. Thus, when done right religious slaughter meets the standards for good animal welfare. Whether more blood is removed during slaughter by religious slaughter remains a controversial question for which more data is needed.

Slaughtered animals are subsequently inspected for visible internal organ defects by rabbinically-trained inspectors. If an animal is found to have a defect, the animal is deemed unacceptable and becomes treife. There is no 'trimming' of defective portions as generally permitted under secular law. This is viewed by some consumers as a reason to choose kosher, i.e., problem animals are eliminated from the kosher food supply. However, the concern in modern times is for invisible pathogens, which both the rabbinical and governmental systems were not designed to detect. The general rule is that the defect would not lead to a situation where the animal could be expected to die within a year. Some rabbis invoke these rules in dealing with issues related to veterinary practices, example, injections into certain parts of the animal's anatomy such as the neck of a chicken.

As the major site of halachic defects, the lungs must always be inspected. Other organs are spot-checked or examined when a potential problem is observed. At this time we do not have a full understanding of what animal handling practices lead to higher incidences of lung adhesions, although pneumonia in the calf is certainly one consideration. However, the concept is that kosher leads to the use of healthier animals. For practical reasons, i.e., because they cannot pass this inspection, culled dairy cows are not generally slaughtered and so the threat of mad cow disease (BSE) is reduced.

Meat and poultry must be further prepared by properly removing certain veins, arteries, prohibited fats, blood, and the sciatic nerve. In practical terms this means that only the front quarter cuts of kosher red meat are used in the US and most Western countries. These are the cheaper cuts and have at times led to the use of higher US Department of Agriculture (USDA) meat grades, example, prime and choice, which are more highly marbled, a possible health concern.

To further remove the prohibited blood, red meat and poultry must then be soaked and salted within 72 h of slaughter. If this is not possible, then under some circumstances the meat may be washed and this wash procedure may be repeated for up to two more times, each time within 72 h of the previous washing. The actual soaking is done for a half hour in cool water, thereafter, the salting is done for one hour with all surfaces, including cut surfaces and the inside cavity of a chicken, being covered with ample amounts of salt. The salted meat is then rinsed three times. The salted meat must be able to drain throughout and all the blood being removed must flow away freely.

Once the meat is properly koshered, any remaining 'red-liquid' is no longer considered 'blood' according to halacha and the meat can be used without further concern for these issues.

Work by Jim Marsden's group at Kansas State University has shown that this salting process does lead to a lower bacterial count so that the process may in fact be an antimicrobial treatment. The other antimicrobial treatments being used for meat safety are receiving mixed reviews from the rabbis. The use of heat (steam or hot water) and some of these chemicals, particularly those that are acidic, before kosher salting and soaking (which usually doesnot occur until after the meat has been chilled) may not be permitted.

The salt used for koshering must be of a crystal size, i.e., large enough that the crystals will not dissolve within the hour and must be small enough to permit complete coverage of the meat. The salt industry refers to this size crystal as 'kosher' salt. Although most salt is religiously kosher, the term 'kosher' in this case is referring to the grain size. The specific process of salting

and soaking meat to make it ready for use is also referred to as 'koshering' meat.

Because of its high blood content, liver cannot be soaked and salted, but must instead be broiled to at least over half cooked using special equipment reserved for this purpose. The liver is then rinsed, after which the liver can be used in any way the user wishes.

Some concern has been raised about the salt level in kosher meat given the interest in low salt diets. Note that only the surfaces are salted, generally using primal cuts, i.e., 20–40 lb pieces of meat, and that the penetration of the salt is less than a half centimeter in red meat (NY Department of Agriculture and Markets, personal communication). Many pieces of meat, as consumed, have therefore not been directly subjected to the salt treatment. If salt content in a diet is a very important consideration, then one should cut off all surfaces and not use any of the drippings that come out during cooking. However, much of the salt that goes into the meat at the surface is lost during cooking.

Any ingredients or materials that might be derived from animal sources are generally prohibited because of the difficulty of obtaining them from kosher animals. This includes many products that might be used in foods and dietary supplement, such as emulsifiers, stabilizers, and surfactants, particularly those materials that are fat-derived. Very careful rabbinical supervision would be necessary to assure that no animal-derived ingredients are included in kosher food products. Almost all such materials are available in a kosher form derived from plant oils. A possible exception might be a normative mainstream gelatin, which is now being produced from kosher beef hides or fish (see the Section Gelatin). Also some rennet, the cheese-coagulating enzyme, is obtained from the dried fourth stomach of a kosher slaughtered milk-fed calf, but the regular supply is not acceptable. Biotechnology derived chymosin is generally kosher (see the Section Biotechnology).

Prohibition of Mixing of Milk and Meat

> "Thou shalt not seeth the kid in its mother's milk." (Exodus XXIII:19, Exodus XXXIV:26, Deuteronomy XIV:21)

This passage appears three times in the Torah and is therefore considered a very serious admonition. As a result, the law cannot be violated even for nonfood uses such as pet food. Neither can one derive benefit from such a mixture. The meat side of the equation has been rabbinically extended to include poultry and religiously slaughtered game (not fish) as both meat and poultry need to be inspected, deveined, salted, and soaked. The dairy side includes all milk derivatives.

To keep meat and milk separate in accordance with kosher law requires that the processing and handling of all materials and products fall into one of three categories:

1. A meat product,
2. A dairy product, or
3. A neutral product called 'pareve,' 'parve,' or 'parev.'

The pareve category includes all products that are not classified religiously as meat or dairy. All plant products are pareve along with eggs, fish, honey, and lac resin (shellac). These pareve foods can be used with either meat products or dairy products. However, if they are mixed with meat or dairy they take on the identity of the product they are mixed with, i.e., an egg in a cheese soufflé becomes dairy.

A special set of rules applies to fish. Fish can be eaten at the same meal at which meat is eaten, but it cannot be mixed directly with the meat. The dishes used with the fish are generally kept separate and rinsed before they are used with meat, or *vice-versa*. The original law in the Talmud speaks of a specific concern that one particular type of fish caused people to get sick when they mixed that fish with meat. This rule is a very specific exception to the generalization that kosher laws are not health laws, but this prohibition for all fish (because no one can determine what was the original fish mentioned in Talmud) is a rabbinical extension and not a Biblical mandate. Another exception with respect to handling fish: One of the very traditional Chassidic Orthodox groups, Lubavitch or Chabad, also has a tradition of not mixing milk with fish, example, not permitting a fish gelatin to be used in yogurt.

To assure the complete separation of milk and meat, all equipment, utensils, pipes, steam, etc. must be of the properly designated category. If plant materials, like fruit juices, are run through a dairy plant, they would be considered dairy under kosher law. Some kosher supervision agencies would permit such a product to be listed as 'dairy equipment (D.E.)' rather than 'dairy.' The D.E. tells the consumer that it does not contain any intentionally added dairy ingredients, but that it was made on dairy equipment. (See the Section Kosher and Allergies) There is also a 'meat equipment (M.E.)' designation. A significant wait is normally required to use a product with dairy ingredients after one has eaten meat. This can range from 1 to 6 h depending on the customs of the area from which the husband came. With the D.E. listing, the consumer can use the D.E. product immediately before or after a meat meal but not WITH a meat meal. Following dairy the wait before eating meat is much less, usually from a 'rinse of the mouth' with water to one hour. Certain dairy foods do require the full wait of 3 to 6 h, i.e., when a hard cheese is eaten, the wait is the same as that for meat to dairy. A hard cheese is defined as a cheese that has been aged for greater than six months or one, i.e., particularly dry and hard like many of the Italian cheeses. Thus, most companies producing kosher cheese usually age their cheese for less than six months, which may limit the quality, although with proper consumer labeling information longer aging can be done as this is not a religious requirement but simply affects the waiting times.

If one wants to make an ingredient or product truly pareve, the plant equipment must undergo a process of equipment kosherization (see the Section Equipment Kosherization). From a marketing stand point, a pareve designation is most desirable because it has the most uses, both for the kosher and for the nonkosher consumer.

Kosher: Special Foods

Grape Products

To be kosher, all grape juice-based products can only be handled by Sabbath-observing Jews from grape-pressing to

final processing. If the juice is pasteurized (heated or 'mevushal' in Hebrew), then it can be handled by any worker, as an ordinary kosher ingredient. If a liquid bottling line, example, a soda line, uses a product with nonkosher grape juice, the line would have to be cleaned (rinsed) out before proceeding to make kosher products.

Jewish Cheese (Gevinas Yisroel)

Similar to the laws concerning kosher wine production, most kosher supervision organizations require that the supervising religious person (mashgiach) add the coagulating agent, i.e., the agent that makes the cheese form a curd, into the vat to ensure that the cheese is kosher. Any cheese that does not meet this requirement is unacceptable.

If all the ingredients and equipment used during cheese making are kosher, the whey will be kosher as long as the curds and whey have not been heated above 120 F (49 °C) before the whey is drained off. This is true even if a mashgiach has not added the coagulant. Thus, there is much more kosher whey available in the US than kosher cheese. To produce kosher whey, several manufacturers of Swiss cheese, which has one of the most desirable, whitest wheys, have reduced the temperature at which they work the curds in the hot whey. Instead of using the traditional 125–127 F (52–53 °C), they are using a temperature under 120 F (49 °C) to work the curds and to obtain a kosher whey. But there are other challenges to be overcome to make kosher whey. Much of the whey is produced using spray driers, which are among the most difficult pieces of equipment to kosherize.

Another problem deals with whey cream. Any cream, i.e., separated from cheese at above 120 F (49 °C) is subject to the restrictions that come with the cheese and is, therefore, generally not considered kosher. This cream has recently been used to produce butter, which is therefore not considered kosher. Most rabbis had traditionally accepted butter as kosher without supervision as is still the case with milk (but not fortified milk as there is a concern about the source of some of the vitamins, particularly Vitamin A (shark liver)). The transition to requiring kosher supervision of butter has been difficult. More detailed articles on this, and closely related kosher dairy issues, have been published in 2002.

Cholev Yisroel

Some kosher-observant Jews are concerned about possible adulteration of milk with the milk of nonkosher animals, such as mare's milk or camel's milk, and therefore require that the milk be watched from the time of milking. This 'Cholev Yisroel' milk is required by some of the stricter kosher supervision agencies for all dairy ingredients and dairy products. Rabbis who accept non-'Cholev Yisroel' milk in the US do so for two reasons. First, they believe that the laws in the US and many other countries are strong enough to assure that adulteration with other milks does not occur. Second, the nonkosher milks are worth more money than kosher milks, so there is no incentive to add nonkosher milk to the milk of kosher species.

Yashon (Old) and Chodesh (New) Flour

On the second day of Passover, Jews traditionally brought a grain offering to the Temple in Jerusalem. This served to bless all of the flour that was 'growing' or had already been harvested on that day. Such flour has attained the status of 'yashon' (old) flour. All wheat for flour that has not started to grow by the second day of Passover is considered 'chodesh' (new) and should not be used until the next Passover. For all intensive purposes, the grain would have had to have been planted more than 14 days before the second day of Passover to be covered by that Passover, the minimum time assumed necessary for the seeds to germinate. All winter wheat from the Northern Hemisphere is automatically considered yashon. It is more difficult to assure the yashon status of spring wheat, which generally is harvested in August.

Early Fruit

Another kosher law concerning plants is the requirement that tree fruits not be harvested for benefit until the fourth year. This has been particularly problematic with respect to papaya, a tree fruit, i.e., often not kept alive for four years! Discussion and disagreement about the status of papaya remains at this time.

Passover

The Passover holiday occurs in spring and requires observant Jews to avoid eating most products made from five prohibited grains: wheat, rye, oats, barley, and spelt (Hebrew: chometz). Those observing kosher laws can only eat the specially supervised unleavened bread from wheat (Hebrew: matzos) that are prepared especially for the holiday. Once again, some matzos, i.e., schmura ('watched') matzos, are made to a stricter standard with rabbinical inspection beginning in the field. For other Passover matzo the supervision does not start until the wheat is about to be milled into flour. Matzo made from oats and spelt are now available for consumers with allergies.

Special care is taken to assure that the matzo does not have any time or opportunity to 'rise.' In some cases this literally means that products are made in cycles of less than 18 min. In continuous large-scale operations, the equipment is constantly vibrating so that there is no opportunity for the dough to rise.

Why 18 min? Note that the word for 'life' is the two letter Hebrew word 'Chai.' The drinking toast among Jews is 'L'Chaim,' To life. Because the Hebrew alphabet is 'mapped' to numbers (e.g., Aleph = 1, Bet = 2), the word 'Chai' equals the number 18! Thus fermentation, 'life' is considered to require 18 min to occur. Anything made in less than 18 min has not fermented and has, therefore, not violated the prohibitions of Passover.

In the middle ages, the rabbis of Europe also made products derived from corn, rice, legumes, mustard seed, buckwheat, and some other plants (Hebrew: kitnyos) prohibited for Passover. In addition to the actual 'flours' of these materials, many contemporary rabbis also prohibit derivatives such as corn syrup, cornstarch, and cornstarch derivatives such as citric acid. A small number of rabbis permit the oil from kitnyos materials, or liquid kitnyos products and their derivatives such as corn

syrup. The major source of sweeteners and starches used for production of 'sweet' Passover items is either real sugar or potato-derived products such as potato syrup.

Rabbis are concerned with other foodstuffs that are being raised in areas where wheat and other Passover grains are grown. Because of possible cross-contamination some crops such as fennel and fenugreek are also prohibited for Passover.

During the long period of time from early in the Common Era to the enlightenment, the Jewish communities within Christian countries did not have regular contact with Jews living in Muslim countries. The laws governing these two separate Jewish communities began to drift apart. This was also the period in which Christianity was developing and when it over time rejected the mandates of Hebrew scripture.

As a result, today's European, or Ashkenazic Jewish community has significantly different laws and customs from the Sephardic Jewish community, which included Spain, North Africa, and the Middle East. Sephardic custom, which is the default in Israel, includes among other rules, no ban on all or some of the kitnyos materials like rice, a 'beit yosef' meat standard of absolutely no lung adhesions for all animals, and a willingness to use hind-quarter that has been correctly subject to nikkur or deveining, including removal of the siatic nerve.

Passover is a time of large family gatherings but requires two additional sets of dishes specifically for Passover, one meat and one dairy. Overall, 40% of kosher sales for the traditional 'kosher' companies such as Manischewitz, Rokeach, and Kedem occur for the week of Passover. Stores generally begin to make Passover products available to consumers between four and six weeks before Passover. Some stores will rent space temporarily just for their Passover sales. Consumers who regularly use products such as dietary supplements, and nonlife threatening drugs will be concerned about obtaining a version of their favorite and/or required product, i.e., acceptable for Passover. For drugs, the prohibition of chometz is of special concern because many Jews do not want any manner of chometz in their home, including drugs, pet feeds (yes, there are kosher Passover pet foods), and nonfood items such as rubbing alcohol.

The most stringent kosher consumers only eat 'whole' unbroken matzos on the first seven days of Passover, the seven days observed by Jews everywhere including Israel. Thus, any prepared food for those seven days (the Biblically commanded time) may need to be made without the use of any matzo meal or matzo flour, i.e., no gebruckts (no broken matzos, no wetting). However, on the eighth day – which is a rabbinical extension of Passover outside of the land of Israel – these people will also eat products made with less than whole matzos.

It is a challenge to make Passover food products that are tasty and have a decent texture with all the limitations of Passover. The kosher community welcomes the assistance of the food scientist and the food industry to develop more and better Passover products.

The need to assure the absence of all chometz in the home meant that most Jewish homes go through an extensive spring cleaning every year. Historically, this was believed to have had a beneficial effect in protecting Jews from the spread of some diseases.

Kosher: Other Processing Issues

Equipment Kosherization

There are three ways to make equipment kosher or to change its status back to pareve from dairy or meat. Rabbis generally frown on going from meat to dairy or *vice versa*. Most industrial conversions are from dairy to pareve or from treife to one of the categories of kosher. There are a range of process procedures to be considered, depending on the equipment's before production history.

After a plant, or a processing line, has been used to produce kosher pareve products, it can be switched to either kosher dairy or kosher meat without a special equipment kosherization step. It can also subsequently be used for halal production (from pareve or dairy lines, not always from meat lines), and then, finally, for nonkosher products. In many cases, a mashgiach, i.e., the rabbinically approved kosher supervisor, is needed on site for equipment kosherization, so it normally is beneficial to minimize the number of change-overs from one status to another.

The simplest equipment kosherization occurs with equipment that has only been handled cold. This requires a good liquid caustic/soap cleaning, i.e., the type of cleaning done normally in most food plants. Some plants do not normally do a wet clean up between runs, example, a dry powder packing plant or a chocolate line, and these would need to seek specific rabbinical guidance for the change-over. In recent years, allergy concerns have eliminated some of these conversions. Materials such as ceramics, rubber, earthenware, and porcelain cannot be koshered because they are considered not 'capable' of releasing the flavors trapped within them even during the equipment kosherization process. If these materials are found in a processing plant, new materials may be required for production.

Most food processing equipment is operated at cooking temperatures, generally above 120 F (49 °C), the temperature, i.e., rabbinically defined as 'cooking.' However, the exact temperature for 'cooking' depends on the individual rabbi, in that it is the temperature at which he must immediately remove his hand when he puts it into hot water. Recently, through an agreement by the major four mainstream American kosher certifying agencies, which has been accepted by most normative kosher supervision agencies in the US have settled on 120 F (49 °C) as the temperature at which foods are cooked and this figure is used throughout this paper. (See the Section Dealing with Kosher and Halal Supervision Agencies.)

Equipment that has been used with cooked product must be thoroughly cleaned with liquid caustic/soap before being kosherized. The equipment must then be left idle for 24 h, after which it is 'flooded' with boiling water being defined as water between 190 F (88 °C) and 212 F (100 °C), in the presence of a kosher supervisor. The details depend on the equipment being kosherized. In some cases, particularly foodservice establishments, a 'pogem' (bittering agent, oftentimes ammonia) is used in boiling water in lieu of the 24 h wait and followed up with an immersion in boiling clean water. All this special activity, therefore, leads to extra cleaning of food plant equipment.

The principles concerning koshering by hagalah (boiling water) or irui (boiling water poured over a surface) are based on an ancient understanding of the movement of 'taam' (flavor) in and out of solid materials. The concepts of taam and its movement between products are also used to analyze the many possible combinations of kosher meat, kosher dairy, and/or nonkosher products interacting accidentally, i.e., for analysis only 'after the fact' ('b'de-eved') and unintentionally. For real accidents, the rabbis are able to be more lenient than they might be for things that are done intentionally ('l'chatchilla,' i.e., planned ahead of time). In modern times, where kosher supervision in the US is active, i.e., the rabbis are operating with a contractual agreement and ongoing inspections, there is less room to use leniencies. In Europe, where rabbis may only make informal visits to plants and report on their visits to their congregants and the greater Jewish community, the rules with respect to 'after the fact' nullification can sometimes be used more freely – because the rabbi cannot control any changes made in the processing after he has left the plant, i.e., between visits.

In the case of ovens or other equipment that uses 'fire,' or dry heat, kosherization involves heating the metal until it glows. Again, the supervising rabbi is generally present while this process is taking place. In the case of ovens, particularly large commercial ovens, issues related to 'odor/vapors' and 'steam' must also be considered. Sometimes the same oven can be used sequentially for alternating pareve and dairy baking. The details are beyond the scope of this paper and require a sophisticated rabbinical analysis to determine which ovens can be used for more than one status without requiring kosherization.

The procedures that must be followed for equipment kosherization, especially for hot equipment, can be quite extensive and time consuming – so the fewer status conversions, the better. Careful formulating of products and good production planning can minimize the inconvenience. If a conversion is needed, it is often scheduled for early Monday morning, before the production week starts.

However, as part of the process, the rabbis often do a very thorough check of the piping systems, and often find errors in the official plant documents. Thus, these inspections may actually uncover problems that plant management was not aware of.

Jewish Cooking and Jewish Baking

In some cases it is necessary for the rabbis to 'do' the cooking (Bishul Yisroel). Often this means turning on the pilot light. As long as the pilot light remains lit, the rabbi does not have to be present; if it goes out, he must return. With electrical equipment and appliances, it is possible to keep electricity on all the time, using the lowest setting when actual heating is not taking place.

Baking generally requires Jewish participation, Pas Yisroel, i.e., the Jew must start the ovens. In addition, if the owner of the bakery is Jewish, there may be a requirement for 'taking challah,' i.e., a portion of the dough is removed and needs to be specially handled. Again, the details need to be worked out with the supervising rabbi.

Note that a company that has greater than 50% Jewish management or Jewish ownership is subject to stricter rules, example, the taking of challah, and the need to observe the Sabbath and other Jewish holidays. To work with the less strict rules, some owners sell their business to a gentile for the period of concern, some even do this each week. This is a legally binding contract and, in theory, the gentile owner can renege on his or her informal agreement to legally sell what was sold back. On Passover, the need to do this can be more critical: Any chometz in the possession of a Jew during Passover is forever prohibited in a kosher home, i.e., if a 'Jewish' grocery store receives a shipment of bread during Passover, that bread, even if marked as kosher, though obviously nonPassover, can never be used by an observant kosher-observing Jew.

'Toveling' (Immersing Equipment Purchased from a Gentile)

When a Jewish company purchases or takes new or used equipment from gentiles, the equipment must be immersed in a ritual bath ('mikvah') before being equipment kosherized. Equipment made from metal and glass requires a blessing; complex items that contain glass or metal may need to be toveled but may not need a blessing. A mashgiach needs to be present for this activity when commercial equipment is involved. A natural body of water can be used instead of the indoor mikvah, especially with large equipment.

Tithing and Other Israeli Agricultural Laws

In ancient times, products from Israel were subject to special rules concerning tithing for the priests, their helpers, the poor, etc. These are complex laws that only affect products from Israel. The land of Israel is also subject to the Sabbath (sabbatical) years, i.e., crops from certain years cannot be used. These additional requirements challenge kosher consumers in the US who are interested in purchasing and using Israeli products. Sometimes the rabbis in Israel arrange for companies to tithe when the products are destined for sale in Israel, but rarely for exports. The details of this process are beyond the scope of this paper.

Kosher and Allergies

Many consumers use the kosher markings as a guideline to determine whether food products might meet their special needs including allergies. There are, however, limitations that the particularly sensitive allergic consumer needs to keep in mind.

1. When equipment is kosherized – or converted from one status to another the procedure may not yield 100% removal of previous materials run on the equipment. This became an issue some years ago when rabbis discovered that the special procedures being used to convert a dairy chocolate line to a pareve chocolate line led to enough dairy contamination that consumers who were very sensitive to dairy allergens were having problems. These lines are koshered without water: Either a hot oil or 'pareve' chocolate is run through the line in a quantity

sufficient to remove any 'dairy' residual as calculated by the supervising rabbi.

Both Islam and Judaism do not permit practices to occur that will endanger life. As a result, rabbis decided that none of the current religiously acceptable methods for equipment kosherization of chocolate are effective enough from an allergy perspective to move between dairy and pareve production. Therefore, mainstream kosher supervision agencies no longer permit this conversion. Among the problems with plants running both types of chocolate is the possible cross-contamination by powdered milk dust. This would not affect the kosher status of the product but is obviously not acceptable for allergenic concerns.

2. Kosher law does permit certain *ex post facto* (after the fact) errors to be negated. Trace amounts of materials accidentally added to a food can be nullified if the amount of 'offending' material is less than 1/60 by volume under very specific conditions, i.e., truly added by accident. However, some items can never be negated, example, strong flavor compounds or enzymes that make a significant impact on the product even at less than 1/60. In deference to their industrial client company's desire to minimize negative publicity many kosher supervision agencies do *not* announce when they have used this nullification procedure to make a product acceptable. When there is a concern about allergic reactions, however, many rabbis are now more willing to alert the public as soon as possible for health and safety reasons.

Products that might be made in a dairy plant – e.g., pareve substitutes for dairy products and some other liquids like teas and fruit juices – may be produced in plants that have been kosherized, but may not meet a very critical allergy standard. Care in consuming such products is recommended.

3. Labels that say Dairy and Meat Equipment: There are no intentionally added dairy or meat ingredients, but the product is produced on a dairy or meat line without any equipment kosherization. The product is considered to be like pareve with some additional use restrictions in a kosher home as previously explained. Again, the more sensitive the allergy, the more caution in using such products is advised.
4. In a few instances where pareve or dairy products contain small amounts of fish, such as anchovies in Worcestershire sauce, this ingredient *may* be marked as part of the kosher supervision symbol. Many certifications do not specifically mark this if the fish in the initial material is less than 1/60 by volume. Someone who is allergic should always read the ingredient label.
5. At Passover there is some dispute about 'derivatives' of kitnyos materials, the nongrain materials that are also prohibited for Ashkenazic Jews. A few rabbis permit items like corn syrup, soybean oil, peanut oil, and similarly derived materials from these extensions. The 'proteinaeous' part of these materials is generally not used. Consumers with allergies to these items can therefore purchase these special Passover products from supervision agencies that do *not* permit 'kitnyos'derivatives. With respect to 'equipment kosherization': supervising rabbis tend to be very strict about the clean-up of the prohibited grains (wheat, rye, oats, barley, and spelt) so these Passover products come closest to meeting potential allergy concerns; this may not be the case with respect to the products subject to the extended kitnyos prohibitions.

Consumers should not assume that kosher markings ensure the absence of trace amounts of the ingredient to which they are allergic. The kosher mark is a useful first screen, but products should be carefully tested before assuming everything is okay, i.e., the allergic person should eat a small portion of the product, and increase the amount consumed slowly, over time, to assure no adverse reaction. People with allergies should get into the habit of checking lot numbers on products and purchasing stable goods with a single lot number in sufficient quantity to meet anticipated needs within the shelf-life expectations of the goods.

How thoroughly are dairy ingredients kept out of a pareve line? The current standard for kosher may not meet the needs of allergic consumers since the dairy powder dust in the air may be sufficient to cause allergy problems? With the new US allergy regulations, a company might indicate that a product is religiously pareve, but may not be sufficiently devoid of dairy allergens for very allergic consumers.

Special Issues

Science

Gelatin

Important in many food products, gelatin is probably the most controversial of all modern kosher and halal ingredients. Gelatin can be derived from pork skin, beef bones, or beef skin. In recent years, some gelatins from fish skins have also entered the market.

Most currently available gelatins – even if called 'kosher' – are not acceptable to the mainstream US kosher supervision organizations as they may be from pork or from non-religiously slaughtered cattle.

A recent development has been the manufacture of kosher gelatin from the hides of kosher slaughtered cattle.

One finds a wide range of attitudes towards gelatin among the lenient kosher supervision agencies. The most liberal view holds that gelatin, being made from bones and skin, is not being made from a food (flesh). Further, the process used to make the product goes through a stage where the product is so 'unfit' that it is not edible by man or dog, and as such becomes a new entity. Rabbis holding this view may accept pork gelatin. Most water gelatin desserts and yogurts with a generic 'K' follow this ruling.

Other rabbis only permit gelatin from beef bones and hides, and not pork. Still other rabbis only accept 'India dry bones' as a source of beef gelatin. These bones, found naturally in India from the animals that fell and die in the fields, because of the Hindu custom of not killing the cows, are aged for over a year and are 'dry as wood.' Again, *none* of these products is accepted by the 'mainstream' kosher or halal supervisions, and are therefore not accepted by a significant part of the kosher and halal community.

Biotechnology

Rabbinical scholars currently accept products made by simple genetic engineering, example, chymosin (rennin) was accepted by the rabbis about a half a year before it was accepted by the US Food and Drug Administration (FDA). The basis for this decision involves the fact that the gene isolated from a non-kosher source is far below 'visible.' Subsequently, it is copied many times *in vitro* and then eventually injected into a host, i.e., then reproduced many times. Thus, the original source of the 'gene' is essentially totally lost by the time the food product appears. The production conditions in the fermenters must still be kosher or halal, i.e., the ingredients and the fermenter, and any subsequent processing must use kosher or halal equipment and ingredients of the appropriate status. A product produced in a dairy medium, example, extracted from cow's milk, would be dairy. Mainstream rabbis may approve porcine lipase made through biotechnology when it becomes available, if all the other conditions are kosher. The religious leaders have not yet determined the status of more complex genetic manipulations and genetic manipulation of animals; such a discussion is, therefore, premature.

Pet Food

Jews who observe the kosher laws can feed their domestic animals pet food that contains pork or other prohibited meats. They cannot feed their animals products that contain a mixture of milk and kosher meat. On Passover their pet food can contain kitnyos, but not chometz.

Health

Although many people believe that the kosher laws are also considered to be among the laws that were given for people's benefit, this is not the case.

Regulatory

Dealing with Kosher Supervision Agencies

In practical terms the food industry works with kosher and halal supervision agencies to obtain permission to use the supervision agency's trademark symbol on their products. In this way the industry can make claims in the marketplace that are legal and more important, credible to those intentionally purchasing these products, i.e., this is a third party audit system. This potential choice provides a significant potential opportunity.

Kosher supervision is taken on by a company to expand its market opportunities. It is a business investment that, like any other investment, must be examined critically in this era of total quality management, just-in-time production, strategic suppliers, etc.

What criteria should a company use to select a supervision agency? Supervision fees must be taken into account, and the agency's name recognition is a consideration. Other important considerations include: (1) responsiveness in handling paperwork, in providing mashgiachs at the plants as needed on a timely basis, and in doing routine inspections at a defined frequency during the year (anywhere from twice a year to every day (including continuous) depending on the nature of the production; (2) willingness to work with the company on problem solving; (3) ability to clearly explain their kosher or halal standards and their fee structure. And, of course, one should consider (4) if the 'personal' chemistry is right, and (5) if the agency's religious standards meet the company's needs in the marketplace.

One of the hardest issues for the food industry to deal with in their day-to-day kosher activities is the existence of so many different kosher supervision agencies. How does this impact the food companies? How do the Jewish kosher or Muslim halal consumers perceive these different groups? Because there has not been a central ruling authority for many years in either religion, different rabbis follow different traditions with respect to their dietary standards. Some authorities tend to follow the more lenient standards, whereas others follow more stringent standards. The trend in the mainstream kosher community today is towards a more stringent standard because some of the previous leniencies were considered undesirable but were tolerated when fewer alternatives were available.

One can generally divide the kosher supervision agencies into three broad categories. First, there are the large organizations that dominate the supervision of larger food companies, i.e., the Union of Orthodox Jewish Congregations (OU), Manhattan, the Organized Kashrus Laboratories (OK), Brooklyn, the Star-K, Baltimore, the Kof-K, Teaneck, NJ, and the Chicago Rabbinical Council (CRC), all five of which are nationwide and 'mainstream.'

A quick digression to explain the concept of 'normative mainstream kosher supervision': The concept of a normative mainstream US kosher standard is the *de facto* kosher standard in the US. There are numerous trademarked kosher symbols, greater than 1000 at last count (KASHRUS Magazine, October, 2010), used around the world. Some are more lenient than the 'normative' standard, whereas others are stricter. The letter 'K' cannot be trademarked; any person or company can put a 'K' on a product for any reason and so it is highly suspect.

In addition to the national supervision agencies, there are smaller private organizations and many local community organizations that provide equivalent religious standards of supervision. As such, products accepted by any of the normative mainstream organizations will, with an occasional exception, be accepted by other similar organizations.

The second category of kosher supervision (more stringent than normative mainstream) is generally associated with the 'Hassidic' communities, i.e., groups with standards beyond the normative Orthodox standard. Many of the products used in these communities require continuous rabbinical supervision rather than the occasional supervision used by the mainstream organizations for production-line products.

The third level is mainly individual rabbis who are more 'lenient' than the mainstream standard. Many of these rabbis are Orthodox; some may be Conservative. Their standards are based on their interpretation of the kosher laws and are almost always well within traditional standards. Employing a more lenient rabbi means that the food processor cuts out more of the 'mainstream' and stricter markets; this is a retail marketing decision that each company makes for itself. More lenient supervisions are sometimes the only ones that will certify a

product with a special problem that causes other supervisions to reject it.

In recent years we have started to see products that have dual halal and kosher certification. The first were the military MREs (meals ready-to-eat).

Ingredient companies should be particularly careful in selecting a supervision agency. They should try to use a 'mainstream' kosher supervision agency because most kosher or halal food manufacturing companies will require such supervision. The ability to sell to as many customers as possible requires a broadly acceptable standard. Unless an ingredient is acceptable to the mainstream, it is almost impossible to gain the benefit of having a kosher ingredient for sale. Ingredient companies need to pay attention to the status of the kosher product, i.e., a pareve product is preferred over a dairy product because it has broader potential use.

A system of certification letters is used to provide information from the certifying rabbi concerning the products he has approved. To prevent fraud, it is helpful if these letters are renewed every year and dated with both a starting and ending date. Obviously a kosher supervision agency will only 'accept' letters from agencies they find acceptable. That decision to accept another agency depends on two components: the actual kosher standards of the other agency, and an assessment of how well they operate and enforce their supervision. To make this process work better, more agencies are automating this process and computer systems are improving.

There are, of course, periodic 'recalls' of specific products for various kosher defects that would prevent their use. KASHRUS Magazine, or its website (www.kashrusmagazine.com, and www.kashrut.com) try to provide up-to-date listings of products with problems, both of consumer items and industrial ingredients covering all of the different supervision agencies. Sometimes these recalls highlight secular (FDA/USDA) problems with products, i.e., when the kosher status is inconsistent with the ingredients labeling, the problem is sometimes with the ingredients label! Unfortunately few of these are followed up by the responsible government agency.

The kosher symbol of the certifying agency or individual doing the certification may appear on the packaging. In some industrial situations, where kosher and nonkosher (or halal and nonhalal) products are similar, some sort of color-coding of product labels and packages may also be used. Most of these symbols are 'trademarks' that are duly registered.

Three additional notes about kosher markings on products:

1. To ensure that labels are marked properly, it is the responsibility of the food company to show its labels to its certifying agency before printing. This responsibility includes both the agency symbol and the documentation establishing its kosher status, example, dairy or pareve. It is the responsibility of the kosher supervision agency to review these labels carefully.
2. The labels for private label products with specific agency symbols on their labels should not be moved between plants and cannot be used if supervision changes.
3. The Toronto Rabbinical Council (COR) requires that each label have a plant number on it. This prevents the movement of labels between plants of the same company. They are the only agency that currently requires this additional safeguard.

State Regulations

Approximately 20 states, some US counties, and a few cities have laws specifically regulating the claim of 'kosher.' Many of these laws have referred to 'Orthodox Hebrew Practice' or some variant of this term, example, reference to specific Jewish documents. The US Supreme Court has ruled (by accepting the decision of the Second Court of Appeals) that this is a violation of the first amendment to the Constitution.

The newer laws focus specifically on 'consumer right to know issues' and 'truth in labeling.' They avoid having the state define kosher. Rather, the food producer defines its use of terms such as 'kosher' or 'cholev yisroel' and is held by the state to that standard. Religious foods supervisors (or anyone else) providing supervision must then declare the information that a consumer needs to know to make an informed decision.

Animal Welfare

Animal welfare issues with respect to religious slaughter are discussed, along with recommendations for auditable religious slaughter standards, in the American Meat Institute Guidelines that have been developed by Dr. Temple Grandin (Colorado State University). These standards require that religious slaughter preferably be done with the animals in an upright position (for mammals), although a proper upside down pen will be accepted. Shackling and hoisting of mammals is not permitted while the standard shackling line for birds is also permitted for religious slaughter.

Conclusion

The Kosher Dietary Laws define the foods a practicing Jew will eat. This is important for the social well-being and spiritual health of those who follow these laws. In addition, many of the issues addressed by the religious supervision authorities are of value to other consumers in the market place, either as directly affecting their health (e.g., allergen issues), their perception of food (e.g., animal welfare) or their spiritual health (e.g., the perception that religiously supervised products have abstract spiritual benefits). Thus the food industry in providing religiously supervised products not only creates new markets, but also takes on a responsibility that needs to be taken seriously. They also need to be thanked for providing these services.

See also: Food Safety Assurance Systems: Good Animal Husbandry Practice. Institutions Involved in Food Safety: World Organisation for Animal Health (OIE). Safety of Food and Beverages: Halal Food Requirements; Meat and Meat Products

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Relevant Websites

www.kashrusmagazine.com
KASHRUS Magazine: List of kosher certifying agencies along with general material and up-to-date alerts.

www.kashrut.com
Kashrut.com: General material on keeping kosher along with up-to-date alerts.

www.kosherfest.com
Kosherfest: The annual kosher trade show.

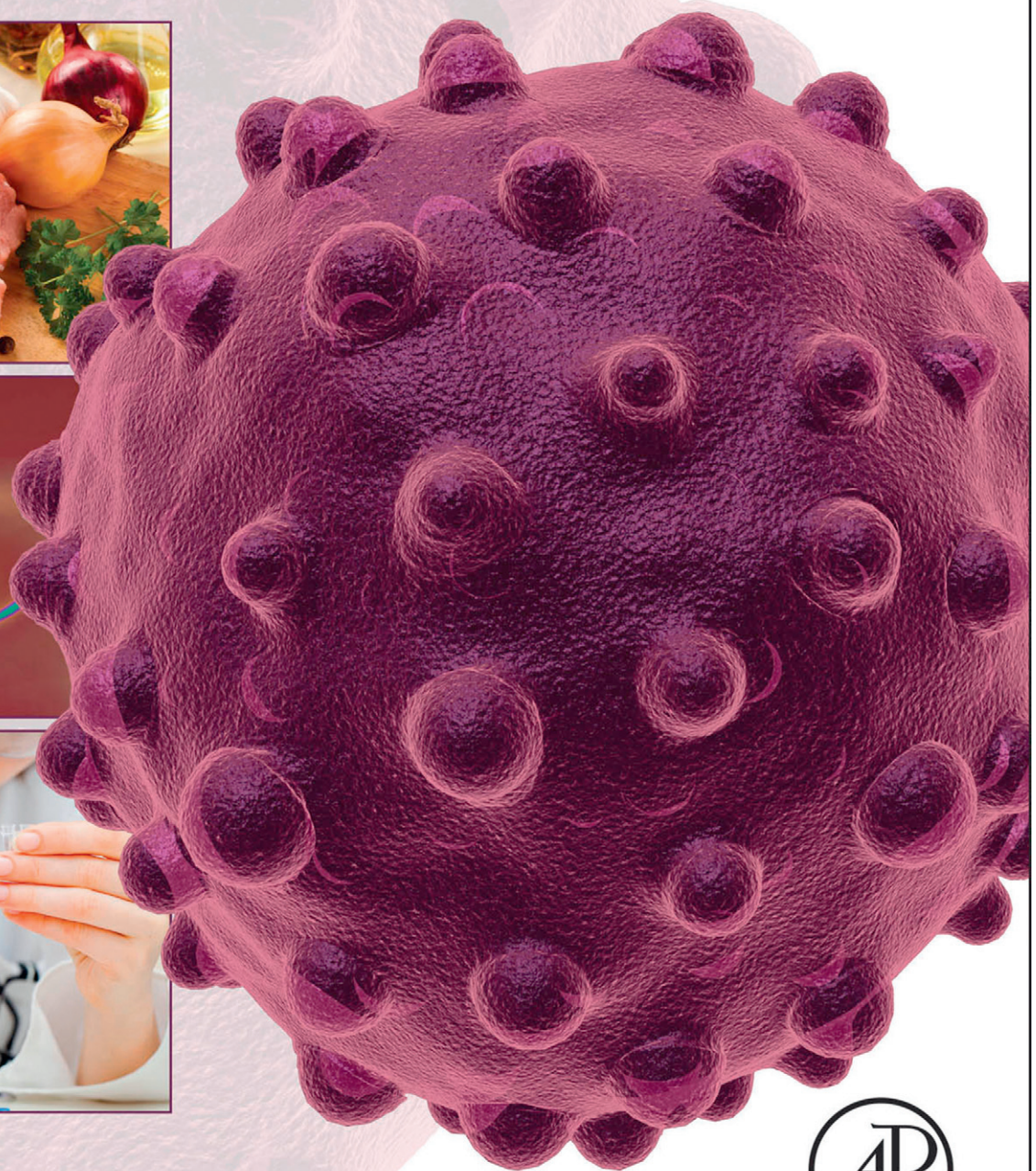
www.koshertoday.com
KosherToday: The business side of kosher.

FOSA

Encyclopedia of Food Safety

ENCYCLOPEDIA OF FOOD SAFETY

Edited by **Yasmine Motarjemi, Gerald Moy, Ewen Todd**



ENCYCLOPEDIA OF
FOOD SAFETY

VOLUME 4

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FOOD SAFETY

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PREFACE

Why an Encyclopedia on Food Safety?

With the world's growing population, the provision of a safe, nutritious, and wholesome food supply has become a major challenge. To achieve this, effective risk management based on sound science and unbiased information is required by all stakeholders, including the food industry, governments, and consumers themselves. In addition, the globalization of the food supply requires the harmonization of policies and standards based on a common understanding of food safety among authorities in countries around the world.

Furthermore, reports of food safety incidents and foodborne disease outbreaks in one country are disseminated almost instantaneously through the 24/7 news cycle to consumers in other countries all over the world. Consequently, food safety managers in government and industry are sometimes called on to respond to queries from politicians, the media, and the general public even before they may be aware of the problem. Taking effective intervention measures and communicating the basis of their decisions and actions are essential for maintaining confidence in the safety of the food supply.

In all the above circumstances, sound scientific information is the key to effectively and efficiently assess, manage, and communicate on food safety risks. Yet, professionals and other specialists working in this multidisciplinary field are finding it increasingly difficult to keep up with developments outside their immediate areas of expertise. The time and staff needed to provide this information are beyond the resources of most individuals and organizations. Therefore, a single source of concise, reliable, and authoritative information on food safety has, more than ever, become a necessity.

This is the role that the Encyclopedia on Food Safety sought to fulfill by gathering all of the world's knowledge and expertise covering the entire spectrum of food safety topics into one comprehensive reference work. This was done with the objective of facilitating the work of those working in the field of food safety and related fields, such as nutrition, food science and technology, and environment. The Encyclopedia also provides a platform for experts to share their state-of-the art expertise and experience with the rest of the food safety community. Furthermore, the Encyclopedia's online feature is designed for rapid search and retrieval of relevant information.

Who Will Benefit from the Food Safety Encyclopedia?

The Encyclopedia will be useful for professionals and other specialists working in, but not limited to, the following institutions:

- Regulatory and enforcement agencies.
- Food industry.
- Trade and industry organizations.
- Audit and certification bodies.
- Academic institutions.
- Private and governmental scientific and research institutions.
- International and nongovernmental organizations with an interest in food.

What Does the Encyclopedia of Food Safety Contain?

With some 280 articles, the Encyclopedia provides comprehensive coverage a broad range of food safety topics, which may be grouped under the following general categories:

- History and basic sciences that support food safety.
- Foodborne diseases, including surveillance and investigation.
- Foodborne hazards, including microbiological and chemical agents.
- Substances added to food, both directly and indirectly.
- Food technologies, including the latest developments.
- Food commodities, including their potential hazards and controls.
- Food safety management systems, including their elements and the roles of stakeholders.

In developing the Encyclopedia, the editors and members of the Editorial Advisory Board have aimed to ensure that the Encyclopedia provides:

- Contributions by the foremost authorities in their fields.
- Unbiased and concise overviews on a multitude of food safety subjects.
- References for further information
- Specialized and general definitions for food safety terminology.

While the editors have made every effort to ensure that the Encyclopedia reflects the most complete and up-to-date information available, new scientific findings, and advances in food safety occur continuously. In undertaking a project of this scale and with the inevitably delays that occur during production, the editors acknowledge that some topics may have been omitted or insufficiently addressed. Therefore, the feedback of readers to point out any such errors or oversights will be greatly appreciated and will facilitate the development of future editions.

Acknowledgments

The lead editors would like to thank the Editorial Advisory Board members, section editors, and particularly, the authors who have generously contributed their time and talent to the development of this Encyclopedia. We are indebted to the Elsevier secretariat, which has assisted in the production of this work since its inception. Finally, a special note of thanks goes to our families whose patience and support are greatly appreciated.

Yasmine Motarjemi
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DEDICATION

This Encyclopedia is dedicated to our children, our grandchildren, and all the world's future generations who we hope will enjoy the benefits of a safe and nutritious food supply, produced with fair management of people working in industry and ethical treatment of animals.

FOREWORD I

Today's food system is one of the humanity's great achievements. It includes millions of commercial actors all over the world who produce, process, transport, store, market, and serve food that feeds billions of people daily. The complexity, diversity, and scope of the food system are almost beyond comprehension – ranging from small producers and processors serving local communities to vast global enterprises producing food for millions and managing extended international supply chains – all aimed at meeting high consumer expectations for safe, nutritious, and affordable food.

For all of its successes, the food system is full of challenges. Food insecurity and hunger remain major problems worldwide, and, for those with ready access to the foods of their choice, it is too easy to choose products high in salt, fat, and added sugar. Food safety – the task of avoiding chemical and microbiological contamination of food that can make people sick – is another persistent and dynamic challenge. In fact, new products in the marketplace, new patterns of production and supply, new consumer behaviors and new bacterial and chemical hazards – coupled with high consumer expectations – conspire to make food safety one of the central challenges of today's food system.

People working in the food system know this. Prominent illness outbreaks and contamination incidents take a toll on the public's health and cause a loss of confidence that can steer consumers away from healthy foods, like fresh fruits and vegetables, and impose big economic losses on food producers and processors. And the food system is responding with a heightened awareness of food safety at all levels of the food system and tremendous effort across the system to improve food safety. Much progress is being made.

One of the most important food safety developments of the last quarter century has been the emergence of a widely shared, science-based understanding of foodborne illness, its causes, and how it can be prevented. This begins with the understanding that the current burden of foodborne illness is unacceptable because it is largely preventable. It is preventable if we see food safety as a food system issue and recognize that microbiological and chemical hazards can enter the food supply at any point in the system along the pathway from the farm through processing, transport, storage, and retail sale. Likewise, opportunities to minimize hazards and help prevent food safety problems exist throughout the system, which means that everyone in the system shares responsibility for the safety of the food we eat.

Fulfilling this responsibility requires that we understand as much as we can about food safety hazards and their causes, devise the appropriate, science-based preventive controls for particular hazards and food production settings, monitor their effectiveness, and adjust the controls as needed based on experience. In short, progress on food safety depends fundamentally on a strong base of knowledge and continuous learning to systematically prevent food safety problems. And participants across the global food safety community are actively seeking and applying the knowledge needed to produce safe food and meet high consumer expectations.

This food safety encyclopedia provides a comprehensive overview of what we know about food safety hazards and control measures. We have more to learn, but the knowledge compiled in this encyclopedia demonstrates that we know a lot and that what we know can help empower participants in today's food system to fulfill their food safety responsibility. Although the food safety challenge is global and continuing, and may seem daunting, it can be met if all who share responsibility for food safety take advantage of the knowledge we have, participate in continuous learning, and place first priority every day on protecting the safety of food. That will be good for the food system – and for the consumers it serves.

Michael R Taylor
Deputy Commissioner for Foods and Veterinary Medicine, US Food and Drug Administration (FDA), Silver Spring, MD, USA

FOREWORD II

Food is one of the most basic requirements to sustain life. However, the safety of food and water cannot be taken for granted. Owing to both manmade and natural processes, an array of chemical and microbiological disease-causing agents find their way into food through multiple routes. When contaminated, such food can endanger or even destroy life. Therefore, from time immemorial, humankind has waged a constant battle against foodborne disease. Over many centuries of human development, people invented technologies that helped them fighting this battle, such as cooking, smoking, sun drying, canning, and freezing, to mention but a few. But like any scientific advance, some of these technologies presented their own food safety issues.

In a number of holy books, religious proscriptions for handling food contributed to food safety. In addition, many centuries ago, some governments already recognized that they had responsibilities in this domain and many laws were enacted to ensure the purity of certain foods. But it was only at the end of the nineteenth century, following scientific developments in the field microbiology and other areas of food science, that 'modern' food regulatory activities started.

In 1948, the availability, accessibility, and affordability of food were recognized as a basic human right by the United Nations in its Universal Declaration of Human Rights (Article 25, 1948). Implicit in this concept is the assumption that the food is first and foremost safe to consume, i.e., absence of health damaging properties. It is therefore not surprising that in the same year, the World Health Organization (WHO) was established as a specialized agency of the United Nations with a broad health mandate that included the specific responsibility to "develop, establish and promote international standards with respect to food…". Subsequently in 1963, WHO together with the Food and Agriculture Organization of the United Nations established an intergovernmental body to develop international standards for food – the Codex Alimentarius Commission. Today Codex stands as a major achievement in the promotion of food safety worldwide with an extensive collection of health and safety recommendations for food that are international recognized and referenced by the World Trade Organization and its member countries.

Thirty years ago, in 1983, WHO, again jointly with FAO, convened an Expert Committee on Food Safety to review the global food safety situation and provide guidance for governments, the food industry and consumers on how to cope with the inherent hazards and risks of our food supply. Based on available data and evidence at the time, the committee concluded that "illness due to contaminated food is perhaps the most widespread health problem in the contemporary world and an important cause of reduced economic productivity."

Unfortunately, this rather alarming statement appears to be still true today. Recent studies examining the morbidity and mortality of foodborne diseases have confirmed the significant public health burden posed by these diseases. In developed countries, one-quarter to one-third of the population are made ill each year because of foodborne diseases. In the developing world, the burden is much more severe. For example, diarrheal diseases are now estimated to cause 2.43 million deaths a year. According to WHO statistics, this is the second leading cause of mortality in low-income countries and kills more people than human immunodeficiency virus infection/acquired immunodeficiency syndrome (HIV/AIDS), malaria, or tuberculosis.

In addition, the large number of food safety crises, which occur with increasing frequency, is contributing to the growing public demand for better health protection from contaminated food. This has prompted governments to strengthen their food safety legislation, improve capacities and infrastructure, and tighten control measures. Examples of governmental measures include the creation of European Food Safety Agency by the European Union in 2002, the Food Safety Modernization Act in the USA of 2011 and, most recently, 2013, the commitment of the Premier of the People's Republic of China, Mr. Li Keqiang, to act with an 'iron fist' to improve food safety.

These positive developments are, unfortunately, contrasted by the fact that in many other countries, mostly developing countries, food safety does not receive the attention it deserves. In this regard, the medical profession and public health community appear to be slow in accepting the role that contaminated food plays in the epidemiology of diarrhea, particularly in infants and young children. The treatment of hospitalized cases and outpatients is rarely seen as an opportunity for educating patients and their families on why foodborne diseases occur and how they can be prevented. Two publications published in WHO's Bulletin in 1993 and 2003 urged the health sector to take steps to correct this oversight. Yet even today progress has been disappointing. For example, in the 2009, United Nations Children's Fund (UNICEF) and WHO published a document entitled 'Diarrhea: Why children are still dying and what can be done,' that again overlooked food safety as one of the most important interventions for these diseases. Consequently, in a recent publication in a prestigious *Medical Journal of Gastroenterology*, the issue had to be raised again and omission corrected. It can only be hoped that the public health and donor communities will eventually adopt a more holistic approach for the prevention of diarrheal diseases, which includes essential food safety interventions.

It is for this and many other reasons that I enthusiastically welcome the initiative of Elsevier to publish this Encyclopedia of Food Safety under the editorial leadership of Drs. Yasmine Motarjemi and Gerald Moy (my former WHO colleagues) as well as Dr Ewen Todd, a world renowned expert in food safety. The laudable collaboration and support of the Editorial Advisory Board, Section Coordinators, and the many authors who have freely devoted their time to advance the cause of food safety through the development of this Encyclopedia is also acknowledged.

With such a collection of information, whoever needs first-hand, reliable, and authoritative information on food safety does not need to consult various books, periodicals, or

websites. All of what is presently known in this domain can be found in this comprehensive work. In particular, the Encyclopedia will be useful for decision-makers, managers, officials, and scientists working in government, the food industry, academia, and nongovernmental organizations.

This Encyclopedia may be particularly important for colleagues in developing countries to not only improve food safety for their people but also convince politicians and other policy makers of the pivotal role of food safety in health and development. Without this awareness, the ultimate goal of safe food for all cannot be achieved.

F Käferstein
Former Director, Food Safety and Food Aid Programme (1980–98), World Health Organization, Wiesbaden, Germany

Further Reading

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EDITOR-IN-CHIEF

Yasmine Motarjemi holds a Masters degree in Food Science and Technology from the University of Languedoc, Montpellier, France (1978) and a Doctoral degree in Food Engineering from the University of Lund, Sweden (1988).

After her research and academic career at the University of Lund, in 1990, she joined the World Health Organization in Geneva as Senior Scientist. In WHO, she was responsible for the surveillance and prevention of foodborne illnesses (including education of professional food handlers and consumers), the development of the food safety assurance systems (e.g., Hazard Analysis and Critical Control Point system), and for assistance to the WHO Member States in strengthening their national food safety programme. She also contributed to the development of the risk analysis process. She has served in the Secretariat of various sessions of the Codex Alimentarius Commission and its Committees.

From 2000 to 2011, she held the position of Assistant Vice President in Nestlé where she worked as the Corporate Food Safety Manager. In this capacity, she has, among others, developed the Nestlé Food Safety Management system and managed various emerging food safety issues and crises.

She is the author, co-author, or editor of numerous peer-reviewed articles, books, training manuals, and other publications. Her latest books are Food Safety Management: A Practical Guide for the Food Industry (Elsevier 2014) and Invisible Things (original in French under the title: Les Invisibles), a book on food safety for children.

CO-EDITORS BIOGRAPHY

Dr Gerald G Moy, since his retirement from the World Health Organization in 2008, is a scientific adviser with Food Safety Consultants International working with numerous governments and international organizations. His expertise includes assessment of national food safety programs, risk assessment of chemical hazards in food, total diet studies, food safety during mass gatherings, and food defense. Dr Moy is the editor-in-chief for recently published book Total Diet Studies (Springer) and is currently preparing a chapter on food and water protection for a book on key public health considerations during mass gatherings. He serves on the International Scientific Advisory Committee of the China National Center for Food Safety Risk Assessment, the Technical Advisory Group of the World Food Program Technical Advisory Group, and the WHO International Virtual Advisory Group on Mass Gatherings. He is the author of numerous book chapters, articles, and publications and serves on the editorial boards for several food safety journals.

He received his BS in chemistry from the University of Wisconsin and his PhD in physical organic chemistry from Oregon State University, followed by a post-doctoral fellowship in biophysics at the University of New Mexico. He is a Fellow in International Academy of Food Science and Technology and a recipient of the 2009 Great Wall Friendship Award for his contributions to food safety during the Beijing Olympics.

Ewen CD Todd is the President of Ewen Todd Consulting and the former Professor in the Department of Advertising, Public Relations and Retailing, and he is also the Adjunct Professor in the Departments of Food Science and Human Nutrition and Large Animal Clinical Sciences at Michigan State University (MSU). He was former directors of the Food Safety Policy Center and the National Food Safety and Toxicology Center at MSU. At both these centers, Dr. Todd coordinated research in microbiology, toxicology, epidemiology, risk assessment, social science, and policy in the area of food safety, distance education programs, and outreach in the community. Previously, he was in the Bureau of Microbial Hazards, Health Products and Food Branch, Health Canada, Ottawa where he was a research scientist for 33 years working on methods development for pathogens in foods, foodborne disease investigation and reporting, costs and surveillance of disease, illnesses caused by seafood toxins, and risk assessment of foodborne pathogens. He also helped develop risk management strategies for the Department including producing videos and pamphlets on food safety education. Some of his recent research has been working on *Listeria* and *E. coli* O157 transfer coefficient and modeling projects, hygiene in child care centers, schools, and elder care facilities. He has also collaborated with government agencies and academia in Spain, Kuwait, Saudi Arabia, Lebanon, Cambodia, Korea, Japan, and China on food safety issues, and is an expert witness in legal suits involving food safety. He has published extensively on many different aspects of food safety, including 11 recent papers on food workers and hand hygiene. He is active in the International Association for Food Protection (IAFP) and other organizations, and speaks and organizes symposia at national and international meetings. He is the associate editor for the Journal of Food Science and is a frequent reviewer of manuscripts submitted to several different scientific journals.

He has received the Government of Canada Distinctive Service Award for extraordinary teamwork and support to the Science and Technology Community; Recipient of the Excellence in Science Award for 1998 by Health Canada; Deputy Minister's Award of Team Excellence for the work done in promoting the Fight BAC! Campaign in Canada; the Professional Institute of the Public Service of Canada Gold Medal for Pure and Applied Science; and he is Fellow of the American Association for the Advancement of Science, the IAFP, and the MSU University Outreach and Engagement. He is also an honorary life member of the IAFP. He is a graduate of Glasgow University with a BSc in Bacteriology and a PhD in bacterial systematics.

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HOW TO USE THE ENCYCLOPEDIA

The material in this encyclopedia is organized into five broad sections, presented in four volumes. The sections consist of:

1. **History, science, and methods**: this section includes papers which help in the understanding of basic sciences underpinning food safety and foodborne diseases and their historical development.
2. **Hazards and diseases**: this section addresses the features of major foodborne hazards be they chemical, microbial, parasitical or physical and their health consequence.
3. **Food technologies**: this section explains the various food technologies and aspects related to their safety, or risks in their application.
4. **Foods, materials, and risks**: similarly, in this section, various groups of food products are described in terms of their risks and measures needed to ensure their safety.
5. **Food safety management**: finally, in this part, the building blocks of food safety management in the private and public sector are explained. The role of major international organizations is also reported.

To help realize the full potential of the material in the Encyclopedia the authors have provided five features to help you find the topic of your choice: a preface giving an overview of the encyclopedia and its objectives, a contents list by subject; an alphabetical contents list; cross-references to other articles; and a full subject index.

1 Contents List by Subject

Your first point of reference will probably be the contents list by subject. This list appears at the front of each volume, and groups the entries under subject headings describing the broad themes of quaternary science. This will enable the reader to make quick connections between entries and to locate the entry of interest. Under each main section heading, you will find several subject areas and under each subject area is a list of those entries that covers aspects of that subject, together with the volume and page numbers on which these entries may be found.

2 Alphabetical Contents List

The alphabetical contents list, which also appears at the front of each volume, lists the entries in the alphabetical order. This list provides both the volume number and the page number of each entry. On the opening page of an entry a contents list is provided so that the full details of any articles within the entry are immediately available.

3 Cross-references

All of the entries in the Encyclopedia have been extensively cross-references. The cross-references, which appear at the end of the entry, serve three different functions:

i. To indicate if a topic is discussed in greater detail elsewhere.
ii. To draw the reader's attention to parallel discussions in other entries.
iii. To indicate the material that broadens the discussion.

Example

The following list of cross-references appear at the end of the entry Characteristics of Foodborne Hazard and Diseases | Drug Resistant Pathogens.

> *See also*: Bacteria: *Listeria monocytogenes*; *Salmonella* Non-Typhi; *Salmonella* Typhi and *Salmonella* Paratyphi; Shiga Toxin-Producing *Escherichia coli* and Other Pathogenic *Escherichia coli*; *Shigella*; *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*. Characteristics of Foodborne Hazard and Diseases: Cost of Foodborne Diseases. Food Safety Assurance Systems: Cleaning and Disinfection. Public Health Measures: Food Safety in Hospitals and Other Healthcare Settings; Surveillance of Foodborne Diseases

Here you will find examples of all three functions of the cross-reference list: a topic discussed in greater detail elsewhere (e.g., *Listeria monocytogenes*; *Salmonella* Non-Typhi; *Salmonella* Typhi, and *Salmonella* Paratyphi; Shiga Toxin-Producing *Escherichia coli*), parallel discussion in other entries (e.g., Other Pathogenic *Escherichia coli*), and reference to entries that broaden the discussion (e.g., Food Safety Assurance Systems: Cleaning and Disinfection. Public Health Measures: Food Safety in Hospitals and Other Healthcare Settings).

4 Index

The index provides you with the page number where the material is located. The index entries differentiate between materials that is a whole entry, is part of an entry, or is data presented in a figure or a table. Detailed notes are provided on the opening page of the index.

5 Contributors

A full list of contributors is listed at the beginning of each volume.

GLOSSARY OF SELECTED TERMS

This Glossary of Selected Terms is a partial list of definitions for terms commonly used in the area of food safety. The terms selected are those that are important for communication among the various disciplines or are often subject to misunderstanding. Most of the definitions are taken from those recommended by international organizations or given by the authors contributing to this Encyclopedia. In cases where there are different definitions for a term, the Glossary presents the definition that is most consistent with usage by the majority of authors. Note that in some instances, slight differences between general definitions in this Glossary and those appearing in the individual articles may occur as the result of the specific context of the articles.

Acceptable daily intake The estimate of the amount of a substance in food or drinking water, expressed on a body weight basis, that can be ingested daily over a lifetime without appreciable health risk to the consumer.

Acute reference dose The estimate of the amount of a substance in food or drinking water, expressed on a body mass basis, that can be ingested in a period of 24 h or less without appreciable health risk to the consumer.

Adulteration (economic) A fraudulent action which is intended to omit a valuable constituent or substitute another substance, in whole or in part, for a valuable constituent; conceal damage or inferiority in any manner; or add any substance to increase its bulk or weight, reduce its quality or strength, or make it appear bigger or of greater value than it is (Note that in the US, adulterated food is generally defined as impure, unsafe, or unwholesome food.).

Antiseptic A substance that inhibits the growth and development of microorganisms. For practical purposes, antiseptics are routinely thought of as topical agents, for application to skin, mucous membranes, and inanimate objects, although a formal definition includes agents which are used internally, such as the urinary tract antiseptics.

As low as reasonably achievable A risk management approach that aims to keep exposure to a substance at the lowest level that is realistically achievable.

Asymptomatic shedder A person who does not exhibit the symptoms of an illness but excrete the pathogen (*see also* carrier).

Benchmark Reference point or standard against which performance or achievements can be assessed. A benchmark refers to the performance that has been achieved in the recent past by other comparable organizations, or what can be reasonably inferred to have been achieved in the circumstances.

Biomarkers Indicators of changes or events in human biological systems. Biomarkers of exposure refer to cellular, biochemical, or molecular measures that are obtained from biological media such as human tissues, cells or fluids, and are indicative of exposure to a substance. Biomarkers of effect refer to biological changes that represent an alteration in endogenous body constituents (e.g., depression of cholinesterase levels as an indicator of exposure to pesticides).

Carrier A person or animal that harbors a specific infectious agent without discernible clinical disease and serves as a potential source of infection. The carrier state may exist in an individual with an infection that is unapparent throughout its course (commonly known as healthy or asymptomatic carrier), or during the incubation period, convalescence and postconvalescence of an individual with a clinically recognizable disease (commonly known as an incubatory or convalescent carrier). Under either circumstance the carrier state may be of short or long duration (temporary or transient carrier, or chronic carrier) (*see also* asymptomatic shedder).

Case-fatality rate Usually expressed as the percentage of persons diagnosed as having a specified disease who die as a result of that illness within a given period. This term is most frequently applied to a specific outbreak of acute disease in which all patients have been followed for an adequate period of time to include all attributable deaths. The case-fatality rate must be clearly differentiated from the mortality rate (Compare with mortality rate).

Colony-forming unit A measure of viable bacterial or fungal cells in which a colony represents an aggregate of cells derived from a single progenitor cell.

Contaminant Any biological, chemical, or physical agent not intentionally added to food, which is present in food as a result of the production, manufacture, processing, preparation, transport, or holding of such food (Compare with hazard).

Control (noun) The state wherein correct procedures are being followed and critical criteria are being met.

Control (verb) To take all necessary actions to ensure and maintain compliance with criteria established in the Hazard analysis and critical control point system (HACCP) plan.

Control measure Any action and activity that can be used to prevent or eliminate a food safety hazard or reduce it to an acceptable level.

Corrective action Any action to be taken when the results of monitoring at the Critical Control Point (CCP) indicate a loss of control.

Crisis A predicted or unpredicted event which represents an immediate or future significant threat to an organization, its employees, consumers, and the public at large.

Critical control point A step at which control can be applied and is essential to prevent or eliminate a food safety hazard or reduce it to an acceptable level.

Critical limit (CL) A criterion which separates acceptability from unacceptability.

Detergent A chemical used to remove grease, dirt and food, such as washing-up liquid.

Disability adjusted life year (DALY) A metric used to express a health gap that extends the concept of potential years of life lost due to premature death to include equivalent years of 'healthy' life lost by virtue of being in states of poor health or disability. The DALY combines in one measure the time lived with disability and the time lost due to premature mortality. One DALY can be thought of as one lost year of 'healthy' life and the burden of disease as a measurement of

the gap between current health status and an ideal situation where everyone lives into old age free of disease and disability.

Disinfectant A chemical agent or a process that destroys, neutralizes, or inhibits the growth of pathogenic microorganisms (*see also* sanitizer).

Dose–response assessment The determination of the relationship between the magnitude of exposure (dose) to a chemical, biological, or physical agent and the severity and/or frequency of associated adverse health effects (response) in the exposed organism, system, or (sub) population in reaction to the agent.

Endotoxin A toxin present in intact bacterial cells and released when bacteria die or the cells are disrupted. A notable endotoxin is lipopolysaccharide, which is a major constituent of the outer cell membrane of Gram-negative bacteria and can cause toxic effect on lysis of bacteria. The term 'endotoxin' is to be differentiated from 'exotoxin', which is a toxin secreted in the surrounding medium and environment of the bacterial cell.

Enterotoxin A cytotoxin produced by bacteria that is specific for the mucous membrane of the intestine and causes diarrhea and/or vomiting associated with foodborne disease. Many infectious microorganisms produce enterotoxins in the gut, but some are produced external to the host (*see also* exotoxin and endotoxin).

Exotoxin A toxin that is secreted by bacteria. There are many different types of exotoxins. They can be released into the susceptible host (after infection and growth) or into the environment, including food (after contamination and growth). Those released into the intestines are typically heat labile (but some *E. coli* strains can produce both heat labile (HL) and heat stable (HS) toxins). *Clostridium perfringens* produces a HL enterotoxin after completion of sporulation in the host's intestines. *Staphyloccocus aureus* and *Bacillus cereus* enterotoxins produced in food are HS and cause vomiting and diarrhea, whereas toxins of *Clostridium botulinum* toxin, also produced in food, are HL and cause systemic neurological symptoms (*see also* exotoxin and endotoxin).

Epidemic The occurrence in a community or region of a group of illnesses which are similar in nature and clearly in excess of normal expectancy, and derived from a common or from a propagated source (Compare with pandemic).

Equivalence The situation where the application of two different food safety management measures lead to the same, or equivalent, public health outcomes.

Equivalence of sanitary measures (import–export of food) Equivalence is the state wherein sanitary measures applied in an exporting country, though different from the measures applied in an importing country, achieve, as demonstrated by the exporting country, the importing country's appropriate level of sanitary protection.

Exposure assessment The qualitative and/or quantitative evaluation of the likely ingestion of a biological, chemical, or physical agent in food as well as exposures from other sources if relevant.

Fecal–oral route A means of spreading pathogenic microorganisms from feces produced by an infected host to another host, usually via the mouth; for example, contact between contaminated hands or objects and the mouth.

Flow diagram A systematic representation of the sequence of steps or operations used in the production or manufacture of a particular food item.

Food Any substance, whether processed, semiprocessed, or raw, which is intended for human consumption, and includes drink, chewing gum, and any substance which has been used in the manufacture, preparation or treatment of 'food' but does not include cosmetics or tobacco or substances used only as drugs.

Food additive Any substance not normally consumed as a food by itself and not normally used as a typical ingredient of the food, whether or not it has nutritive value, the intentional addition of which to food for a technological (including organoleptic) purpose in the manufacture, processing, preparation, treatment, packing, packaging, transport, or holding of such food results, or may be reasonably expected to result, (directly or indirectly) in it or its byproducts becoming a component of or otherwise affecting the characteristics of such foods.

Food allergy A form of food intolerance in which there is evidence of an abnormal immunological reaction to the food (Compare with food intolerance).

Food establishment Any building or area in which food is handled and the surroundings under the control of the same management.

Food handler Any person who handles, prepares, or serves food, be they domestic food handlers, such as preparing family food, or professional food handlers, such as those working in food service establishments (cooks and waiters), retail stores, supermarkets, etc. (*see also* food worker).

Food hygiene All conditions and measures necessary to ensure the safety and suitability of food at all stages of the food chain.

Food industry The term includes primary manufacturing and processing industry as well as some other establishments involved in the food chain.

Food intolerance A reproducible, unpleasant reaction to a food or food ingredient, including reactions due to immunological effects, biochemical factors, such as enzyme deficiencies and anaphylactic reactions that often include histamine release (Compare with food allergy).

Food poisoning (or acute foodborne intoxication) A disease caused by a toxin or a chemical in food with symptoms usually appearing within 24 h after ingesting the agent. This term is commonly misused as a synonym for foodborne disease, which covers both infections and intoxications.

Food safety Assurance that food will not cause harm to the consumer, when it is prepared and/or eaten according to its intended use (Compare with food suitability and food hygiene).

Food safety hazard A biological, chemical, or physical agent in, or condition* of, food with the potential to cause an adverse health effect (Compare with contaminant). *Refers also to property of a food.

Food safety objective (FSO) The maximum frequency and/or concentration of a hazard in a food at the time of consumption that provides or contributes to the appropriate level of protection (ALOP) (Compare with Performance objective).

Food suitability Assurance that food is acceptable for human consumption according to its intended use (Compare with food safety and food hygiene).
Food worker Individuals who harvest, process, prepare and serve food, i.e., across the whole food chain to retail/foodservice; it is broader than that of a food handler, who typically works in foodservice establishments typically foodservice; however, the two terms are used interchangeably in the literature (*see also* food handler).
Foodborne disease Any disease of an infectious or toxic nature caused by or thought to be caused by consumption of contaminated food, including drink.
Foodborne pathogens Disease-causing microorganisms that are transmitted to humans via foods.
Generally recognized as safe Status of a substance that is generally recognized, among qualified experts, as having been adequately shown to be safe under the conditions of its intended use (used mainly in the USA).
Genomics The study of an organism via decoding the entire genetic sequence of the organism.
Genetic modification A process of altering the genetic makeup of an organism by techniques of modern biotechnology.
Genetically modified organism (GMO) AGMO or genetically engineered organism is an organism whose genetic material has been altered using genetic engineering techniques.
Good animal husbandry practice A system of management controls that need to be adopted at the level of primary producers to ensure production of food of animal origin that is safe for human consumption and acceptable for trade in the world market.
Good hygienic practice A system of management controls that need to be adopted at production, processing, storage, distribution, and preparation to ensure safety and suitability of products of consumption.
Good laboratory practice A system of management controls for laboratories and research organizations to ensure the quality, integrity, consistency, and reliability of results.
HACCP plan A document prepared in accordance with the principles of HACCP to ensure control of hazards which are significant for food safety in the segment of the food chain under consideration (see also HACCP).
Hazard A biological, chemical, or physical agent in, or condition*; of, food with the potential to cause an adverse health effect (Compare with contaminant). *Refers also to a property of a food.
Hazard analysis The process of collecting and evaluating information on hazards and conditions leading to their presence to decide which are significant for food safety and therefore should be addressed in the HACCP plan.
Hazard analysis and critical control point system A preventive system which identifies, evaluates, and controls hazards which are significant for food safety.
Hazard characterization The qualitative and/or quantitative evaluation of the nature of the adverse health effects associated with a biological, chemical, or physical agent which may be present in food. For a chemical agent, a dose–response assessment should be performed. For a biological or physical agent, a dose–response assessment should be performed if the data are obtainable.
Hazard identification The identification of the type and nature of adverse effects that a biological, chemical, or physical agent in food is capable of causing in an exposed population.
Incidence rate The number of new cases of a condition arising in a defined group within a given period or the number of new infections per unit of person–time at risk (Compare with prevalence).
In vitro In an artificial environment outside the living organism.
In vivo Within a living organism.
Lethal dose 50% The dose of a substance that would be expected to kill half of a population of exposed organisms.
Margin of exposure Ratio of the no-observed-adverse-effect level or benchmark dose lower confidence limit for the critical effect to the theoretical, predicted, or estimated exposure dose or concentration.
Maximum residue limit The maximum concentration of residues resulting from the use of a pesticide or veterinary drug that is acceptable in or on a food.
Minimum infective dose The lowest number of microorganisms required to cause an infection in the host.
Monitoring (CCP) The act of conducting a planned sequence of observations or measurements of control parameters to assess whether a CCP is under control.
Monitoring (general) Continuous or repeated observation, measurement and evaluation of health, and/or environmental or technical data for defined purposes, according to prearranged schedules in space and time, using comparable methods for sensing and data collection.
Morbidity rate An expression of the number of illnesses in a population at risk over a given period of time (usually one year).
Mortality rate An expression of the number of deaths in a population at risk over a given period of time (usually one year).
Nanomaterials Materials engineered at the nanoscale to have novel functionality or properties. Such properties will typically, but not exclusively, be demonstrated in the size range 1–100 nm, but this size range should be considered approximate.
Nanoparticles Particles with one or more external dimensions in the range 1–100 nm, but this size range should be considered approximate.
Nanotechnology The manipulation or materials at the nano level.
Notifiable disease A disease that must, by law or by ministerial decree, be reported to a government authority.
Outbreak A group of at least two cases of a single illness that are demonstrated by epidemiological investigation to have a single common exposure or source.
Pandemic Epidemic occurring over a very wide area, crossing international boundaries (often more than one continent) and usually affecting a large number of people.
Pasteurization A process involving heat treatment at a prescribed time–temperature combination to kill vegetative forms of pathogens that may be present, while causing minimal changes in the composition, flavor, and nutritive value of food. However, with advances and the development

of new food technologies, the term is sometimes used for nonthermal technologies leading to the same effect.

Pathogen An organism capable of causing disease.

Pathogenesis The course of a disease from its origin to its manifestation; more specifically it refers to the cellular events and reactions, and other pathologic mechanisms occurring in the development of the disease.

Pathogenicity Ability of a microorganism to cause disease in a host (Compare with virulence).

Performance criterion The effect in frequency and/or concentration of a hazard in a food that must be achieved by the application of one or more control measures to provide or contribute to a performance objective (PO) or an FSO.

Performance objective The maximum frequency and/or concentration of a hazard in a food at a specified step in the food chain before the time of consumption that provides or contributes to an FSO or ALOP, as applicable (Compare with Food Safety Objective).

Pesticide Any substance intended for preventing, destroying, attracting, repelling, or controlling any pest including unwanted species of plants or animals during the production; storage; transport; and distribution and processing of food, agricultural commodities, or animal feeds or which may be administered to animals for the control of ectoparasites. The term includes insecticides, herbicides, fungicides, rodenticides and algicides as well as plant growth regulators, defoliants, desiccants, and agents for thinning fruit or preventing the premature fall of fruit.

Prerequisite program Practices and conditions needed prior to and during the implementation of HACCP and which are essential to food safety.

Prevalence The number of persons in a population who have a disease at a specified point in time or over a specified period of time (Compare with incidence rate).

Primary production Those initial steps in the food chain up to and including, for example, harvesting, slaughter, milking, and fishing.

Processing aid Any substance or material, not including apparatus or utensils, and not consumed as a food ingredient by itself, intentionally used in the processing of raw materials, foods, or its ingredients, to fulfill a certain technological purpose during treatment or processing and which may result in the nonintentional but unavoidable presence of residues or derivatives in the final product.

Processing contaminant Undesirable contaminants that are formed during the treatment of food as a result of the interaction of their natural components or their ingredients.

Provisional maximum tolerable daily intake (PMTDI) The health-based reference value used for contaminants with no cumulative properties. Its value represents permissible human exposure as a result of the natural occurrence of the substance in food and in drinking water. In the case of trace elements that are both essential nutrients and unavoidable constituents of food, a range is expressed, the lower value representing the level of essentiality and the upper value the PMTDI.

Provisional tolerable monthly intake The health-based reference value used for a food contaminant with cumulative properties that has a very long half-life in the human body. Its value represents permissible human monthly exposure to a contaminant unavoidably associated with otherwise wholesome and nutritious foods.

Provisional tolerable weekly intake The health-based reference value used for food contaminants such as heavy metals with cumulative properties. Its value represents permissible human weekly exposure to those contaminants unavoidably associated with the consumption of otherwise wholesome and nutritious foods.

Quality management Quality management includes all the activities that organization use to direct, control, and coordinate quality. These activities include formulating a quality policy and setting quality objectives. They also include quality planning, quality control, quality assurance, and quality improvements.

Recommended dietary allowance The average daily dietary nutrient intake level that is sufficient to meet the nutrient requirements of nearly all (97–98%) healthy subjects in a particular life stage and gender group.

Reservoir An animal species that specifically harbors an infectious agent over long periods, often without harm to the host.

Risk A function of the probability of an adverse health effect and the severity of that effect, consequential to a hazard(s) in food.

Risk analysis A process of decision making (usually government) for managing food safety, consisting of three components: risk assessment, risk management, and risk communication.

Risk assessment A scientifically based process for evaluating risks associated with foodborne hazards, consisting of the following steps: (i) hazard identification, (ii) hazard characterization, (iii) exposure assessment, and (iv) risk characterization.

Risk assessment policy Documented guidelines on the choice of options and associated judgments for their application at appropriate decision points in the risk assessment such that the scientific integrity of the process is maintained.

Risk characterization The qualitative and/or quantitative estimation, including attendant uncertainties, of the probability of occurrence and severity of known or potential adverse health effects in a given population based on hazard identification, hazard characterization, and exposure assessment.

Risk communication The interactive exchange of information and opinions throughout the risk analysis process concerning risk, risk-related factors, and risk perceptions, among risk assessors, risk managers, consumers, industry, the academic community, and other interested parties, including the explanation of risk assessment findings and the basis of risk management decisions.

Risk estimate The quantitative estimation of risk resulting from risk characterization.

Risk management The process, distinct from risk assessment, of weighing policy alternatives, in consultation with all interested parties, considering risk assessment and other factors relevant for the health protection of consumers and for the promotion of fair trade practices, and, if needed, selecting appropriate prevention and control options.

Risk manager A person or an organization (usually government) with the authority to decide on the acceptability of risk and, if necessary, measures needed for their management.

Risk profile The description of the food safety problem and its context.

Safe (food) A level of risk that is deemed to be acceptable by some standard. The question of safety always involves the question of to whom the risk is acceptable, and by what criteria that party judges it so.

Sanitizer Type of antimicrobial (disinfectant) that kills or irreversibly inactivates microorganisms present on a surface, especially designed for use on food-processing equipment. The US Environmental Protection Agency further defines a sanitizer as providing at least 99.9% reductions of all microorganisms on a surface (*see also* disinfectant).

Shelf-life The predicted time at which a product will change from acceptable to unacceptable quality. It is influenced by factors such as raw ingredient quality, processing conditions, packaging practices, and storage conditions. Typically, shelf-life is determined by a combination of microbial, sensory, and chemical methods. 'Shelf-life' can be expressed on food labels by a variety of dates, including 'expiry', 'use by', 'sell by', 'best before', and 'consume by', depending on the applicable legislation.

Step (HACCP) A point, procedure, operation, or stage in the food chain including raw materials, from primary production to final consumption.

Strain An isolate of the same type of microorganism possessing different properties.

Surveillance The systematic, ongoing collection, collation, and analysis of data on specific diseases in a defined population, to guide public health decisions.

Surveillance (active) Public health surveillance that regularly reaches out to diagnostic laboratories or to clinicians to actively collect reports of specific diagnoses of infections.

Surveillance (passive) Public health surveillance that collects reports of specific diagnoses from clinicians or diagnostic laboratories, which they are required or requested to submit because of notifiable diseases regulations.

Time–temperature abuse A situation where food has not been cooked for long enough or at a sufficient high temperature to reduce contaminants to safe levels, or food has been stored for a time or at a temperature that permits bacteria to proliferate.

Traceability/product tracing The ability to follow, forward as well as backward, the movement of a food through specified stage(s) of production, processing, and distribution.

Uncertainty In risk assessment, imperfect knowledge concerning the present or future state of an organism, system, or (sub) population under consideration.

Validation (analytical methods) Practice undertaken to substantiate or confirm methods or procedures perform as expected and in a reliable manner and consistently meet expectations.

Validation (control measures) Obtaining evidence that a control measure or combination of control measures, if properly implemented, is capable of controlling the hazard to a specified outcome.

Validation (HACCP) Obtaining evidence that the elements of the HACCP plan are effective.

Variability Heterogeneity of values over time, space, or different members of a population. Variability implies real differences among members of that population.

Verification (general) The application of methods, procedures, tests, and other evaluations, in addition to monitoring and to determine whether a control measure is or has been operating as intended.

Verification (HACCP) The application of methods, procedures, tests, and other evaluations, in addition to monitoring and to determine compliance with the HACCP plan.

Veterinary drug Any substance applied or administered to any food-producing animal, such as meat or milk producing animals, poultry, fish, or bees, whether used for therapeutic, prophylactic, or diagnostic purposes, or for modification of physiological functions or behavior.

Virulence The degree of pathogenicity of a microorganism as indicated by case-fatality rates and/or its ability to invade the tissues of the host; the competence of any infectious agent to produce pathologic effects. The virulence of a microorganism is a measure of the severity of the disease it causes (Compare with pathogenicity).

Waterborne disease A disease resulting from the contamination of water either by pathogenic viruses, bacteria or protozoa, or by chemical substances. These agents are directly transmitted to people when the water is used for drinking, preparing food, recreation, or other domestic purposes.

Withdrawal period (veterinary drugs) The interval between the time of the last administration of a veterinary drug and the time of the collection of edible tissue or products from a treated animal that ensures the concentration of residues in food comply with the maximum residue limit for the drug.

Zoonosis A disease that can be passed directly or indirectly from animals, whether wild or domesticated, to humans. Also called zoonotic disease.

ABBREVIATIONS OF TECHNICAL TERMS

This is a nonexhaustive list of commonly used abbreviations in the area of food safety.

ADI	Acceptable daily intake.
ADME	Absorption, distribution, metabolism, and excretion.
AI	Adequate intake.
ALARA	As low as reasonably achievable.
ALOP	Appropriate level of protection.
ARfD	Acute reference dose.
BMD	Benchmark dose.
BMDL	Benchmark dose at lower confidence limit.
CCP	Critical control point.
CFR	Case fatality rate.
CFU	Colony forming unit.
CIP	Cleaning in place.
DALY	Disability adjusted life year.
DGGE	Denaturing gradient gel electrophoresis.
DNA	Deoxyribonucleic acid.
EAR	Estimated average requirement.
ED_{50}	Effective dose 50%.
ELISA	Enzyme linked immunosorbent assay.
EMRL	Extraneous maximum residue limit.
FSO	Food safety objective.
GAHP	Good animal husbandry practice.
GAP	Good agricultural practice.
GHP	Good hygienic practice.
GAqP	Good aquacultural practice.
GC	Gas chromatography.
GC–MS	Gas chromatography–mass spectrometry.
GHP	Good hygienic practice.
GLP	Good laboratory practice.
GM	Genetically modified.
GMO	Genetically modified organism.
GMP	Good manufacturing practice.
GPVD	Good practice in the use of veterinary drugs.
GRAS	Generally recognized as safe.
HAB	Harmful algal bloom.
HACCP	Hazard analysis and critical control point.
HPLC	High performance liquid chromatography.
HPLC–MS	High performance liquid chromatography–mass spectrometry.
HPP	High pressure processing.
HTST	High temperature short time.
HUS	Hemolytic uremic syndrome.
IEDI	International estimated daily intake.
IESTI	International estimated short term Intake.
LD_{50}	Lethal dose 50%.
LOAEL	Lowest observed adverse effect level.
LOD	Limit of detection.
LOQ	Limit of quantitation.
MFFB	Moisture on a fat free bases.
ML	Maximum level.
MLST	Multilocus sequence typing.
MLVA	Multiple locus variable number tandem repeat analysis.
MOE	Margin of exposure.
MRL	Maximum residue limit.
mRNA	Messenger ribonucleic acid.
MS	Mass spectrometry.
NEDI	National estimated daily intake.
NOAEL	No observed adverse effect level.
NOEL	No observed effect level.
OPRP	Operational prerequisite programme.
PC	Performance criterion.
PCR	Polymerase chain reaction.
PDCA	Plan do check act.
PEF	Pulsed electric fields.
PFGE	Pulsed field gel electrophoresis.
PMTDI	Provisional maximum tolerable daily intake.
PO	Performance objective.
PRP	Prerequisite program.
PrP	Protease resistant protein.
PTMI	Provisional tolerable monthly intake.
PTWI	Provisional tolerable weekly intake.
QPS	Qualified presumption of safety.
RDA	Recommended dietary allowance.
RNA	Ribonucleic acid.
SMEs	Small- and medium-sized enterprises.
SOP	Standard operating procedure.
SPS Agreement	Agreement on the Application of Sanitary and Phytosanitary Measures.
TBT Agreement	Agreement on Technical Barriers to Trade.
TDI	Tolerable daily intake.
TDS	Total diet study.
TEF	Toxic equivalency factor.
TEQ	Toxic equivalence.
TMDI	Theoretical maximum daily intake.
TSE	Transmissible spongiform encephalopathy.
UHT	Ultra high temperature.
UL	Upper limit.
UV	Ultra violet.

PUBLIC HEALTH MEASURES

Contents

Modern Approach to Food Safety Management: An Overview

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Glossary

As low as reasonably achievable (ALARA) A risk management approach that aims to keep exposure to a substance at the lowest level that is realistically achievable.

Food Any substance, whether processed, semi-processed, or raw, which is intended for human consumption; it includes drink, chewing gum, and any substance which has been used in the manufacture, preparation, or treatment of 'food', but does not include cosmetics, tobacco, or substances used only as drugs. (In the context of this topic, drinking water is considered as food).

Foodborne disease Any disease caused by infectious or toxic agent thought to be transmitted by consumption of food (including drinking water); often wrongly referred to as food poisoning.

Food hygiene All conditions and measures necessary to ensure the safety and suitability of food at all stages of the food chain.

Food industry The term includes primary, manufacturing, and processing industry as well as some other establishments involved in the food chain, for example, large-scale catering establishments and retailers.

Food safety Assurance that food will not cause harm to the consumer when it is prepared and/or eaten according to its intended use.

Food safety objective (FSO) The maximum frequency and/or concentration of a hazard in a food at the time of consumption that provides or contributes to the appropriate level of protection.

Food suitability Assurance that food is acceptable for human consumption according to its intended use.

Hazard analysis and critical control point system (HACCP) A system which identifies, evaluates, and controls hazards which are significant for food safety.

Performance criterion The effect in frequency and/or concentration of a hazard in a food that must be achieved by the application of one or more control measures to provide or contribute to a performance objective or an FSO.

Performance objective (PO) The maximum frequency and/or concentration of a hazard in a food at a specified

step in the food chain before the time of consumption that provides or contributes to an food safety objective or appropriate level of protection, as applicable (compare with food safety objective).

Precautionary principle A notion which supports taking protective action before there is complete scientific proof of a risk; that is, action should not be delayed simply because full scientific information is lacking.

Risk A function of the probability of an adverse health effect and the severity of that effect, consequential to (a) hazard(s) in food.

Risk analysis A process consisting of three components: risk assessment, risk management, and risk communication.

Risk assessment A scientifically based process consisting of the following steps: (1) hazard identification, (2) hazard characterization, (3) exposure assessment, and (4) risk characterization.

Risk communication The interactive exchange of information and opinions throughout the risk analysis process concerning risk, risk-related factors, and risk perceptions among risk assessors, risk managers, consumers, industry, the academic community and other interested parties, including the explanation of risk assessment findings and the basis of risk management decisions.

Risk management The process, distinct from risk assessment, of weighing policy alternatives, in consultation with all interested parties, considering risk assessment and other factors relevant for the protection of consumers' health and for the promotion of fair trade practices, and, if needed, selecting appropriate prevention and control options.

Verification (general) The application of methods, procedures, tests and other evaluations, in addition to monitoring, to determine whether a control measure is or has been operating as intended.

Introduction – Historical Background

Although protecting food from spoilage and degradation has been a concern for mankind since the dawn of history, it is mainly in the past few decades that food safety management has received its due scientific attention.

The history of food safety goes back to the time of the earliest humans when the consumption of poisonous foods was a threat to their survival. Presumably, they used their sensory perceptions, i.e., taste and smell, together with memory to evaluate the safety of their food. In other words, consumers would directly taste the food and would learn through trial and error, avoiding those foods which presented an acute risk for health. The cold climate during the ice age period also contributed to the preservation of food and the prevention of microbial organisms. Later, the discovery of fire was a major milestone in the history of food safety, as cooking of food, particularly meat, over fire contributed to its nutritional quality and microbial safety. Today, we know that it may also have introduced some process contaminants such as polycyclic aromatic hydrocarbons, acrylamide, food safety concerns discovered in modern times.

As civilization progressed, religious beliefs in many cultures prescribed food handling practices, many with food safety implications. As societies grew to include specialized food-related groups, like the butcher, the baker, and wine maker, governments regulated certain practices and their enforcement set the basis for food safety management. For the most part, these regulatory requirements were focused on the prevention and control of fraud.

The work of several scientists has been instrumental to the progress in the microbial food safety. Among these, Anton von Leeuwenhoek (1632–1723) first reported seeing microbes under the microscope and called these as *animalcules*. Later in the nineteenth century, the work of scientists such as Louis Pasteur (1822–95) who developed the germ theory and Robert Koch known for the criteria which establish the causal relationship between a pathogen and an illness, set the basis of modern food microbiology and food hygiene.

However, until recent history, i.e., the 1970s, unsanitary practices such as the use of unsafe water were the main concern as many prevalent infectious diseases, for example, cholera, shigellosis, typhoid fever, were attributed to the use of unsafe water or poor sanitation. The management of food establishments was also heavily based on visual inspection, and sometimes on subjective judgments. As laboratory techniques developed, microbiological or chemical testing of food products came to complement the visual inspection and formed the basis for food safety management.

In the 1960s, awareness on the chemical aspects of food safety grew from the concern associated with the increasing use of food additives in the food manufacturing industry as well as the use of agrochemicals in the food production. Advocates such as Rachel Carlson, author of the famous book 'Silent Spring' drew the attention of the general public on the environmental risks associated with the use of dichlorodiphenyltrichloroethane, greatly contributed to this awareness.

On the microbiological side, it was only in the mid 1980s that the concern for food safety came to the focus of public health authorities and the decade of 1990s was a period of growing awareness about foodborne illnesses. In countries monitoring foodborne diseases and outbreaks, authorities noted an increase in a number of foodborne diseases such as salmonellosis, campylobacteriosis, and the occurrence of large-scale foodborne disease outbreaks. These, together with the emergence of new pathogenic agents such as enterohemorrhagic *Escherichia coli*, *Campylobacter* spp, *Listeria monocytogenes*, abnormal prions (agent of bovine spongiform encephalopathy (BSE)), and an array of food safety incidents, such as the case of feed contaminated with dioxin in Belgium in 1999, just to mention one as an example, increased consumers' concern and undermined their trust in the safety of the food supply and the capability of regulatory authorities in their governance role. With regard to the developing countries, the role of food

contamination in infant diarrhea and its association with malnutrition received increasing recognition at the international level; however, it was the cholera epidemic in Latin America and Africa in the 1990s, which really raised the awareness of the governments of the developing countries on the importance of food safety management as countries who were affected by cholera saw their food export embargoed for food safety reasons. Later, the European Union extended their food import embargo to all those countries that could not demonstrate capabilities in controlling the safety of their food supply.

Consequent to the increased awareness of the general public on the existence of gaps in food safety management, which were particularly notorious in the management of the BSE crisis in 1996 and 2000 and the dioxin crisis in 1999 in Belgium, a general atmosphere of mistrust developed in Europe. This also affected the acceptance of technologies in food production and processing, for example, the use of food additives, biotechnology, food irradiation, etc. On the other side of the Atlantic in North America and also in other countries like Japan, large scale outbreaks of foodborne diseases associated with foodborne pathogens such as Salmonella, enterohemorrghic *E.coli, Listeria monocytogenes*, alerted the general public on the importance of food safety and a number of programmes for its improvement were initiated (e.g., the Clinton Initiative).

Concomitantly with the increased public concern, the finalization of the Uruguay Round of Multilateral Negotiations in Marrakesh and the establishment of the World Trade Organization (WTO) in 1995 paved the way for increased trade in food and feed, and thus for concern about the import of contaminated food and feed. To provide countries with the right for protection without establishing unnecessary discriminatory regulations, 2 WTO agreements, 'Agreement on the Application of Sanitary and Phytosanitary Measures (SPS)' and the 'Agreement on Technical Barrier to Trade,'" came into force when WTO was established. These agreements also included concepts such as appropriate level of health protection, risk assessment, equivalence, etc, which would require further clarification.

It was in this climate of food safety problems, increased trade in food and feed and increased consciousness about the role of food safety in public health, that a number of fundamental questions were raised among stakeholders:

- How safe should food be?
- What should the Appropriate Level of Protection be?
- To what level should hazards in food be controlled?
- Who decides and what data should be considered in the decision-making process?
- How are consistency, objectivity, acceptability, and efficiency ensured?
- How efficient and cost-effective is a control measure and at what point of the food chain should a hazard be controlled and at what cost?
- How are feasibilities and other risks or factors considered in the decisions?
- How will food safety be controlled in the global market?

Meanwhile, consumers raised a number of questions from their perspective:

- Who is deciding? On what basis?
- How is the uncertainty considered in the decision-making processes?
- How are stakeholders' views taken into account?
- How are the societal values considered?

These questions give rise to principles and concepts such as transparency, precautionary principles, involvement of stakeholders in the decision-making process, and the balance between scientific and social values.

The food safety incidents such as BSE and dioxin mentioned above also raised the importance of:

- The 'Farm to Fork' approach,
- The consideration of science and uncertainty in science in the process,
- Transparency in decision-making,
- The impact of perception on the food supply,
- Defining responsibilities.

The WTO/SPS Agreements already answered a number of questions and contributed to the development of the modern approach to food safety management; required that regulations follow a certain number of principles, namely:

- Be based on sound scientific assessment of the risk,
- Be non-discriminatory,
- Be transparent, and
- Accept equivalent approaches to achieve the same level of health protection.

Additionally, WTO referred to the standards, practices and other recommendations of the Codex Alimentarius Commission (CAC) as the representing international consensus regarding health and safety requirements for food. In other words, the SPS Agreement recognized CAC as a reference for international requirements for food safety. This meant that countries that rejected food which complied with the Codex food safety standards had to provide scientific evidence that the food in question posed a specific risk for their population. Indirectly, this article also encouraged countries to align their legislation with the standards of Codex Alimentarius. Short of this, they could be challenged by an exporting country to provide scientific evidence, i.e., risk assessment, for having a legislation that is more stringent than the standards and recommendations of Codex. A illustrative example of the application of this article was the case of hormones in beef for which European countries had to provide evidence of risk before a WTO Dispute Settlement Panel. Eventually they lost the case based on the risk assessment evidence provided in part by experts serving on the Joint FAO/WHO Expert Committee on Food Additives (JECFA). In this regard, note that Codex bases all of its health standards on the recommendations of JECFA and other FAO/WHO expert bodies.

These developments at the international level and the increased awareness of the general public led to numerous changes in the management of food safety. In the food industry, the application of hazard analysis and critical control point (HACCP) system and traceability received heightened attention and in some countries, it became mandatory. At the

governmental level, risk analysis emerged as a decision-making process. In some countries or regions, it led to the restructuring of governmental institutions. For instance, in Europe, the European Food Safety Authority was established in 2002 as a result.

Concept of Food Safety and its Definition

Today, the subject food safety has become a discipline in its own right and a formal definition was elaborated in 1997 by the CAC.

According to the CAC, 'food safety is the assurance that food will not cause harm to the consumer when it is prepared and/or eaten according to its intended use.'

This definition embodies several important notions:

1) The notion of harm which separates safety aspects of food from other quality aspects that make food unfit for human consumption without necessarily presenting a danger to health. The aspects of food which make it unfit for human consumption, even though it is safe, are referred to by CAC as food suitability.
2) The concept of assurance, i.e., food safety and its management should be based on measures that are in place to provide assurance that food is safe. In other words, food safety depends on the conditions in which food is produced and prepared, and not on the results of the end-product testing, which for many contaminants cannot be a reliable method for food safety assurance. The conditions for ensuring both safety and suitability are referred by the CAC as 'food hygiene.'
3) Preparation and/or use of a food product should be considered in the design of the safety, and *vice versa*. A food product is considered safe if it is prepared and/or used according to its intended use. Subsequently, the intended use should be considered by the producer, manufacturer or, those selling the product in the design of the product as well as in their information to their customers, if necessary. Customers must also follow the instruction of manufacturers. In this way, the definition promotes interaction between the stakeholders of the food chain.

The definition also stipulates that food will not cause harm. However, it is also generally recognized that 100% safety does not exist as, realistically, foods cannot be totally void of the presence of the multitude of hazards in the environment. Even for agrochemical and food additives that undergo extensive testing before they are permitted in food, there is always a chance that toxic effects can occur. This brings in the notion of risk that is also defined by CAC as a function of (a) the probability of an adverse health effect and (b) the severity of that effect, consequential to a hazard(s) in food. Over and above the exposure, the risk depends also on three factors: the susceptibility of the individual, the nature of the agent, i.e., the degree of pathogenicity and virulence (e.g., the dose-response relation, bioavailability of an agent), and also the food matrix. The dose-response relationship for certain pathogens may be different according to the food matrix. For instance, it has been experienced that in a fatty matrix such as chocolate, a lower number of pathogens can trigger illness than in some other foods.

As one tries to analyze the notion of safety, one can see that safety is a complex subject, and often very difficult to communicate. A frequent misconception and error by the general public is not realizing the difference between hazard and risk, i.e., the mere presence of a hazard at a certain low level does not necessarily mean that food is unsafe. It is the quantity of the hazard in the food and the amount of food ingested, in other words the likely dose of exposure, which determines the risk posed by a product.

On a scientific basis, risk assessors can qualitatively and quantitatively evaluate the risks associated with the presence of a given hazard in foods. However, the question remains as to when a food is considered as safe. Today, it is generally accepted that a food is safe when it does not present an unacceptable risk for the population. At the same time, it is recognized that what is considered as an unacceptable risk in a society depends on the perception of the risk by the population, and on a number of other factors such as the cultural and societal values (e.g., animal welfare, religion, and ethics), other health risks such as (nutritional, microbial, or chemical), other societal risks (e.g., environment, reputation), feasibility and costs (which itself impacts on the price of food), consumer preferences (organoleptic quality of food).

Risk communication experts have identified a number of factors that can influence the acceptability of risk. These are:

- The prospects of significant benefit for 'me',
- Whether the risk is voluntary (i.e., if there is consent) or involuntary,
- If the risk is familiar,
- The 'dread' factor
- If the risk and benefit are 'fairly' distributed,
- If the risk is part of an unethical activity,
- If the risk assessor and risk manager are trustworthy,
- Unnatural versus natural risk.

The consideration of these factors is essential in risk management: i.e.,

- Who is assessing the risk,
- How it is carried out,
- Who, how, and what is communicated,
- Which factors are considered in the decision-making process.

These considerations led to a change in the process of decision-making and the introduction of the risk analysis process in food safety management.

Food Safety Management: Shared Responsibility

One of the key functions in the management of any subject is the determination of responsibilities. For the management of food safety at the societal level, in the 1990s the WHO coined the concept of shared responsibility (Figure 1), according to which a concerted effort of the different sectors, i.e., government, industry, consumer, and academia, was needed to ensure that food up to the point of consumption is safe, wholesome, and benefits consumers nutritiously. The concept was represented as a building (temple) where each sector formed a supporting

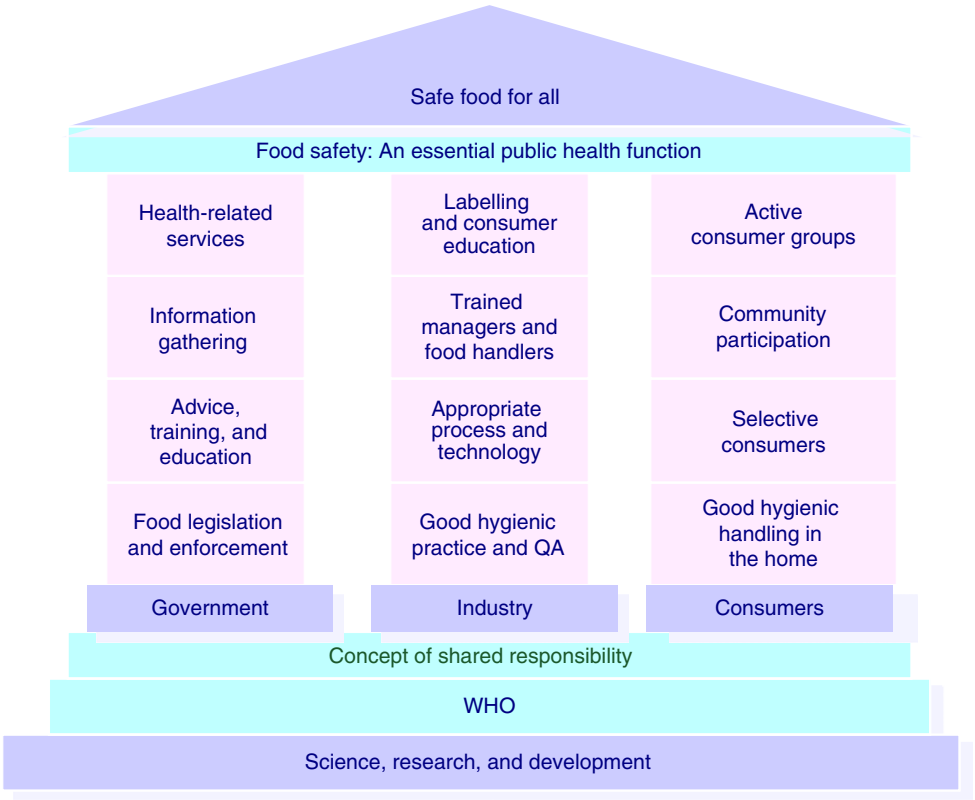


Figure 1 Concept of shared responsibility as proposed by the World Health Organization (WHO). Reproduced from WHO (2000) *Foodborne Diseases: A Focus on Health Education*. Geneva: World Health Organization.

pillar. The building blocks of the pillars describe the roles and functions of each sector. While the building block of the temple has greatly evolved subsequently, the concept as such is still valid.

Principles of Food Safety Management

The developments and events of the 1990s led to a rethink of the principles and procedures of food safety management, both at the national and international level.

The fundamental principle in the new approach to food safety management was the recognition that protection of human health should be the primary objective in food safety management.

Additionally, the following key principles gained prominence:

1. Integrated approach, i.e., consideration of the risks and control measures along the entire food chain up to the point of consumption.
2. Shared responsibility, which as mentioned above, is the recognition that all sectors, including consumers, have a responsibility in ensuring food safety.
3. Multi-disciplinary approach, which comes from the understanding that ensuring food safety requires different types of scientific and operational expertise.
4. Evidence-based and risk-based decision-making to ensure objectivity and the most efficient use of resources in food safety management. This principle is important to assure stakeholders or trading partners that measures are based on scientific and technical evidence, are effective and commensurate to the degree of risk. The principle also facilitates the implementation of the WTO/SPS article on equivalence mentioned below.
5. Equivalence: This principle allows countries to deviate from the requirements of importing countries, if they can demonstrate the equivalence of measures on a scientific basis. This principle was an additional impetus for the concept of validation of control measures.
6. Transparency, uncertainty, and precautionary principles: Transparency is an obvious consequence of the above-mentioned principles on the evidence-based decision-making process. However, it gains particular importance when there is uncertainty in data or when data are lacking. The value of transparency is that, in absence of full scientific information or variation in the degree of risk, the uncertainty and variability are declared; evidence of the adequacy of protective measures is provided. Transparency also increases trust in stakeholders and trading partners. The precautionary principle stipulates the need for taking protective action before there is complete scientific proof a risk that is action should not be delayed simply because full scientific information is lacking.
7. Structured approach: i.e., while risk managers and risk assessors should maintain an active interaction, there should be a functional separation between risk assessment and risk management to ensure objective and unbiased

decision-making, balancing scientific consideration with societal values and economic interests, as well as considering the risk perception of consumers.

8. Harmonization of food standards, although a goal of the CAC, de facto has become a consequence of the WTO/SPS agreement.
9. Continuous improvement: As in any quality management system, a plan, act, and review process should be applied in food safety management in order to incrementally but continuously improve the safety of foods to reduce risks to as low as technically achievable. This principle does not apply to foods which present an immediate and/or an unacceptable risk to consumers' health and where a crisis management procedure should be implemented. The principle applies both in governmental functions that should progressively drive the contamination of food supply and the incidence of illnesses to as low a level as technically and reasonably achievable (as low as reasonably achievable principle), as well as to industry where it is expected to have a yearly objective for improving the food safety assurance system.

Most importantly, there was the recognition that food safety cannot be solely measured by the number of incidents or the end-product testing but should be evaluated in terms of measures put in place to prevent contamination. With regard to the former it should be remembered that a past record of safety is not a guarantee for the future. As to end-product testing this presents several limitations in providing food safety assurance with regard to microbiological hazards:

- End-product testing is of limited value for assessing and verifying safety due to the large number of samples that would be required to be tested to have a reliable evaluation.
- As alluded before, to have a reliable information, a great number of samples need to be tested and this will be costly and thus often not feasible.
- Even if the agent is detected, the approach leads to loss of time and product. In some conditions, for example, in the airline catering business, the food may even be consumed by the time the result becomes available.
- Finally the method leads to the detection of the problem without any information as to its cause.
- With this principle, the limitation of end-product testing as a control measure, in particular for certain hazards such as microbial hazards or mycotoxins, was recognized.

With the trend to move away from end-product testing and statistics on incidence as evidence of safety, emphasis is put on preventive measures and a clear differentiation is made between preventive and control measures versus measures for verification purposes.

Elements of Food Safety Management

Management of food safety in today's world has become a very complex and intricate task. On the one hand, a mind-boggling number of chemical, microbiological, and physical hazards may find their way into the food supply, and this at any stage of the food chain. The control of these *per se* is a major challenge, as each presents a different set of characteristics. On the other hand, measures necessary for ensuring food safety are often intertwined with a number of other considerations of social, environmental, cultural, and economical nature. Awareness of this complexity combined with a number of major food safety crises which hit the society in the past few decades have been an impetus for major changes in the management of food safety and the development of procedures and infrastructure. Parallel to the development of food safety management systems, the food defense system has also been strengthened. The food defense system has been developed with the specific objective to protect food safety from bioterrorism and other types of sabotage.

Figure 2(a) and **(b)** illustrate the functions of different sectors as described below.

Government

Public health and food control authorities have the leading role in managing food safety and have the responsibility of overseeing that food supply is safe up to the point of consumption. With this responsibility, they have to do the following:

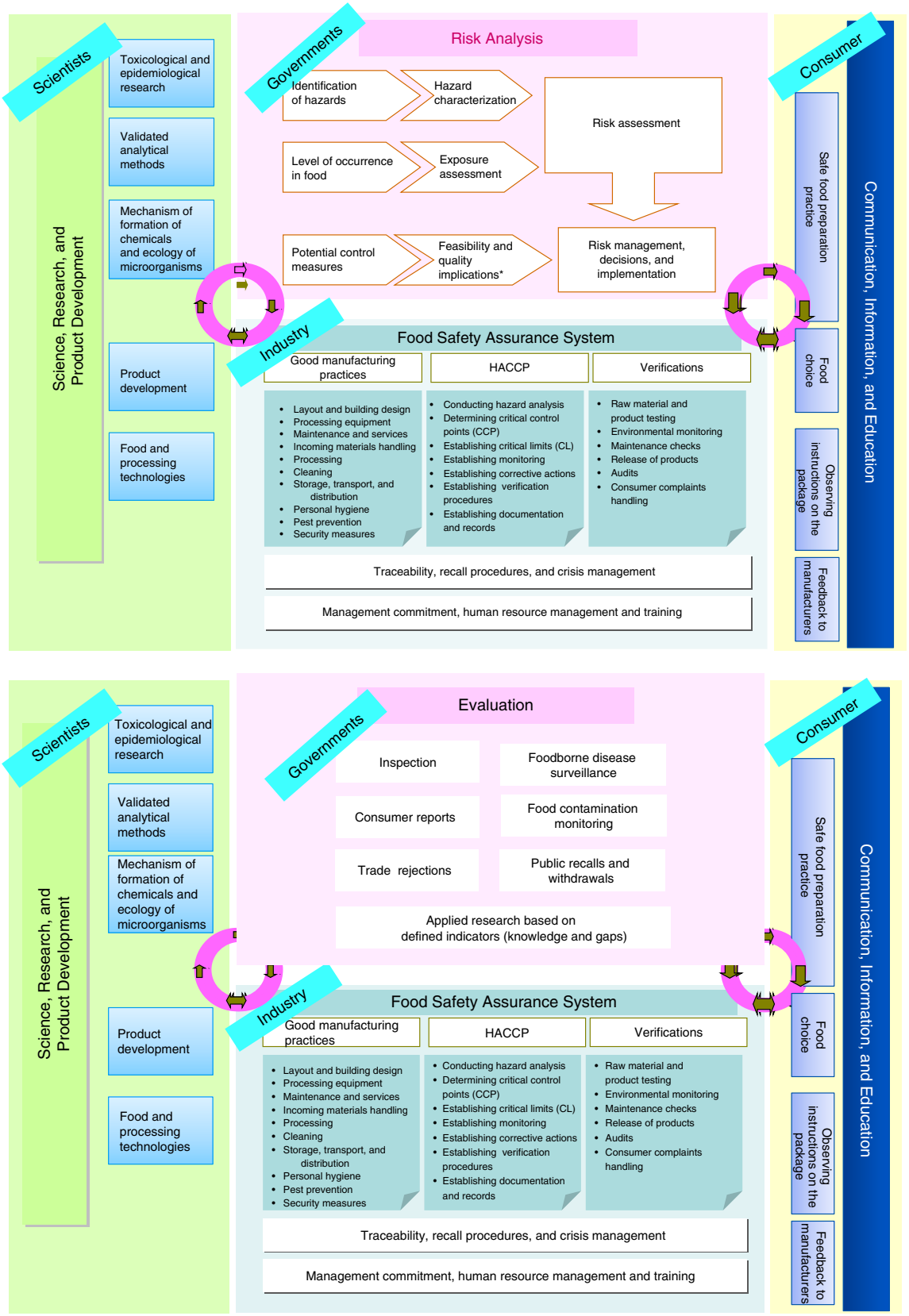
a) Foresee all infrastructures and public health services that are necessary for a good food safety management, such as public health laboratories, water supply and sanitation, etc.,
b) Promulgate laws and regulations, which give priority to public health but which can also meet other societal and environmental requirements,
c) Enforce legislation through inspection, monitoring food supply as well as the provision of advice to the trade and commercial sector, and
d) Provide education to care givers, consumers, travelers, health professionals, and the public at large.

Decisions on measures required to manage risks today are taken in the context of the risk analysis process. There are different types of models for describing the risk analysis process. **Figure 3** depicts the process according to Codex Alimentarius. The process includes: risk assessment, risk management, and risk communication.

As risk managers, regulatory authorities are, among others, responsible for (1) driving the risk analysis process, (2) setting public health goals, and (3) deciding on risk management priorities.

The risk management process itself comprises a number of steps which are briefly discussed here. For more in-depth review, the reader is referred to specific articles in this encyclopedia.

The first step is referred to as preliminary activities. As part of this, governmental risk managers will commission a risk profile for a given hazard or hazard/food. Based on the outcome and in the light of existing data, they will decide if a risk assessment is required, or if it is possible to evaluate various control options. Should risk managers find that a risk assessment is justified, bearing in mind the resource and time investment, they may decide to commission a qualitative or a quantitative risk assessment. In this case, they are responsible



* Include nutritional, microbiological, chemical safety as well as cost and consumer acceptance

Figure 2 (a) Overview of food safety management and the role of different sectors – illustrating the risk analysis process by governmental authorities. (b) Overview of food safety management and the role of different sectors – illustrating the various evaluation activities by the governmental authorities.

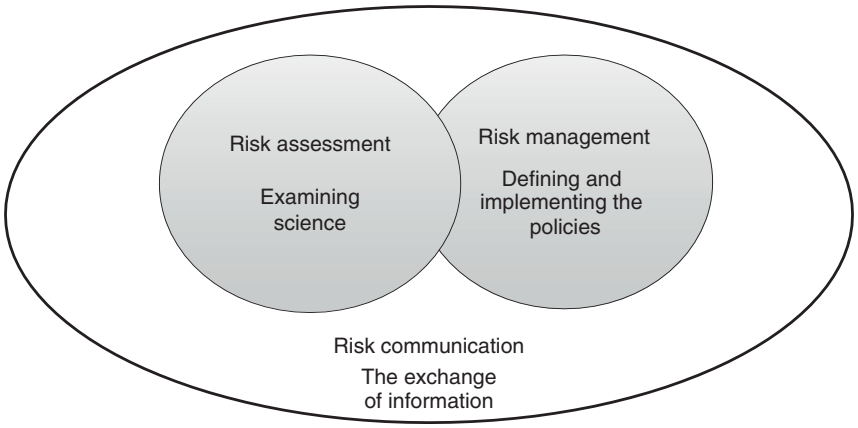


Figure 3 Risk analysis process.

for elaborating a risk policy assessment in consultation with risk assessors and other interested parties. A risk assessment policy is a guidance to risk assessors, outlining information such as:

a) The purpose and scope of risk assessment, for example, sector of the food-chain, types of food, and products to consider.
b) Target populations or subpopulations.
c) Key scientific judgments, particularly when a high degree of uncertainty in existing data or in data gaps exists.
d) The type and sources of data to be considered.
e) How the data should be presented, in particular the types of assumptions and uncertainties.

The process of risk assessment and risk management follows an iterative interaction between risk assessors and risk managers, during which these need to foster mutual understanding and refine the risk assessment so that it responds as closely as possible to the questions posed by risk managers. In deciding on the appropriate control measures, risk managers need to take into consideration a number of other factors, sometimes also referred to as 'other legitimate factors.' These factors vary according to the nature of the hazard under consideration, and can include costs, feasibility, benefits, other risks (e.g., environmental or nutritional), consumer preferences and societal values, such as animal welfare. At times, risk assessment may be required to advise the efficiency of control measures, to develop an understanding of the public health outcome according to different levels of contamination, to have an estimation of the risk of various food/hazards combination, etc.

In managing a risk, depending on the nature and degree, and on other factors mentioned above, risk managers have different options at hand. These range from taking a regulatory action such as those listed below to taking no action.

- Compliance with certain standards (e.g., setting a norm for a chemical hazard or a food safety objective (FSO) or microbiological criteria for a microbiological hazard),
- Labeling,
- Testing and/or certification of foods,
- A specific processing of foods to inactivate pathogens,
- Application of a code,
- Recalling a product in case of an incident.

Alternatively, they may decide to manage the risk by providing education to consumers or requiring the training of food handlers in food service establishments. It can also happen that they decide not to take any action (e.g., if the risk is low or negligible). In any event, the food safety authorities have the responsibility to communicate and explain their decision to the stakeholders.

To identify possible food safety problems and to review the implementation of the risk management decisions and to evaluate the need for any revision in decisions or implementation, the collection of various types of data could be considered. Examples are:

- Inspection reports and evaluation of implementation of risk management decisions by the food industry,
- Monitoring of chemical contaminants,
- Surveillance of foodborne diseases (data from different types of surveillance methods need to be considered),
- Consumer complaints,
- Trade rejections,
- Public recalls, withdrawals and/or incidents, and
- Applied research based on defined indicators (knowledge and gaps).

Other types of information may also be required for planning, improvement, or preventive actions. Examples are trends in incidents and alerts, whether occurring in a country or outside the national boundaries, adequacy of resources, as well as various changes in the society, for example, changes in climate, demography, international trade and travel, or the emergence of new agents.

Industry

The industry is responsible for ensuring that the food that it puts on the market or served in food service establishments is safe, fit for human consumption, and meets the regulatory requirements of the country where it is marketed. They have to consider the regulatory norms for hazards as food safety

standards and ensure that their products are not violating these limits. To meet these responsibilities, the food industry is required to have an integrated food safety assurance system.

A model for this system consists of combining three sets of measures according to three lines of defense (Figure 2):

The first line of defense is the implementation of codes of good practices. These are a set of general measures and principles that have been identified through past experience as necessary to ensure safety and wholesomeness of produced foods; with some adaptation, they are generally applicable to all categories of foods and products and/or establishments regardless of location, specific conditions, and type of business. Depending on the sector, they are referred to as Codes of Good Agriculture Practice, Codes of Animal Husbandry, Codes of Good Manufacturing Practice, Codes of Good Transport or Storage Practice, etc. Very often, such codes are voluntary, but at times they are legally established by regulatory authorities. However, where they do not exist or are not stringent enough, the industry may also develop such codes. The Codex Alimentarius Commission has developed a large number of codes of practices. The recommended International Code of Practice – General Principles of Food Hygiene is one of the 'horizontal' codes with wide application in the food industry.

The second line of defense is the application of the HACCP system. During this process, hazards specific to a food and/or process are proactively analyzed and control measures specific to the identified hazards are identified. For steps that are considered critical for ensuring the safety of the food product, monitoring parameters and critical limits are established and the steps are monitored to ensure that the critical limits are respected at all times. As for codes of practices or any other regulatory requirement where governments have provided limits for a hazard (norms or FSOs) or performance criteria intermediary processes, these need to be considered during product/process design or respected during operations. Needless to say that during the development of a HACCP plan, measures identified for controlling the hazards and the parameters and limits to be respected have to be validated, short of which the HACCP study will become a simple paper exercise. HACCP also has other elements such as corrective actions in case the process is going out of control, and as explained below, verification and documentation.

A strategy that has been used by some governments to assist small or less developed businesses in applying the HACCP system is to develop HACCP-based codes of practices for specific categories of food products. Such an approach is important for small or less developed businesses as they often lack expertise in food safety, and unless assisted by a trade organization, they may not be in a position to carry a HACCP study by themselves. A HACCP-based code of practice for a specific sector combines both the general principles of food hygiene, and the considerations and requirements specific to a given sector.

Frequently, the question is raised about the difference between the code approach to food safety assurance versus the HACCP system and their respective benefits. Originally, a code approach was viewed as a general and prescriptive system of management of food safety in a business. Subsequently, HACCP was recommended by public health authorities to promote a proactive approach based on the analysis of hazards in foods or processes, before these lead to an incident. When applying the HACCP system, hazards specific to a particular food product, process, and to the conditions in which the food is prepared, are identified and control measures specific to the hazard in question are devised. In this way, as opposed to codes which are for general guidance, through the HACCP system, control measures are targeted at hazards specific to the product (raw material or conditions of production). However, with experience, it became evident that both approaches have their respective values, and that HACCP would be more efficient if some basic hygienic conditions and preventive measures were in place. Today, in food safety assurance systems of the food industry, these are referred to as prerequisites, and it is recognized that it is by combining both approaches that the optimum conditions of food safety management are attained.

Very often, the documentation required as part of HACCP has given the system a negative image of being burdened by paper work. However, records and documentation are essential as support material for communication between members of the HACCP team and/or with time, for the maintenance of the plan, i.e., for the HACCP team to be able to consider the need for any change in the plan and thus ensure that the system remains valid and up-to-date. Also, records are required to provide evidence to customers and/or inspectors on the adequacy of measures.

The third and last line of defense is verification activities. These are also part of the HACCP application, but to delineate between measures implemented for prevention and those required for verifying that preventive measures are effective and performing correctly they are presented separately.

As for the governmental evaluation process, verification activities include all tests and other data collected to verify that preventive measures are implemented and achieve the objectives set. Verification should not be mistaken for validation, which is a process to ensure that control measures are effective to achieve the objectives desired. The validation process is usually implemented during the product and process design stages, or when a change has been made in product design during its manufacturing. If verification data indicate that a product is not meeting a set standard, even though the plan has been implemented, validation of control measures may be put in question.

In principle, where codes of good practices and the HACCP system are optimally implemented, a high degree of safety can be assured. Nevertheless, verification measures are important to detect any dysfunctionality in the system. They also provide evidence of compliance with the food safety standard and should not be stopped on the grounds that data on contamination are negative, as data are needed for proving the performance of the food safety assurance system, at all times. Examples of verification measures are:

- Raw material and end-product testing
- Environmental monitoring
- Calibrations and other maintenance checks
- Release of products
- Audits
- Consumer complaints handling

Should verification data indicate non-compliance, the adequacy of the implementation of the HACCP system and the prerequisites must be examined in the first place. In absence of any non-compliance, the validation of elements of the HACCP study can then be questioned.

At times, in spite of all measures, it can happen that a raw material used in a product is contaminated or a product which is contaminated is marketed. Through a traceability system, i.e., information on the source of raw materials or the customers who have received the product, a contaminated product can be traced and recalled. Regulatory authorities in some countries require that the traceability system of an establishment ensure that information on the source of a raw material or the destination of a final product be available for one step up or one step down. With the globalization of food supply and the passing of food ingredients through various traders, it is sometimes difficult to ensure precise or valid information on the condition of the production of raw materials. Where information on traceability is lacking, the investigation of outbreaks and identification of implicated food becomes more difficult as observed in an outbreak of *Salmonella* in paprika in the US, originally attributed to tomatoes, and subsequently consumers may be more exposed to a contaminated product for a longer period of time. The weaker the traceability, the larger the scale of the outbreak. This was demonstrated in the dioxin incident in Ireland where a full product recall was conducted for pork meat, whereas for beef meat it was possible to limit the recall to the contaminated product because after the BSE crisis, a traceability system was established for beef products. Similarly, in the food manufacturing industry, the finer the traceability, for example, indicating the date and time the product was produced, the smaller the product waste in case of recall.

Finally, the entire food safety assurance system should be supported by a well-performing crisis management system to protect consumers from exposure to contaminated products.

Fundamental to all these systems are the training and education of the staff as well as the management commitment. Therefore, fostering a culture of food safety for people, from their training to their motivation and understanding of their perception and constraints, constitutes one of the most important pillars of food safety management in industry and in governmental functions. The importance of an organization culture cannot be emphasized enough. Reporting of any non-compliance or of a risk-prone situation at an early stage can contribute to preventing 'near misses' situations, and thus the likelihood that one of these resulting in an incident.

Consumers and Informal Sector

Consumers at large and domestic and professional food handlers in particular, also have an equally important role in food safety. These include, but are not limited to:

- Observation of good hygienic practice in the preparation of food.
- Reading information (e.g., use 'by date' of products, target consumer) on the labels of products and observing the instruction for the preparation and storage of products.
- Reporting defective (unsafe) products to the public health authorities and/or manufacturer.
- Be discriminatory in the selection of products and establishments to exclude those that may present a risk for health and do not respect food hygiene, do not meet regulatory requirements or have unethical practices.

To enable consumers to assume their responsibility in hygienic handling of food as well as to judge potential risks with certain products, practices or establishments, consumer information, and education is the key. This is best carried out by professionals who are both trusted by the general public and who also have contact with the pubic in the framework of their work. An example of such a professional group is health professionals, or teachers in the schools. Unfortunately, these professionals are not always aware of or attentive to the importance and magnitude of the problem; many perceive foodborne illnesses as benign self-limiting illnesses, or are misled by the poor statistics in this area or their erroneous interpretation.

Academia

Scientists in general, whether they work in academic institutions, in governments, or in industry, also have an important function. With the trend in evidence-based decision-making and taking science into consideration, be it life or social sciences, the role of this sector in the risk analysis process has increased during recent years. Their integrity, excellence, and relevance make them ideal communicators to managers (e.g., report of their results, articles) or to the general public (e.g., interviews in the mass media). As such, they play an important role in both the management of food safety (in particular risk assessment and risk communication) and the management of a crisis. However, they may also be the source of a crisis, should they fail in their risk assessment or have an inappropriate risk communication practice.

On the technical aspects, scientists contribute to food safety management by providing different types of scientific data and their assessment, which is necessary for decision-making. Examples are:

- Toxicological information, mechanisms of contamination of foods with chemicals, or their formation,
- Ecology of microorganisms and epidemiology of foodborne diseases,
- Validated analytical methods,
- Process and technologies to control hazards,
- Consumer perception, beliefs, and practices.

In industry, scientists can minimize risks associated with products and processes by designing them out during product development and defining necessary control measures to manage the operational risks during the production or manufacturing of foods.

Additionally, scientists can further contribute to the management of food safety by creating tools to make information on food safety easily accessible to all the actors in the society. The participation of several hundred scientists from different sectors in the preparation of the Encyclopedia on Food Safety is an example of such a concerted effort and of contribution of scientists to the management of food safety.

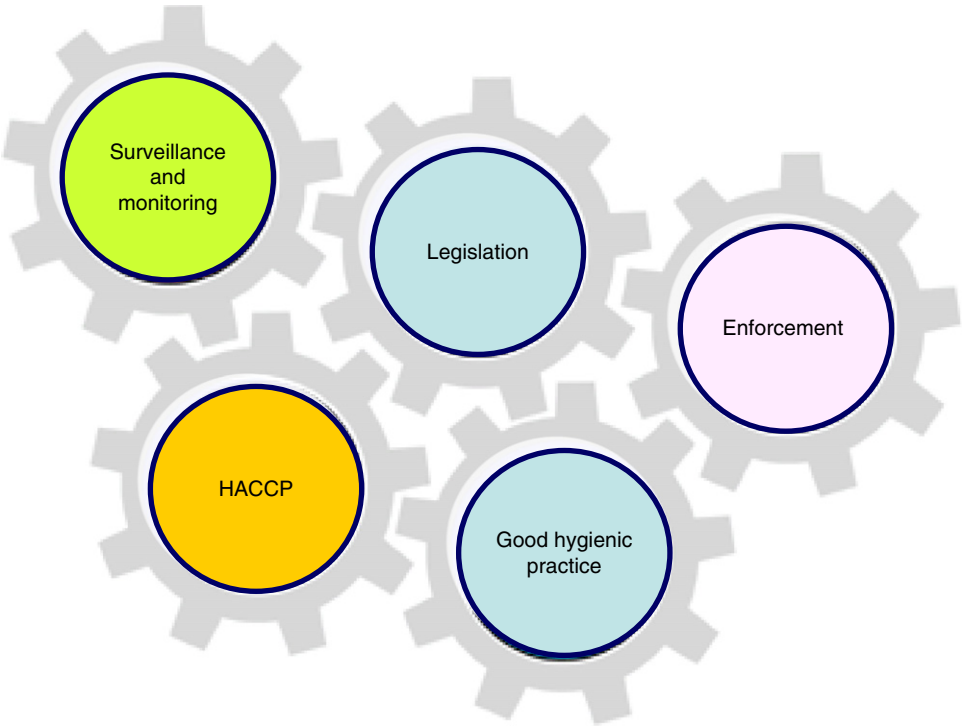


Figure 4 The need for an interconnected food safety management system.

Conclusion

In summary, the history of food safety can be considered in three major eras:

- A time where consumers were managing the safety of products directly,
- An era where governments were managing food safety by testing products and removing contaminated or non-compliant products from product; food was considered safe unless tests would indicate otherwise.
- An era where the food business has become responsible for providing evidence that they have taken necessary measures to prevent contamination of foods and ensure the safety of products. Since then, the role of governments has shifted from identifying potentially unsafe food or unsafe food practices to supervising and verifying the implementation of a food safety management system by industry.

In the contemporary world, due to the huge number of risks associated with food, the complexity of operations and the food chain, international trade in food, the diversity of food products as well as consumer practices and perception, the management of food safety has become very complicated. These have required the establishment of an objective and a transparent system of decision-making, taking into consideration both science and the views of stakeholders.

To manage food safety efficiently, a concerted effort is undoubtedly needed on the part of all sectors, i.e., governments, industry, consumer, and academia. Each sector has a specific role and responsibility; however, to have a well-functioning system at the national or international levels, it is important that different elements of food safety be interlocked, like the gears in a machine, that different sectors work in a concerted and coordinated effort (Figure 4). Today, there is adequate science and technical know-how to ensure the safety of the food supply, at least with regard to most hazards that are known to science. It is weakness or neglect in the implementation of the know-how, past experience, and policies that are often the source of major adverse events. However, while collaboration between sectors is needed, the independence in research, in the decision-making process at governmental level and in implementation is the corner stone of successful and sustainable food safety management. In doing so, consumers' health should be the first priority.

See also: Food Safety Assurance Systems: Essentials of Crisis Management; Good Animal Husbandry Practice; Good Practices in Fisheries and Aquaculture; Hazard Analysis and Critical Control Point System (HACCP): Principles and Practice. Public Health Measures: Evaluation of the Efficacy of National Food Control Programs; Food Inspections and Enforcement Systems; Fundamentals of Food Legislation. Risk Analysis: Estimating the Burden of Foodborne Disease; Food Safety Training and Health Education: Principles and Methods; Risk Analysis of Hazards in Food: An Overview; Risk Assessment: Chemical Hazards; Risk Assessment: Microbiological Hazards; Risk Assessment: Principles, Methods, and Applications; Risk Communication: Biological Hazards; Risk Communication: Chemical Hazards; Risk Communication: Diet, Nutrition, and Health; Risk Communication: Novel Foods and Novel Technologies; Risk

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PUBLIC HEALTH MEASURES

Risk Governance

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Glossary

Concern assessment A systematic scientific process of gathering and analyzing data on social responses to food risks, insights on risk perception, and other impacts related to downstream consequences of food consumption.

Framing Relates to risk assessment policy (in the terminology adopted by Codex Alimentarius) and includes the initial analysis and scoping of a risk problem and the setting of terms of reference on which the assessment authority will base the assessment.

Risk assessment Primarily a scientifically driven process aimed at understanding risk and concluding what we know about risk. It comprises the task of identifying and exploring the types, severity, and likelihood of the (normally undesired) consequences related to a risk source.

Risk evaluation The process of determining the value-based components of making a judgment on risk. This includes risk–benefit balancing or incorporation of quality-of-life implications and may also involve looking at such issues as the potential for social mobilization or at prerisk issues such as choice of technology and the social need of the particular operation giving rise to the risk.

Risk management An intrinsically political process primarily aimed at deciding what to do about risk. It includes generation, selection, and implementation of management measures as well as monitoring of how these measures perform in practice.

Stakeholder Socially organized groups that are, or will be, affected by the outcome of the event or the activity from which the risk originates and by the risk management measures taken to address the risk.

Food Risks as a Contemporary Governance Challenge

Food safety is essential for people's health and well-being. In many parts of the world, mainly in the highly developed industrialized countries, it is regulated as part of the public health policy. People here, expect to be widely protected from the contamination of the food supply by the competent food safety authorities. This expectation is met with frustration when food-safety scares occur of the kind that afflicted Europe between the late 1980s and late 1990s, most notably mad cow disease with its most wide-ranging implications in the economic, political, and institutional arenas. Another more recent example of a food-safety scare that gained global attention is the Chinese milk powder incident disclosed in 2008, where milk and infant formula adulterated with the substance melamine resulted in a large number of sick children and several fatalities. These negative experiences do not necessarily – and presumably only in exceptional cases – result in a constant worry for consumers about the safety of their food. However, consumers have become more sensitized to externally imposed food risks because their trust in the competent authorities has been shattered.

By the late 1990s, the prevailing diagnosis in European policy circles was that the series of food-related scares and controversies, most notably dioxin contamination, bovine spongiform encephalopathy (BSE), beef hormones, and genetically modified organisms (GMOs), had seriously reduced the level of public trust in both food safety and the competence of the authorities formally entrusted with assessing and managing food risks. In Europe, this diagnosis was the main reason why policy-makers decided to give top priority to risk governance. The European Union (EU) and many of its Member States subjected the rules and routines of food safety regulation and the roles and responsibilities of public and private actors in food safety management to a thorough review. Restoration of trust has been one of the key objectives of the resulting institutional and procedural reforms.

The so-called BSE crisis also had international repercussions. In Japan, for instance, it gave impetus to the establishment of a new food safety commission. Food safety incidents in combination with enhanced public awareness of safe food have triggered reform in threshold countries such as China where the food safety regulation system has undergone substantial legal and institutional change since 2009. Clearly, in a comparative perspective, food safety governance has been given exceptionally high priority in contemporary Europe. However, for several reasons we can assume that in many more parts of the world, political and public attention to food safety governance may grow or continue to grow in the near future. These include: the significant increase in the diversity of the food supply including

entirely new or fundamentally modified products for which there exists no or only very little experience in safety matters; the progressing trade liberalization in an ever growing and increasingly competitive global food market and the heightened concerns of consumers faced with this development; and the intense and persistent transatlantic trade disputes over hormone-treated beef and GMOs which, subjected to the dispute settlement mechanism of the World Trade Organization (WTO), have been attracting global attention.

All this has made risk governance in the food safety area a particularly interesting field of empirical risk research. A number of studies dealing with the aspects of food risk governance have been carried out over the past decade in research fields such as science and technology policy, risk regulation politics, participatory governance, and risk/benefit perception and communication. This article, will focus on three themes that are dealt with prominently in recent publications. These are: (1) the demarcation and coordination between risk assessment and risk management; (2) the opportunities and challenges of enhancing stakeholder and public participation in risk governance; and (3) the particular challenge of value conflicts for the governance of food risks. In the face of the legitimacy and trust crisis in Europe and the fierce transatlantic trade disputes, these themes have shaped regulatory debates and policy at national, EU, and transnational levels over the past 15 years. Many scholars identify them as critical dimensions of an innovative risk governance regime, which can help to improve scientific and democratic legitimacy of food safety regulation. We will address these themes as major governance challenges that public authorities face in dealing with food risks, and use mainly the European situation and problems as examples. Before doing so, we shall first outline the concept of risk governance.

The Concept of Risk Governance

In the past decade, the term 'governance' has experienced tremendous popularity in various research fields. These include international relations, comparative political science, policy studies, sociology of environment and technology, and also risk research. Although there is no generally agreed definition of what constitutes 'risk governance', this concept is typically understood as involving the translation of the substance and core principles of the governance term to the context of risk and risk-related decision-making. This implies that the concept of risk governance pays special attention to collective decision-making on risk as a multiactor and multilevel process involving new modes of regulation and collaboration. Within a broad notion, risk governance refers to the complex web of actors (governmental and nongovernmental), rules, conventions, processes and mechanisms concerned with how the relevant food risk information is collected, analyzed, and communicated and how decisions on risk management are taken and implemented at different policy levels (national, transnational, global). Food risk governance (or the similar term 'food safety governance'), in this perspective, is not only understood to include but also to extend beyond the three conventionally recognized interrelated components of risk analysis. These are risk assessment (aimed at understanding risk), risk management (aimed at acting on risk), and risk communication (aimed at informing all relevant actors and exchanging messages about risk and meanwhile predominantly understood as an activity cross-cutting assessment and management). Food risk governance also involves the collaboration of and coordination between public authorities and the commercial and civil society actors and wider contextual factors such as institutional arrangements, regulatory styles, legislative procedure, and the political culture. In the food safety literature, the concept of risk governance implies not only a scientific dimension but also a political, institutional, and a cultural dimension.

The Relationship Between Risk Assessment and Risk Management

The way in which science and policy are interrelated in processes of collective decision-making around risk issues is an aspect to which the concept of risk governance pays special attention. A particular focus here is the way in which this interrelation could and should be accounted for in the organization of the relationship between risk assessment and risk management.

Since the mid-1990s, the question of how to demarcate and coordinate between food risk assessment and management activities has substantially gained in importance as a research aspect. This has mainly been due to the mad cow scare, which made this question a salient policy issue in wide parts of Europe. The BSE crisis was interpreted as a result, at least partly, of a regulatory regime marked by a nontransparent and inappropriate intermingling of the roles of assessment and management, and of scientific and nonscientific considerations. The Committee of Inquiry into BSE, set up by the European Parliament, in its report deemed a blurred relationship between science and policy to have been one of the major shortcoming of the European Communities policy – in the years before 1996 – as well as of the approach of the UK from where the BSE crisis originated. It concluded that the EU institutions had given precedence to national interests of agriculture and industry at the expense of public health protection. Suspected of abetting partiality and obscurity in dealing with food risks, the traditional approach of rather seamless scientific and political activities became a subject of intense debate, scrutiny, and reform. Up to the mid-1990s, both EU institutions and EU Member States were neither systematically differentiating between activities of risk assessment and risk management, nor did they structurally separate organizational or institutional responsibilities. It was normal for the responsibility of assessment and management to be handled by a single institution, for those responsible for risk management to be closely involved in preparing and deciding risk assessments, and for scientific advisors to be expected to provide specific advice on particular policy issues. With the BSE event, the appropriateness of this approach became heavily contested.

The use of mechanisms designed to assure a stricter separation of the risk assessment function from decision-making on management measures formed the core of the reform of existing food safety institutions in Europe. In terms of loss of public trust and social legitimacy, the remedy resorted to, in this approach is the supposed trust-generating power of what is represented as independent risk assessment. Safeguarding

scientific analysis against distortion by inappropriate policy influences and considerations is intended to re-establish and assure the credibility of risk assessment activities and results by which risk management decisions are to be made. This reform approach is especially pronounced at EU-level and in countries such as Germany and France where responsibility for the functions of risk assessment and risk management have been allocated to different institutions.

Food safety governance reform in Europe did not, however, settle all the disputes over the assessment–management relationship. Rather it has tended to accentuate them. This has been described and discussed in a number of recent empirical studies which deal with theoretical and practical aspects of this relationship. These studies indicate that there exists wide appreciation – within and beyond Europe – that the revised EU food safety system has its basis in the risk analysis framework outlined by the World Health Organization (WHO) and the Food and Agriculture Organization (FAO). Very broadly, this framework is comprised of the three interrelated but functionally separated components: risk assessment, risk management, and risk communication. Nowadays, this framework underlies and informs food safety regulation at the EU-level (and at national level in many European countries) as well as in the USA and also at the global level in the form of the standard-setting activities of the Codex Alimentarius Commission (a joint World Health Organization and Food and Agricultural Organization standard-setting body) whose standards have acquired legal authority with the creation of the WTO in 1995. The risk analysis framework advocates the functional separation of assessment and management on the basis of the conception that regulatory actions rest on two basic components: first, knowledge about the seriousness of risks; and second, legally prescribed procedures, norms, or standards of how to handle risks. Even if the same knowledge is processed by different regulatory agencies, the prescriptions may vary in many aspects concerning selection rules, interpretative frames, action plans for dealing with evidence and uncertainty, and so on. National culture, political traditions, and social norms influence the mechanisms and institutions for specifying the role of knowledge for policy formulation in the respective political arena. The separation of risk assessment and risk management is a reflection of this fundamental difference between the rather universal and intersubjectively testable knowledge claims about the seriousness of a given risk and the more idiosyncratic procedures to design policy options on how to handle risks once they are identified and assessed, most preferably in quantitative terms whereas qualitative terms are often a reasonable addition or the only possible option.

In the Western world and also in Japan, the functional separation of food risk assessment and food risk management appears to be largely beyond dispute. In contrast, the practice of institutional separation of the respective responsibilities has been critically discussed in both policy and academic circles. The growing food safety governance literature has, in particular, criticized the idea that often underlies the practice, that the envisioned performance of assessment and management tasks in strict separation and sequence could safeguard risk assessment as a purely scientific exercise. Recent studies in this field assert that the concept of strict segregation has never represented real practice anywhere in risk regulation, not even in the revised EU food safety system. It was exactly the institutional and geographical divide here that had made it increasingly clear to the risk professionals that there are interfaces between assessment and management at which exchange and coordination was vital. It is obviously more of a challenge to organize this interaction if this implies coordination across different institutions located in different parts of Europe. In this view, a certain extent of interaction between those responsible for risk assessment and those responsible for risk management is not merely admissible, it is even conducive to dealing effectively and legitimately with food risk problems.

This concept of a cooperative relationship is in accordance with the Working Principles for Risk Analysis of the Codex Alimentarius Commission. These Principles emphasize that food risk analysis is an iterative process and interaction between risk managers and risk assessors is essential for its practical application. Scholars of risk regulatory policy and science and technology policy have expressly supported this view. They have persuasively argued that there are inherent interlinkages between the scientifically orientated activity of assessing food risks and the politically orientated task of managing these risks. The specific approach to a particular assessment inevitably includes nonscientific considerations and value judgments (be they explicit or implicit), which cannot be derived from and determined on purely scientific grounds. Such considerations include, for instance, the selection of impacts to assess, the disciplinary perspectives to shed light on these impacts, the choice of more or less conservative safety factors, and the ways to deal with different sources and types of uncertainty. So far, the concept of a risk assessment performed in a political vacuum and based on pure science is misleading even in theory. This is a point that the National Research Council of the USA had already made in its famous 1983 book on dealing with environmentally induced health risks (*Risk Assessment in the Federal Government: Managing the Process*). While underlining the need to separate risk assessment from management, this seminal work in the field of risk research emphasizes, at the same time, that it was an erroneous belief that full separation at the organizational level would be a way to effectively separate science from policy. Both factual and normative judgments play a role in risk assessment. Therefore, mutual communication between assessors and managers would be desirable and should not be disrupted. In the USA, the organizational conditions are more conducive to this interaction than in the EU. The US Food and Drug Administration (FDA) is a full-blown regulatory agency which has the mandate to deal with both the scientific and policy aspects of risk appraisal and decision-making. There is no institutional divide between assessment and management in the US food safety system. However, within FDA there are different departments that deal with assessment and management separately.

At the EU-level, there exist clear tensions between efforts to rebuild trust by assertion that with the new two-institution solution, scientific assessment would be insulated from political judgment and the practical requirements of coordinating assessment and management activities. Recent studies putting forward this problem diagnosis have also discussed ways to

account for the interlinkage between the scientific and the political aspects of food safety governance without compromising the generally agreed functional differentiation between assessment and management.

One proposal is to organize those steps in risk governance in which knowledge and values are closely intertwined and which are, therefore, considered as lying at the interface between assessment and management as separate additional governance stages within specified procedures and structures, ideally performed jointly by assessors, managers, and also key stakeholders. Two of these interface tasks are identified. The first refers to activities carried out before the risk assessment starts. The main task here is the definition of the problem in question (in consideration of possible different perspectives of how to conceptualize the food issue) and, related to that, the determination of the significant context conditions, the assessment boundaries, and the selection of conventions (scientific, political, and legal) and procedural rules needed for the assessment. This task is variously referred to as framing, preliminary assessment, preassessment, definition of risk assessment policy, or similar terms. The second task refers to activities carried out after the risk assessment has been performed. They are oriented toward arriving at a judgment on the acceptability of the risk based on balancing pros and cons, testing potential impacts on the quality of life, discussing different development options for the economy and society, and weighing the competing arguments and evidence claims in a balanced manner. This task is typically referred to as evaluation. Under current regulatory arrangements, these two sets of activities are often exercised in a manner which lacks transparency, and the associated responsibilities are unclear and accountability is not given.

There is a clear need to clarify and render more explicit terms for cooperative interaction between managers and assessors (and also key stakeholders). Crucially, the formalization of preassessment and evaluation activities is considered a means to achieve greater transparency and accountability in the ways in which knowledge and value inputs are articulated in risk management decisions.

Stakeholder and Public Participation in Risk Governance

One of the defining features of the governance term is that it refers to the interaction between various actors in public problem-solving. Accordingly, food risk governance emphasizes that analysis and management of food risks cannot be confined to public food safety authorities. It denotes the involvement of a wider array of actors in pursuing risk governing purposes. This also includes policy-makers, economic actors, scientists, and other experts not directly affiliated to food safety authorities, and the affected and interested civil society actors, besides regulators and official expert advisors.

Although the multiactor perspective is generally understood as an inherent element of the governance concept, there is an emerging literature focusing specifically on promises and pitfalls of various actors in the processes of public food safety handling under the heading of 'participatory governance' or 'inclusive governance'. Several scholars have argued that the governance process as a whole should be rendered more sensitive and responsive to the relevant knowledge (systematic, practical, and experiential) and the preferences and values of affected and interested parties. They claim that mutual exchange around framing assumptions, knowledge claims, and acceptability judgments in relation to food risks and ways to manage them may be able to substantially improve the final decisions. In this view, purposive multiactor dialog is a tool to shed light on the different dimensions of a food safety problem as well as to stimulate reflection, mutual learning, and more balanced judgments. Such an inclusive approach is also meant to improve trust. More inclusive and cooperative forms of food risk policy-making and regulation are often part of theoretical and empirical analyses of the trust and legitimacy crisis in Europe and the reform efforts that followed.

Current approaches to food risk governance at EU-level and in a number of European countries indicate a greater role for the stakeholder and public involvement in achieving appropriate risk-handling solutions. At the EU-level, there have been growing efforts during the past couple of years to involve stakeholders in both the management and assessment of food risks. Declarations of the value and the need for connecting with citizens and stakeholders, open dialog, and understanding and addressing the concerns of the stakeholders and consumers now present a standard part of the official rhetoric of many European policy-makers, regulators, and expert advisors. Empirical research into practical implementation of the reform objectives has critically commented that reform in the participatory policy in Europe has been largely confined to a broadening of consultation activities using, also, new mechanisms such as internet-based tools. There is continuity in organizing public involvement in most part as the elicitation of responses to predesigned proposals. Some high-profile dialog-based participation procedures have been carried out in the past decade, including, for instance, the GM Nation-debate in the UK. However, such exercises are often stand-alone events, mainly intended to elicit citizens' attitudes toward a new technology or a specific issue, and only very loosely (if at all) coupled to the official policy-making and regulatory processes. In view of this, scholars of science and technology policy and risk policy have asserted that food safety governance activities should be communicated by symmetrical two-way exchanges with the potential to really empower different social actors to provide knowledge, evaluations, and policy advice. Such deliberative efforts are particularly helpful in situations in which the scientific uncertainty and social controversy around a food safety case make risk governance especially challenging.

Some studies have pointed to the complex relationship between participation and trust. They have stressed that increased participation may actually destroy the public trust if applied in inappropriate circumstances. One context variable that deserves special attention, in this view, is the level of trust in the food safety authorities. If a regulatory system wants to draw on this trust resource, too much openness and participation in the risk assessment and management processes may jeopardize this goal. Inputs by stakeholders may be seen as compromising scientific objectivity and independence and as challenging the claim that authorities act in the best interest of

all people. The resistance of the Swedish National Food Administration to extend participation in the food safety regulation when the BSE crisis shook Europe has been interpreted as a purposeful and reasonable way of acting in a situation of high-trust that continued during and after the crisis. However, empirical studies demonstrate that even highly trusted organizations need to document that they are open to public demands and be transparent about their assessment and evaluation procedures. Transparency may not always help to gain trust but being secretive and hiding information has most often contributed to destroying trust.

Other general challenges and pitfalls of participatory risk governance that have been discussed in relation to dealing with food risks are over- and under-representation of certain actor groups, cumbersome decision-making leading to undue delays in the regulatory processes, and an overkill of participatory procedures abusing the scarce resources of both the responsible institutions and those invited to become involved. Stakeholder fatigue has developed into a buzzword in the academic as well as stakeholder circles in the past few years. There seems to be general agreement among scholars that exhaustive levels of participation on every food safety issue are definitely not desirable. Recent studies have stressed that the level as well as the form of participation would need to be matched with the level and nature of intractability of the respective risk issue. This was essential for ensuring practicality, efficiency, and legitimacy of participatory risk governance.

The Neglected Challenge of Value Conflicts

Recently published research work has underlined the need to distinguish, generally more carefully, between different aspects and contexts in food safety governance. This work offers suggestions on how to achieve greater integration of science, precaution, and public involvement in the current arrangements for European food safety governance. The main argument is that strongly contrasting implications are presented by food risks that may be seen (respectively) as: routine in nature, definitely prohibitive in their consequences, or in some way intractable – either because their implications are scientifically uncertain or are evaluated very differently by political and societal actors. Each of these broad aspects and contexts of food safety, the argumentation goes, demands different kinds of attention and different modes of coordination between the specialists, political decision-makers, and the corporate and civil society actors. Each in turn, therefore, also requires at least partly distinct technical methodologies, deliberative processes, and institutional configurations.

To devote different kinds of attention to food risk problems includes a differentiated scoping of the assessment exercise. Although in most cases routine health risk assessment will be sufficient, in other cases an additional analysis of the environmental, social, economic, and ethical impacts might be required. Recent studies have emphasized that a broader assessment is required in relation to food safety-related issues which are associated with deeply held value-orientations. They have underlined that for most people, food safety is not merely about absence of hazardous substances or unsafe levels of substances in the food they buy. Instead, in many instances, individuals, social groups, and different cultures also link wider concerns and expectations to a risk issue. These may be cultural, religious, or philosophical beliefs, concerns regarding the welfare of animals used in food production, or the desire for less high-tech in food production and more natural foods. Currently, such concerns are part of discussions in relation to topics such as the use of animal cloning for food production and the application of nanotechnologies for food-production purposes. The persistent controversy over GM food is an often cited example in this respect. Several studies dealing with the GM debate have suggested that divergent interests concerning the material risks and benefits relating to food safety or nutritional quality and disputes on the relevance, quality, and sufficiency of knowledge have also fueled this conflict but may not have acted as the prime driver. In this perspective, the main motives of opposition and criticism are rather assumptions or convictions that GM food does not meet a social need, encourages human hubris to model everything as one sees fit, and is opposed to one's own idea of life or the world. In short, the prime motives are identified as normative or ethical in nature.

Earlier conceptual work in risk research has classified conflicts about worldviews and value systems as the third (most challenging) level of risk debates. The first level refers to the factual evidence, probabilities, and uncertainties; the second level to the institutional performance, expertise, and experience. Recent empirical research suggests that current regulatory regimes in the Western world – including the revised EU regime – usually address issues of levels 1 and 2. They might be likely, however, to fail to address the third level, i.e., the level of values and visions, because such orientations were usually not addressed in a proactive, systematic, and direct manner. Suggestions for how to put such an innovative approach into practice include the following procedural arrangements. First and foremost, food safety issues should be screened for value-based conflict potential at an early stage in the governance process (when the terms of reference for the assessment are set). If this potential is identified, the advice is to complement the assessment of the physical risk(s) with a concern assessment. Concern assessment is defined as a systematic process of gathering knowledge about the concerns, expectations, and perceptions that individuals, groups, or different cultures may link to a certain risk and about the associated potential of social controversy and conflict. This knowledge can be used to assess the likeliness of wider socioeconomic and sociopolitical impacts related to the source of a food risk or indeed risk management practices.

If the results of this appraisal confirm the potential or existence of a critical value-based conflict, a public participation procedure should be designed to provide more reliable information on the question of framing of the issue concerned and on the dimensions of the value conflict that need to be addressed in the decision-making process on management measures. These procedural devices are not presented as means to resolve conflicts based, for instance, on divergent notions of the nature and underlying worldviews. The idea is rather that they can guide risk managers in the choice of the management instruments. With labeling, these include a powerful (however, also trade sensitive) tool for letting citizens and consumers make their own value judgments about food issues and incorporating sociocultural

perceptions and preferences across lifestyles (and countries) into food safety regulation.

Whether there is a necessity to address the third level of risk debates and deal with value conflict depends on the broader cultural context in which a specific food issue is embedded. In the case of GMOs, the need has been much greater in the EU than, for instance, in the US where the issue has never been as controversial as in Europe. Scholars of the regulatory policy have traced the different degrees of public controversy and the different regulatory responses (in contrast to the EU, GMOs are not subjected to specific regulation in the US regulatory system) partly to the higher value that European consumers and regulators attach to traditional food practices than US consumers. In the EU, it is a widely held belief that food should be produced in the most natural way possible and the criterion of naturalness enjoys great popularity.

Outlook

Some scholars assume that attributes such as natural, authentic, and traditional will continue to gain in importance across Europe as motives of consumer choices. Under these conditions, the controversy over GM foods may establish a precedent on how Europe will debate and regulate novel food production, processing, and packaging technologies that are present in addition to challenging scientific issues complex normative and ethical questions. More culturally informed strategies to handle food risks could gain in importance in European food safety governance. These are, however, limited by an international framework for the setting, arbitrating, and harmonizing of the world food standards. This framework is made up of the WTO dispute procedures, the Agreement on Sanitary and Phytosanitary Measures (SPS) which is part of the Final Act of the General Agreement on Tariffs and Trade (GATT) and stipulates global harmonization of standards for which the Codex Alimentarius Commission plays a pivotal role, and the Technical Barriers to Trade agreement. The SPS Agreement requires members to justify the food safety regulations that they apply and demonstrate that any trade-distorting effects are proportionate. Justification may be done by the adoption of international standards or through risk assessments which must satisfy certain requirements. The global framework in which European food safety regulation is embedded essentially encourages the reliance on a standardized, international, and science-based approach to regulation. In this approach, risk assessment is a technical, nonpolitical, and value-free exercise. Accordingly, cultural factors may enter the stage of choosing risk management measures, but not the stage of determining whether there is a food safety risk.

Institutional responses to wider concerns, however, must not be restricted to legislative and regulatory reforms. As the GM food conflict shows, the corporate sector may be prepared to react to such concerns more easily than governmental or supranational authorities. GM food was first boycotted by British supermarket chains before retailers across Europe followed the example. The responsiveness of the food industry and the retail sector – for instance, by increasing differentiation of products, production methods, and channels of distribution – will depend, among others, on the extent of mobilization of consumer, environmental, and other civil society organizations affected by and interested in food safety related matters. At any rate, social analysis should not be restricted to how public actors deal with the multifaceted challenges of many food safety issues. Instead, it should give equal importance to the roles of the corporate sector and civil society and also partnerships between the public and private actors. All of these actors contribute to food safety governance. In Europe, decline in public trust in food safety authorities and increased public awareness about food-related incidents have given a significant impetus not only to regulatory reform but also to major changes in governance structures in the private sector. The enactment of public mandatory standards has been paralleled by a shift toward more so-called self-regulation mainly in terms of the development of private food safety and quality standards. The tightening of both the public and private standards has affected access of developing countries to export markets. This has been recognized as one of the major challenges for future food safety governance at both national and global levels.

See also: Institutions Involved in Food Safety: FAO/WHO Codex Alimentarius Commission (CAC). Public Health Measures: Challenges of Industrialized Countries in Food Safety Management. Risk Analysis: Risk Analysis of Hazards in Food: An Overview

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Challenges of Developing Countries in Management of Food Safety

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Introduction

The past few decades have posed tremendous challenges to developing countries in ensuring adequate supply of safe and quality foods for the domestic and international markets. The major priority in management of food safety has been to ensure access to the international markets so as to generate valuable foreign exchange. Unfortunately, less attention has been paid to prevention of foodborne disease locally.

The structure of international trade in agricultural products has changed significantly over the past decades for developing countries. There has been a substantial increase in exports of fresh produce, compared with manufactured or processed products. In the current global food market, the protection of human, plant, and animal life and health is a challenge that must be addressed by developing countries through development of internationally recognized food safety systems. However, the developed countries, which are major markets, have been tightening their controls so as to provide a 'high level' of protection for their consumers.

Most food safety challenges facing developing countries can be attributed to the management system's inability to detect potential risks and gaps, share information, plan together, and identify appropriate strategies for collaborative management of food safety in the supply chain and protect the consumer. In developing countries, the prime responsibility of ensuring food safety in the food supply chain is regarded as a responsibility of government.

Prominent food scares, caused by bacterial (e.g., *Salmonella* and *Listeria monocytogenes*) and chemical (e.g., mycotoxins) contaminations and changes in the international trading environment have elevated food safety to the forefront of international agri-food policy and public health concerns. These concerns were exacerbated during the 1990s by 'mad cow disease' and the 'dioxin crisis' and forced regulators to rethink food safety strategies, integrating the various components of the value chain and introducing traceability requirements. In the new millennium, food production and distribution have become even more complex and market choices for consumers even wider. The media and consumers have developed a much greater interest in food safety issues following a number of food scares internationally.

The changes in the international trading environment include an increased emphasis on food safety regulations in trade, introduction of strict food safety standards, reorientation of food quality techniques toward preventive management, and a shift by regulatory agencies toward process-based standards and mandatory Hazard Analysis Critical Control Point (HACCP). These changing trends raise several important food safety management challenges and opportunities for developing countries. The most obvious concerns are the impact these have on the heavily relied on agricultural commodity export markets and domestic food sector.

Many developing countries are pursuing value addition strategies which are geared toward expanding their food export markets. The 1999 statistics shows that approximately 50% of agricultural imports in the USA were from developing countries, an increase of 38.37% from 1995. There is a growing public and scientific community perception that incidences of foodborne disease are on the rise. However, the high standards can only be effective when they are enforced through an efficient food control system. Therefore, developing countries need to establish food safety management systems that are responsive to recent changes in the international food safety system. The most important issues are how the export sector is coping with new international requirements and how the governments, producers, and consumers are coping with the evolving food safety challenges.

Food Production System

Food production, processing, and marketing systems in developing countries are complex. The food production systems are highly fragmented and dependent on a large number of small-scale producers. The current farm structure constrains farmer's capacity to meet domestic and international food safety standards. Although this may have socioeconomic benefits as large quantities of food pass through a multitude of food handlers and middlemen, the risk of exposing food to unhygienic environments, contamination, and adulteration increases. Literacy rate for most farmers and food handlers in developing countries is low; this limits the number of farmers capable of adopting more sophisticated modern agricultural practices, food hygiene, and good food handling practices necessary to meet more stringent food safety requirements.

However, innovative interventions such as organizing farmers into producer groups, establishing collection centers, contract farming arrangements, and creating public–private partnerships to assist farmers can overcome the farm size constraints. Contract farming and farmer groups' arrangement has been a success in meeting stringent food safety and quality standards in Kenya's fresh fruit and vegetable and India's

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spices, gherkin, and fruit and vegetable export sectors. This arrangement can also assist farmers in obtaining the capital required to make on-farm improvements, improving farming skills through joint extension provision and assistance in acquiring the required certifications.

During the Green Revolution, use of high-yielding seeds together with chemical intensive agriculture that uses massive quantities of fertilizer and pesticides created diverse effects on soil and environmental contamination. This trend had serious implication on the current international food production system, which emphasizes on minimum use of chemical fertilizers and pesticides, thus posing serious challenges to management of food safety by developing countries. In the past decades, the structure of food production systems has radically changed with many small scale producers venturing into production of export crops with some successes and failures. Major food safety problems occur as a result of poor post harvest handling and storage of food and also due to inadequate facilities and infrastructure such as the absence or shortage of safe water supply, electricity, storage facilities including cold stores, transport networks, etc.

Food Processing Industry

In developing countries, food processing industries range from sophisticated state-of-the-art facilities to small artisanal operations producing food for the local community. The size of these processing units varies from a few large plants to a many small and cottage-scale units with very limited resources for effective technological inputs. In most scenarios, the premises are not equipped to deal with the maintenance of food safety and quality in a scientific and sustainable manner. The challenge for developing countries is how to provide incentives for the effective expansion of these small units so that they adapt better technologies to ensure food safety and quality. Governments often support these small units as they provide employment and generate income for their operators.

Food processors in developing countries also face problems with the reliability and timely delivery of safety and quality raw materials. Raw materials are produced by smallholder farmers who lack infrastructure for their conservation and preservation, resulting in variability in the quality and safety of the raw materials. This calls for greater vigilance by the food processing units and food control activity at all stages along the food supply chain. Food security and safety have synergic effects as technologies used for food preservation also promote food safety both at industrial and household level. Hence, food safety aspects should not be neglected from public health and agriculture policies in developing countries.

Recent Trends in International Food Safety Management

Recent trends in food safety management have had serious impact on management of food safety in developing countries. International food markets provide significant opportunities for these countries to develop their agricultural exports and domestic markets. However, agricultural food products in developing countries are likely to contain food safety risks and encounter sanitary and phytosanitary (SPS) measures as barriers to market access. In addition, extensive media attention and increased awareness on the consequences of food contamination on health have heightened consumer concerns. Food safety and agricultural health standards differ across countries due to differences in tastes, diets, income levels, and perceptions, which influence people's tolerance to these risks. Differences in climate and available technologies (refrigeration, irradiation, etc.) affect the incidence of food safety and agricultural health hazards.

To fully utilize gains from international food trade, developing countries need to overcome these barriers and develop capability to guarantee the safety of their food products. The implications of these trends mean that an effective food safety management system is a challenge but a prerequisite for enhancing food exports from developing countries. The adopted food safety management practices should not only improve their international competitiveness but also heighten domestic food safety awareness. A well-coordinated food safety management system that involves all stakeholders within the supply chain is necessary for prevention of potential risks as experienced in Kenya's fresh horticultural produce and fish sector.

Food Safety Standards and International Trade

The General Agreement on Tariffs and Trade (GATT) international trade rules to protect human, animal, or plant health were so vague that many countries used 'health requirements' as barriers to trade. Changes to the GATT rules embodied in the Uruguay Round of multilateral trade negotiations in 1994 that brought food and agricultural products into the fold of international trading rules addressed these concerns. This lead to the adoption of the Agreement on Application of SPS measures (laws, regulations, and procedures) and an updated Agreement on Technical Barriers to Trade (TBT) that provided an opportunity to ensure fair and efficient international trade based on considerations of equity and fair access to global food markets. These agreements were designed to lay out conditions for transparency, equivalence, regionalization, harmonization, and national sovereignty when countries establish their regulatory measures to ensure food safety, consumer protection, and plant and animal health. The use of unjustified health-related measures as barriers to trade was discouraged unless such measures have science-based evidence and risk assessment principles. Through the World Trade Organization (WTO), there is a scientifically based approach to negotiation and conflict resolution to keep food safety from being an intractable barrier to trade.

The management of SPS measures to reduce food-related health risks poses clear, specific challenges for developing countries which are handicapped by more limited access to scientific and technical expertise and information needed to meet these new requirements. Their difficulties do not appear to influence the international legislative process as most developing countries lack the necessary financial facilitation to participate in the activities of international organizations. The prevailing food production and marketing conditions are

highly fragmented and dependent on a large number of small scale producers. Thus, they are incompatible with SPS requirements such as traceability. Preliminary estimates show significant negative economic consequences of more stringent trade barriers, with millions of dollars lost in commodity trade. Henson *et al*. reported that the number of technical notifications for developing countries to the WTO and its predecessor, the GATT, doubled between 1990 and 1998.

Therefore, any collaborative and development cooperation should support developing countries in the management of food safety for domestic and export markets. With the assistance from international organizations measures to enhance food safety control systems have been implemented in many developing countries with some success, but greater efforts are still needed to allow these countries to effectively build their systems into a sustainable structure.

Strict International Standards

Food safety standards have become a more prominent issue for global trade in agricultural food products. International food trade in high-value food products from developing countries to high-income countries has diversified enormously over the past decades. Owing to increasing consumer concerns, regulators in developed countries have been raising the standards that exporters need to meet to sell on their markets. However, the proliferation and strengthening of food safety and agricultural health standards at international level and in individual supply chains have caused considerable concern among developing countries due to the effect they might cause on the magnitude of the expanded and diversified food exports. There is greater scrutiny of the production or processing techniques employed along the food supply chains.

However, this situation is creating a scenario for competitive repositioning and enhanced export performance of developing countries. Although recognizing that food safety standards (as barrier to trade) can act to impede food exports and prevent new entrants into the high-value food trade, there is a need to view them as catalysts toward improvement of food safety standards for international trade.

Private food safety standards, which fall outside of the WTO, are playing a more prominent role in governing food markets. The costs of compliance, multiplicity of different standards, increasing specificity of those standards, and lack of harmonization among them are major concerns for developing countries. However, evidence indicates that, in many instances, the costs of compliance are less than assumed, especially relative to the value of exports. The growing complexity and lack of harmonization between countries impedes the efforts of developing countries to gain access to potentially lucrative markets in industrialized countries. The World Bank report findings indicated that there is adequate capacity to meet emerging food safety requirements where substantial progress has been made in relation to quality assurance and logistics management. But many developing countries lack the administrative, technical, and scientific capacities to comply with dynamic and increasingly strict food safety standards, presenting potentially insurmountable barriers to the development of high-value food products market opportunities. Several international agencies have been exploring ways to assist developing exporting countries build the necessary national capacity to meet these international safety and quality standards.

The most serious challenges for developing countries have been the costs of failing to meet strict international food safety standards. Evidence of continuing trouble is clearly apparent from Import Refusal Reports issued by the EU and the US. These incidences reported on international alert systems causes drop in price, economic losses due to loss of business, and longer term reputation damage. In 1997, the detection of salmonella in fish and fishery product exports to the EU from India, Kenya, Uganda, and Tanzania were banned. This was attributable to noncompliance of hygiene standards in fish processing plants. However, these countries addressed the hygiene-related problems, and on re-inspection in 2002, approved facilities meeting the requirements of the EU hygiene legislation concerning structure, maintenance, and hygiene, and the countries were added to the list of 'approved' countries.

There are examples of well-organized and well-managed supply chains in developing countries that have maintained or even enhanced their competitiveness and market share during this period of more stringent standards. This is well demonstrated by the case of Thai and Kenyan horticulture (fruit and vegetable) sector, the Kenya Nile perch sector, Thai and Nicaraguan shrimp, and Indian spices. New or more stringent standards also provide a stimulus for investments in supply chain modernization; increased incentives for the adoption of better safety practices and help clarify the appropriate roles of government in food safety and agricultural health management. Significant changes have been introduced in food safety management systems by the leading export companies by adopting and refining HACCP systems.

Evolution in Food Quality Systems

The past decades have witnessed a revolution of food quality systems in food safety management. These include the Good Practices guidelines, HACCP principles, various guidelines for total quality management such as the ISO 9000-2000 set of standards and 'farm-to-fork' strategies. The lack of good agricultural, manufacturing, and hygiene practices in developing countries remain a major challenge for improving food safety both for the domestic and export market. However, in the recent past, there have been efforts to promote good practices inline with GlobalGap or EurepGap. Although the quality management systems remain voluntary, the usefulness of HACCP has been recognized and it is becoming a legislative requirement in many developing countries. A rise in mandatory HACCP for some agricultural sectors (fresh fruit and vegetable, meat, and fishery products) has become a license requirement to export these products. The fragmented and large number of small-scale producers, middlemen, and retailers in the food system poses a big challenge to the management of the 'farm to fork' aspect of food safety for both domestic and export market.

The industries and regulatory bodies in developing countries have also shifted away from the traditional largely reactive focus on end-product testing to process-based quality management (prevention of quality failures before they occur) and

involvement of all personnel in providing customer satisfaction. Process-based regulations shift the primary responsibility for safety from the government to the industry sector, with the government acting as the auditor of the industry's own programs, a move from a 'command and control' approach to one stressing responsibility of private industry actors.

Food Control Infrastructure and Resources

Food regulatory infrastructures in developing countries are inadequate due to limited resources and a multiplicity of agencies under different laws and government departments or ministries (Agriculture, Health, Commerce and Industry, and Fisheries). The multiplicity of agencies makes the management and coordination of control measures difficult. Each agency operates independently to fulfill the function for which it was established. Concerns have been raised that these agencies have no clear vision or precise strategy, and the overlapping of activities and divergences on priorities has a negative impact on the efficiency of the control system. Therefore, there is need for all the food safety activities to be integrated into a single independent and coordinated system. Collaborative planning and effective communication is, therefore, required in the whole food supply chain to efficiently respond to market requirements.

A lack of overall strategic direction means that limited resources are not properly utilized. Food control systems suffer from poorly or inadequately developed compliance policies. Most of the legislations are outdated and have regulatory vacuums in several fields for several products. The goal should be to introduce procedures into the legislation and regulatory texts for preventive control operations and administration. The human resource capacity is limited and not focused in their training. Upgrading of their knowledge and skills in current developments in food safety and quality, food and disease surveillance, HACCP, and risk analysis will enhance their understanding of food safety.

Food control laboratories do not cover the whole country and are limited in scope of testing, are understaffed, few in number, poorly equipped, and lack suitably trained staff. This is accentuated where multiple agencies are involved in food control. Most of the available laboratory equipment are outdated and cannot test to the same level of detection accuracy as in common in developed countries and unable to detect certain emerging sensitive chemicals hazards. Testing for safety of foods derived from biotechnology (genetically modified organisms (GMOs)) is of concern in developing countries. Modern food control systems call for science-based and transparent decision-making process. The preventive approach requires access to qualified and trained personnel in all disciplines. A few laboratories are in the process of modernizing the laboratories to improve their capacity and respond to the increasing demand for the services.

Poor Infrastructure and Services in the Marketing System

Poor infrastructure in developing countries challenges their abilities to meet either public or private standards. Reducing food safety risks from the farm to domestic and export markets is constrained by inadequate infrastructure and facilities, particularly at the farm level, distribution level, and wholesale markets. The infrastructure and facilities in these markets are limited and rudimentary. Waste management and pest control in the markets are very weak. Reducing food safety risks will require significant public and private investments to upgrade the markets infrastructure and services. For regulated markets, this will also require improving the operational and fiduciary in order to build consumer and customer trust.

Food Safety and Domestic Market

Consumers in developing countries face a higher level of exposure to contaminated foods than those in developed countries. The climate in most developing countries favors proliferation of pests, disease, and naturally occurring toxins; the water for cleaning equipments and utensils and processing food are frequently unsafe. There are no regulatory standards, whereas existing standards are poorly enforced. The control of food products on domestic market is essentially repressive and consists of checking that finished products for sale adhere to regulations and fair business practices are respected. This leaves a noticeable void at the level of the production and primary processing stages. Thus, this approach does not always guarantee quality and safety. The rural–urban migration, in past few decades, has contributed greatly to an increase in urban and periurban agriculture. This urban and periurban farming may led to microbial and chemical, especially heavy metal, contamination of food, thus posing a risk to public health.

Owing to poverty, the rural masses do not constitute an important domestic market and cannot stimulate profitable economic activity for the food industry. In most developing countries, increasing incomes, urbanization, literacy, improved infrastructure, and closer ties to global trends, especially during the past decade, have resulted in an expanding domestic consumer-based concerns about food quality and safety. Rapid urbanization and middle class with high income have complicated the situation by changing population's traditional ways of handling food with more people depending on food markets or food prepared outside of the home.

Increased vigilance by non-governmental organizations (NGOs), consumer groups, and local research institutes is also raising consumer awareness and advocacy and spurring action among consumers and policy makers to address food safety risks. These trends have brought increased attention to safety concerns in the handling, processing, and marketing of foods. There is also growing consumer preference for convenience shopping, increased exposure to the media (television, cable, and the Internet), and ownership of durables such as refrigerators, which demand greater efficiency and food quality and safety standards in the supply chain.

There is need to shift to the modern control system, which is characterized by reduction of analyses and their cost to the regulatory bodies. The industry takes on the responsibility of self-inspection and the government replaces inspection of the finished product with validation of industry self-inspection and self-evaluation systems. Some analyses can be carried out within the framework of voluntary self monitoring by the industry. The control system should set up permanent dialogue with industry representatives in order to inform them of

their obligations and changes in regulations. However, export products follow a codified methodology essentially dictated by the requirements of the export market.

Street Foods

The rural–urban migration in developing countries has contributed to an increase in street vended, ready-to-eat, and convenience foods. Studies in developing countries have shown that up to 20–25% of the household food expenditure is incurred outside the home and some segments of the population depend entirely on street foods. As one of the consequences of rapid urbanization, millions of populations largely depend on street foods for their daily sustenance. In most developing countries, street food vendors are an important component of the food supply chain. Being reasonably priced and conveniently available, street food satisfies a vital need of the urban population. These ready-to-eat foods and beverages are prepared and/or sold by vendors or hawkers mainly in streets or other convenient public places such as around places of work, schools, hospitals, railway stations, and bus terminals. Food safety is major concern within the street foods supply chain. These foods are handled, prepared, and sold under unhygienic conditions with limited access to safe water, sanitary services, or garbage disposal facilities. Hence, street foods pose a high risk of food poisoning due to microbial contamination, improper use of food additives, adulteration, and environmental contamination.

Foodborne Diseases and Surveillance Systems

Foodborne diseases are a problem in developing countries. Food is prepared under unhygienic conditions at household and food service establishment level. This results in foods frequently being contaminated with foodborne pathogens and thus major cause of diarrheal disease. In developing countries, infectious diarrhea is the second leading cause of death, especially in children less than age of 5 years, after respiratory diseases. The cause of diarrheal diseases has traditionally been attributable to untreated water supply and unhygienic sanitation. It is widely assumed that many pathogens associated with infectious diarrhea are transmitted through the fecal–oral route. Efforts to prevent these diseases have been focused on and sometimes limited to improving water supply and sanitation. However, data show that food, being a favorable medium for growth of microorganisms, is the most important factor in the transmission of diarrhea illnesses. In developing countries, the role of food as vehicle for acute infectious diarrhea and in the epidemiology of infectious diarrhea diseases are ignored and preventive measures do not adequately include food safety considerations. Many outbreaks associated with foods are sporadic cases and often go undetected. In developing countries, risks associated with food contamination and need for improving food safety are frequently not included among strategies for the prevention of diarrhea illnesses by public health authorities and other agencies. In majority of these countries, attention given to foodborne illnesses is more reactive rather than proactive.

Most of the developing countries lack or have weak surveillance system. Investigations into foodborne disease causative factors and magnitude of exposure are inadequate. This results in significant underreporting of foodborne illness. Available data are not well documented and analyzed during risk management. This often leads to wrong decision making and thus reoccurrence of foodborne illness with negative impact on public health and food trade. The management of infectious diarrhea in these countries is the responsibility of public health, whereas food safety is associated with food regulation and food control agencies under different ministries. This results in food safety receiving marginal attention from public health sector. In the past few decades, food safety has been recognized within the agriculture and trade sectors, instead of it being at the forefront to ensure that human health is adequately considered in food safety policies and standards.

Policy and Regulatory Environment

Because of low consumer awareness, the private sector engaged in food production, processing, distribution, and retailing in developing countries has not taken the necessary steps to improve the quality and safety of food products. The government is responsible for ensuring food safety through enacting and enforcing legislation and setting standards. Therefore, addressing food safety issues in developing countries requires the adoption and enforcement of more appropriate food safety policies, legislation, and standards suitable to local food risk conditions and preferences and consistent with international requirements.

Weak Extension Systems

The agricultural extension systems are very weak and have not effectively focused to the changing needs of food safety and agricultural health and market safety requirements. Food safety concerns have been partly addressed through the integrated pest management programs. Farmers primarily depended on personal observation or on other farmers for information about food production and post harvest practices and pesticide use. The weakly coordinated research at the national and international level further increases the difficulty of ensuring effective research–extension–farmer linkages at the national level. Private extension provision (fee for service) is emerging within farmers producing high-value export crops. There are an increasing number of input suppliers, traders, contract buyers, and exporters who provide extension services to farmers as an integral part of their trading arrangements.

Consumer Knowledge and Awareness

The level of knowledge and awareness on food safety issues among the domestic consumers is still very low and in fact their main concern is more on food security rather than food safety. Majority of people in developing countries have low incomes and are faced with a big challenge of limited choice, a situation worsened by the limited supplies of most essential food categories during seasons of limited supply.

Inadequate Food Safety Standards and Poor Enforcement

The domestic food safety standards need to be aligned with international standards. However, for some commodities, it may not be possible to align domestic standards with international standards as there are no established international standards. In these instances, conducting research is important to set appropriate standards for the domestic market. Some national bodies provide third party certification quality certification scheme as an assurance of quality and safety.

It has been recognized that the gains in improving food safety practices in the export sector should spillover into the domestic market. However, because majority of production is consumed in the low-priced domestic market, farmers do not see any advantages for altering their production practices. Until domestic consumer awareness and willingness to pay for improved food safety becomes more widespread, it is unlikely that addressing food safety concerns will become standard practice. International experience shows that modernization of the food retail sector is an important driver for change not only in the structure of production and wholesale marketing of produce but also in fostering adoption of improved food safety standards.

Lack of Proactivity in Addressing Food Safety Issues

Domestic food safety scares and problems in agro exports reveal absence of proactivity within developing countries in addressing food safety concerns. Several factors contribute to this. In the case of agro exports, the emerging SPS measures and international standards are widely viewed as not scientifically based and as representing unfair 'barriers to trade.' These measures are viewed as efforts to protect foreign farmers or processors from competition or are being fueled by unreasonable consumer fears in developed countries and improved technologies for detecting hazard. The approach of developing countries has been to try to negotiate away the problems with trading partners and failing to address the various measures in international standard setting. Insufficient attention has been devoted to monitoring the requirements of standards, interpreting their implications, and using current and anticipated requirements as catalysts to upgrade existing operations and strengthen supply chain management.

This absence of proactivity has meant that developing countries either have to adopt a 'defensive' strategy by avoiding markets with more stringent food safety and agricultural health standards or have to launch into a fire-fighting mode when faced with potential disruption or loss of trade due to noncompliance with standards. The absence of proactivity is well illustrated through examples of problems faced with exports of fish products to the EU from Kenya and India in the late nineties. In both cases, although there were signs of potential problems for a considerable period of time, the food safety problems were not given serious attention until these countries were faced with a ban crisis.

Collective Action

Cases of successful experiences in fruit and vegetables, fishery, and spices sectors using collective action have highlighted its importance within the food sector. It is aimed at promoting awareness of food safety matters, finding solutions to emerging challenges, providing a degree of self-regulation, and reducing government agencies enforcement roles. Joint and shared efforts by the government and private sector is needed in better risk management, promotion and adoption of good practices, greater collective action, and some targeted public investments. Although there are many critical regulatory, research, and management functions that are carried out by governments, the private sector also has an important role in the actual compliance with food safety requirements.

Technical Assistance and Role of International Agencies

The need for technical assistance in strengthening food control systems in developing countries is well recognized. Both the SPS Agreement (Article 9) and Technical Barrier to Trade (TBT) Agreement (Article 11) specifically refer to need to provide technical assistance to developing countries. Food and Agriculture Organization of the United Nations (FAO), United Nations Industrial Development Organization (UNIDO), and World Health Organization (WHO) are the main international agencies involved in food quality and safety technical cooperation programs with developing countries. Such assistance is in areas of processing technologies, research and infrastructure, establishment of national regulatory bodies, etc. In particular, developed countries are required on request to provide technical assistance to the exporting developing countries to enable them to meet their WTO/SPS/TBT obligations. This opportunity to access technical assistance under the WTO/SPS/TBT Agreements has not been fully utilized by developing countries. Technical assistance in the food control area may also be obtained through the World Bank and bilateral donor agencies. Access to such funds is dependent on the priority that developing countries attach to strengthening their food control systems as reflected in their national development plans.

Conclusion

The challenges for ensuring food safety in the domestic and export food market in developing countries still remain large. Improving domestic food safety in developing countries, for domestic market or export trade, is hampered by a number of structural, policies, institutional, technical, and cultural challenges. Addressing these food safety concerns will require adoption of appropriate legislation, strengthening capacity to enforce laws, promoting adoption of good agricultural, manufacturing, and hygiene practices, greater collective action, and some targeted investments. Implementing these actions will require joint efforts by the government and private sector. The challenge for the future should be to adopt a more strategic rather than crisis management approach. This will be essential to ensuring the sustainability and cost effectiveness of these efforts. Hence, there is a need for continued technical assistance and dissemination of relevant information to developing

countries to help them to meet the ever-increasing and more complex challenges posed by international markets.

See also: Foodborne Diseases: Overview of Biological Hazards and Foodborne Diseases; Overview of Chemical, Physical, and Other Significant Hazards; Overview of Emerging Food Technologies; Prevalence of Foodborne Diseases in Africa; Prevalence of Foodborne Diseases in South East and Central Asia; Prevalence of Foodborne Diseases in Western Pacific Region

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PUBLIC HEALTH MEASURES

Challenges of Industrialized Countries in Food Safety Management

LM Crawford, USA

Glossary

Apertization French term for canning; preservation of food by sealing in cans or jars.
Organoleptic Inspection of food through the use of the five senses.
Robert von Ostertag German veterinarian who devised the organoleptic system of food inspection.
Salmonella A bacterium that is one of the leading causes of foodborne disease in humans and animals.

Introduction

Industrialized countries vary greatly in food safety system's organizational structure and effectiveness. This article is designed to provide some common ground on the essentials of a national food safety program. Inherent differences in the physical characteristics and the culture of different countries complicate a precise harmonization of food safety measures and requirements. Nonetheless, international trade in food requires that there be a core set of rules and procedures that trading partners hold in common. Moreover, scientific consensus on the importance of certain key requirements demands a certain degree of agreement between nations.

Modern food safety had its origins in international trade. The German approach to food safety was to establish rules. This grew out of the success of their Bier und Brot (beer and bread) law which was effectuated in the thirteenth century. That law forbade additives and demanded pure water to be used in food manufacture.

In the 1880s and 1890s, a brisk trade in pork was established between Germany and the US. This led to regulations from the US Department of Agriculture (USDA) requiring inspection of US meat consigned to international destinations. Ironically, there was no such law requiring inspection of meat destined for the US market.

The Germans were primarily concerned about *Trichinella spiralis,* the causative agent of trichinosis which was prevalent at that time in pork. And in 1900, Germany passed into law the Imperial Law Concerning the Inspection of Meat and Food Animals. This proscriptive law set the standard for similar laws in the Western world. Most notable among these was the US Wholesome Meat Act of 1906.

More important than the laws was the method of inspection that fulfilled the dictates of the Imperial Law. Robert Ostertag was a Renaissance man who was a veterinary parasitologist at the Berlin School of Veterinary Medicine. His 1904 book, *Handbook of Meat Inspection*, which described the organoleptic food inspection system, remained the standard throughout the world until comparatively recent times. In fact, this system which involved the use of the five senses in the systematic inspection of food remains the standard and the basis of food inspection in much of the world.

In the US and certain other developed nations, the food inspection system has embraced, to varying extents, the hazard analysis and critical control point system (HACCP). Critical control points are points in the food production process that can potentially contaminate food with pathogenic microorganisms. Many experts believe that HACCP is sufficient as a food control system without the addition of the organoleptic system. The US meat and poultry inspection system, however, includes elements of both systems.

This article then addresses the challenges inherent in a national food safety program in the industrialized world. The discussion of those challenges must perforce begin with the acknowledgment that there is no failsafe or perfect system. This acknowledgment recognizes the unpredictability and the biological difficulty of controlling, with precision, foodborne pathogens. Societal and political considerations will likewise be considered. Chief among these are public expectations of food safety programs.

Essentials of a National Food Safety Program

The first essential is a strong food safety law. Laws are enacted in different manners throughout the world. Some are dictated, others are the product of a more or less democratic process. Ideally such laws will have been the subject of serious debate and careful planning. The result of such a process results in a law that represents the national conscience as well as the expectations of the people. With a comprehensive and workable statute, the leadership of the national food safety program will have a blueprint for accomplishing his/her mission. Not only that, the political leadership of the respective country will have approved and ratified the law. Moreover, the law should have enforcement provisions describing the responsibilities of food

producers, consumers, and the government. Also, the law should provide legal authority to take punitive action along with penalties for violations of the law. The strength of the law reposes in its specificity and the appropriateness of the prescribed penalties.

The second essential of a national food safety program is the presence of a state-of-the-art scientific laboratory system. Food safety concerns can be reduced to two categories: microbiological contamination and chemical contamination. Control efforts are thwarted if detection methodology is inadequate. This frustrates monitoring and surveillance of the food supply and it can invalidate enforcement actions. It is difficult to police the food supply if you cannot detect what is in it. A modern food laboratory system is expensive and it requires expert personnel. The quality of these laboratories varies from country to country and this complicates communication between trading partners with the end result being factual disputes that could be avoided.

The third essential element is having a staff that is adequately trained and credentialed. Food safety is essentially an applied scientific endeavor. The scope of that endeavor is broad and the required knowledge base is demanding. The key to proper staffing lies in determining the required disciplines. This inevitably invites comparisons between food scientists, chemists, biologists, veterinarians, microbiologists, and physicians among others. Experience has shown that access to the expertise of all these is needed. Probably the most versatile discipline at the baccalaureate or bachelor's level is that of food scientist. The food science curriculum presents some exposure to chemistry, microbiology, statistics, and food manufacturing. It is currently popular to have the head of food safety be a physician or someone else with a doctoral degree. This is probably good if the incumbent has experience in the field of food safety. To put it differently, the training of a food safety staffer largely refers to their collegiate training and experience in the field of food safety. Credentials can be said to represent validation of expertise through graduate training in related fields, awards, honors, and the like. The presence of a thoroughly trained and experienced staff is mandatory in order to establish authority and to effectively carry out the responsibilities of national food control. Such a staff is difficult to maintain, expensive, and terribly hard to retain, but it must be done. Errors in judgment, mistakes in regulatory determinations, and unimpressive job performance will effectively invalidate the performance of a food safety agency. The agency does not have to be a university but it must, nonetheless, be competent.

The next essential of a national food safety system is to have an adequate number of personnel. The strongest law and the greatest commitment cannot make up for a deficiency of personnel. The level of food protection will dangerously decline if a country does not have enough people to get the job done. Proper staffing numbers should be determined using practical work efficiency models taking into consideration travel logistics and work product success rates. No two countries that have populations of 4 million will require the same number of personnel. Notwithstanding that, exchange of staffing information and discussion of staffing adequacy between nations can be a useful starting point in determining the optimal staff size. Most national food safety agencies pride themselves in having cordial and productive relationships with counterpart organizations in other countries. These relations not only can lead to useful models but also are important in regulating as well as encouraging international trade.

Fifth and finally, it is essential to have the optimum organizational structure. First and foremost, the person in charge of food safety in a national government must have the authority to make public health decisions. And that person should report to a scientist or physician if at all possible. Experience has shown that the best locus for a food safety agency is the department of health, not agriculture. Decisions made by the food safety chief are often matters of life and death. They must not be dealt with lightly. If the chief is not in a position of authority, then the leadership of the food safety agency can degenerate into a committee which is a situation incompatible with public health. Of course the minister of health or similar leader should and must have the prerogative to overrule the food safety official and to relieve him or her from office if necessary. Agriculture is an improper location for food safety not so much because agriculture departments must promote farmers, but because ministers and subministers often have no food safety or disease experience. The food safety agency must be organized economically in that the fewer leaders the better. Ideally, food safety agencies should be divided into inspection and enforcement branches. Therefore, the core administrators should be the Chief of Food Safety, the Deputy Chief for Food Inspection, and the Deputy Chief of Food Safety Enforcement. The names and the titles may of course be altered consistent with national usage in governments. A third tier of leadership can include assistant chiefs for finance or budget, outreach, political affairs, etc. The assistant chiefs should be staff officers and not line officers. Further branching of authority may be incorporated into the organization but should be kept to a minimum.

The Ideal Inspection System

HACCP is, at the present time, the state-of-the-art system for food safety management. This is because HACCP focuses on preventing contamination of the food supply with disease causing organisms. The Ostertag or organoleptic system focuses on detection of contamination after the fact. If the contamination has already occurred, it is too late to prevent foodborne disease. Even continuous inspection will overlook virtually all contamination. The organoleptic system was designed to look for the main food safety concern of that day–parasites. Today, the principal food safety concern is disease causing microorganisms that cannot be seen by the naked eye. The visible manifestations of bacterial and viral infection–pus, hemorrhage, swelling, and discoloration can sometimes be seen but usually are not. Thus, the organoleptic system is outdated and inadequate.

HACCP is a systematic approach to the identification, evaluation, and control of food safety hazards. The Pan American Health Organization (PAHO) describes HACCP, simply, as a preventive system for assuring food safety. The PAHO manual on HACCP further elaborates: (HACCP) is based on a common sense application of technical and

scientific principles to the food production process from field to table. The principles of HACCP are applicable to all phases of food production, including basic husbandry practices, food preparation and handling, food processing, food service, distribution systems, and consumer handling and use.

Scientists, the world over, always seek to improve public health and therefore the tools of public health including HACCP. The question is what is beyond HACCP. Future improvements may be marginal given the elegance of the HACCP system. Such improvements may or may not emanate from HACCP.

The hazards that HACCP is designed to prevent fall into two categories: microbiological and chemical. That is to say, HACCP only exists to aid in the control of these contaminants. And, admittedly, neither HACCP nor any other system is perfect. Therefore, future changes in HACCP or development of a new system will be dependent on scientific developments that point the way to a new system or improvements in HACCP. Because HACCP is, in fact, a system and not a scientific development, the basic structure of HACCP may not be improved on. What will change will be the science of contaminant detection and the means of reducing or destroying contaminants.

Elucidation of the genome of various pathogenic microorganisms could lead to better systems of detection and control. Transgenic microorganisms could be developed that target and destroy pathogens. Transgenic organisms likewise could be created that scavenge chemical contaminants and microbial toxins.

Decontaminants of both pathogens and chemicals could be developed that are more effective than today's armamentarium. Findings borne out of serendipity could vastly improve control of both categories of contaminants through mechanisms not yet discovered.

But in fine, all these imagined break-throughs would not force a change in the fundamental structure of HACCP. Thus, its status as the ideal food safety system may not change in the foreseeable future.

The Ideal Enforcement System

Ideally, the enforcement system must be grounded in a strong, enforceable national food safety law. Enforcement cannot be based on borrowed authority. The law must explicitly be designed to ensure the availability of safe food to the extent possible. Weak laws may be successfully challenged in court resulting in disarray in the food safety enforcement system. Also, it is important to design the law correctly the first time. Amending the law based on experience is time-consuming and difficult to accomplish.

Serious enforcement requires strict adherence to the national food law. This requires well-worded regulations (rules) and clearly written directives or guidances to the regulated industry. These likewise should be sensible, defensible, and enforceable. Public confusion over the requirements of the law and the resultant regulations leads to an untenable situation which can only lead to an erosion of authority and an inability to accomplish the objectives of the enforcement division.

Enforcement personnel must be well-trained. There likewise should be continuing training and refresher courses in their duties and responsibilities. The morale and enthusiasm of enforcement personnel is bolstered by strong administrative support and adequate resources to accomplish the mission.

In some countries, the effectiveness of enforcement is weakened by uncertain authority between national, state, and local authorities. Optimally, the national food safety agency should have clear authority over state and local authorities. If a regulated product is not traded nationally, authority may devolve to subsidiary authorities. In order for effective communication to occur between state and national authorities, comprehensive written agreements should be constituted. These, commonly known as memoranda of agreement, declare the articles of delegated authority as well as the expectations of the specific parties.

Facing Unexpected Food Safety Challenges

The Centers for Disease Control and Prevention in the US estimates that there are 5000 deaths, 76 million illnesses, and 325 000 hospitalizations from foodborne disease each year in a country with 300 million people. This means that foodborne disease is not rare and is a major problem in a country that is considered highly developed. This, then, is the expected food safety challenge – a high level of debilitating disease that materially affects US citizens. The unexpected challenge comes in the form of major outbreaks that often have no clear source and which result in some serious illnesses, deaths, and even permanent injury. And the US is not alone among developed nations that continue to have unacceptable levels of foodborne disease.

The major outbreaks of food related disease affect public opinion and often bring about changes in food safety procedures. Outbreaks are generally a small fraction of the actual number of illnesses in a given country and the outbreak itself can be surprisingly small. Nonetheless, major outbreaks receive enormous publicity and political interest. A cursory review of some of the major outbreaks in recent US history follows.

The year 1985 was the year of perhaps the two most important food disease outbreaks. First came the Jalisco cheese outbreak in Southern California. The causative agent was *Listeria monocytogenes*. There were 142 cases and 47 deaths. Business at the cheese plant had increased dramatically. To meet the demand, the company overwhelmed the Pasteurization equipment with raw milk and thereby the cheese became contaminated with *Listeria*. Next came the great Chicago milk outbreak. The Centers for Disease Control and Prevention (CDC) estimated the total number of illnesses in six Midwestern states from 16 000 to 200 000. The numbers of deaths were estimated from 2 to 7. The milk was placed in large cooling vats one of which had a small tear in the inner lining. Milk leaked into the space between the inner and outer lining where it remained for an extended amount of time contaminating each new batch of milk with the *Salmonella typhimurium* that was proliferating in the tank.

In 1993, a fast food company in the Pacific Northwest introduced a large new hamburger named the Monster Burger.

Although never conclusively proved, some of the Monster Burgers may not have been thoroughly cooked. *E. coli* 0157:H7 caused the deaths of four children and sickened hundreds of others.

1994 was the year 3000 fell ill in 41 states subsequent to eating a particular brand of ice cream. A tanker truck was used to convey liquid, raw eggs on one leg and pasteurized ice cream on the return trip without cleaning and disinfecting the truck. The eggs were contaminated with *Salmonella* which contaminated the final product – ice cream.

Salmonella contaminated irrigation water in Mexico was found to have contaminated serrano peppers that were used as ingredients in various US foodstuffs. This caused at least 1017 illnesses, 203 hospitalizations, and at least one death in the US in 2008.

Salmonella newport contaminated peanuts were sold as an ingredient in numerous other foods in 2009. Nine people died and an estimated 22 500 were sickened. The vector of the pathogen was thought to be rodents that had easy access to the stored peanuts.

In all these devastating outbreaks, there was a simple cause that common sense and vigilance would have prevented. As a result of obvious disregard for public health, thousands were made seriously ill and many died or were hospitalized. Public confidence in the food supply has eroded, and millions of dollars have been spent on food safety by government, industry, and the public.

Some years ago, the Food Safety and Inspection Service of the US Department of Agriculture produced a film titled, The Anatomy of a Recall.

The essence of the script was that it is much better to prevent a recall than it is to go through one. If large amounts of food have to be recalled and consumers must be subjected to the publicity of doubt, fear, and accusation centered on something so basic to the diet as legumes, meats, nuts, eggs, etc., the system has failed and much harm will ensue. Each food manufacturer, each consumer, and each government regulator must be sharply focused on food safety on a continuing basis. This must start with the leadership of all affected organizations. Today, the officers of most food companies in the industrialized world receive regular media training. They also should have regular food safety training. The previous narrative on historically significant food safety outbreaks does not mention that many of the companies affected by these outbreaks no longer exist or that their demise was directly due to the outbreak. Sadly, many of these experienced in their existence, only one food safety outbreak and it was the one that put them out of business.

If a recall is required, it means that the system has failed and that the consequences will be great. The company has failed to produce safe food, the government has failed to detect or prevent unsafe food from reaching the market, and the public has an obligation to return the unsafe food.

Public and Political Considerations

There can be little doubt that food safety is an international movement. In the twentieth century, citizens found out about food safety problems from newspapers, television/radio, and books. The twenty-first century is the century of the internet. Food safety information reaches the public instantaneously. Major foodborne disease issues have convicted the public to demand an absolutely safe food supply. The fact that this is not practically possible has not dissuaded the people. Unexpected, new food safety problems have been mainly responsible for the current profound concerns.

All principal paradigmatic developments developed in the latter part of the twentieth century. Cardinal among these is bovine spongiform encephalopathy (BSE). No foodborne disease has ever struck such a fear and it was such a startling development. Second has been genetic engineering of food and food animals together with cloning and bovine somatotropin. Although it is obvious that the manipulation of genes is not a foodborne disease and that these technologies are safe, the newness and the novelty have caused concerns about the food supply that may exceed those of BSE. Likewise, the spate of a group of pathogens not generally familiar has wreaked havoc with public confidence in the food supply. *E. coli* 0157:H7 is obviously a mutant of *Escherichia coli* that causes a potentially fatal form of hemorrhagic enteritis in man. Particularly alarming to the public is the fact that this organism appears not to cause disease in the carrier species, cattle. *Salmonella enteritidis* which was found in the 1970s and 1980s to have adapted to the oviduct of laying chickens again causing no disease in the layer but causing serious enteritis and sequelae has further concerned the public. *Campylobacter spp.* likewise was found in the 1980s to be a serious pathogen of man and to be present in a significant percentage of chickens and to be a factor in the development of Guillain–Barre disease in humans. These developments, all occurring in the space of a quarter of a century, have been one of the reasons for the increasing interest in food safety in industrialized nations.

Beleaguered officials in government and industry have generally tried to explain the aforementioned developments by apologizing for their own missteps. Certainly legislators have blamed government and industry for most of the present day food safety problems. The language of food safety should not be so much tinged with recrimination. Although there are miscreants in food manufacturing and production, the vast majority will do everything possible to prevent food safety outbreaks. The answers no doubt lie in science. As surely as mathematics is the language of science, then science must be the language of food safety. For example, a Canadian vaccine company has developed an *E. coli* 0157:H7 vaccine for cattle that may prevent bovines from being the carrier of this organism thus preventing infection in humans. Thus, a new scientific development may accomplish what disinfectants and antibiotics and inspection and enforcement, and not even HACCP could accomplish. Research should and must be the future of foodborne disease and therefore food safety itself.

The Food Safety Imperative

Food safety is located in various different departments of government throughout the industrialized world. But food safety is special. It should be placed among agencies that deal with public health and the emphasis should be on the human health consequences of food safety that is to say on foodborne disease.

This is because the department that houses food safety must perforce ascribe to the food safety imperative. This dictum embraces the following principle: Food safety is a public health undertaking and all its efforts are dedicated to preventing disease in human beings. It is important that the food safety imperative be the working credo of food safety agencies. This is because its employees and the public it serves must know what the agency's mission is and where its priorities lay. Nonpublic health functions should be looked on as distractions. Therefore, the first great challenge is to have the food safety agency located in the national health department.

The second great challenge is foodborne disease itself. Food safety agencies, with guidance and input from the public it serves, must rank the foodborne diseases in order of priority. This must be done analytically using the science of risk assessment. If the greatest risk to the population is *Listeria*, then the most effort should be expended in counteracting *Listeria*. Such a ranking should place the organism at the forefront of inspection, research, and outreach priorities. Also, the citizens should be aware that the food safety agency had targeted *Listeria* as its lead priority and that *Listeria*, in the view of the agency, represents the greatest risk to the population as a whole.

The third challenge is chemical contamination. As late as the 1980s, concerns about chemical contaminants were greater than for microbiological concerns. But the era of pesticide and antibiotic residues in food has diminished and in the current era, industrialized countries are more concerned about microbiological hazards. This concern must not be allowed to overwhelm concerns about chemical hazards. Many of the same historical concerns about chemicals still are troublesome. To be sure, mercury levels in fish remain a potential public health threat in most countries. Heavy metals can be found in much of the food supply at various levels. Newer chemicals are being introduced to the environment on a regular basis with little concern for food safety implications. Moreover, older chemicals become of great concern because of new toxicological findings. A case in point is the plasticizer Bisphenol A or BPA. Although it has been known to have estrogenic properties for decades, it is now suspected of being a fetal toxicant and more. Acrylamide, a compound produced by some cooking procedures now appears to be more toxic than originally believed. Diacetyl, used in the manufacture of popcorn and certain other foods has been shown to cause serious lung disease under some circumstances. Because diacetyl, BPA, and acrylamide are of human health concern, efforts should be underway to determine the threat that food contamination might play, but there appears to be little research in this particular area.

Fourth in this cascade of challenges is environmental contamination. Degradation of the environment can affect the food supply both directly and indirectly. The greatest concern now is, and always has been, sewage. Fecal contamination from both man and animals can introduce pathogenic microorganisms and parasites to the food supply. Population increases, water shortages, floods, and many other factors affect the presence and location of this pernicious contamination. Population increases and the source of the food are major contributing factors. Other forms of environmental contamination such as industrial run-off remain of concern.

The fifth great challenge is international trade. Increased food trade is a good thing that it inevitably results in better nutrition over the seasons for most countries and it is a useful source of revenue for individual countries. Some countries, such as South Korea, import most of their food supply, so food trade is essential. Unchecked international food trade can nonetheless lead to adverse effects. Apart from the obvious and omnipresent threat from bioterrorism, travel time and conditions aboard vessels of conveyance pose the risk of spoilage and microbe proliferation. Rejection of food shipments at ports of entry can lead to port shopping and reentry to the country that rejected the shipment as well as to other countries. In these instances, adulteration of the food with toxic substances that are used in an attempt to prevent spoilage or to mask the odor of decomposition can occur and sometimes creates a hazard greater than the initial concern. Trans-shipment of unfit food through one or more countries sometimes results in confusion about the country of origin. These examples bespeak the need for regulation of international food commerce. It is the dual responsibility of the country of origin and the recipient country to ensure that foodstuffs from other countries are safe.

Challenge number six is the further development of food safety science. In industrialized countries, there is often a more or less well-developed scientific body of knowledge in food manufacturing, sensory physiology, food preservation, and the like. But institutes of food safety medicine and food public health are rare and if they exist, they are often underfunded. Among the many reasons for this is the constellation of disciplines that are requisite in understanding and preventing foodborne disease. Cardinal among the required disciplines is medicine, including veterinary medicine. Food science, microbiology, virology, toxicology, parasitology, and others all constitute the necessary body of knowledge and experience in addition to medicine. The CDC in the US is well known and respected for its epidemiological expertise and diligence. But, CDC is not a basic research institution. Other federal and private research programs are limited in food safety basic research programs. Notwithstanding, the microorganisms continue to evolve and to develop new and different means of circumventing natural defense mechanisms and external control programs. Each industrial nation must have a well-funded and well-planned program in basic food safety research. It is well to remember that before the 1970s, *E. coli* 0157:H7 was not known to exist. *S. enteritidis* had not been found in chicken eggs. The role of *Campylobacter spp.* in foodborne disease and in Guillain–Barre syndrome was poorly understood. HACCP was unfamiliar to the food industry. Control programs for *Listeria* and most of the zoonotic foodborne organisms were inadequate. And virtually every control scheme for pathogenic foodborne organisms was developed after outbreaks in humans. That is to say many food control systems are retrospective and the ability to predict the next serious pathogens in the food supply is elusive. The means of dealing with foodborne disease lies in research. Research that is targeted toward existing disease and the pathogens to come.

Conclusion

The current annual CDC estimates of the incidence of foodborne disease is 5000 deaths, 325 000 hospitalizations, and

76 million illnesses in the US. If these estimates are correct and if they are true in similar countries, foodborne disease is uncontrolled and a leading cause of sickness in the industrialized world. Moreover, current control programs, including HACCP, should be regarded with suspicion.

It is generally accepted that HACCP is the most practical and efficient food control system extant. In point of fact, practical and efficient imply a certain compromise. HACCP is neither absolute nor failsafe. All food processed under HACCP is reasonably safe but calamities do happen.

What is beyond HACCP? No one knows at this point and practically no research is undergoing on systems that would potentially be superior to HACCP. Few public health programs are conducted with such disregard to the possibility of progress. In cancer research and in neurological research as well as in genetic disease of all sorts, the understanding is that control programs are but a first step on the steady pathway to eradication. HACCP experts should be impatiently looking to replace HACCP with a system that is more effective.

Often, great progress in public health programs has been preceded by technological break-throughs such as was the case with Pasteurization and with water purification. But two of the greatest triumphs in foodborne disease prevention occurred as a result of national competitions. When Napoleon needed a method for preserving food for his troops, he heavily funded a prize to the first person to come forward with an effective method. History was made when a baker, Nicholas Apert, presented the Emperor with apertization (canning). When the National Aeronautics and Space Administration (NASA) of the US wanted a food safety system for space travel, they invited applications for a major grant designed to stimulate the development of such a system. The Pillsbury Company under the leadership of Howard Bauman got the grant and developed HACCP. Perhaps it is time to fund a grant for the development of the next generation of food safety systems.

In closing, it must be said that the control of foodborne disease is a public health undertaking that affects every man, woman, and child on this planet. The food safety imperative is guided and energized by the simple truth that it should be the right of all people to expect and depend on a safe and nutritious food supply. Let no one believe that the food safety imperative has been accomplished. There is much work to be done.

See also: Institutions Involved in Food Safety: FAO/WHO Codex Alimentarius Commission (CAC); Food and Agriculture Organization of the United Nations (FAO); World Health Organization (WHO). Public Health Measures: Modern Approach to Food Safety Management: An Overview

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PUBLIC HEALTH MEASURES

Fundamentals of Food Legislation

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Key Objectives

When a society believes that it needs protection, it is usual for the national government to respond by passing legislation, which provides that protection. Early laws on food control can be traced back many centuries, but the modern concepts of food law are mainly based on ideas developed in the mid-nineteenth century. With industrialization and urbanization, the food producers and consumers had become separated not only in distance but also in personal contact. This led to widespread abuses that even affected all urban dwellers, including the elite. At the same time, advances in science, including microbiology and chemical toxicology and analysis, made it clear that the food supply was frequently contaminated and often harmful. Action followed and led to the adoption of general food laws – for example, the UK Act of 1860 is claimed to have been the first general pure food law in the English-speaking world.

Today with continuing advances in food science and technology and the rapidly growing urbanization of much of the world's population, the food supply chain has become increasingly complex and globalized. Although the advances have enabled most people to enjoy a regular and varied food diet, many consumers however are still reluctant to accept certain aspects of modern science and technology in the food supply. The rejection in some parts of the world of irradiated and genetically modified foods are clear examples where the advances have not only met consumer resistance and the legislators attempt to adopt controls which assure safe food but also allow clear consumer choice.

Food legislation is only one part of the system used to try and provide consumer protection. While the scientist or technologist may be focussed on the content of the legislation – what level of additive is permitted, for example – the effective implementation of a national food control system depends on the smooth and efficient operation of various elements. A modern and well-drafted set of legal documents may appear to be sufficient but in practice, without an effective administrative team within government, without a committed and professional enforcement team, and without the support of a well-resourced laboratory service, the legislation is likely to be ineffective.

Food law can usually be identified as belonging to one of two key objectives of protection – food safety and food quality. A third objective, the use of legislation to enhance the nutritional quality of the diet is a more recent development. These are indicated in Figure 1.

Food Safety

To protect consumers from adverse health effects, legislation usually places responsibility on the food suppliers to ensure that the food is safe. To provide for ease of enforcement, detailed legislation has been developed to provide clear statements of what constitutes safe and unsafe food. Examples of these include controls on the hygiene of production and distribution, limits on the levels of chemicals in the food (whether added on purpose or found as contaminants), approval systems for the control of new processes (e.g., food irradiation or genetically modified foods), and specifications for food-contact materials, including packaging.

Food Quality

Food can be perfectly safe but may not be satisfactory. As an example to illustrate the difference, consider a container of milk. The milk may have been subject to detailed hygiene controls and may be entirely safe for consumption. However, if during the collection and/or distribution of the milk, some water had been added, but the milk was still being sold as pure milk, the consumer buying the milk would in fact be paying for a mixture of milk and water. If the product was sold as milk, then the consumer is not only being defrauded but also deprived of nutrients. Food law is therefore developed to provide consumers with protection in the compositional quality of the food they are buying. The manner of these controls varies and different approaches are possible.

- At one extreme, it is possible to provide detailed specifications for a wide range of food products. Manufacturers are then required to only produce products meeting these legal specifications. This method, sometimes termed as 'recipe law' ensures that consumers can be confident that the foods they buy will meet nationally approved standards. However, this approach limits the manufacturers, stifles product development, and restricts consumer choice.
- At the other extreme, it is possible to allow manufactures total freedom to manufacture any food product (a *'laissez faire'* approach), but for the legislation to require fully informative labeling so that the consumer can then decide whether or not to buy the food based on the information on the label.

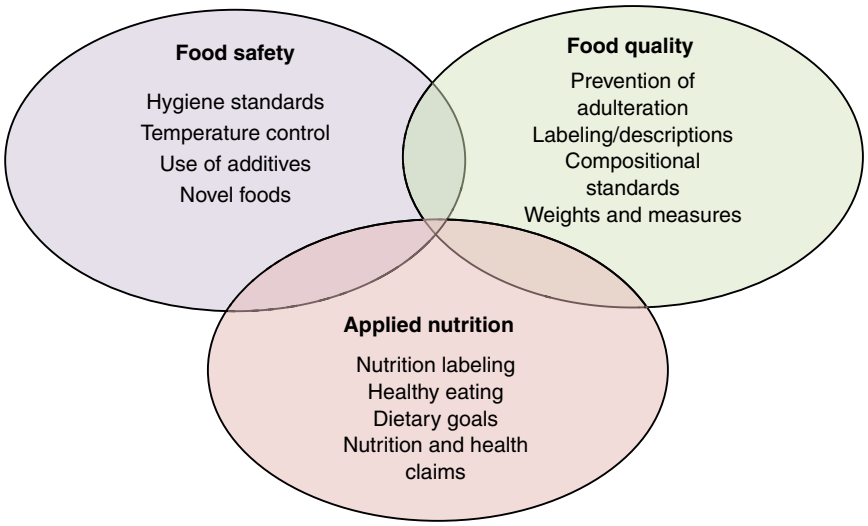

Figure 1 Areas of protection by food legislation.

In practice many countries have adopted a combination of these two approaches. It is common to provide a number of controls based on the product standards for products which are considered of importance to the national diet – for example, bread and milk. When manufacturing other products, manufacturers have much more freedom, but must comply with the labeling rules. The balance between these two approaches does vary. In developing countries with low literacy rates or a lack of consumer awareness, reliance on labeling may not provide sufficient consumer protection. In these countries there tends to be a greater reliance on product standards. In many economically developed countries or regions, the approach to recipe law, which was prevalent during the mid-twentieth century, has largely been replaced by the use of informative labeling controls.

Applied Nutrition

Limited attempts to protect or improve health by food law have been adopted for some time. The main control has been the requirement for fortification of certain foods so as to restore or enhance the consumption of key micronutrients. Examples include the addition of vitamins and minerals to margarine, milk, flour and bread, and the iodization of salt. These targeted dietary interventions have usually been successful in achieving their limited objectives.

On a larger scale though, many countries are now facing a significant public health problem due to changes in diet and lifestyle. Examples of these are the increasing levels of obesity, diabetes, and of cardiovascular disease. The issue though is how to tackle the causes. Encouraging people to adopt a healthier lifestyle may be possible, but adopting policies which can lead to a healthier diet are also being considered and adopted. Possible options include a return to some vertical controls, but an alternative is the use of more detailed labeling and advertising controls including enhanced nutritional guidance and tighter controls on nutrition and health claims.

Other Objectives

These three different objectives cover most issues which are normally regarded as food law. However, as food can have a great significance within a country's culture and economy, often laws are passed which have an impact on the food supply situation, but which have their origins outside the objectives described above. For example, countries with a large agricultural economy may seek to protect their farmers or rural industries by adopting certain protective measures, whereas some countries use food law to ensure compliance with religious rules. Often general controls applied to protect the society from a wide range of risks will also impact businesses in the food chain – for example, weights and measures, health and safety, customs and taxes, plant and animal health, animal welfare, or environmental controls. These wider controls are not normally considered as 'food law' but food businesses need to comply with all relevant legislation.

Written Food Legislation

This article relates to 'food legislation.' An alternative phrase is 'food law.' However, there can be a confusion as the terminology can vary between countries. The following definitions have been provided by the FAO (2006, p. 39) (Strengthening National Food Control Systems – Guidelines to Assess Capacity Building Needs):

> **Food legislation** (or food law) is the complete body of legal texts (laws, regulations and standards) that establish broad principles for food control in a country, and that governs all aspects of the productions, handling and trade of food as a means to protect consumers against unsafe food and fraudulent practices.
>
> **Food regulations** are subsidiary legal instruments (usually issued by a minister rather than by parliaments) which prescribe mandatory requirements that apply to various aspects of food production, handling, marketing and trade, and provide supplementary details that are left open in the main parliamentary-level legislation.

Food standards are nationally or internationally accepted procedures and guidelines (voluntary or mandatory) that apply to various aspects of food production, handling, marketing and trade to enhance and/or guarantee the safety and quality of food.

Even the above definitions are not fully satisfactory and, in particular, leave the boundaries to some of the terms rather vague. One main difficulty is the use of the term 'standards' in different situations. The International Organization for Standardization adopts and recommends standards. However, in most countries these are only considered advisory unless made mandatory by a legal provision (such as a regulation). Care is therefore needed when discussing national legal structures.

It can also be mentioned that the written legal documents are subject to interpretation. Although those drafting the legislation may have attempted to ensure clarity and precision in the text, those attempting to comply with the provisions may not be certain how to interpret the requirements. When first written, a general concept may sound sensible, but its application may cause difficulties. Words such as 'misleading,' 'legible,' 'misbranded,' or 'hygienic' are often used, but need interpretation. This may happen by additional guidance being issued by the authorities or the adoption of detailed codes of practice (which may, like standards, be either voluntary or mandatory). Alternatively, a complex issue may result in a case being taken to court resulting in a judgment or adjudication which can be applied more generally. Over time these judgments ('case law') can become an important component of the food legislation framework.

The creation of a legal basis for food control is vital if it is to be effective in protecting consumers. It is to be expected and hoped that the vast majority of food suppliers will wish to ensure that the food they supply is safe and meets consumers' requirements. However, there will be others who are prepared, either through negligence or a desire for personal gain, to put consumers at risk or to defraud them. Legislation is, therefore, necessary to provide a deterrent to minimize the numbers who are prepared to take this risk.

The written legal documents need to provide a comprehensive structure for food control. Key elements which are usually found in the main food legislation are:

- *Introductory provisions*: Legislation works best if there are clear definitions and it is common to find key terms defined in the law. Common definitions are for 'food,' 'food business,' 'safe food,' 'food adulteration,' and 'hazard.'
- *Enabling and administrative provisions*: This will identify which public authorities have responsibility under the law and may establish an agency or board to act on behalf of government. The law will also specify the enforcement authority and the powers of the inspectors to enter the premises and to take samples.
- *Offenses and penalties*: Key offenses are usually created which provide general protection from unsafe food or food which is labeled in a misleading manner. The penalties have to be sufficient to deter potential offenders.
- *Specific provisions on food*: Depending on the priorities in a country, the law is likely to contain a number of specific controls. These could relate to import and export conditions or to the requirements relating to the registration of a product or licensing of the premises. More generally, the law is likely to provide the authority for the adoption of secondary legislation in the form of food regulations.

Although the fundamental components of the main food law may not need to be changed very often, it is necessary to ensure that it remains an effective legal document. This requires a regular review of its provisions.

The secondary legislation in the form of food regulations will contain the main details required for effective food control. The more technical requirements contained in the food regulations are likely to be more regularly updated as new information becomes available on potential hazards or as technological advances introduce new substances or processes into the food supply chain.

These food regulations can be categorized into three main types:

Regulations Affecting Specific Food Products

To protect consumers from adulteration, legislation frequently sets out to define the acceptable components of food products and to establish acceptable processing procedures for them. Example of these can be bread, chocolate, coffee, and baby foods. This type of control, referred to above not only as 'recipe law' but also frequently termed as 'vertical' legislation, provides a high level of protection for consumers because manufacturers are forced to manufacture only to recipes which meet the legal requirements. The disadvantage is that the consumer is provided with a limited range of foods and choice is restricted.

Regulations Affecting Food Products in General

An alternative approach to control is to concentrate on more general issues and to establish criteria which can be applied to any type of food. For example, controls on food hygiene or food labeling can be adopted, which require all foods to be manufactured under specified hygienic conditions and to be packed with labels which provide a detailed list of ingredients. To distinguish these controls from the product-specific regulations, these are often termed 'horizontal' controls and allow much greater flexibility for the food manufacturer to be creative in their product development giving consumers a much wider choice of products on the market. However, consumers have to be aware that the composition of the products may not be regulated and hence will need to consider the information provided on the label while making a purchase decision. Additional areas covered by these more general regulations can be food additives (although specific requirements for food additives may also be found in the product-specific regulations), food contaminants (including environmental contaminants and pesticide, and veterinary drug residues), and controls on food-contact materials (whether packaging, food processing equipment or tableware and utensils).

Regulations for Organizational or Coordinating Purposes

The third type of regulation covers a broad range of supporting controls which give more detail as to the manner in which the legal controls operate. Example might include, the procedure for the issuing of licenses or of the taking of samples. There may need to be minimum standards of training for the food inspectors or the analysts working in laboratories. Specific controls may be applied to imported or exported foods to ensure compliance with national legislation or international obligations. These administrative controls may be considered routine, but can be just as necessary for the effective application of the food legislation.

The Application of Food Legislation

The effectiveness of food legislation is only confirmed by inspection and monitoring.

Inspection

A definition of 'food inspection' has been provided by the FAO (2006, p. 66) (Strengthening National Food Control Systems – Guidelines to Assess Capacity Building Needs):

> **Food inspection** is the examination of food or systems for the control of food, raw materials, processing and distribution, including in-process and finished product testing, in order to verify that they conform to requirements. Food inspection can be operated by government agencies, as well as independent organizations that have been officially recognized by national authorities.

Inspection at all stages in the food supply by official enforcement officers is necessary to provide the public with an assurance that those who fail to meet the legal requirements are detected and prevented from continuing in such a manner. It is also helpful to the food businesses who are complying with the law to know that competitors who fail to meet the same standards will not be permitted to continue.

Modern inspection systems use a professional and systematic approach built on a risk-based program of inspections. Examples of key components of the food inspection system are:

- Documented policies and procedures for risk-based inspection.
- Database of food premises categorized by risk.
- Adequate professional food officers with appropriate training, qualifications, and experience.
- Access to resources including facilities, equipment, transport, and communications.
- Procedures for the collection and handling of food samples.
- Procedures for handling food emergencies, outbreaks of foodborne disease and consumer complaints.

Monitoring

Once the enforcement officers have taken a sample, it is essential that it is subject to the appropriate testing. This requires an official laboratory to be available which can provide the officer with a clear and accurate statement of the physical, chemical, or microbiological status of the food sample. The result of the analysis should be capable of being used as evidence in any subsequent court proceedings. As such, the analysis needs to be conducted to a high level of integrity ensuring that the methodology is appropriate and it meets national or international standards. Beside enforcement samples taken in cases of suspected violation, random sampling is essential for providing an overall assurance that the food supply is safe, particularly from chemical hazards.

To help ensure that the laboratories are appropriately staffed and using appropriate techniques, it is common for them to participate in proficiency schemes in which their performance is independently judged in comparison with other official laboratories. The maintenance of high standards of analytical procedures linked to the use of standardized methods aids the accuracy of the results.

Although many routine analyses can be conducted with a fairly limited range of analytical equipment, advances in technology and the need to detect low levels of contaminants (e.g., pesticide residues or aflatoxins) have increased the range and sophistication of the equipment needed for official laboratories.

The Regionalization of Food Legislation

As described in section Key Objectives, governments of individual countries have responsibilities to their citizens to provide adequate protection. However, they also have responsibilities to promote economic development, and the promotion of trade is seen as an effective way to achieve this. Adopting national legislation may be important for consumer protection, but if every country adopts its own set of controls, the result can be to create non-tariff barriers which make it complex and expensive for businesses to trade – whether for imports or exports. One of the main objectives in working together as regional blocks is to try and adopt harmonized controls which enable unnecessary barriers to be removed and for trade to take place smoothly and with only limited interruption when crossing frontiers.

Various different regional groupings now exist around the world and they have different roles and objectives – some go well beyond the desire to seek harmonization but they still incorporate the general objective of harmonization and the abolition of barriers. One such example, the European Union (EU), will now be considered in more detail.

Although originally founded in 1956 as an 'economic' community, the EU has evolved over the years into a more extensive 'political union.' The scope of EU food law has evolved during this transition. Originally food legislation was focussed on removing barriers to trade by the adoption of harmonizing measures. Early food law was adopted for products such as jam, chocolate, sugar, and dried milk. Additional barriers caused by differences in food additive legislation led to the adoption of positive lists of EU food additives (e.g., colors, antioxidants, and emulsifiers) and the creation of the food additive identification 'E-number' system. Food hygiene legislation was adopted to ensure that harmonized meat

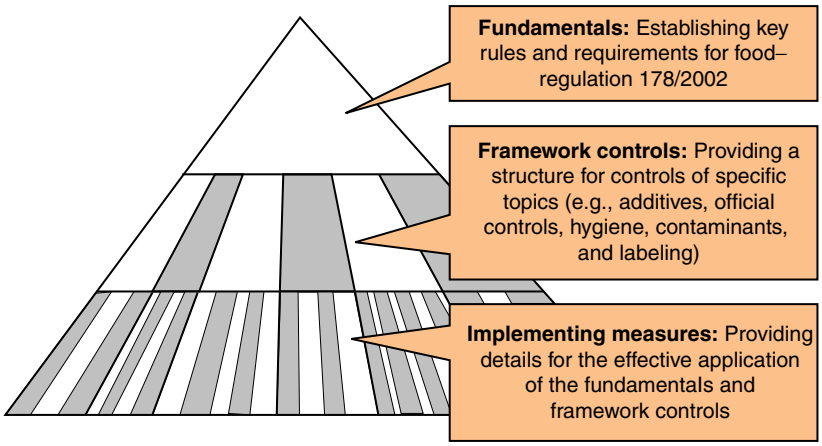

Figure 2 Structure of EU food legislation.

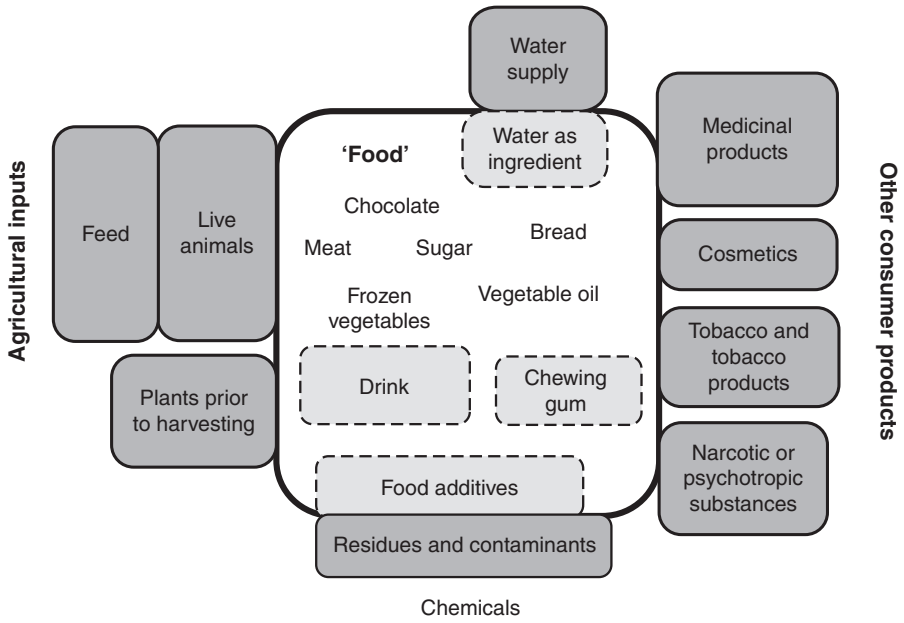

Figure 3 Boundaries related to food legislation.

inspection procedures were employed usually based on veterinary supervision. These were progressively developed to cover a wide range of animal-based products (e.g., poultry, red meat, eggs, milk, and fish).

Attempts were made during the 1970s and 1980s to provide a more systematic approach to this body of legislation. However, it was the food safety crises of the 1990s (in particular, the combination of the bovine spongiform encephalopathy epidemic in cows and the finding of dioxin residues in animal-based products) that led to the creation of a more comprehensive structure for EU food legislation. This is illustrated in Figure 2.

It was mentioned earlier that it is necessary to have detailed definitions included in the legislation. This can be illustrated with a diagram summarizing the definition of 'food' now used in EU food legislation. A detailed provision in Regulation 178/2002 provides the exact definition for EU purposes, but the aim of the definition is to define the boundaries to the concept of 'food' and to ensure that those subject to the controls can determine whether they are included, subject to a different set of controls, or excluded completely.

With the definition of food, the major areas of debate are the exact point at which the primary food producers (e.g., farmers or fishermen) become subject to the controls and the distinction between 'food' and 'medicines'/'drugs.' Other boundary issues involve the supply of water and environmental contaminants. This is presented schematically in Figure 3.

Most EU food legislation is now in the form of EU 'Regulations' which has a specific meaning as defined in the Treaties establishing the EU. It does not, unfortunately, correspond with the FAO definition of 'food regulation' given earlier. EU

Regulations can contain fundamental aspects of the controls or the much more detailed technical requirements contained in implementing measures. An alternative form of EU legal document is the 'Directive' which requires national implementation before it becomes a law in each member state. Although this was the preferred form used in early EU legislation, nearly all controls are now adopted as EU Regulations which define legal requirements in all member states from their date of application. Defining enforcement and penalties are still left to each member state according to their national legal rules.

The EU has now managed to create a body of food legislation which covers all key aspects of food production, manufacture, distribution, and sale. Where there are gaps, national rules still apply. Differences in these national rules can still create barriers to trade, although in general these are often limited to minor technical aspects or differences in interpretation of the EU requirements. The willingness to work together to move from a multitude of national controls to a common set of binding controls was difficult to achieve and took many years. Ultimately, a recognition that in a world where trade is now global and the safety of our food supplies are only defined by the weakest link in the chain led to the EU national governments being willing to move to a set of common rules. Similar moves are happening in other regions of the world although there is a wide variation in the stages reached.

See also: Institutions Involved in Food Safety: FAO/WHO Codex Alimentarius Commission (CAC). Public Health Measures: Evaluation of the Efficacy of National Food Control Programs; Food Control and Public Health Laboratories; Food Inspections and Enforcement Systems; International Standards and Harmonization of Food Safety Legislation; Modern Approach to Food Safety Management: An Overview

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International Standards and Harmonization of Food Safety Legislation

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Glossary

Compliance A situation in which products and/or practice meet regulatory requirements (to be differentiated from conformity, which means that activities are carried out according to the established procedures).

Food legislation Laws and regulations intended to protect and promote the identity, integrity and safety of the food supply and to facilitate trade.

Harmonization of food safety legislation A process by which appropriate national food laws and regulations are established, amended or administered with the view of ensuring the safety of food and facilitating domestic and international trade.

Risk analysis A process of decision-making (usually by government) for managing food safety, consisting of three components: risk assessment, risk management and risk communication.

Risk assessment A scientifically-based process for evaluating risks associated with foodborne hazards, consisting of the following steps: (i) hazard identification, (ii) hazard characterization, (iii) exposure assessment, and (iv) risk characterization.

Risk communication The interactive exchange of information and opinions throughout the risk analysis process concerning risk, risk-related factors and risk perceptions, among risk assessors, risk managers, consumers, industry, the academic community and other interested parties, including the explanation of risk assessment findings and the basis of risk management decisions.

Risk management The process, distinct from risk assessment, of weighing policy alternatives, in consultation with all interested parties, considering risk assessment and other factors relevant for the health protection of consumers and for the promotion of fair trade practices, and, if needed, applying appropriate prevention and control options.

Introduction

International trade in food and feed, international travel, and development of mass communication media have all contributed to the globalization of food safety risks to virtually every country in the world. This globalization of risks includes not only real risks, such as those posed by microbial or chemical hazards, but also perceived risks, such as those generated by media coverage rather than objective assessments of the risk. Globalization presents a unique challenge to governments and the food industry and calls for a more harmonized approach to the management of food safety risks at the international level. As food safety legislation is the foundation for food safety management, harmonization of national laws and implementing regulations will serve to protect consumers everywhere and facilitate international trade in food.

Food safety legislation can cover a broad range of subjects, including food additives, pesticides, veterinary drugs, food contact materials, contaminants, nutrition, labeling, claims, novel technologies, requirements for safe operation, and procedures for the administration of food control. This article focuses on the contributions of international organizations, in particular, the Codex Alimentarius Commission (Codex) and its two parent bodies, the World Health Organization (WHO) and the Food and Agriculture Organization (FAO), to the harmonization of food safety legislation.

At the outset it should also be pointed out that harmonization of food legislation is not necessarily a process of developing one law for all nations. Rather, it is a process by which appropriate national law is established and administered with the view of ensuring safety standards taking into account the national situation and at the same time, the facilitation of domestic and international trade.

Purpose of Food Safety Legislation

Food safety legislation is an essential public health function of governments. The term legislation encompasses basic food law and implementing regulations as well as any adjudication by the courts. In most countries, food law lays out the basic mandate for compliance and administrative policy and the operational programs to be implemented by the competent national food control authority. However, food safety regulations provide specific and detailed information needed to comply with the provision of the law, such as various standards. They also provide the basis for specific administrative,

procedural, and technical requirements to ensure food safety. The main goals of food safety legislation are to:

- protect and promote public health;
- protect consumers from products that are spoiled, fraudulent, or otherwise unfit for consumption; and
- provide consumers with relevant and accurate information so that they can make informed choices with regard to safety and nutrition.

Although not often duly acknowledged, food safety legislation is also of great importance to the food industry as it serves to:

- promote fair trade by ensuring a consistent standard among competing businesses;
- increase the confidence of consumers in the food supply;
- provide guidance and norms on matters related to food safety;
- provide data for validating food safety assurance systems of the industry; and
- ensure that all stakeholders of the food chain, i.e., both suppliers and customers, fulfill their roles.

Importance of Global Harmonization

Since the mid-twentieth century, the world trade in food had begun to increase dramatically. By 2010, global food trade was estimated to exceed USD$1 trillion and to include not only primary food commodities but also an ever growing number of food ingredients and processed food products. Globalization has also had consequences on food safety. Many food safety incidents have shown how domestic problems can have global implications. For example, the melamine problem in milk (China, 2008) and the bovine spongiform encephalopathy (BSE) outbreak (UK, 1996) had international repercussions for health and trade. Furthermore, the complexity of the international food trade network has resulted in a situation where the origin of a food (and a food safety problem) cannot always be easily traced, such as the cases of *Salmonella*-contaminated Serrano and Jalapeño peppers imported from Mexico (USA, 2008) and fenugreek seeds from Egypt contaminated with enterohemorrhagic *Escherichia coli* O104:H4 (France and Germany, 2011). Nor is it possible to easily trace forward a problematic product that has entered international commercial channels, as was the case with sauces made from chili imported from India that was adulterated with Sudan Red (UK, 2003). Problems with contaminated food moving in international commerce are occurring with disturbing regularity. Additionally, the rapid dissemination of information through the 24/7 news cycle and social media is raising consumer concerns for food safety and placing greater pressure on both industry and national food safety authorities to address food safety problems promptly.

For many countries, food export is a major source of foreign exchange earnings and an important factor for economic development. Underdevelopment is a major cause of poverty, which itself is the source of many of society's ills, including foodborne diseases and marginal practices affecting food safety. The latter exacerbate the food safety situation and jeopardize food exports. Whereas food standards should be health based and adequate to protect the health of consumers, food standards that cannot be justified on sound scientific grounds will create nontariff barriers to trade, often damaging the economies of developing countries. For food businesses, the harmonization of food legislative is also of primary importance as producing food according to the different requirements of countries further complicates food production operations that are already inherently complex. This not only decreases efficiency in production but also increases operational risks and costs.

There is also another fundamental reason for which global harmonization of food legislation is important. In their World Declaration on Nutrition, the ministers and the plenipotentiaries representing 159 states of the then European Economic Community recognized that 'access to nutritionally adequate and safe food is the right of each individual.' This universally accepted principle maintains that all individuals have a basic human right to the same standard of food safety and the same degree of health protection. Global harmonization of food safety legislation is therefore a major step in recognizing this right.

Historical Background

The development of food legislation started in the early period of human civilization, as many cultures and religions made provisions with regard to foods to ensure they were authentic, hygienic, and fit for human consumption. For example, the Persians standardized certain food commodities within their empire as a result of concerns for food adulteration and fraud. However, in those times, the basis for food safety was not really understood and many measures were not effective. Beginning in the Middle Ages in Europe, food producers in some countries formed trade guilds to promote the quality and safety of their products. However, it was not until the industrial revolution that countries began to establish science-based food safety legislation.

In the mid-1800s, Louis Pasteur unequivocally demonstrated the validity of germ theory and extended this knowledge to the field of food science and technology. This scientific approach led to safer, better quality food products and arguably, to our modern food supply system. Also during this time, growing urbanization increased the distance between producers and consumers, which created new opportunities for fraud and contamination. For example, the gross hygienic practices in the large stockyards in Chicago in the early 1900s outraged the population and led to the first US food safety legislation in 1906. By the mid-twentieth century, international trade, facilitated by advances in transport and food-processing technologies, started its enormous growth and underpinned not only the need for national food safety legislation but also its harmonization.

At the international level, some of the earliest international standards were those for milk and dairy products, which were elaborated by the International Dairy Federation in 1903. In the late 1940s, there were several attempts to establish regional food codes. In 1949, Argentina proposed a regional Latin American Food Code, the 'Codigo Latino-Americano de Alimentos.' In the

mid-1950s, Austria also pursued the creation of a regional food code, the 'Codex Alimentarius Europeaus.' The establishment of the FAO in 1945 and the WHO in 1948 was the foundation for food safety at the international level. Whereas FAO was given the mandate to establish international standards for quality and composition of foods and WHO was given the mandate to establish health and safety standards for food. These compatible mandates were the basis for the establishment of the Joint FAO/WHO Food Standards Programme and its Codex Alimentarius Commission, which is described below.

Codex Alimentarius Commission

The premier international body overseeing harmonization of food safety legislation is the Codex Alimentarius Commission (Codex) which was established as an intergovernmental body by FAO and WHO in 1963 under the Joint FAO/WHO Food Standards Programme. Codex develops harmonized international food standards, guidelines, and other recommendations to protect the health of the consumers and ensure fair practices in the food trade. These are compiled in a set of documents known as the 'Codex Alimentarius' (Latin for 'food code'). The Commission also promotes coordination of all food standards work undertaken by international governmental and nongovernmental organizations. Member countries of Codex now include 99% of the world's population. Many developing countries actively participate in the work of Codex – in many cases assisted by the Codex Trust Fund, which strives to finance and train participants from such countries. Being an active member of Codex helps these countries to compete in sophisticated world markets and to improve food safety in their own countries.

The Secretariat of Codex is housed at the FAO Headquarters in Rome and operates through a number of commodity committees and general subject committees. The procedures for adoption include an eight-step process of discussion and consultation with member countries, concluding with the formal adoption by full commission, which meets every year, alternating between Rome and WHO headquarters in Geneva. Most of the early work of Codex related to recommendations for international standards for food commodities, which were vital for facilitating the growing international trade in food. For example, such standards allow exporters know what importers demand, and importers are protected from substandard shipments. Some of these standards included sections that dealt with food safety issues, such as permitted food additives and maximum residue levels for pesticides. However, the real food safety harmonization work of Codex is now carried out by its horizontal committees, which address issues that apply across all foods. These include the following Codex Committees:

- Food Hygiene;
- Food Additives;
- Contaminants in Food;
- Pesticide Residues;
- Residues of Veterinary Drugs in Food;
- Nutrition and Special Dietary Uses;
- Labeling; and
- Methods of Analysis and Sampling.

In addition, Codex has convened several task forces on topics of interest to food safety, including animal feeding, foods derived from biotechnology, and antimicrobial resistance. A summary of the achievements of Codex in international harmonization is given in **Box 1**.

Until the mid-1990s, the 'Codex Alimentarius' was considered only as recommendations to its member states who were free to accept, modify, or reject them. Although the work of Codex was often used as a reference for national legislation, adoption of Codex recommendations was often inadequate. For example, a study comparing the regulatory requirements of the European Union (EU) with those adopted by the Codex Alimentarius Commission found many discrepancies. Furthermore, the study estimated EU standards resulted in a considerable loss of revenue from exports of cereal, edible nuts, and preserved fruit. For a number of African countries, this reduced their exports by USD$670 million per year. Thus, food safety standards by importing countries that are too stringent can create unjustified trade restrictions and contribute to underdevelopment and poverty in developing countries.

Box 1 Standards, guidelines, and other recommendations of the Codex Alimentarius

Number	*Topics*
186	Commodity standards
46	Commodity-related texts
9	Food labeling provisions
5	Food hygiene provisions
3	Food safety risks assessment
15	Methods of sampling and analysis
8	Inspection and certification procedures
6	Animal food production provisions
153	Maximum levels for contaminants including detection and prevention methods
1112	Food additives provisions covering 292 food additives
7	Food additives-related texts
2930	Maximum limits for pesticide residues covering 218 pesticides
441	Maximum limits for veterinary drugs covering 49 veterinary drugs
3	Regional guidelines

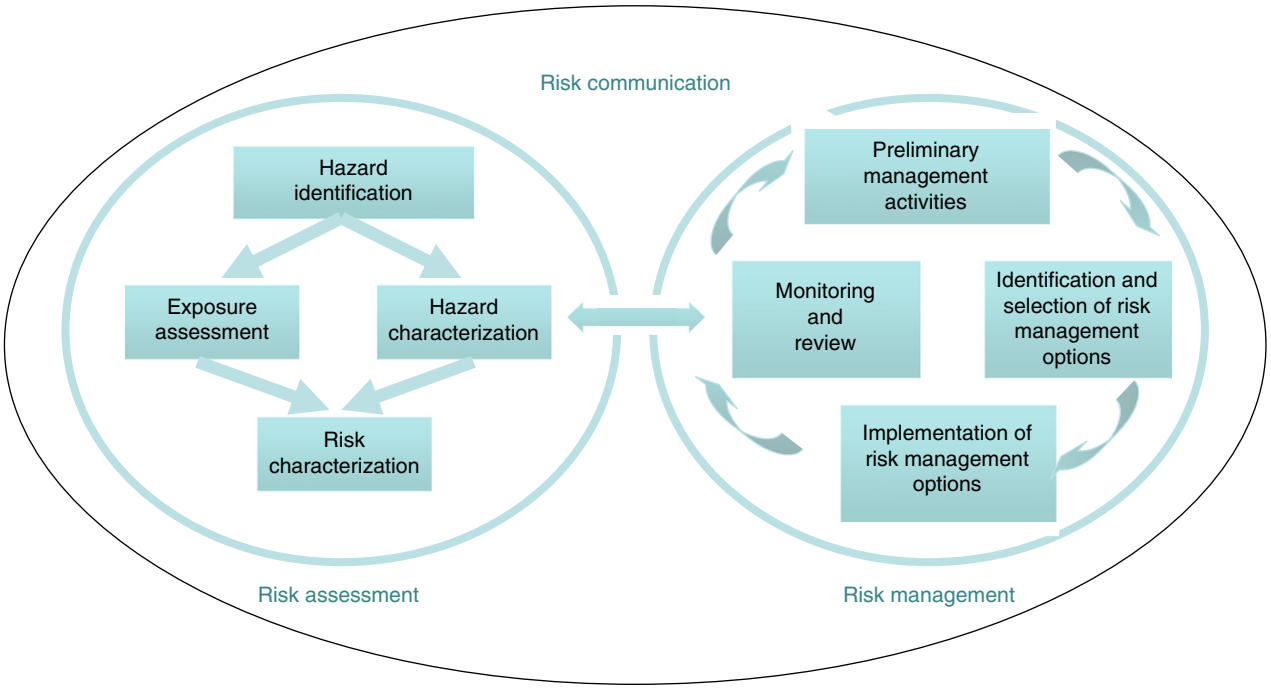

Figure 1 Generic framework for risk analysis.

Consequently, global harmonization of food safety regulations is of paramount importance in the twenty-first century.

Codex and World Trade Organization (WTO) Agreement

The Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement) of the WTO recognizes that governments have the right to take sanitary measures, but it specifies that such measures should be based on sound science to the extent necessary to protect human health, and that such measures should not discriminate unjustifiably between member countries where identical or similar conditions prevail. The SPS Agreement recognizes the food safety standards, guidelines, and other recommendations of Codex as representing the international consensus on health and safety matters. As such, member countries that comply with Codex are considered to be in compliance with the SPS Agreement. It is also expected that WTO member countries accept the sanitary measures of others as being equivalent if the exporting country demonstrates to the importing country that its measures meet the importing countries' appropriate level of health protection. New terms, such as 'food safety objectives' and 'performance objectives,' have been coined to determine appropriate levels of health protection in relation to food safety and facilitate the principle of 'equivalence' in the context of the SPS Agreement. Although these concepts are still being debated internationally, the SPS Agreement has nevertheless played a major role in encouraging countries to bring their legislation into line with international food standards, guidelines, and recommendations. Note that if a country adopts a higher level of protection than that afforded by Codex, the country must do so based on an appropriate assessment of the risk.

The WTO's Agreement on Technical Barriers to Trade (TBT Agreement) is also important to food trade and particularly to food labeling. It provides member countries with the right to consider other legitimate factors in their decision-making process. Examples of such factors are considerations regarding the environment, animal welfare, and consumer preferences.

After the SPS Agreement came into force in 1995, FAO and WHO convened a series of three consultations on risk assessment, risk management, and risk communication that are the components of risk analysis. Reports of these consultations were subsequently considered by Codex, which developed a set of principles and recommendations related to the use of risk analysis in standard setting. Although these principles and recommendations were directed at member countries, Codex itself began a process to integrate them into its own procedures. One of the principles was the functional separation between risk assessment and risk management, yet with adequate risk communication between them. However, this already existed in Codex because its risk assessment advice is provided by FAO/WHO expert meetings, including the Joint FAO/WHO Expert Committee on Food Additives, the Joint FAO/WHO Meetings on Pesticide Residues, and the Joint FAO/WHO Meetings on Microbiological Risk Assessment. Other recommendations relate to the process of food safety management from scientific evaluations to the risk management decisions, including consideration of other legitimate factors as well as communication with all stakeholders. An overview of this process is shown in Figure 1.

These principles and recommendations are also reflected at national and regional levels. For example, the European Food Safety Authority was created with the responsibility of conducting risk assessments for risk management, which remained with the European Commission. Although these

principles have been adopted nationally in many countries, notably in North America and Europe, there are differences in their implementation, in particular, with regard to risk management decisions and how uncertainties in risk assessment and other legitimate factors should weigh in the decision-making process.

In Europe, past experience with BSE and consumer reaction to the BSE crisis have influenced the process of risk analysis so that the decision-making process is:

1. more conservative in its approach, including the application of the 'precautionary principle' in times of uncertainty and
2. more attentive to consumer perceptions and preferences regarding food safety risk and ethical and environmental issues.

In North America, the general public is less averse to modern methods of food production and processing; however, large-scale foodborne disease outbreaks, in particular, outbreaks of *E. coli* O157:H7 affecting children, have led to the strengthening of food safety legislation.

WTO Dispute Settlement

In addition to the international agreements on trade, the WTO also provides judgment in disputes related to the SPS Agreement and may require members to modify or withdraw their noncompliant sanitary measures or face punitive measures. The WTO can authorize countries affected by the violation of the SPS Agreement to take retaliatory measures. The unsuccessful intervention of the WTO in resolving the US–EU hormone-treated beef dispute is an example of the difficulties in harmonizing food legislation even with the SPS Agreement in place.

International Health Regulations (IHR)

Concomitant with the increasing industrialization of the world in the early 1800s, international travel and migration also expanded, and along with this, a greater risk of spreading infectious diseases. In 1830 and again in 1847, major cholera epidemics struck Europe caused by imported cases and catalyzed international cooperation in public health that led to the first International Sanitary Conference in Paris in 1851. Exactly 100 years later and 3 years after the establishment of WHO in 1948, WHO member states adopted the International Sanitary Regulations, which were later renamed the IHR. The IHR is an international legal instrument, binding 194 countries to fulfill the obligations in regard to disease outbreaks of international public health concern. The aim of IHR is to prevent and respond to public health risks that have the potential to cross borders and threaten people worldwide. The latest revision of IHR came into force on 15 June 2007 and greatly expanded the scope and coverage of the IHR, which explicitly include food. The IHR requires countries to report certain disease outbreaks and public health events of international concern to the WHO. Under this requirement, WHO member states are requested to report a contaminated food product that may be, or is suspected to be, of international public health concern. To support exchange of information on contaminated food products entering international trade, in 2004, WHO in collaboration with FAO established the International Food Safety Authorities Network, which includes a food safety emergency component coordinated with IHR.

International Organizations

In the context of harmonizing food safety legislation, a number of international organizations merit mentioning. Some of these are covered in more detail in separate articles.

WHO

WHO is the directing and coordinating authority for health within the United Nations system. It is responsible for providing leadership on global health matters, shaping the health research agenda, setting norms and standards, articulating evidence-based policy options, providing technical support to countries, and monitoring and assessing health trends. With headquarters in Geneva, WHO is one of the more decentralized of the United Nations agencies with six regional offices located around the world. These offices are staffed with regional food safety advisors who work directly with the countries to strengthen their food safety programs. Given its mandate in food safety, some of its earliest publications of WHO in the 1950s were on the food safety topics, such as milk pasteurization, meat hygiene, safety of food additives, and safe use of pesticides. The Department of Food Safety and Zoonoses (FOS) in WHO Headquarters provides leadership in WHO's global efforts to lower the burden of diseases from food and animals. In carrying out its activities, FOS focuses on

- providing evidence-based scientific options for policy development to protect consumer's health and manage food safety;
- developing mitigation strategies to prevent, control, and contain risks;
- setting international standards and promoting their implementation;
- coordinating international efforts in food-related outbreak surveillance, detection, and response;
- ensuring clear risk communication in support of foodborne and zoonotic disease prevention and control; and
- providing technical support to assist member states in building sustainable capacity.

WHO works closely with the FAO as well as the World Organisation for Animal Health (OIE) and other international organizations to address food safety issues along the entire food supply chain from production to consumption.

FAO

Based in Rome, FAO's primary mandate is to raise levels of nutrition, improve agricultural productivity, better the lives of rural populations, and contribute to the growth of the world economy. The food safety activities in FAO are mainly located in the Food Safety and Quality Programme under the Nutrition and Consumer Protection Division. The activities of the program include

- providing independent scientific advice on food safety and nutrition which serves as the basis for international food standards;
- developing institutional and individual capacities for food control and food safety management in countries, including the management of food safety emergencies;
- supporting processes for the development of food safety policy frameworks;
- facilitating global access to information; and
- encouraging and supporting the development of food safety and quality networks.

This division also houses the secretariat of the Joint FAO/WHO Food Standards Programme and its Codex Alimentarius Commission, which is the most important body for international food safety harmonization.

WTO

The successful conclusion of the Uruguay Round of Multilateral Negotiations led to the establishment of the WTO in 1995. Based in Geneva, the mandate of the WTO is to undertake negotiations to liberalize international trade. The organization deals with regulation of trade between participating countries by providing a predictable framework for negotiating and formalizing trade agreements. It also offers a dispute resolution process aimed at enforcing participants' adherence to WTO agreements, which are signed by representatives of member governments and ratified by their parliaments. Most of the issues that the WTO focuses on derive from previous trade negotiations, especially from the Uruguay Round. In regard to food safety, two agreements had major implications for the harmonization of food legislation. These were the SPS Agreement and the TBT Agreement. In regard to the SPS Agreement, sanitary measures relating to animal and human health are referred to the OIE and Codex Alimentarius Commission, respectively, whereas phytosanitary measures relating to plant health is referred to the International Plant Protection Convention.

OIE

The OIE is an intergovernmental organization that was established in 1924 as the result of an international incident involving the transmission of rinderpest. The organization is based in Paris and still uses its French acronym OIE (from Office International des Epizooties). The primary mandate of OIE is to improve animal health worldwide. OIE currently has 178 members and maintains liaison with 35 international and regional organizations. The OIE is referred to in the WTO SPS Agreement in regard to animal health issues. Among its activities is the timely dissemination of information regarding animal disease outbreaks, which is facilitated by its World Animal Health Information System (WAHIS). Under WAHIS, rapid notification and follow-up reports are provided to countries in response to exceptional and/or international animal disease events. In this regard, OIE has worked closely with both WHO and FAO in responding to the global outbreak of avian influenza. OIE was also very involved in the control and eradication of BSE.

International Organization for Standardization (ISO)

In operation since 1947, the role of ISO is to facilitate the international coordination and harmonization of industrial standards to promote international exchange of goods and services. ISO is a nongovernmental organization, and as such, its standards are voluntary and not enforceable as regulations. Nevertheless, some of its standards, in particular those related to health, safety, and environment, have been adopted in some countries as part of their regulatory framework or are referred to in their legislation. ISO standards are developed by experts in the industry or business sector. They are therefore referred to as private standards. Standards with regard to food are developed under the Technical Committee on Agriculture (ISO/TEC 34). Under this committee, over 500 international standards have been adopted and over 100 more are underdevelopment. With regard to food safety, the ISO 22000 standard entitled 'Food safety management systems – requirements for any organization in the food chain' has received attention in regulating the customer–supplier relationship.

In addition to these international organizations, other efforts to promote harmonization of food safety legislation have been undertaken by nongovernmental organizations. Some of these are described below.

Global Harmonization Initiative (GHI)

The GHI is a nongovernmental initiative with the goal to specifically promote harmonization of food safety legislation. In the context of this initiative, since 2004 food scientists from all over the world have been working to achieve a global consensus among scientists on food safety issues and to provide tools for facilitating the global harmonization of food legislation.

Safe Food International (SFI)

SFI is a project designed by and for consumer organizations that want to improve food safety on a global scale. SFI aims to unify and focus the efforts of consumer organizations worldwide that are working to ensure a safer food supply by assuring that their national food safety legislation addresses common food safety problems. At a conference in 2005 cosponsored by the WHO and FAO, consumer organizations shared their food safety expertise and developed a common vision of how to strengthen national food safety systems, including legislation. Based on consensus and endorsed by the WHO and FAO, SFI published its 'Guidelines for Consumer Organizations to Promote National Food Safety Systems,' which have become the basis for training and advocacy by consumer organizations around the world. Consumers International and the International Association of Consumer Food Organizations – both international umbrella organizations composed of consumer organizations – also provided important assistance in the development of the guidelines.

There are also a number of other international nongovernmental organizations that directly or indirectly promote harmonization of food safety legislation, including the

International Union of Food Science and Technology, Confederation of European Food Industries, International Life Sciences Institute, and International Commission on Microbiological Specifications for Foods. These are covered in separate articles related to those organizations.

Future Challenges

Despite its importance, global harmonization of food safety legislation will remain a major challenge for the twenty-first century. The international community will have to reach an agreement on a multitude of food safety issues that provides adequate health protection, but at the same time

- meets the expectations of consumers;
- allows economic and trade growth;
- advances food science and technology; and
- ensures fair competition between large and small industries as well as between industrialized and developing countries.

See also: Institutions Involved in Food Safety: Consumer Organizations; FAO/WHO Codex Alimentarius Commission (CAC); FoodDrinkEurope; Global Harmonization Initiative (GHI); International Food Information Council (IFIC) and Other Food Information Organizations; International Life Sciences Institute (ILSI); International Organization for Standardization (ISO); International Union of Food Science and Technology (IUFoST); National Industry Organizations – Case of UK Food and Drink Federation; World Organisation for Animal Health (OIE). Public Health Measures: Modern Approach to Food Safety Management: An Overview. Risk Analysis: Risk Analysis of Hazards in Food: An Overview

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PUBLIC HEALTH MEASURES

Food Inspections and Enforcement Systems

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Glossary

Adulteration A legal standard applied to distinguish food that is inferior, contaminated, produced under insanitary conditions, or different from an identity standard; or food that contains harmful, less valuable, or prohibited substances.

Civil penalties Civil fines can be applied administratively or by a court for lesser violations of law, providing a more flexible response to corporate misconduct that can be tailored to the violation.

Codex Alimentarius A collection of internationally recognized standards, codes of practice, guidelines, and other recommendations relating to foods, food production, and food safety.

Criminal penalties Criminal fines or incarceration are applied to individuals or firms following conviction on charges of criminal behavior in a court of law.

Detention An order, typically issued by a government authority, to temporarily hold a shipment of food so that it cannot enter commerce pending a more thorough examination.

Inspection The examination of food or systems of food control in order to ensure that food meets the requirements set by government authorities.

Recall Recalls are actions taken by a firm to remove a product from the market. Recalls may be conducted on a firm's own initiative, by request from a food safety agency, or, in the case of a mandatory recall, by order under a food safety agency statutory authority.

Traceability The ability to follow the movement of a food or ingredient through multiple stage(s) of production, processing, and distribution.

US Department of Agriculture (USDA) The agency that regulates production and importation of all raw beef, pork, lamb, goat, horsemeat, chicken, turkey, duck, and goose as well as processed meat and poultry products and liquid, frozen, and dried egg products in the United States.

US Food and Drug Administration (FDA) The US agency that regulates domestic and imported food products (except for most meat, poultry, and egg products), including processed foods, dairy, shell eggs, seafood, and products which contain minimal amount of meat.

Introduction

Inspection is used to verify that food, and the systems used to produce it, meet the requirements established to protect consumers from foodborne hazards and control deceptive marketing practices. Since ancient times, inspection has been an essential component of regulatory control programs to ensure the safety of food products.

Inspection services give government regulators, customers, and consumers consistent information regarding conditions throughout the food chain. Serving as in-house experts, inspectors have essential information about the food production system that can help in responding to food safety emergencies.

Food inspection services can cover many aspects of the food supply. Many inspection functions are focused on food processing facilities (including slaughter facilities) and at import facilities. Restaurants, farms, storage, and packing facilities can also be subject to inspection. Street vendors are subject to oversight in many places.

A variety of components contribute to the effectiveness of inspections. The number of inspectors and facilities will impact the frequency of inspection. Many programs focus most of their resources on risky foods and higher risk sectors of the food supply, which can leave other sectors without oversight. Inspector training and ethics are also critically important to ensure effective inspections.

Inspection Models, Past and Present

One of the purposes of food inspection is to deter adulteration of food. Where inspection has been mandated by law, there are several models:

- Carcass-by-carcass inspection for slaughter plants has been in place for more than 100 years in the meat industry. Some countries require government inspectors to be present in all slaughter facilities during their operation to inspect each carcass for signs of illness, disease, or filth.

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- The US and other countries require daily inspection for many high-risk processing facilities such as processed meat, poultry, and egg products.
- Many governments worldwide use random or periodic inspection to check for unsanitary conditions that could lead to adulteration as well as to verify a food facility's food safety system.

When food-related disease outbreaks or other food crises occur, government agencies can be subject to significant criticism. Some governments may practice a reactive approach to inspection, when, for example, they send inspectors to inspect facilities in response to an outbreak or contamination event linked to foods from a similar type of facility. Several governments are working to identify and define the components of a risk-based inspection system for periodic inspections. Under a risk-based inspection system, food facilities are inspected regularly on a schedule that reflects the likely risk to consumers from the type of food produced, not just in response to outbreaks. Consultation with all stakeholders on the elements of the inspection system can help to inform those both inside and outside of government.

Modern Inspection Systems

According to the Codex Alimentarius, inspection is the examination of food or systems of food control in order to ensure that food meets the requirements set by government authorities. Inspection can be applied at many points along the food chain, encompassing raw materials, processing, and distribution. It can also include both in-process and finished product testing.

The World Health Organization (WHO) and the Food and Agriculture Organization (FAO) of the United Nations, in advising governments that are developing or modernizing food safety systems, have defined the responsibilities of inspection systems as follows:

- Inspecting premises and processes for compliance with hygienic and other requirements of standards and regulations.
- Evaluating Hazard Analysis and Critical Control Point (HACCP) plans, their development and their implementation.
- Sampling food during harvest, processing, storage, transport, and sale to establish compliance, to contribute data for risk assessments, and to identify offenders.
- Recognizing different forms of food decomposition, or food that is adulterated, misbranded, or otherwise unfit for consumption.
- Providing evidence and other assistance in law enforcement activities.
- Conducting inspection, sampling, and certification of food products for import/export purposes.
- Examining how rework, noncompliant raw material, products, incidents are managed.

According to FAO/WHO, the effectiveness of the inspection control system is highly dependent on the inspection force, including workforce qualifications, training, and honesty. FAO/WHO recommends that inspection personnel be trained in food science and technology sufficient to allow them to analyze a HACCP program and identify safety or quality problems. Additionally, inspectors must have a wide variety of skills in order to inspect premises, collect samples, conduct an overall evaluation, understand and apply food legislation, collect evidence, and handle HACCP audits.

The Frequency of Inspections

Inspections can be daily (as some countries require for meat and poultry) or periodic. In either case, to maximize consumer confidence, Codex guidance recommends that inspection or other appropriate controls should extend to all stages of production, manufacture, importation, processing, storage, transportation, distribution, and trade.

For periodic inspections, managers should apply principles of risk to ensure an adequate frequency of inspection is maintained, i.e., foods and facilities should be evaluated to ensure high-risk foods are inspected more frequently than those presenting a low risk.

Codex outlines a number of components to consider when assessing the risk of imported foods, many of which apply equally well to domestic products:

- The scientific determination of the food safety risk, based on foodborne illness outbreaks and epidemiologic evidence, risk assessments, and contaminant or residue information, where available.
- The adequacy of processing controls in a country, as evidenced by the laws, infrastructure, and enforcement capabilities.
- The compliance history of the segment of the food industry.
- The compliance history of specific suppliers.
- Reports from other entities such as a foreign government or other local or national agencies, where available.

Regular review of the level of assigned risk helps ensure that the nature and frequency of inspection is appropriately matched to the specific products. The review should be documented, with changes over time recorded.

Other Elements of Legislation that Support Inspection

A number of elements of a food control system can make the inspection program more effective as well as more efficient. For example, an inventory of food facilities operating in or supplying food to a particular country or region facilitates inspection. Here are some provisions in use in different countries:

- Facility registration to provide food control authorities with an inventory.
- Rapid alert systems to ensure effective dissemination of findings of adulteration or misbranding, nationally or regionally.
- National systems to approve HACCP plans and controls, equipment design and use, and standardized training of inspectors.
- Export certification to provide assurance that the specific facility or food meets the standards of the importing country, which can facilitate the trade of foods between countries.

- Laboratory systems that provide the ability to sample and test foods and facilities for specific contaminants.
- Active foodborne outbreak surveillance systems to inform regulators about the foods most likely to cause illness.
- Utilization of state, provincial, or potentially private inspectors to augment the national management scheme.

Enforcement

The role of enforcement is to provide deterrence and to promote the use of best practices. Government sanctions are the primary means of enforcing food safety standards. However, private lawsuits can also serve to promote compliance. Enforcement is important to deter noncompliance in countries where market forces alone do not provide sufficient incentive for businesses to produce safer food.

Important government enforcement tools include:

- Detention: An order, typically issued by a government authority, to temporarily hold a shipment of food so that it cannot enter commerce pending a more thorough examination.
- Seizure: The government takes unsafe food, which may occur without compensating the owners, to prevent it from reaching consumers. Seizures may require a court order.
- Injunction: An agency may seek a court ordered injunction to stop a food establishment from producing or distributing food.
- Civil penalties: A government agency may impose civil fines administratively or go through the courts. Civil penalties provide a flexible response targeted at restoring compliance with food safety requirements.
- Criminal penalties: The government may prosecute individuals or firms, seeking court ordered fines and/or prison sentences. Statutes normally specify the criminal penalties for specific violations.

Other authorities that are useful in enforcing food laws include:

- Business records access: Reviewing process-related business records provides important information to regulators. For example, access to records documenting preventive controls, monitoring and verification during inspections, or tracking information to facilitate recalls in an emergency assists government authorities.
- Mandatory recall: Removing food from the market when a firm violates the law is essential to protect consumers. The threat of a costly recall order can have a strong deterrent effect.
- Tracking food products: Mandating traceability can minimize the impact of foodborne outbreaks by allowing for more rapid identification of food linked to an outbreak. It can also provide evidence of where the problem occurred for purposes of taking corrective action.
- Employee protections: Food handlers and plant employees can provide valuable information to government agencies regarding unsanitary conditions. They may only come forward if they have protection from the threat of dismissal for helping in an investigation.

Government Enforcement

Government actions often begin after an inspection reveals problems in a food establishment, or may occur in response to an outbreak of illness linked to a food. Government agencies may utilize a multistep approach to ensure compliance. For example, they may begin by issuing notices of deficiencies and conducting reinspections to check on a business's corrective actions. If problems are not corrected or the noncompliance is especially egregious, the agency may issue a warning letter. If the product has been shipped to distributors, a business may be asked to voluntarily withdraw or recall the adulterated or misbranded product. Agencies also publicize their findings through a public health warning and through formal administrative procedures.

Some agencies may utilize more direct approaches such as detaining suspect food or levying civil monetary penalties. If the food is subject to continuous inspection or requires approval by government inspectors before being marketed, the agency may withdraw inspectors, which effectively shuts down the business until it demonstrates that it has come into compliance.

Governments may also take firms or individuals to court to seize unsafe food or issue an injunction that stops the firm from manufacturing or distributing their products. The government may seek criminal sanctions if appropriate. Prosecution may target the person who was in a position of responsibility, regardless of whether that person intended to commit a crime. Punishments can include a fine, prison, or both.

Governments can enlist food establishments to enforce standards. For example, the Food Safety Act of 1990 in the United Kingdom provides a due diligence defense to encourage food establishments to take responsibility for their products. A person who can prove he took all reasonable precautions and exercised due diligence to avoid the commission of the offense can escape conviction. One result of having the due diligence defense available has been the emergence of private food safety standard setting and certification bodies.

Private Action

Private enforcement occurs in some countries when a person has been injured by an adulterated or misbranded food product. Lawsuits involving food safety claims can arise from negligence, product liability, or breach of warranty, depending on the facts of the case. In the case of food, product liability claims may follow 'strict liability' doctrine in which there is no need to prove the defendant's conduct contributed to the injury. Although government action focuses on compliance, private lawsuits seek to compensate victims. Even so, the threat of lawsuits can improve compliance.

Conclusion

Inspection is a core function of a national food safety system. It gives regulators, the regulated industry, and consumers information regarding conditions in food facilities and

throughout the food chain that can affect food safety. Advancements in food safety practices over time have changed the scope and purpose of inspections and have put more emphasis on prevention rather than enforcement.

See also: Food Safety Assurance Systems: Audits of Food Safety Management Systems; Documentation and Record Keeping; Hazard Analysis and Critical Control Point System (HACCP): Principles and Practice. Institutions Involved in Food Safety: FAO/WHO Codex Alimentarius Commission (CAC). Public Health Measures: Challenges of Developing Countries in Management of Food Safety; Challenges of Industrialized Countries in Food Safety Management; Food Control and Public Health Laboratories; Fundamentals of Food Legislation; Modern Approach to Food Safety Management: An Overview

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PUBLIC HEALTH MEASURES

Alerts and Early Warning Systems

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Glossary

Emerging risk 'A risk resulting from a newly identified hazard to which a significant exposure may occur or from an unexpected new or increased significant exposure and/or susceptibility to a known hazard' (as defined by European Food Safety Authority's Scientific Committee).

Holistic Focusing on whole systems rather than specific parts of it. In this article, 'holistic' refers to systems and approaches that take account of developments outside the food manufacture chain so as to be able to detect changes that eventually will impact on this chain.

Indicator A qualitatively or quantitatively measurable and sensitive parameter that is indicative of a certain condition of interest. In this article, for example, holistic systems utilize indicators within influential sectors surrounding the food manufacture chain in order to detect developments within these sectors that may eventually impact on the chain itself.

Influential sector A subdivision of society (sector) that exerts influence on other sectors, the latter in this case being the food manufacture chain.

Signal A detected change in the nature or magnitude of the indicator that is sufficiently large to signify changes in the conditions with which the indicator is linked.

Food Safety and Rapid Alert Systems

The repeated occurrence of food safety issues in the last decade has evoked a public concern about the safety of our food production systems and has resulted in a decline in the consumer confidence in the food safety regulation and management inside and outside Europe. In Europe, for instance, the European Commission responded by issuing a new food safety regulation (Regulation (EC) No. 178/2002/EC) often referred to as 'General Food Law.' This regulation clearly describes the food safety framework in European Union (EU) including the responsibility of the different stakeholders in the food production chain. With this law the independent European agency for risk assessment for all categories of food hazards (European Food Safety Authority (EFSA)) was established. In the USA, for instance, the Food and Drug Administration and the Human Health Services launched a joint initiative, the Food Protection Plan, in 2007. With the plan, these federal authorities responded to the increasing globalization of food trade and changing consumption patterns, in order to further safeguard a healthy food supply. This plan extends on the existing food safety and food defense initiatives. It addresses both intentional and unintentional threats, and imported and domestic foods, whereas promoting further cooperation between national (state and interstate) authorities as well as public–private cooperation. It employs advanced information technology tools and aims at addressing risks in a more targeted way. The plan consists of three major pillars, of which one important pillar is prevention, such as through identifying vulnerabilities, introducing good manufacturing practices, and filing prior notices of export to the USA for foods produced abroad. The other two pillars are: (1) intervention, such as through rapid detection methods for signals of contamination and through the application of information technologies; and (2) response, such as through cooperation between authorities. In Japan, the Food Safety Commission, an independent body advising the government on food safety issues, such as the safety of genetically modified foods, pesticides, feed and food additives, contaminants, and antimicrobial resistance in food pathogens, was established in 2003 as laid down in the Japanese Food Safety Basic Law.

It is apparent that food safety risks can be reduced if food safety hazards are identified at an early stage and is followed by proper management actions to prevent the hazard to develop into an unacceptable level of risk. Many information and monitoring systems on food safety have been put in place, both nationally and internationally, which has been reviewed recently by Marvin *et al*. These authors divide early warning systems into three main categories:

1. Reactive systems which are endpoint or hazard focused,
2. proactive predictive systems (often referred to as emerging risk systems), and
3. systems based on a holistic approach. This category of systems uses information from inside and outside the food production chain to predict the emergence of a food safety risks.

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Reactive Systems

The vast majority of the early warning systems in place fall into the first category: reactive early warning systems. These systems collect, analyze, and interpret data from running monitoring and surveillance programs and can be either hazard or disease recording systems. Hazard-focused systems focus on the identification of hazards as they occur in the food production from farm to fork. A typical example is the European Rapid Alert System for Food and Feed (RASFF), which is used by its members for notifying hazards to food and feed safety that they have identified and that are relevant to the other members as well. These members include the national food safety authorities of EU member states and associated countries, as well as the European Commission, which hosts the system. The notifications usually contain information obtained from food inspection and monitoring programs and cover many different kinds of hazards in food and feed, such as microbiological and other biological hazards, chemical contaminants and residues, physical hazards, and fraud-related issues. Both food and feed products are included in the RASFF system. The system also contains information on the origin of the product, the level of contamination, the risk management actions that have already been taken, and to which extent the product has already been distributed or not. A previous investigation examined the possibility of using trends in RASFF data for the purpose of identifying potentially emerging food safety issues. The authors found that the EU and national authorities had already responded with risk management measures in the cases identified, which also highlights important contribution of the system to adequate risk management of food safety.

Foodborne disease outbreak monitoring systems includes health surveillance and other epidemiological tools, such the electronic notification system for animal diseases (World Animal Health Information System) set up by the World Organisation for Animal Health (OIE). National veterinary authorities inform OIE of the occurrence in their country of animal diseases from a list of notifiable diseases in the frame of the terrestrial animal health code and the aquatic animal health code. This network has global dimension involving veterinary offices from its 175 member countries. Another example of disease monitoring system that functions as an early warning system is the European Centre for Disease Control (ECDC). This institution was established in 2004 to support the control of communicable diseases and other serious health threats, and is among others, based on input from national health protecting agencies of the EU member states. It also coordinates EU-wide surveillance networks for foodborne pathogens, and links to international initiatives in this field. The activities of the Enter-Net network for detection of international outbreaks of salmonellosis and verocytotoxin producing *Escherichia coli*, for example, was continued within the ECDC's Food and Waterborne Disease Unit. Enter-Net, which was a continuation of the previous Salm-Net network, was able to identify various international outbreaks related to specific serotypes of pathogens identified via pulse-field gel electrophoresis. PulseNet International is an example of an international network on foodborne pathogen-related disease surveillance in which ECDC participates. This network provides protocols for the subtyping of pathogens under surveillance, such as *Campylobacter jejuni*, *E. coli* (O157:H7 and non-O157), *Salmonella*, *Shigella sonnei*, *Listeria monocytogenes*, *Vibrio parahemolyticus*, and *Vibrio cholerae*, which allows for standardization and comparison of deoxyribonucleic acid (DNA) profiles of pathogens across participating laboratories. Using these protocols, a number of outbreaks have been linked to contaminated products and sources of contamination. These and similar networks for pathogen surveillance are also reviewed by Kleter and Marvin.

Proactive Predictive Systems

Proactive predicting early warning systems, however, are not focused on existing public health threats but are aiming to predict food safety risks that may emerge in the (near) future. Examples of such systems that are hazard-specific include the mycotoxin warning systems and the forecast system for harmful algal blooms, which produce marine biotoxins that can accumulate in shellfish and other seafood, based on satellite imagery, observations by workers in the field, computer models, and laboratory analysis. Other systems are more broadly oriented such as 'horizon scanning,' and information collecting systems, such as the Global Information and Early Warning System on Food and Agriculture run by Food and Agriculture Organization (FAO) or European Media Monitor (EMM) operated by the Joint Research Centre (JRC) in Europe. In the following section we will discuss the recently published study of the Emerging Risk (EMRISK) Unit of EFSA, which evaluated the applicability of EMM as an early detection tool for food and feed hazards.

New Developments and Improvements

The role of the early warning systems in the process of risk analysis, mentioned in the previous section, can be to aid the identification of hazards that need further being assessed scientifically for the actual risks involved and that also need being managed so as to prevent or mitigate the risks, for example, by recalls of implicated products or the implementation of controls in the food supply. Moreover, monitoring programs could include measurement of early warning signals and detection of signals that signify potential emerging risks.

Various developments in the field of emerging food safety risks have recently taken place at the level of national authorities and international organizations. In the USA, for example, the US Department of Agriculture's Animal Plant and Health Inspection Service transformed its Center for Emerging Issues into the new Center for Animal Health Information and Analysis. The new center's activities include the scanning of open-source media for information on emerging animal diseases, including zoonotic diseases in livestock that can be transmitted to consumers, as well as the use of geospatial data and models to predict the spread of diseases and their economic impact. In addition, Food Standards Australia New Zealand commits itself to providing information on emerging issues to stakeholders, including consumers and food industry, for example, through the recently initiated Monitoring of

Emerging Issues Newsletter. Although recognizing the global dimension of the recent developments in the field of emerging food safety issue identification, in the section that follows we focus on the EU and the FAO/World Health Organization (WHO) as advanced examples of these developments.

Establishment of the EMRISK Unit of EFSA

As mentioned in the Section Food Safety and Rapid Alert Systems, EFSA was established by the General Food Law, Regulation (EC) No. 178/2002, in 2002. This law also provided directions on which activities and tasks EFSA had to perform, including the provision of independent, objective, and transparent scientific advice on food and feed safety, as well as a number of related fields, such as plant health, animal welfare, and environmental release of genetically modified organisms. Moreover, EFSA was supposed to collect data on food consumption, biological risks, contaminants, and residues, as well as to conduct scientific studies within the remit of its mission. The identification of emerging risks was also specifically mentioned as a task of EFSA's, namely in article 34 of the General Food Law. This article specified, among others, that EFSA should establish monitoring procedures in order to systematically retrieve information on emerging risks. Information that is received by EFSA as part of its activities should be used for this purpose as well. The General Food Law also mentions that EU member states and various EU-wide institutions are to be contacted with a request for relevant information in the case there is a suspicion of an emerging risk. After EFSA has evaluated the emerging risk, it should inform the European Parliament, the European Commission, and the EU member states.

To perform its tasks related to emerging risks, EFSA has established the EMRISK unit. This unit's activities include the collection of relevant data and monitoring of information sources, as well as the development of procedures for the analysis and evaluation of these data. It has oversight over a number of groups dealing with emerging risks, such as an internal task force that monitors data available to EFSA for emerging risks, and the EFSA Scientific Cooperation Working Group on Emerging Risks. The latter, for example, has identified indicators and information sources, including a range of EU institutions and global initiatives, that could be used for the monitoring for signals that flag up an emerging risk.

The EMRISK unit has also explored the possibilities of using various data sources for the identification of emerging risks, including the RASFF system, EMM (see the following section), and trade data. With regard to RASFF data, routine monitoring of these data was considered useful, and the unit has established a system for collecting and presenting RASFF data, including a geographical map which highlights RASFF alerts pertaining to products from a specific country. Trade data collected by EU and FAO were also considered useful, for example, to detect certain changes in the volume or nature of food and feed products entering the EU. Such data, if combined with other data, could then be used for the identification of emerging risks. The unit has also established a Stakeholder Consultative Group, which contains experts with a wide-ranging experience in the fields of food and feed safety and stakeholders covering the whole food manufacture chain. It is considered that these stakeholders can provide useful data on emerging risks. The EMRISK unit also leads scientific projects on emerging risks, including a recently initiated project on the impact of climate change on the occurrence of aflatoxin B1 in cereals.

The EMM

The European Commission's JRC has developed a software tool (EMM) that collect publications and reports from news portals worldwide that are available through the World Wide Web. The system retrieves real time over 40 000 reports on a daily basis in 43 languages from more than 4000 websites from 1600 key news portals, 20 commercial news feeds, and some specialist sites. The collected information is classified, filtered, aggregated, and alerts are issued and presented in a user friendly manner via graphs, maps etc. EMM contains four portals, the NewsBrief, the NewsExplorer, the Medical Information System (MedISys), and the EMM-Labs. MedISys presents articles and reports related to public health, categorized into 100 different categories (e.g., diseases, symptoms, chemical agent, geographic regions, organizations, etc.) based on predefined keyword combinations and warns the user with automatically generated alerts. EMRISK has evaluated the work required to customize MedISys on food and feed hazard detection and to determine its performance as a monitoring and early warning system. The following evaluation was performed in this analysis:

- Identification of ways to increase the sensitivity of the system to food and feed hazards (i.e., category definitions and identification of gaps in media coverage).
- Evaluation of the efficiency of MedISys as monitoring tool (comparing reporting time of a hazard by MedISys with other systems such as the internet-based reporting system Program for Monitoring Emerging Diseases (ProMED-mail) and RASFF.
- Efficiency of MedISys as early detection tool using nine case studies (comparing timeliness between MedISys, ProMED-mail, and RASFF).

Comparing the monitoring data generated by MedISys, RASFF, and ProMED-mail in a fixed time frame (from January to April 2009) showed that MedISys was more successful and often earlier in retrieving food safety hazard reports than the other two systems. Only three of the ten hazards reported by MedISys were also notified in RASFF. This difference may be explained by the fact that RASFF is only reporting hazards on the European market. It was concluded that MedISys is an efficient monitoring and early detection tool, but its sensitiveness to food and feed hazards needs to be increased, for example, by developing multilingual categories related to food and feed.

Emerging Risk Systems based on a 'Holistic' Approach

A holistic host environment analysis of a series of food safety cases revealed that factors from outside the food production chain (such as human behavior, trade, climate, and

regulation) may directly or indirectly influence a food safety risk to develop. It was advocated that such factors should be taken into account when developing a proactive emerging risk identification system. With such an identification system, risks can be anticipated at an early stage of development so that timely preventive and/or mitigating actions can be taken in order to protect public health and abate negative effects in consumers. For this purpose, indicators have to be selected and measured within these influential sectors. A change in these indicators is a signal that flags up a potential development outside the food production chain that will eventually have its impact on food production and, consequently, food product safety. Also the inputs from experts are considered an important element in selecting indicators and determining whether signals warrant further follow up. Next, we describe an initiative toward the development of a system that mimics the reasoning of experts triggered by signals consisting of observed changes in indicators. This way, the system, once operational, can comprehensively scan a vast array of data.

In 2004, a team of researchers started a project called 'Emerging risk in the Dutch Food Chain' aiming to explore the potential of such holistic approach as a basis for an emerging risk identification system. Within this project a prototype system (called Emerging Risk Detection Support System (ERDSS)) was built. ERDSS is a knowledge-based system that uses expert knowledge and (external) signals (e.g., changes in a predefined indicator/database) to reason. The system mimics the reasoning of human experts using forward chaining to deduce new facts unknown using three basic elements: concepts (also known as things), facts (gives a meaning to a concept), and rules (often in the form of if–then rules). Once a new fact (e.g., change in a database, publication, etc.) is entered to ERDSS, the reasoning engine will use the expert knowledge (stored in the computer database as concept and facts) and reasoning rules to deduce new facts, some of which may be an emerging risk. The feasibility of this approach was demonstrated for salmon production. Risk managers in the Netherlands have recognized the potential of the ERDSS concept and it is foreseen that a functional working system will be developed in the near future using information data collected from the Internet as input to identify new emerging food safety risks.

International Exchange of Information: FAO/WHO

In the last years much effort has been given to the improvement of the information exchange on food safety-related issues between nations to combat the development and spreading of food safety risks.

FAO has recently established the Emergency Prevention System (EMPRES) Food Safety. Before that, it already had EMPRES systems in place for the detection of – and coordinated action against – transboundary threats to animal and plant health. For plant health, for example, the EMPRES system collects information on locust movement, whereas, for animal health, it does so for rinderpest, including the geographical dissemination of the pest or disease, as well as the preventive measures that are being taken. EMPRES also collects such information for a number of other plant and animal diseases, such as wheat stem rust and highly pathogenic avian influenza. EMPRES Food Safety is intended to act as a similar resource for food safety, not only identifying sources of potential food safety threats but also selecting those that require risk mitigation actions, and formulating strategies toward risk mitigation, whereas stimulating a dialog among the stakeholders involved and lending support to regional and national authorities in their risk management measures. Besides its cooperation with the animal and plant health components, EMPRES Food Safety will also collaborate with other institutions, such as the International Food Safety Authorities Network (INFOSAN) hosted by the WHO. The INFOSAN network aims to rapidly exchange information on food safety incidents and to share information of common interest to its members. It in turn has a relationship with the WHO Global Outbreak Alert and Response Network (GOARN), which focuses on human disease outbreaks. Other parallel global online initiatives through which health professionals report infectious disease outbreaks to each other include the International Society for Infectious Diseases' ProMED-mail and the commercial Global Infectious Diseases and Epidemiology Online Network (GIDEON) web application and database for health professionals, each with frequent updates. In addition, GIDEON also provides information on possible therapies to be applied, background information on microbial taxa and toxic substances in the workplace. The JRC's MedISys system, which also continuously provides updates on disease incidents, has been discussed in the Section 'The EMM' in more detail.

Conclusion

Both reactive and proactive early warning systems are in place that could be used for the identification of emerging risks to food safety. Several recent developments within the EU and at the global level of FAO/WHO indicate that there is an increased interest in developing proactive systems that use information from the Internet and from the environment of the food manufacture chain, i.e., the influential sectors linked with it, such as the ERDSS system.

See also: Food Safety Assurance Systems: Investigation of Incidents in Industry. Public Health Measures: Environmental Assessment in Outbreak Investigations; Food Defense: Prevention of Sabotage and Bioterrorism; Food Inspections and Enforcement Systems; Foodborne Disease Outbreak Investigation; Surveillance of Foodborne Diseases. Safety of Food and Beverages: Risks of Food Adulteration

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PUBLIC HEALTH MEASURES

Monitoring of Contaminants

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Glossary

Contaminant It is a substance which is not intentionally added to food. It is present in food as a result of production (including operations carried out in crop husbandry, animal husbandry, and veterinary medicine), manufacture, processing, preparation, treatment, packing, packaging, transport, or holding of food or as a result of environmental contamination. The term does not include insect fragments, rodent hairs, and other extraneous matter.

Monitoring of contaminants The ongoing testing of food for the presence and concentration of contaminants.

Risk analysis A process consisting of three components: risk assessment, risk management and risk communication.

Introduction to Monitoring of Chemical Contaminants in Food

The presence and concentrations of contaminants in food is less predictable than that for chemicals resulting from human intervention, for example, food additives and pesticide residues, and direct information on expected levels in different food types is not usually available. Monitoring of these contaminants in the food supply is, therefore, crucial in order to assess any potential risk to food safety and provide information to underpin risk management responses.

Food chemical contaminants include, but are not confined to, the following classes: heavy elemental metals, naturally occurring toxicants (e.g., cyanogenic glycosides, mycotoxins, and biotoxins), and radionuclides. Contaminants frequently arise from environmental sources during the growth of the food. In some cases, human activity has added to the natural levels of environmental contamination, for example, dioxins, PCBs, radionuclides, and heavy metals.

Food can be contaminated with certain chemicals by the action of microorganisms, for example, the growth of two *Aspergillus* species on food can result in production of aflatoxins, which are of toxicological concern. Chemical contaminants can also result from the processing of food, for example, acrylamide is formed when some foods are prepared/cooked at high temperatures or result from other stages in its production, packaging, transport, or holding. Occasionally, chemical contamination can result from tampering or the deliberate addition to foods, for example, the addition of melamine to milk in China.

The Role of Monitoring in Risk Analysis

Risk analysis comprises three components: risk assessment, risk management, and risk communication. The risk assessment component for a hazard in food has the following four subcomponents: hazard identification, hazard characterization, exposure assessment, and risk characterization.

The dietary exposure assessment of food chemicals is the qualitative and/or quantitative evaluation of the likely intake of a chemical agent via food with exposure from other sources considered relevant. In the case of food chemicals, dietary exposure assessment takes into consideration the occurrence and concentrations of the chemical in the diet, consumption patterns of the foods containing the chemical, and likelihood of consumers eating large amounts of the foods in question (high consumers) and of the chemical being present in these foods at high levels. Usually, a range of intake or exposure estimates will be provided (e.g., for average consumers and for high consumers) and estimates may be broken down by subgroups of the population (e.g., infants, children, adults, and gender).

Monitoring data on the concentration and distribution of contaminants in food, therefore, provide a crucial input into the dietary exposure assessment component of the risk assessment. In the absence of monitoring data, the exposure assessment may have to rely on worst-case assumptions or may not be possible to undertake.

Types of Government Monitoring Activities and Other Considerations

National governments of developed countries, and many developing countries, have established monitoring programs to provide information on which regulatory decisions are made, including the establishment of food standards.

Food standards that aim to control contaminant levels by setting maximum levels (MLs) as a risk management measure are usually established for two reasons. MLs may be set for those foods that are known to be a major source of dietary

exposure to the contaminant (e.g., cadmium in potatoes) and/or for foods that may be consumed in small quantities by the general population but have high concentrations of the contaminant such that individual high consumers may be at risk (e.g., cadmium in liver and kidney). As a general principle, regardless of whether or not an ML exists, the levels of contaminants and natural toxicants in all foods should be kept as low as reasonably achievable (the as low as reasonably achievable (ALARA) principle).

The type of monitoring and surveillance of contaminant levels in food undertaken will depend on requirements to evaluate national food standards and/or monitor imported food, local knowledge about contaminants likely to be in food, the need to assess the presence of newly identified contaminants in food, analytical capability, capacity to use biomonitoring, and the resources available.

Ongoing Monitoring versus *Ad Hoc* Surveillance

The decision to undertake ongoing monitoring of the food supply as well as *ad hoc* surveillance by a government will depend to a large extent on government support and resources available for food collection, analysis, and regular reporting of results, as well as public expectations. The establishment of an ongoing system also requires the identification of key contaminant/food combinations to track, based on the available information. The reasons for *ad hoc* or less regular surveys will vary. For example, a total diet study may be undertaken every few years or in response to a contamination incident.

National Monitoring Surveys

The main aim of national monitoring surveys of the food supply is to collect concentration data on contaminants and other food chemicals, such as pesticide and veterinary drug residues, to assess if samples are in compliance with national legislation. The representativeness of the samples included will vary with the purpose of the survey and resources available, because ongoing surveys are expensive to maintain. For example, in the UK, the national food surveillance survey runs via an independent committee to give credibility to the data and covers contaminants and pesticides. By contrast, in Australia, the National Residue Survey of agricultural chemicals and contaminants is supported by the government and certain sectors of the food industry that export produce. Hence, sampling tends to reflect food from these sectors.

Total Diet Studies

Countries may opt to run total diet studies (TDSs) in addition to national monitoring surveys. The main aim of TDSs is to assess chronic dietary exposure to food chemicals by analyzing selected foods in the form commonly eaten by the population of interest, i.e., 'as consumed.' Published results include chemical concentration levels for the foods analyzed as well as estimated dietary exposures and contributions to that exposure from different foods. However, the food lists for TDSs are often limited to 80–200 foods, with analytical results 'mapped' to similar foods to estimate exposure from the whole diet. For example, concentration data for strawberries might be assigned to all berries consumed. An alternative approach used is to composite similar foods before analysis to reduce analytical costs.

WHO GEMS/Food Program

Since 1976, the Global Environment Monitoring System–Food Contamination Monitoring and Assessment Program (GEMS/Food) has informed governments, the Codex Alimentarius Commission (Codex), and other relevant institutions as well as the public on levels and trends of contaminants in food, their contribution to total human exposure, and significance with regard to public health and trade. The program is implemented by the World Health Organization (WHO) in cooperation with a network of more than 30 WHO Collaborating Centers and recognized national institutions located all around the world. The GEMS/Food program also involves national experts in more than 100 countries working to collect and analyze data and information to support the food risk assessment process.

Several important streams of data related to food consumption and food contamination are included in GEMS/Food:

1. Concentration of chemicals in food: Database on the level of chemicals in raw food commodities as well as in food consumed by final consumer.
2. Per capita food consumption: The GEMS/Food Cluster Diets based on similarities between dietary patterns of different countries and dietary patterns derived from Food and Agricultural Organization of the United Nations (FAO) food balance sheet data.
3. Individual food consumption: Compilation of individual levels of food consumption for average and high consumers on the basis of national surveys. This information is used mainly to assess exposure to food chemical presenting a potential acute risk to human health.

Food contamination monitoring is an essential component of assuring the safety of food supplies and managing health risks at the international level. The GEMS/Food database is open to competent authorities to submit and share their data on food monitoring and surveillance. Data are checked for consistency and completeness before being accepted by the WHO. Hence, GEMS/Food provides reliable information for setting priorities for consideration by Codex.

Within Codex, the chemical contamination of food is addressed by the Codex Committee on Contaminants in Food (CCCF). Risk management decisions are highly dependent on comparable and reliable exposure assessments, and GEMS/Food has provided assistance on a range of chemical issues to CCCF as well as the Joint Expert Committee on Food Additives (JECFA), the FAO/WHO scientific expert body with respect to contaminants in food.

Sampling Considerations

There are two types of monitoring and surveillance data: random and targeted. Targeted data are often collected from

foods likely to contain the contaminant of interest in response to specific problems that enable a quick risk management response to be taken. However, targeted data should be used with caution to estimate dietary exposure for use in risk assessments, as they may not be representative of all the food available for sale. For this reason, random sampling is preferable for these purposes. This is particularly true for acute dietary exposure assessments, as the fact that only a small proportion of any commodity entering the food chain is tested, means that there are significant limitations in ensuring the potential range of contaminant levels captured.

Contaminant data from monitoring and surveillance on food samples that are closer to the point of consumption in the food chain generally provide a better characterization of chemicals in food as purchased or eaten by consumers and hence provide a more accurate estimate of dietary exposure. Such sampling, therefore, accounts for chemical changes during transit, processing, and storage. Total diet studies are unique in that foods are prepared 'as consumed' before analysis, for example, meat is cooked and vegetables boiled, roasted, or fried according to local custom, if not usually eaten raw.

Deliberate contamination may elicit a more immediate response with targeted surveys necessary to determine the extent of the problem before developing an appropriate risk management response. For example, during the melamine incident, MLs for melamine were established by some countries on the basis of risk assessments, such as that undertaken by an FAO/WHO expert meeting, where available monitoring data, both from clinical cases and food analysis, were used. An example of a country's response to this incident has been described for Australia, where foods likely to be imported from China and foods containing milk powder were targeted for surveillance, for example, chocolates, biscuits, confectionery, and milk based beverages.

Analytical Testing Issues

In general, it is preferable to use the most sensitive method of analysis available, with the lowest level of detection as these data are more useful for dietary exposure assessments. However, the purpose of the monitoring program needs to be noted by the data user because some monitoring programs are designed to measure compliance with a given standard only and may, therefore, not use the most sensitive methods of analysis. Concentration levels in the food as consumed may not be reported if marker organs are used. For example, levels of heavy metal contamination may be analyzed only in the liver, rather than muscle meat.

Monitoring of Imported Food and Compliance

Some countries have extensive import food monitoring programs to test compliance with national food regulations, often deemed to be important where a large proportion of food consumed is imported. For example, in Hong Kong and Singapore, food regulatory agencies publish food monitoring results on a daily basis.

In other countries, a risk-based approach may be established on the basis of risk assessment information, as a means of prioritizing resources, such that foods with higher risk of chemical or microbiological contamination are tested for compliance at a higher rate than low-risk foods. For example, in Australia, 100% of consignments of high-risk foods are initially tested at the border, whereas only 5% of low-risk foods are tested on a random basis.

Biomonitoring

Biomarkers of exposure to food contaminants include biological changes to the body that are measurable, subclinical, and reversible and therefore do not depend on either food consumption or concentration data because they are 'downstream' from consumption. However, this means that the source of exposure cannot necessary be determined from the biomarker levels, that is, whether it is from the diet or other sources such as smoking or occupational activities. Although biomarkers can be used to assess if control measures have been effective in altering exposure levels for a given population, it is often difficult to interpret results or characterize the relationship between biomarker levels and health risk.

Increasingly, countries are looking to include biomedical surveys in conjunction with national nutrition surveys, for example, the US National Health and Nutrition Examination Survey (NHANES), the Canadian Health Measures Survey, the Australian Health Survey, and the New Zealand Nutrition Survey. These surveys could, in the future, provide a valuable data source for assessing exposure to contaminants. Blood and urine samples are normally obtained from individuals in these surveys for testing for chronic disease factors and nutritional status. In some cases, tests for environmental chemicals are already undertaken in addition to the standard health-related tests, for example, more than 600 chemicals are now tested in the NHANES survey, which expanded in 1999 to include more environmental chemicals and more than 200 in the more recently established Canadian biomedical survey. It was data derived from early NHANES that provided the first concrete evidence that among Americans blood levels of lead were increasing. As a result, the Environmental Protection Agency called for a reduction in production and sales of consumer products containing relatively large amounts lead, most notably gasoline and household paints. The UK Biobank is different in that it is an independent program collecting additional samples such as saliva as well as blood and urine. Also, from 2012, the Biobank is open to application from researchers to study these samples.

Human milk has long been recognized as a unique biological matrix, and the WHO, through the GEMS/Food program, has monitored levels of contaminants in human milk over several decades, in particular persistent organic pollutants (POPs). These chemicals are recognized as a good example of exposure biomarkers as they are known to accumulate in the food chain. Human milk monitoring, therefore, gives information about POPs in the environment and our bodies.

National Government Programs for the Monitoring of Food for Contaminants

National governments undertake a range of monitoring activities. These monitoring activities serve a number of

different purposes as outlined above, including the identification of emerging issues, data generation for risk assessments, and facilitation of prioritization of enforcement activity. Some examples of these national monitoring activities are given.

European Union

The European Union has a comprehensive set of monitoring and import controls for chemicals and microbiological hazards in food. The official controls are undertaken primarily by the relevant competent authorities of the member states and are laid down in a number of regulations relating to both food of animal and nonanimal origin. The Food and Veterinary Office check compliance with the requirements and contributes to the implementation of effective control systems.

With respect, specifically, to contaminants, the basic principles are in Council Regulation 315/93/EEC of 8 February 1993. MLs are set for the contaminants of greatest concern to EU consumers, due to either their toxicity or their potential prevalence in the food chain. These include aflatoxins, heavy metals (such as lead and mercury), dioxins, and nitrates and thus the MLs are set out in Commission Regulation (EC) No 1881/2006. These levels are set on the basis of scientific advice provided by the European Food Safety Authority (EFSA).

Other monitoring data are generated by member states in accordance with their own national priorities or as a result of European Commission recommendations. For example, for acrylamide, such a recommendation requests further investigations into the production and processing methods used by food producers in cases where high levels of acrylamide are found in the testing.

US

In the US, since the early 1960s, the key program of the monitoring of contaminants in food is the Total Diet Study conducted continuously by the US Food and Drug Administration (FDA). This involves the analysis of a group of foods that reflect the average food consumption patterns of a given population. Results of the analyzes are then used to estimate the average dietary exposure of contaminants from eating those foods. This information also provides a tool for supporting the regulatory actions and, over time, tracking the impact of the regulations. The FDA is generally responsible for testing imported food.

The US FDA also conduct a number of discrete surveys targeted at specific contaminants. For example, an exploratory survey of perchlorate in range of food and beverages was undertaken in 2004–05 and a dietary exposure assessment was undertaken using this data.

However, the testing of imported meat and poultry for contaminants is undertaken by the Food Safety and Inspection Service of the US Department of Agriculture.

Canada

Health Canada assesses the risks posed to Canadians by environmental contaminants in food. In support of these risk assessment activities, scientists monitor the concentrations of various environmental contaminants in foods through Canada's ongoing Total Diet Study. A number of other contaminant surveys are undertaken by Health Canada, such as the ongoing Human Milk Survey, which has the goal of examining the exposure of Canadian infants to a variety of contaminants, chemicals, nutrients, and immunoprotective constituents in breast milk. When necessary, Health Canada sets MLs for contaminants in foods.

Testing of food for contaminants is also undertaken by the Canadian Food Inspection Agency. For example, in 2010–11, 628 samples were collected and analyzed in a targeted survey of aflatoxins in dried fruits, nuts and nut products, and corn products.

Australia and New Zealand

Food Standards Australia New Zealand (FSANZ) in Australia, the Ministry for Primary Industries in New Zealand, and other government agencies monitor the food supply to ensure that it is safe and to ensure that foods comply with food standards.

The analytical testing of contaminants in food occurs either as specific discrete surveys, as periodic analytes in national Total Diet Studies, or as part of compliance activities. An example of a specific discrete survey would be the Australian survey of polycyclic aromatic hydrocarbons in food conducted by FSANZ in 2010.

Japan

Japan has a comprehensive monitoring scheme in place for imported food inspected by the Imported Foods Inspection Services. The published list of tests to be applied to imported food in 2012 includes a number of contaminants, for example, mycotoxins and shellfish toxins in specified foods.

Japan also undertakes monitoring of domestically produced food. This has included radionuclides in food. Japan implemented an enhanced monitoring program following the damage to the Daiichi nuclear power plant in Fukushima. In the year following the event, more than 120 000 food products were tested, predominantly meat and fishery products and vegetables, and the total number of cases that exceeded the provisional regulatory limit of 500 Bq kg^{-1} was 1162.

Emerging and Developing Countries

Other countries undertake the monitoring of contaminants in food. However, the size and scope of the monitoring programs varies considerably between countries. In some developing countries with limited economic and laboratory resources available, very little monitoring of contaminants in food is undertaken. In contrast, a number of emerging economies have extensive monitoring programs.

Case Studies – The Use of Research and Monitoring Data for Contaminants in the National Government Management of Food Safety Risk

Methylmercury in Fish

Mercury is released into the environment from both natural and anthropogenic sources and occurs in food as methylmercury or organic mercury. Health-based guidance values have been set for methylmercury, for example, a provisional tolerable weekly intake of 1.6 μg kg^{-1} body weight (bw) was set by JECFA in 2006 and a tolerable weekly intake of 1.3 μg kg^{-1} bw, expressed as mercury, was set by EFSA in 2012.

As for many other contaminants, it is important for governments to generate monitoring data to help to inform the development and review of risk management strategies for restricting methylmercury in the diet. The aim would be to ensure that potential exposures to methylmercury do not exceed the health-based guidance values.

Fish and seafood are the major source of methylmercury in most populations, with predatory and long-living fish such as marlin, swordfish, shark, ling, pike, and rays having the highest concentrations. Typically, governments may set MLs for fish and seafood and/or develop advisories on fish consumption. An additional risk management measure may be to restrict the sale or size of some species, the latter because mercury content increases with age of fish, assuming the size of the fish is a proxy for age. However, it is a complex risk management decision because fish are considered a good source of some nutrients (in particular protein, omega-three fatty acids, and iodine) and are low in saturated fat. So populations, especially pregnant women, are often encouraged by health professionals to regularly include fish in their diet. Although food standards can be set to restrict the level of methylmercury in fish, it is undesirable to set them so low that it restricts availability of fish in the market place. As a result, although food standards for MLs for total mercury or methylmercury can be set at an international (Codex guideline) or national level, they do not necessarily protect high or regular consumers of fish and seafood.

It is, therefore, important that governments monitor the effectiveness of the selected risk management strategies, particularly for vulnerable populations such as young children and pregnant women, and communities with high fish consumption levels. The effectiveness of food standards can be evaluated via regular monitoring surveys and total diet studies. Laws restricting fish size for sale are increasingly difficult to monitor and enforce as more fish are caught and processed at sea before sale at market.

At an international level, contaminant data are collated by the WHO GEMS/Food Program. The concentration data obtained from the monitoring surveys can then be used to estimate dietary exposure to total mercury, inorganic mercury, and/or methylmercury as part of the total diet study or other risk assessment process.

An example of monitoring data reported by EFSA from various countries is given in Table 1.

Available monitoring data on mercury or methylmercury levels in specific species of fish and seafood are critical to developing appropriate advisories on fish consumption. The concentration data are first combined with national food consumption data to give dietary exposure estimates for methylmercury from each species of fish, and then the number of serves of different types of fish that can be consumed per week or per month is calculated, taking background exposure from other foods into account. Consumption advisories vary with country because fish consumption habits and fish species differ between countries. For example, the USA, Canadian, Japanese, UK, and Australian governments have issued advisories highlighting the fish species relevant to their own populations that are of concern at high level of consumption.

Table 1 Range of total mercury and methylmercury reported in fish and shellfish from European countries, Canada, China, Ghana, Hong Kong, India, Malaysia, Papua New Guinea, Persian Gulf, and the USA

Type fish/seafood	*Total mercury μg kg^{-1}*	*Methylmercury μg kg^{-1}*
Freshwater fish	10–2 950	5–2 630
Marine fish	0–18 000	0–16 000
Shellfish	40–830	2–220

Evaluating the effectiveness of advisories requires quantitative and/or qualitative consumer research to assess whether the message has been received, understood, and implemented in people's lives. An example of a national advisory is given in Table 2.

Acrylamide in Certain Cooked Foods

Acrylamide is an industrial chemical whose primary use is the synthesis of polyacrylamide. In April 2002, the Swedish National Food Administration and researchers from Stockholm University announced their findings that acrylamide, a toxic and potentially cancer-causing chemical, is formed in many types of food prepared/cooked at high temperatures. As a result of concern expressed by member countries, an expert Consultation was convened jointly by the FAO and the WHO in June 2002.

The FAO/WHO Consultation concluded that the information on the levels of acrylamide in food (i.e., monitoring data) is far from complete and the magnitude of the cancer risk posed by acrylamide in food could not be quantified. Nevertheless, the consultation recommended a number of principles aimed at reducing the risk, for example, food should not be cooked excessively.

Following the consultation, research confirmed that the primary mechanism of formation is the Maillard Reaction of glucose and/or fructose with the amino acid asparagine, during heating. In the subsequent years, research has focused on generating monitoring data to determine levels in foods and toxicological studies to better characterize the hazard.

Monitoring data have been generated by a large number of countries in wide range of foods. A high level of variability in the levels of acrylamide has been found in individual foods, reflecting different formulation and processing conditions. In addition, the acrylamide levels vary with product, manufacturer, and country.

Table 2 Australian advice on mercury in fish (last updated September 2011). Number of serves of different types of fish you can safely eat

Pregnant women and women planning pregnancy	*Children (up to 6 years)*	*Rest of the population*
1 serve equals 150 g[a]	1 serve equals 75 g[a]	1 serve equals 150 g[a]
2–3 serves per week of any fish and seafood not listed below		2–3 serves per week of any fish and seafood not listed in the column below
OR		OR
1 serve per week of orange roughy (sea perch) or catfish and no other fish that week		1 serve per week of shark (flake) or billfish (swordfish/broadbill and marlin) and no other fish that week
OR		
1 serve per fortnight of shark (flake) or billfish (swordfish/broadbill and marlin) and no other fish that fortnight		

[a]A 150 g serve for adults and older children is equivalent to approximately two frozen crumbed fish portions. A 75 g serve for children is approximately three fish fingers (Hake or Hoki is used in fish fingers). Canned fish is sold in various sizes, for example, the snack size cans of tuna are approximately 95 g.

Table 3 Typical monitoring levels of acrylamide in example foods

Food	*Acrylamide concentration ($\mu g\ kg^{-1}$)*
Potato chips (crisps)	117–4215
French fries (potato chips)	59–5200
Bakery products and biscuits	18–3324
Bread	<10–397
Breakfast cereals	<10–1649
Chocolate products	<2–826
Roasted coffee	45–935
Coffee extract/powder	87–1188

In Europe, the EFSA have been publishing annual reports of acrylamide monitoring for several years. In a consolidated report for 2007–10 twenty-five European countries submitted a total of 13 162 acrylamide results. A similar monitoring research effort has been undertaken by many other countries, the USA, New Zealand and China. Typical monitoring levels of acrylamide in example foods are given in Table 3.

National monitoring levels have been used by the JECFA, the EFSA, and national governments to conduct dietary exposure assessments. At its 72nd meeting, the JECFA calculated mean dietary exposure to be 0.001 mg kg^{-1} bw day^{-1}, leading to a margin of exposure (MOE) of 180 and 310 for Harderian gland tumors in mice and mammary tumors in rats, respectively. The JECFA concluded that these MOEs indicate a health concern, a conclusion that could not have been reached in the absence of monitoring data.

Conclusions

Monitoring data for contaminants in food are important in allowing the assessment of the risk posed by these contaminants in the diet. These data also play a crucial role in establishing appropriate food standards and ensuring compliance with these standards. Many national governments have extensive monitoring programs for contaminants in food.

See also: Analytical Methods: Overview of Methods of Analysis for Chemical Hazards. Foodborne Diseases: Overview of Chemical, Physical, and Other Significant Hazards. Processing Contaminants: Acrylamide. Public Health Measures: Alerts and Early Warning Systems; Modern Approach to Food Safety Management: An Overview. Risk Analysis: Risk Assessment: Chemical Hazards; Risk Management: Application to Chemical Hazards. Toxic Metals: Mercury

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Assessment of Novel Foods and Ingredients

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Glossary

Allergen Any substance that can cause an allergy.
Antinutrient Natural or synthetic compounds that interfere with the absorption of nutrients.
Bioactive compound Inherent non-nutrient constituent with anticipated health promoting/beneficial and/or toxic effects when ingested.
Genetic modification The direct manipulation of an organism's hereditary information using biotechnology.
Glycoalkaloid A family of poisons commonly found in nightshade plant species.
Nanotechnology The manipulation of matter with at least one dimension sized from 1 to 100 nm.
Superfruit A marketing term referring to a fruit which combines exceptional nutrient richness and antioxidant quality with appealing taste.
Toxin A poisonous substance produced within living cells or organisms.

Introduction

New foods or foods with new ingredients are introduced in their thousands each year to worldwide supermarket shelves, but are they novel? At first glance such a question seems to involve a tautology and requires an explanation.

First to the definition of new that in some dictionaries are specified as 'not existing before; made, introduced, or discovered recently or now for the first time.' With this definition most of the 'new' foods presented to customers are not new at all. They might be a mix of two existing products with some spices added, ready to cook, or just presented in new packaging material or a new package size for convenience. But they are basically the same food as before. New to the customer but not to the food safety risk manager (Figure 1).

So what about novel? Here the dictionaries have much the same definition specifying it as 'new and not resembling something formerly known or used, original or striking especially in conception or style.' This is not surprising because they both relate to the Latin *novellus,* with the diminutive of *novus* through the Old English *nīwe.* But in the food area there is a clear distinction between what is new and what is novel with novel foods or food ingredients captured in some specific national or regional legislation requiring authorization before being introduced into the market. However, as will be seen, a distinction between new and novel is not always easy to make.

In most countries, general food law requires that all food should be safe for consumption, novel or not. In the USA, the concept 'generally recognized as safe' by qualified experts is used. Although very few traditional foods and food ingredients in the market have been the subject of systematic toxicological or nutritional assessments, they are generally regarded as safe to eat because of their long history of use without evidence of harm when applying customary preparation methods. This is despite the fact that the food may contain antinutrients, toxins, or allergens that may require special knowledge of preparation or processing methods for safe consumption. Such knowledge has been built over their long history of use.

The introduction of novel foods or food ingredients in a market where they are unfamiliar or the use of novel processes in food production might pose a problem in that their immediate safety cannot be assured. This has required the introduction of approaches to determine their safety. However, such tasks are not trivial in that toxicological paradigms most often require studies using levels of administration orders of magnitude above the anticipated real-life intake, which is not possible for ordinary food. Instead, a

Figure 1 The large range of new foods introduced annually onto supermarket shelves are not necessarily novel (Photo: Alalsacienne).

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comparative approach has been developed for their safety assessment in that they are matched with similar traditional foods that have a history of safe use to prove substantial equivalence.

Novel Foods Defined in Legislation

The special focus on novel foods or food ingredients was initially in response to the appearance of genetically modified foods or ingredients in the market and clearly different food production technologies like the use of nanotechnology. There was also a concern that unfamiliar products promoted as healthy alternatives, like chia seeds and so called superfruits like goji berries, might have unintended effects. Lately products from cloned animals have been included in the novel food concept. However, although the previous specific examples are clearly novel alternatives, novel foods or food ingredients can be difficult to define in a legal sense. In a general sense they can be seen as a type of food or food ingredient that does not have a significant history of consumption or is produced by a method that has not previously been used for food. However, such a general definition poses significant problems in that it does not account for geographical patterns and differences in cooking traditions. Specific legislation in place in Europe, Canada, and Australia/New Zealand have all attempted to further define novel foods (Figure 2).

In Europe, novel foods were originally defined as all foods and food ingredients not used for human consumption to a significant degree within the European Union (EU) before 15 May 1997. Thus, the legislation includes a geographical and a time limitation. In addition, these foods and food ingredients had to fall into six categories:

- containing or consisting of genetically modified organisms (GMOs);
- produced from, but not containing, GMOs;
- with a new or intentionally modified primary molecular structure;
- consisting of or isolated from microorganisms, fungi, or algae; or
- consisting of or isolated from plants, or food ingredients isolated from animals, except for foods and food ingredients obtained by traditional propagating or breeding practices, and having a history of safe use; or
- include a production process not currently used, where that process gives rise to significant changes in the composition or structure of the food or food ingredient, which affects its nutritional value, metabolic effect or level of undesirable substances.

Figure 2 Chia seeds introduced as a novel food on new markets (Photo: Stacey Spensley).

Originally, the European novel food legislation was conceived in response to the arrival of food products derived from genetically modified crops, but these are now controlled under a separate regulatory regime.

In Australia and New Zealand novel foods are defined in legislation as nontraditional foods with no history of safe use. The characteristics of a food that make it novel means scientists cannot be certain they are safe to eat, so they are supposed to be rigorously assessed before being allowed to be sold. Novel foods are required to be listed in the Food Standard before they may be sold in Australia or New Zealand. This means that any food manufacturer wanting to sell a novel food or ingredient must ask for an amendment of the Standard to include it in the list. If the food passes a safety assessment the manufacturer can go ahead and sell it, so long as it complies with any conditions specified. A specific committee has been appointed to adjudicate whether or not a food or food ingredient is novel.

In Canada, novel foods are regulated under the Novel Foods Regulations. The regulations define novel food as products that have never been used as food, foods that result from a process that has not been previously used for food, or, foods that have undergone genetic modification and have new traits. There is a requirement that a company wanting to sell a novel food notify Health Canada before marketing or advertising the product. Premarket notification permits Health Canada to conduct a thorough safety assessment to demonstrate that a novel food is safe and nutritious before it is allowed in the Canadian marketplace.

Safety Assessment of Novel Foods and Food Ingredients

The general principles to be applied in the safety assessment of novel foods or food ingredients have been outlined internationally by the Organisation for Economic Cooperation and Development and the World Health Organization. They specify as a fundamental requirement that a rigorous, science-based risk assessment be carried out before putting novel foods and ingredients on the market. To facilitate such an assessment, a comparative approach should be adopted wherever possible to prove substantial equivalence with traditional foods that have a history of safe use. Although this principle was originally introduced for assessing genetically modified foods, the concept is feasible for all safety assessment of foods from novel sources and produced by novel processes.

In cases where detailed testing and analysis prove necessary it should focus on any potentially critical difference between the novel and the traditional food that could become a hazard. In this way, assurances are obtained that novel foods are

as safe as the traditional foods with which they are compared. Although not fully conclusive, it provides a pragmatic approach to assure relative safety.

If a food ingredient used as the source of a novel food has a history of safe consumption in traditional use then any toxicological concerns about proposed new uses are reduced. However, it is still important to evaluate the food production method and expected consumption patterns in detail because hazardous components might survive new industrial processes although it is of no concern in traditional use. The safety assessment should include analytical, compositional, and nutritional data as well as previous and predicted exposure and results from animal and human studies if appropriate.

If substantial equivalence to a traditional food can be proven and no new hazards identified this will be sufficient for introduction of the novel food onto the market. A postmarket survey might be required to confirm the preliminary conclusion. If doubts persist it will be necessary to conduct a risk assessment by identifying potential hazards and describing dose-response details. Public exposure to the hazard should be ascertained and the health impact of the risks posed by ingesting the hazardous substance at these exposure levels evaluated.

Identifying Public Health Hazards

To assess the safety of a novel food, initially it will be important to identify the traditional food to which it could be compared. If there is no similar food with a history of safe use a more elaborate safety assessment will be necessary. To document the history of safe use the Canadian guidelines requires proof of significant human consumption of food over several generations and in a large, genetically diverse populations for which there exist adequate toxicological and allergenicity data to provide reasonable certainty that no harm will result from consumption of the food. In the EU guidelines, information on past and present use of the food source in other parts of the world would be an essential piece of information required to assess the safety of the novel food. According to the Australia and New Zealand legislation foods or food ingredients may only be considered novel, if they are first considered nontraditional. The definition of 'nontraditional food' does not include foods produced using new processes or foods derived from novel sources unless there were some altered characteristics. An external panel has been set up to adjudicate whether or not a food is novel based on information supplied by the applicant.

Various databases can be used to help to establish whether a particular product has a history of safe use as a food or food source. These include national food survey reports and global, regional and national surveys of plant and animal products with food uses. If an equivalent traditional food exists with similar use, a comparison with the novel food is performed as a second step and includes chemical composition, methods of production and use, intake patterns, nutritional value, and target groups. The hazard identification should focus on antinutrients, toxins, bioactive compounds, and allergens.

Figure 3 There are as many as 5000 different varieties of potato around the world (Photo: US Agricultural Research Services).

Chaco Potato Example

Assume you wanted to introduce a new potato species currently not on your market called Chaco or *Solanum chacoense* in Latin, a wild relative of the cultivated potato to be used in a similar fashion to traditional potatoes. You definitely do not want to repeat the mistake made by the cooks of Queen Elizabeth I when Sir Walter Raleigh according to popular legend introduced potatoes for the first time to Ireland. Unfamiliar with the plant, they threw out the edible tubers and cooked the stems and leaves which contain high volumes of solanine. Everyone fell deathly ill (Figure 3).

Currently there are approximately 5000 potato varieties worldwide. Three thousand of them are found in the Andes alone, mainly in Peru, Bolivia, Ecuador, Chile, and Colombia. Apart from the 5000 cultivated varieties, there are approximately 200 wild species and subspecies of which you have settled on one because of its different taste and perceived benefits in coming from an extensive agricultural source without chemical inputs. You know that traditional potatoes naturally produce solanine and chaconine, a related glycoalkaloid, as a defense mechanism against insects, disease, and predators. A normal potato might have 12–20 mg kg^{-1} of glycoalkaloid content and this is no threat to public health. However, some noncommercial varieties can have much higher levels. This would typically be the hazard you have to study in detail during different growing and storing conditions.

Characterizing the Identified Hazards

Having identified a possible equivalent product to the novel food and potential hazards a detailed analysis should follow. The novel food and the traditional equivalent if available should both be fully characterized and results compared. This includes a precise biological identification using appropriate methodologies. The origin, geographical distribution, and genetic diversity of the food source should be described. For purified ingredients, focus should be given to chemical identity and potential impurities that could be formed or introduced during manufacture. Particular attention should be given to compounds that may have implications for the health

of more vulnerable population groups, which could include infants, children, elderly, and pregnant women.

The potential adverse health effects attributable to a specific hazard should be described, the mechanisms by which the hazard might exert its toxic effects should be detailed, and the associated dose, route, duration, and timing of exposure established. This should include the qualitative and, wherever possible, quantitative description of the inherent properties of the hazard or the situation having the potential to cause adverse effects. In addition, when feasible, a dose–response assessment and its associated uncertainties should be calculated.

Back to the Chaco Potato Example

Coming back to the Chaco example, you are aware that in general the glycoalkaloid compounds in potato plants are concentrated in leaves, stems, sprouts, and fruits. However, exposure to light, physical damage, and age will increase the glycoalkaloid content within the tuber itself. The concentration of glycoalkaloid in wild potatoes are often much higher than in cultivated potatoes because selective plant breeding has reduced the levels of glycoalkaloids in the latter.

Solanine poisoning is rare and in most cases benign but can become serious. You have to review the symptoms that might include nausea, diarrhea, vomiting, stomach cramps, burning sensation in the throat, cardiac dysrhythmia, headache, and dizziness. In more severe cases, hallucinations, loss of sensation, paralysis, fever, jaundice, dilated pupils, hypothermia, and death have been reported. It has been suggested that the toxic mechanism of solanine is caused by the chemical's interaction with mitochondrial membranes.

According to official statistics solanine in potatoes has killed at least 30 people and made over 2000 very sick over the years. Case history reports cover incidents in Cyprus and North Korea although the real number of cases dismissed as just ordinary gastroenteritis is probably much higher. It is believed that doses of 2 to 5 mg kg^{-1} bodyweight can cause toxic symptoms, and that doses of 6 mg kg^{-1} bodyweight might be a fatal threshold. Those will be your inputs for the risk characterization.

Estimating Exposure

Exposure assessment is the estimation of the likelihood that a consumer will be exposed to a substance and to quantify the extent of that exposure, when and if it occurs. To do so, exposure assessments combine data on concentrations of a chemical substance present in food with data on the quantity of those foods consumed. To be able to combine these different types of data it is necessary to consider standardization of associated metadata, in particular harmonization of the food description.

Exposure assessments are most often conducted at the general population level, but subgroups can also be explored that show particular exposure patterns, for example, due to a high level of consumption or particular physiological or pathological conditions. Assessments can be made either for acute or chronic exposure, where acute exposure is estimated for a period of up to 24 h whereas chronic (long-term) exposure covers the average daily exposure over several years or an entire lifetime (Figure 4).

Figure 4 Calculating exposure for the general population and subgroups of particular interest like children (Photo: Sean Dreilinger).

Exposure assessments are often performed according to the fit-for-purpose principle with the consequence that there is little harmonization across disciplines with each having their own agreed international guidelines. For potential hazards in novel foods it will be important to assess other sources of the same hazard to put the exposure into context. The actual exposure calculation for a novel food is simple in that it can focus only on the consumption of this food. However, the situation is a bit more complex for novel food ingredients because they can be used in the production of a range of different foods at different levels. Calculation of worst-case scenarios can be useful for such cases.

Chaco Potato Consumption

Potatoes were introduced outside the Andes region four centuries ago, and are now a common food all over the world. There is thus ample evidence of prior consumption among a variety of population groups without appreciable harm. The annual diet of an average global citizen in the first decade of the twenty-first century included approximately 33 kg (73 lb) of potato or an average of 90 g day^{-1}. However, the local importance of potato is extremely variable and rapidly

changing. It remains an essential crop in Europe (especially eastern and central Europe).

According to the food consumption statistics published by the European Food Safety Authority an average portion of potato for adults might roughly be estimated at 200 g whereas a high consumer might eat a portion of 400 g. At an average solanine level of 15 mg kg^{-1} in cultivated potatoes an average person would be exposed to approximately 0.05 mg solanine per kg bodyweight from a portion, which can double for high consumers.

Being a wild variety, average solanine levels in Chaco potato has been estimated to be approximately 150 mg kg^{-1}. This would result in solanine exposure of 0.5 mg per kg bodyweight from a portion for an average consumer and 1.0 mg kg^{-1} for a high consumer.

Risk Characterization

Risk characterization is the final phase of the food safety assessment process. It is in risk characterization that the results of the risk assessment are presented. This phase determines the probability of an adverse effect to a human population in consuming the novel food or food ingredient and outlines permissible exposure levels from which standards of exposure are set. These results are provided in the form of risk estimates and risk descriptions that provide answers to questions risk managers might pose during the authorization process or information necessary for the food producer in managing food safety.

Safety of Chaco Potato

Although commercially grown potatoes have a safety margin of at least 50 times between estimated exposure and the first signs of toxic effects, this would be reduced to five times for Chaco potatoes. A further review of Chaco potato consumption in the Andes region showed that this variety is mainly used as an appetizer and not for the main meal. Typical consumption levels would be limited to a portion of not more than 30 g. You thus propose to use your Chaco potatoes as a delicatessen only to be sold in small portion packs to reduce any threats to public health as much as possible.

Authorization Process

The authorization process will vary depending on the geographical region and the respective regulations in place. At a minimum the food producer must ascertain that in their own judgment the novel food or food ingredient comply with general food safety provisions. If the 'history of safe use' of a novel food or the traditional food used as a benchmark to assess the safety of a novel food can be sufficiently described, limitations identified, and the intended conditions of use are the same, then few further safety data should be required. If the novel food is taken out of the cultural, processing, and intended use context of the novel food or comparator, which provides its acceptable 'history of safe use,' then more safety data and a more rigorous evaluation may be required.

There are two possible routes to comply with the EU legislation for authorization of novel foods – a full application or a simplified procedure. A simplified application is possible when a national competent authority believes that the novel food or food ingredient is substantially equivalent to an existing food or food ingredient. In such cases the commercial company can just notify the European Commission of its intention to market the novel food or novel food ingredient. However, other member states are given an opportunity to object should they wish to do so. All other cases require a full application that is initially assessed at the national competent authority level with a recommendation for approval required by the Standing Committee on Food Chain and Animal Health.

Examples of novel products approved during 2012 include:

- Docosahexaenoic acid and Eicosapentaenoic acid-rich oil from the microalgae *Schizochytrium* sp. to be used in food supplements, cereal products, and spreadable fats;
- Synthetic vitamin K2 that essentially comprises all-trans menaquinone-7;
- Extension of the use of Antarctic krill oil to also include bakery products, nutrition bars, nonalcoholic beverages as well as milk-based drinks and dairy analog drinks.

An example of a rejected application cover the Nangai nut (*Canarium indicum* L.). It is native to Vanuatu. It was concluded that the nut was not previously consumed in the European Union and the requesting company, even after having been invited to submit a full assessment report, failed to do so.

In Canada, government scientists use data submitted by the companies seeking approval of their products to assess their safety. This data must be of the same high quality that is required by scientific journals for publication and peer review. The data are thoroughly analyzed, as are the rules used to ensure the validity of results. If the data is not scientifically sound, if it is incomplete, or if it is inadequate, government regulators require that the product developer address these problems before the assessment can continue. To date, more than 90 novel foods have been approved for sale in Canada including a number of canola, corn, cottonseed, and flax crop lines. Some of the novel traits include herbicide tolerance and pest and disease resistance. In spite of the benefits brought about by the development of novel foods, there are also a number of concerns that have been raised concerning their potential impact on the environment and on human health and safety.

In Australia and New Zealand, an Application Handbook assists the process of assembling required information for an authorization. It specifies a number of categories of novel foods with customized information for each. In general, an application should address the history of use as a food in other countries, the composition of the novel food, particularly the levels of antinutrients and naturally-occurring toxins and the method of preparation and specifications of a novel food ingredient. It should describe the potential for allergenicity of the novel food and present results from metabolism/toxicokinetic studies on the novel food ingredient, as well as animal toxicity studies and human tolerance studies.

Postmarket Monitoring

Once a novel product has reached the market it will be very important, when certain assumptions about its use were made, to follow up market penetration and potential negative reactions. This is commonly called postmarket monitoring and will provide additional reassurances regarding long-term safety of products, as well as their impact on the food supply. It is an additional tool to complement the premarket risk assessment when some uncertainties remain.

Postmarket monitoring should be used as a means of addressing specific questions to be useful. It could include an evaluation of actual intake of a food by target consumers deemed most at risk in the premarket assessment to increase the probability of detecting adverse health effects in everyday life. It is normally not a routine requirement for the approval of novel foods, but there are some examples when it might be necessary.

A commercial company requested authorization for the use of plant sterols in yellow fat spreads. It has since been expanded to cover other product types also. Plant sterols are structurally related to cholesterol and can be divided into phytosterols and phytostanols, the latter being the saturated version of the former. Scientific studies indicate that consumption of 1.5–3 g of plant sterols per day can significantly reduce the level of low-density lipoprotein cholesterol in individuals if consumed as part of a healthy diet. The products would target people with high cholesterol levels. Unfortunately, the consumption of high doses of plant sterols might also significantly reduce the blood levels of carotenoids. As a prudent precaution it has thus been suggested that intakes of plant sterols should not exceed 3 g day^{-1}.

As part of the approval process the applicant was asked to undertake postmarket monitoring to check whether or not products with added plant sterols were purchased exclusively by the target group and if multiple products were used by consumers increasing the risk of exceeding the 3 g per day of plant sterols. The research found that more than 60% of the consumers of food products with added plant sterols had high blood cholesterol levels and a large majority belonged to the over 45 age group. There was some leakage with whole families consuming the products without belonging to the target group, including a few children. However, reassuring authorities, that there seemed to be little over-consumption of food products with added plant sterols, rather the average consumer exposure to plant sterols was on the low side of what is considered an effective dose.

Even if not required by the authorization, postmarket monitoring is often used by industry on a voluntary basis to monitor the performance of novel foods or food ingredients in the marketplace.

See also: Foodborne Diseases: Overview of Emerging Food Technologies. Risk Analysis: Risk Communication: Novel Foods and Novel Technologies; Risk Communication. Safety of Food and Beverages: Probiotics and Prebiotics; Safety Consideration in Developing Functional Foods; Safety of Genetically Modified Foods; Safety of Irradiated Foods

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Health Canada: Guidelines for the safety assessment of novel foods.

PUBLIC HEALTH MEASURES

Food Defense: Prevention of Sabotage and Bioterrorism

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Introduction

The world is challenged with nourishing a rapidly expanding population with increasingly limited resources. Although globalization of the food system offers many benefits, it also presents new challenges to safe food production and distribution. The increasing complexity of food supply chains, global nature of production, and widespread distribution of food products from a single manufacturing facility all increase the food supply to the potential of an attack.

The intentional contamination of food to cause harm, and the consequent concern of food defense, has a very long history. There are several examples of the military use of food contamination in the historical record. One such example includes the Athenian contamination of drinking water for the city of Kirrha with the plant root helleborus from 590 to 600 BC. This water contamination event reportedly caused severe gastrointestinal illness, rendering the city defenseless for the ensuing attack. The Carthaginian General Maharbal reportedly contaminated wine with mandagora, and there are various historic instances of plague infested animal/human bodies dumped into water and food supplies during Roman times. The Japanese army, during World War II is known to have experimented with the use of food as a delivery vehicle for several pathogens. These pathogens include *Vibrio cholerae*, *Salmonella enterica* serovar Paratyphi, *Shigella* spp., and *Yersinia pestis*.

For the purposes of this article, the following definitions will be used. Food security refers to access to sufficient calories to meet a population's demand. Food Safety is defined as the prevention of accidental hazards. Food defense refers to the prevention of intentional contamination. Food Protection is used as an umbrella term to encompass both food safety and food defense.

Intentional versus Unintentional Contamination

As stated previously, the food system is an extremely complex network of networks. Movement of food products is generally not linear, and products can move through multiple suppliers, warehouses, or distribution centers before reaching the final consumer. In such a complex network of systems, prevention of contamination of any kind, be it intentional or accidental, is a challenge.

When a contaminant of any kind can result in human illness, both intentional and unintentional acts are of concern. Extensive efforts have been undertaken by the food industry, government, and academia to address the food safety concerns associated with accidental contamination. However, food safety failures are still known to occur. A 2009 outbreak of *Salmonella* associated with peanuts was identified which ultimately led to more than 700 illnesses in 46 states. The company implicated had sold peanut butter products to more than 2100 accounts, and at least 431 peanut butter-containing products needed to be recalled from 54 different companies. Although food safety incidents can be anticipated as a result of system failure, the nature of intentional contamination is fundamentally different. Individuals who intentionally contaminate the food supply are purposefully trying to avoid the prevention and detection systems normally in place. In addition, individuals who are purposefully trying to cause harm will also be purposefully trying to avoid detection before the incident.

There are several actors to consider when looking at the risk of intentional contamination. These actors include the disgruntled insider, the disgruntled outsider, and an individual who is a terrorist. The motives of these three actors are different and should be considered when developing a food defense program. The motives of a disgruntled insider or outsider may be to cause brand damage to a specific company. This is a stark contrast to the motives of a terrorist. Terrorism, as defined by the Federal Emergency management agency, is the use of force or violence against persons or property in violation of the criminal laws of the United States for purposes of intimidation, coercion, or ransom. Terrorists are generally seeking to cause harm to a large number of individuals, rather than any specific brand damage to a company.

Risk Management

For either intentional or unintentional contamination of food, risk management strategy includes identifying the contaminant, risk or vulnerability of insertion of that contaminant, and subsequent insertion of controls to reduce the likelihood of the contaminant entering the food supply. The control strategies can be inserted at any point of the food chain, from production and preharvest inputs, through consumption. There are three control strategies which historically have been used for food defense. These include hazard analysis critical control points (HACCP), operational risk management (ORM), and the criticality, accessibility, recuperability, vulnerability, effect, and recognizability (CARVER) tool.

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In general, modern food-producing facilities have been designed to control for the accidental introduction of known microbiological threats. The HACCP system, is one example of how threats which are deemed 'reasonably likely to occur' can be controlled. When considering intentional contamination, one begins with selection of the actual contaminants of concern, includes where and how they would be introduced, to what food, the level of introduction, and many additional factors. A one size fits all approach is difficult to apply when considering intentional contamination. One limitation to the HACCP-type approach is that HACCP programs are designed around hazards deemed reasonably likely to occur. Terrorism is an asymmetrical threat making it difficult to determine how and when a food terrorism event may occur. It is important to note, however, that the HACCP approach may also provide benefits to food defense.

ORM originated in the United States by the National Aeronautics and Space Administration and the United States Department of Defense. The purpose of ORM was to reduce the risk failure of aircraft, space missions, and weapons. ORM was adopted by the US Food and Drug Administration – Center for Food Safety and Applied Nutrition for early food system risk assessments. ORM is a five step process for identifying and managing risks. These five steps include indentifying the hazards, assessing the potential consequences of the hazard, determining which risks to manage and with which interventions, implementing the interventions, and finally assessing the success of interventions and modifying as necessary.

ORM is a function of the severity and the probability of the failure. For the purposes of food defense, probability can best be considered as the probability of success if an appropriately skilled person or group tried to contaminate the food system. For any unit of operation in the food supply, one can conduct the ORM analysis to compare the severity with the probability and focus interventions on where both of these factors are high. ORM has historically been used to reduce threats to food; however, in recent years ORM has been replaced by the new CARVER + shock methodology.

CARVER and the newer iteration of CARVER + shock is another strategy for completing food defense risk assessments. This tool is now used by both the Food and Drug Administration (FDA) and the United States Department of Agriculture (USDA). CARVER + shock risk assessment is composed of seven elements, which are used to evaluate the vulnerability of a system by analyzing each node of the system. Modified to address the concern of intentional contamination the seven elements are listed below.

- Criticality: the degree to which the public health or economic consequences are nationally significant. High scores equate to catastrophic morbidity, mortality, or economic harm.
- Accessibility: physical access to the target; the ability of the perpetrator to gain access to the point of contamination and escape undetected.
- Recuperability: overall system resiliency as measured by the time required to bring the system back into operation, with low scores for only days to recover and high scores for recovery going on a year or longer.
- Vulnerability: attack feasibility as viewed by the potential for a successful attack. This includes both the ability to introduce enough of a material of concern to cause harm and the potential for subsequent processing to reduce the risk.
- Effect: direct loss from the attack as defined by the fraction of the food system that has been impacted by the attack.
- Recognizability: ease of target identification is a measure of the degree of specialized knowledge needed in order to identify the point for the intentional contamination.
- Shock: combined health, economic, and psychological impact of the attack, which is a measure of the overall impact. Importantly, the economic and psychological impacts of an attack may not require any morbidity or mortality if they result in a substantial lack of public confidence in the food system or government.

Each of these seven steps is evaluated and a score for each element is assigned. A team of experts is generally required to complete this facilities evaluation. A composite score is then compiled. The score can then be used for comparisons across the section of the food system under consideration.

Assessing the risk through ORM requires less training than CARVER + shock and in some ways is simpler than CARVER + shock. ORM only uses two rating elements for ranking risk, the severity and the probability. In some cases, a combined approach uses both CARVER + shock, and ORM may be desirable. Once risks are identified, then a risk management approach such as HACCP may be incorporated into the facilities design.

Prevention Strategies

Preventing intentional contamination of food is difficult; however, there are several approaches one can take to deter an intentional food contamination event. In the private sector, several steps can be taken to reduce the threat. Some of the simplest steps include locking external entryways to facilities and limiting access inside buildings to only employees. Employee background checks can also be performed to identify any employee with a potentially suspicious history.

Employees should also only be allowed to access those areas of a facility in which they are assigned to work. Different colored clothing can be assigned to employees working in specific areas of a facility, thus making it easy to identify if an employee is out of his or her assigned area.

Industry best practices call for restricting access to dry ingredient storage. Access should only be allowed to those employees that are designated for that area. An observation system to further reduce the risk of contamination of ingredients is also beneficial. Entry requirements for increased physical security of facility are also necessary. Standardized entry and exit procedures should be adhered to, with checks and verifications of personal identification, equipment, and any other items entering the facility. Furthermore, locks should be used on all doors in a facility to limit access. Employees should all come through a common entryway, where credentials can be checked.

In the public sector, governments have also become increasingly aware of the potential threat of an intentional attack

on the food supply. The World Health Organization has published 'Terrorist Threats to Food,' which is intended primarily for policy makers in national governments with responsibilities for ensuring food safety and designed to assist them in incorporating considerations of food terrorism into existing food safety systems. The document received favorable comments from governments, the food industry, and consumers and has been one of the most requested WHO documents in the field of food safety. Within the United States, the Food and Drug Administration as well as the USDA both have developed groups looking specifically at food defense issues.

Food defense working groups have also been established in consumer organizations and food industry professional organizations. These working groups have served to initiate a dialogue among interested parties working to ensure the safety of the food supply from all possible threats. The private and public sectors both have roles to play in food defense, and both sectors have been taking steps to enhance food defense.

Summary

Intentional attacks on the food supply are not a new paradigm and present a very real and dangerous threat. Food has historically been used as a vehicle to cause illness, and it can be expected that food may continue to be used as a vehicle to cause illness in the future. What has changed is the potential footprint of an intentional attack on the food supply, due to the concentrated manufacturing with widespread distribution of food products. The modern food system is complex and rapidly changing, making it an attractive target for terrorism.

Building a robust food defense plan will also have the ancillary benefit of ensuring food safety. A good food defense program will ensure the health of the end consumer and will ensure a safe and abundant food supply for all.

See also: Food Safety Assurance Systems: Tampering

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Evaluation of the Efficacy of National Food Control Programs

WA Anderson, E O'Leary, D Cousins, R Evans, and A Reilly, Food Safety Authority of Ireland, Dublin, Ireland

Glossary

Monitoring Conducting a planned sequence of observations or measurements with a view to obtaining an overview of the state of compliance with feed or food law, animal health and animal welfare rules (European Food Law Definition).

Surveillance The continuous monitoring of the food supply to ensure consumers are not exposed to components in foods, such as chemical contaminants or biological hazards, which pose a risk to health (WHO definition).

Introduction

National food control programs (FCPs) are designed and implemented to ensure a high level of public health protection with respect to the food supply in a particular country. Their aim is to protect public health by providing a legal framework for the production, distribution, and sale of safe food, while facilitating the development of the food industry within a system of oversight by the competent authority. FCPs must cover all aspects of food production throughout the food chain from farm to fork, including both the domestic food industry and the imported food. Equity of treatment is an essential cornerstone of a fair FCP, where domestic and imported foods are subjected to the same risk-based controls. This ensures better protection of public health and facilitates global trade.

In practice, few countries actively design their whole FCP before its inception. Most FCPs have evolved over time around a basic legal framework using a combination of activities carried out by one or more state bodies. Generally, food control legislation and official control activities tend to expand in response to changes in technology, resource allocation, and emerging food safety problems. This trend for expansion can lead to inefficiencies over time and even reduce the effectiveness of the control program when resources are finite. Therefore, at some point in time the control program may need to be reviewed and, if necessary, a new framework developed. For example, after several decades of food law development in the European Union and after several well-documented food scares, it became necessary to redesign and simplify the legislative framework to reflect the modern food safety requirements and protect the consumer's health in a better way. The underlying principles of this redesign were articulated by the European Commission (EC) in the White Paper on Food Safety in 2000 and provide a good example of a reevaluation process.

As food safety programs develop and change, it is also essential that competent authorities evaluate their effectiveness to establish if they remain fit for purpose, to identify gaps and overlaps, and to enable the development of corrective action plans that can improve the protection of public health if implemented. This article provides an overview of methodologies used to evaluate the efficacy of national food safety control programs.

Essential Elements of an Effective National FCP

The basic elements of an FCP are the same today as earlier, a legislative framework combined with coordinated inspection of food businesses and testing of food to monitor safety and compliance. However, control programs have progressed over the past decades from hazard-based systems to risk-based systems, although this transition is still in progress around the world. Early FCPs were focused on the control of food hazards through the implementation of visual inspection of food premises and food-testing regimes designed to monitor microbiological and chemical contamination. They then progressed with the introduction of audits of food businesses, food safety management system, and testing of food that was linked, wherever possible, to the verification and validation of Hazard Analysis Critical Control Point systems.

Today, effective FCPs need to be based on risk control rather than hazard control. Food control systems that constantly focus on tighter controls to eliminate hazards tend to become burdensome for both the food industry and the competent authorities. Such programs increase the cost and complexity of compliance for food businesses and stretch the limited resources of the competent authorities by providing little basis for prioritization. Effective FCPs should ideally be designed with the risk analysis paradigm in mind. This concept has been defined by the Codex Alimentarius as consisting of three

interlinked elements; risk assessment, risk management, and risk communication.

Ideally, the risk assessment process should be independent of the risk management process to ensure transparency and fairness, for example, risk assessments provided by the European Food Safety Authority for the EC. The risk management function, which in the context of FCPs, consists of the development of food legislation and the monitoring and enforcement of food business compliance, should be based on science and informed by risk assessment. For example, in the context of food safety, legislation should be introduced only where it is necessary to control a risk to public health. Additionally, the requirements of food legislation should only be as stringent as necessary to control the risk, for example, microbiological criteria that tolerates low numbers of *Listeria monocytogenes* in ready-to-eat foods that do not support its growth rather than zero-tolerance policies (<100 colony-forming units per gram during the shelf life). Similarly, food control activities within this legislative framework also need to be informed by an assessment of risk. For example, the risk posed by a certain food business or business sector needs to be evaluated based on the nature of food handling and production, the risks posed to public health, and the history of compliance of the business or business sector. This allows for an FCP that focuses its limited resources on the parts of the food chain where risks to public health are greatest. This should lead to better protection of public health rather than a system that attempts to eliminate all hazards at all times, an impossible and arguably futile task.

For an effective FCP, the risk assessment and risk management functions must be involved in good risk communication. For example, the food control legislation should not only be based on risk assessments that are published and which have been communicated effectively but also developed under a transparent system of engagement with all stakeholders. Similarly, food control activities on the ground must take risk into account. For example, enforcement requirements, such as food recall, need to be based on an assessment of the risk and complimented by a risk communication approach that enables food businesses to understand the basis for enforcement action as well as protecting public health by communication to the public.

Finally, an effective FCP, based on the elements of risk analysis, has to be focused on an understanding of the whole food chain because this reflects the exposure pathway that modulates the transfer of a hazard to the consumer and hence modulates risk. An effective FCP is holistic in this respect and should ensure coordination of the different state bodies involved in food control activities at different parts of the food chain. Ideally, the activities of national FCPs should be collated and published in an accessible format, for example, national control programs published under the requirements of European Union food law. This facilitates transparency, but importantly, it acts as the focus for an evaluation of the effectiveness of the control program, which will now be further elaborated.

Purpose of FCP Evaluation

The success of any FCP relies on a thorough evaluation process. This process should measure outputs from the FCP and the results achieved, and establish whether the system, activities, and/or processes as defined are fit for purpose and deliver satisfactory results. In other words, the evaluation process confirms whether the system is effective and that the planned arrangements have been realized (see Figure 1). The effectiveness or 'efficacy' of an FCP differs from its 'efficiency,' although they are somewhat linked. Efficacy looks at whether the outputs of the program lead to the desired outcomes, whereas efficiency considers the relationship between inputs and outputs (see Figure 2).

Other factors that may also need to be taken into account when evaluating an FCP include:

- Relevance – The rationale for adopting policies, strategies, etc. in response to the health needs and other important factors.
- Adequacy – The strategies and policies suitable or capable of meeting consumer needs and requirements.
- Necessity – Is it a legal obligation?
- Progress – The comparison of actual with scheduled activities to ensure that operations are proceeding as planned and scheduled.
- Impact – The overall effect on health and related socioeconomic development.

Measuring Output and Outcomes in Food Safety Programs

The efficacy of an FCP is characterized by two elements; its outputs and its outcomes. Examples of outputs would include the numbers of inspections, numbers of samples tested, or numbers of incidents managed by the control agencies. Examples of outcomes would include reduction in foodborne disease, reduction in complaints, improvements in compliance levels, or reduction in the contamination of the food tested.

Evaluation of efficacy can be achieved by a number of approaches, alone or in combination. Evaluation methodologies rely on verification techniques in order to qualify the selected information and hence inform evaluation conclusions. The main techniques used are:

- auditing,
- monitoring and surveillance,
- review of data and information, and
- peer review.

Irrespective of the technique employed, it is essential that the purpose and objectives of the evaluation process are specified and clearly defined and that the methodology employed is carried out in a structured way. Figure 3 provides a logic sequence for the performance of evaluations and which employs a systematic approach for consistency and thoroughness.

Key factors that need consideration in order to optimize the success of the evaluation itself are the selection of appropriately skilled and competent evaluators as well as the gathering and checking of information to inform the assessment.

It is essential that assessors are suitably qualified and that the appropriate level and type of expertise is available to the evaluation team. Professional judgments form a significant part of the evaluation process and/or in reviewing their results

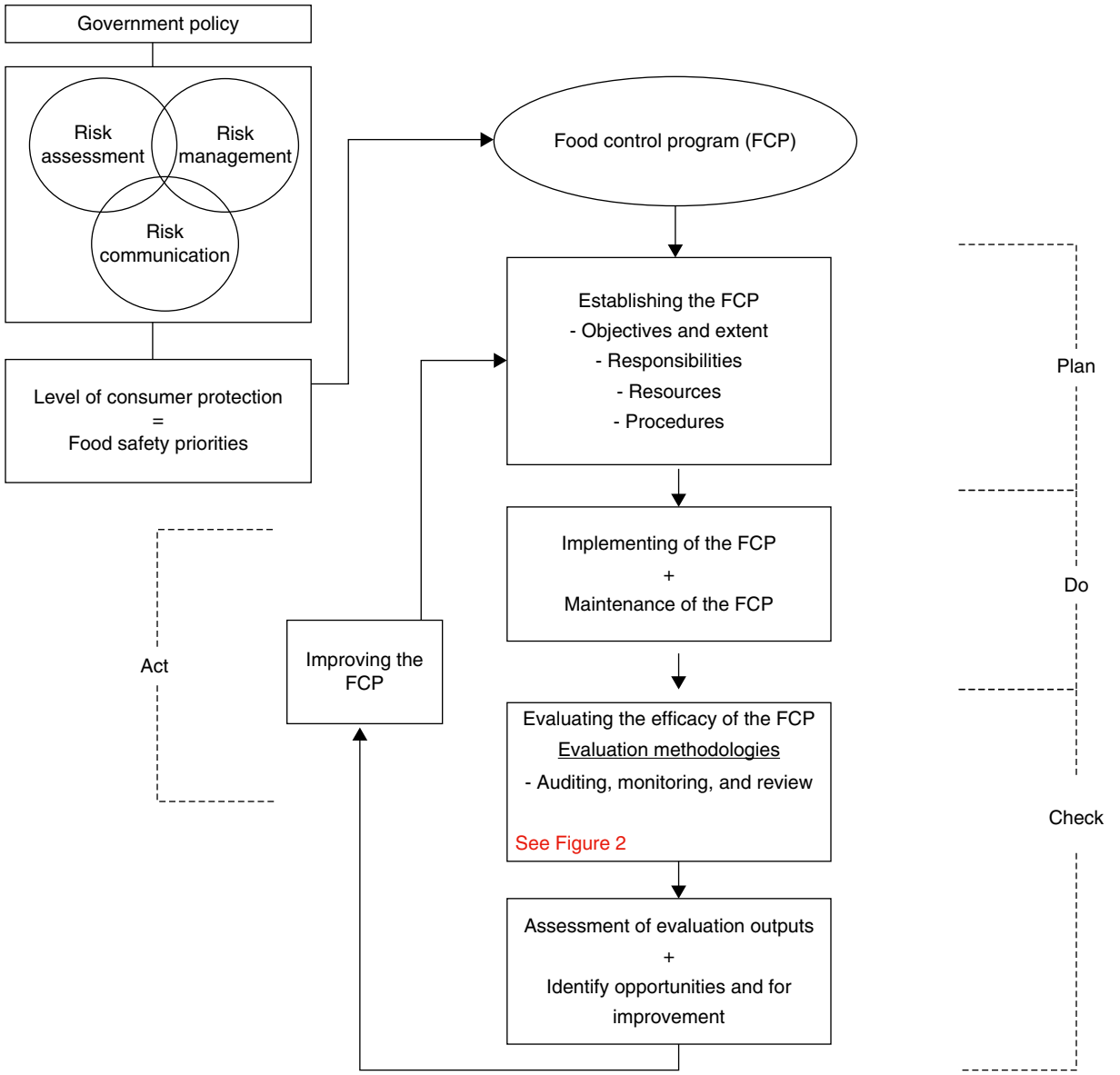

Figure 1 Evaluating the efficacy of FCPs. Reproduced from International Standards Organisation – Guidelines for Quality and/or Environmental Management System Auditing (I.S. EN ISO 19011:2002). Geneva: ISO, with permission from National Standards Authority of Ireland.

and it is essential that these responsible tasks are performed to high standard and are overseen by competent team leaders.

Equally of importance to the success of the evaluation process is the gathering of quality information about the performance of the FCP. Consequently, it is essential that as part of the evaluation process, the information collected is checked and verified for its accuracy and reliability before it is accepted as objective evidence.

Auditing

As an evaluation tool, audits can provide objective assessment of the efficacy of the FCP. Audits of the FCP can provide an evaluation of performance of the system as a whole and/or of the individual processes, elements, or activities. Unlike other evaluation techniques, audits allow an assessment of the design of the system as well as its implementation. Other evaluation techniques tend to focus directly on the outputs of the FCP and their results.

The audit process by nature is a probing technique and has the capability to be tailored to provide targeted evaluations. The audit process is flexible enough to allow horizontal assessment across several common, different interdependent elements or vertical appraisal focusing entirely on one aspect in its entirety.

As a process, audits can be performed by internal assessors as a part of ongoing internal verification that the system and procedures are working efficiently and effectively or they can be carried out exclusively by external auditors. For example, this may be necessary for certification of the FCP to a recognized standard, for example, to ISO 9001:2008. Additionally, external examination may be carried out for regulatory assessment of

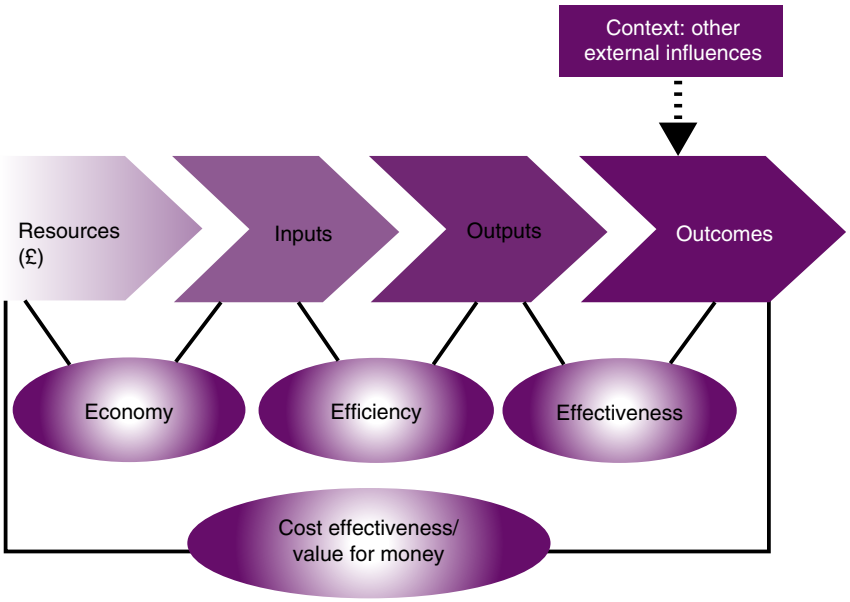

Figure 2 Performance information: inputs, outputs, and outcomes. Reproduced with permission from *Choosing the Right Fabric*, HM Treasury, Cabinet Office, National Audit Office, Audit Commission, Office for National Statistics. http://archive.treasury.gov.uk/performance_info/fabric.pdf

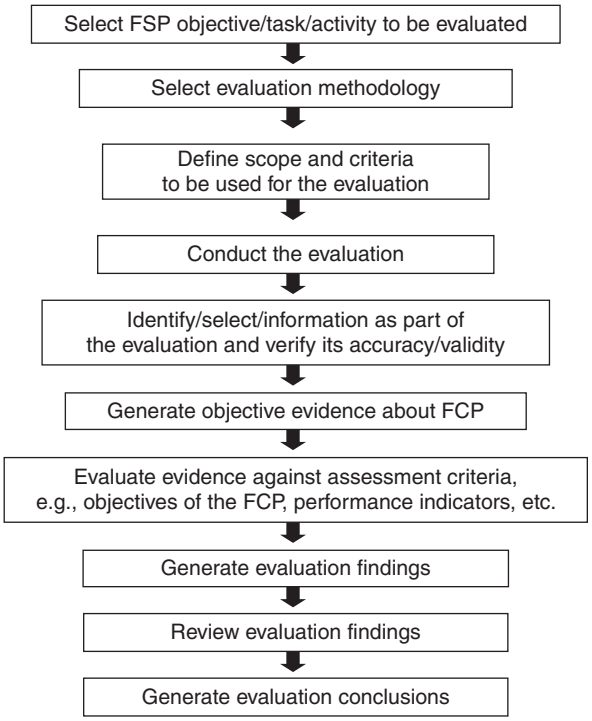

Figure 3 The evaluation process – logic sequence. Reproduced from International Standards Organisation – Guidelines for Quality and/or Environmental Management System Auditing (I.S. EN ISO 19011:2002). Geneva: ISO, with permission from National Standards Authority of Ireland.

compliance of official controls in various countries. For example, missions conducted by the audit section of the EC's Food and Veterinary Office assess the efficacy of FCPs in both European member states and/or Third Countries.

Consequently, audits can provide a wide range of information as to the performance and efficacy of FCPs. For example, they can provide information as to whether:

- the system as designed is capable of achieving its specified objectives,
- the system is being implemented effectively and that planned arrangements are being carried out as intended,
- the system is being properly maintained,
- the degree of conformity attained by the FCP as judged against specified and predefined criteria, for example, compliance with statutory, regulatory, and contractual requirements,
- the system is meeting the needs of interested parties and is delivering a high level of food safety,
- there is a good level of coordination and cooperation within the FCP and interdependent stakeholders,
- the entire food chain is adequately controlled and covered and that there are no gaps or overlaps, and
- the enforcement activity, action, or method is adequate, appropriate, and consistent.

Monitoring and Surveillance

Monitoring as an evaluation technique tends to focus on the performance of the system, as implemented, and its results. The EC has established a definition of monitoring in European food law:

> Monitoring means conducting a planned sequence of observations or measurements with a view to obtaining an overview of the state of compliance with feed or food law, animal health and animal welfare rules.

Incorporation of monitoring into every aspect of FCPs contributes to the establishment of effective evidence-based public health programs. Monitoring is used to assess if FCP objectives are being achieved and if specific control activities are having the desired impact.

Performance indicators are used to monitor performance of programs and as a basis for a review of effectiveness. According to the World Health Organization (WHO), the ideal indicator should be:

- readily determined (data can be obtained without undue difficulty),
- valid (they should actually measure what they are supposed to measure),
- objective (the answer should be the same if measured by different people in similar circumstances),
- sensitive (to changes in the situation), and
- specific (they should reflect changes only in the situation concerned).

Thorough and systematic consultation with a full range of stakeholders, such as consumers, industry, government policy makers, legislators, and international organizations, will provide useful information about which indicators are most meaningful and how indicators can contribute to a more effective program. By way of general examples, the Pan American Health Organization with the objective of examining the overall performance and status of a food safety program developed the following indicators:

- Foodborne illness – number and incidence of suspected and confirmed cases, by month. Later, cases will be categorized into acute and chronic as well as microbiological and chemical.
- Laboratory analysis – number carried out by each primary food safety organization and findings (e.g., proportion unsatisfactory or proportion positive). As far as possible, reasons should be given for unsatisfactory findings.
- Inspections – number of inspections and compliance rate (by type of establishment).
- Consumer complaints – number of complaints reported, number of complaints found to be justified (by commodity).
- Health education – for consumers, the number of 'programs' conducted (slide shows, TV/radio, newsletters, etc.). For food handlers, the number of course conducted and the number of people trained.
- Exports/imports – annual number of export consignments rejected by commodity and volume (weight). For imports, the quarterly number of rejections by commodity, volume (weight), reason, and source country.
- Legal enforcement – number of notices issued, number of notices complied with, number of prosecutions initiated, number of convictions, number of seizures (by commodity and volume), number of destructions (by commodity), and number of establishments closed.

Monitoring can be complemented by surveillance, which in the context of FCPs, tends to focus on detailed studies of zoonotic disease in animals and humans as well as chemical and microbiological contamination of the food supply. WHO has defined surveillance as:

> the continuous monitoring of the food supply to ensure that the consumers are not exposed to the components in foods, such as chemical contaminants or biological hazards, which pose a risk to health.

In essence, surveillance is an extension of monitoring where the intention is that the information collected is utilized for the purpose of applying active control measures. Effective surveillance requires the timely collection, analysis, interpretation, and feedback in order to take appropriate action; for example, coordinated multiagency surveillance systems will identify unusual strains of pathogens in humans and link these to food and animal isolates. Surveillance systems should be simple and sustainable, tailored to country needs, and maintained and expanded when already in place, while being coordinated at international level through common protocols, analytical tools, and databases.

Review of Data and Other Information

The reviewing and interpretation of data and information is essential to any evaluation of an FCP. Collection and collation of relevant data underpins strategy development, with stakeholders reaching consensus on objectives, priorities, policies, roles of different ministries/agencies, industry responsibilities, and timeframe for implementation. In particular, major problems associated with the control and prevention of foodborne diseases are identified so that effective strategies for the resolution of these problems can be implemented. Analysis of data is a process of inspecting, cleaning, transforming, and modeling data with the aim of retrieving useful information so as to inform decision making and arrive at conclusions. Ongoing analysis of official control data is an integral part of the management of food control programs. A well-coordinated national FCP should ensure the integration of systems to allow for the establishment of links between contaminants and zoonoses in animals and food to foodborne disease in humans. Investment in the development of national reference laboratories is essential for the detailed study of microbiological and chemical contaminants and the development of methods of analysis.

Access to reliable and current intelligence on the incidence of foodborne illness is also critical. It is, therefore essential, that effective linkages are established between food control agencies and the public health system, including epidemiologists and public health doctors. In this way, information on foodborne diseases may be linked with food monitoring data and lead to appropriate risk-based food control policies. This information includes annual incidence trends, identification of susceptible population groups, identification of hazardous foods, identification and tracking the causes of foodborne diseases, and the development of early warning systems for outbreaks and food contamination.

Peer Review

The peer review process is another form of evaluation that can be employed by countries (or organizations) as an additional means of benchmarking their activities and improving performance.

Peer review can be described as the systematic examination and assessment of the performance of a state or organization (the reviewee) by another state or organization (the reviewer), with the ultimate goal of helping the reviewee improve its policy making, adopt best practices, and comply with established standards and principles. An established system of benchmarking is currently operating within the network of European Medicines Agencies and could serve as a model for the food safety sector. At European level, the Food Law Enforcement Practitioners working group have also considered that the peer review/benchmarking approach could also provide a useful methodology in evaluating the performance of FCPs.

Peer review is conducted on a non-adversarial basis, and it relies heavily on mutual trust among the parties involved in the review, as well as their shared confidence in the process. It can provide information on the performance of the FCP as well as the quality of evaluations. Additionally, this technique can be used as part of the capacity building process for developing countries or as a refining tool/process in the pursuit of best practice and excellence for those countries where the FCP is well established.

All verification methodologies have the potential to provide objective assessment of system performance as well as identification of opportunities for improvement. Consequently, a useful outcome of the evaluation process is the identification of weaknesses, which once corrected can improve the efficacy of the FCP.

Assessment of Evaluation Outcomes and FCP Improvement

An equally important stage in identifying and improving the performance of FCPs is the assessment of the outcomes of the evaluation process and a review of their results. Experienced assessors are again critical for the interpretation of evaluation results and in deciding on appropriate recommendations and the necessary corrective and/or preventative actions that should be taken.

In making decisions about FCP, it is essential that reviewers also take into account possible uncertainties in the evaluation process. For example:

- Audits in general take a sample of activities to obtain the overview of the FCP activity or of the system; consequently, they do not necessarily look at every component or element but take a 'snapshot' of compliance.
- Monitoring may indicate varying levels or degrees of performance; however, without on-site verification, this may be incomplete.

Indeed a range of evaluation techniques may need to be employed in unison or in tandem in order to provide accurate oversight and in order to inform appropriate corrective actions. At their most idealistic evaluation, strategies have the potential to correct poor design and highlight inadequate or unsatisfactory performance and lead to appropriate corrective action.

Examples of corrective actions could include:

- reallocation or redeployment of resources due to risk priorities,
- the changing or introduction of a high level FCP strategic objective to deal more effectively in protecting vulnerable groups, and
- adjustment of surveillance programs to cater for new pathogens, chemical contaminants, etc.

Conclusion

Evaluations of the FCP can be used to obtain objective evidence that the system is fit for purpose, is delivering the planned arrangements, and it is achieving predefined goals and strategies.

The quality of the evaluation process and its review is of critical importance. If incorrectly performed, it can misinform and lead to unsuitable strategies and incorrect readjustments of the FCP – in effect, it can do more harm than good.

Arguably, no one evaluation tool is often sufficient in order to gauge effectiveness due to their singularly narrow field of vision; however, collectively, they can bring the true merits and performance of the system into sharper focus and allow objective assessments to be made.

See also: Food Safety Assurance Systems: Audits of Food Safety Management Systems; Hazard Analysis and Critical Control Point System (HACCP): Principles and Practice. Public Health Measures: Food Control and Public Health Laboratories; Food Inspections and Enforcement Systems; Fundamentals of Food Legislation; Modern Approach to Food Safety Management: An Overview; Monitoring of Contaminants; Surveillance of Foodborne Diseases. Risk Analysis: Risk Communication

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PUBLIC HEALTH MEASURES

Surveillance of Foodborne Diseases

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Glossary

Active surveillance Public health surveillance that regularly reaches out to diagnostic laboratories or to clinicians to actively collect reports of specific diagnoses of infection.

Cluster of disease A group of cases of a single illness that are suspected to have a common source, because they are caused by the same strain of a pathogen, or have other features in common.

Food vehicle of transmission The specific food to which an outbreak of foodborne illness is attributed by an investigation. That food may be either simple, in which case the investigation narrows it to a single ingredient that was presumed to be contaminated (for example the undercooked eggs used in an omelette), or complex, in which case the investigation is not able to determine the specific ingredient that was a source of contamination (e.g., a fruit salad)

Outbreak of disease A group of at least two cases of a single illness that are demonstrated by epidemiological investigation to have a single common exposure or source.

Passive surveillance Public health surveillance that collects reports of specific diagnoses from clinicians or diagnostic laboratories, which they are required or requested to submit because of notifiable diseases regulations.

Serosurveillance Using a systematic serological survey of a population to determine the frequency of a specific infection in that population.

Surveillance (public health) The systematic collection, analysis, and interpretation of data on specific diseases in a defined population, to guide public health decisions.

Introduction to Public Health Surveillance

Public health surveillance is the systematic collection, analysis, and interpretation of data on specific diseases in a defined population, to guide public health decisions. For infectious diseases, the data come largely from the diagnoses made in the clinical health care system, sometimes strengthened by further study of the microbes themselves. Surveillance can define the magnitude of a health problem, driving policies that will prevent it. Surveillance can identify an increase in the number of reported cases above the expected baseline which may be an outbreak, triggering an investigation so that the source can be identified and controlled. Surveillance also can provide a platform for more detailed studies to improve management and prevention. Finally, surveillance can track trends over time, measuring the impact of control and prevention efforts (see Figure 1).

Surveillance data are usually expressed as the number of cases of a disease occurring in a defined population over a defined period of time. For acute infections, the incidence is expressed as the number of new cases occurring in a population, typically the number per 100 000 in a year. For chronic conditions, the prevalence is usually reported, which is the number of cases that exist in the population at a particular point in time.

The history of foodborne disease surveillance began with the reporting of a few severe clinical illnesses and localized events that were identified as problems with a social gathering, or within an individual county or city. As surveillance grew to cover larger populations, and as microbiological diagnoses became common, more widespread outbreaks became apparent. With standardized molecular subtyping, highly dispersed outbreaks are routinely detected. Surveillance is linked to both the health care systems that produce the diagnostic data, and to the public health systems that refine, analyze and act on it to control or prevent future cases of illnesses.

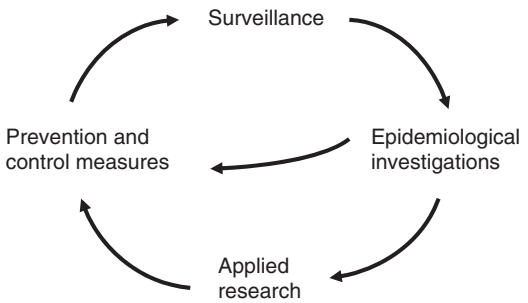

Figure 1 The cycle of public health prevention depends on surveillance to identify problems, investigations to find what went wrong, applied research to devise solutions, control and prevention interventions, and then surveillance to confirm whether the interventions are successful.

Most surveillance is passive, which means that clinical offices and laboratories are requested to report specific diagnoses to public health authorities when they make them. This depends on their remembering which conditions are reportable, how to report them, and on committing the resources to do so. Surveillance can be active, in which case the public health authorities regularly contact laboratories or healthcare providers to ask if there have been any recent cases. Active surveillance is more labor intensive, but also gives more complete and detailed information. Reporting can be mandatory or voluntary. Automated electronic laboratory reporting could make much surveillance reporting instantaneous.

Surveillance can gather information from the entire population, or from a representative group of sentinel sites. Sentinel site approaches make it possible to collect more detailed information at a fraction of the cost of gathering that information from the entire population. For estimating the burden, conducting studies and tracking trends, the sentinel approach may be very useful if the sites represent the entire population. Similarly, surveillance done to track incidence and burden, can take the form of periodic surveys, rather than continuous data collection. However, as outbreaks can occur anywhere and at anytime and can be localized or dispersed, surveillance to detect them needs to cover the entire population continuously.

Surveillance typically gathers basic information about the disease that was diagnosed, the date it began, the date of the first clinical visit, whether illness led to hospitalization or death, the demographic characteristics of ill person, such as their age, gender, occupation and location of residence, and history of recent travel. For some rare and serious infections (e.g., in the US, for botulism, vibrioses, and listeriosis), additional information about severity, treatment and possible exposures is gathered routinely. In addition, the use of molecular subtyping strategies for outbreak detection (see The Power of Standardized Molecular Subtyping in Surveillance) means it is important to supplement this information with characteristics of the bacteria, virus, or parasite that caused the infection, as determined in public health laboratories. This means that clinical laboratories must send the infecting strains they isolate or clinical specimens to the public health laboratories for characterization.

The public health and medical community in each jurisdiction and country decide what conditions should be made notifiable, and how they should be reported. In the US, this authority rests with each state, and the Council of State and Territorial Epidemiologists (CSTE) determines what conditions shall be reported at the national level. The decision to add a foodborne infection to the list of notifiable diseases often follows a large outbreak. For example, in 1988 the state of Washington was the first in the US to make *E. coli* O157 infection notifiable after a large outbreak was traced to ground beef. In 1993, a large multistate outbreak of these infections was first detected in Washington State, and was also linked to ground beef. Following this outbreak, many states made the disease notifiable, and CSTE added it to the list of nationally notifiable diseases.

In Belgium, the medical association operates a sentinel physician surveillance system of great flexibility and efficiency. Each year the association itself chooses which condition are of interest, and a voluntary panel of primary care physicians then supplies information about those diagnoses for the following year. Trends over time are followed by collecting data on a given condition every five years. The system is now being prepared for convertion to electronic reporting.

Most surveillance depends on some level of voluntary effort, whether it be to send a strain of *Salmonella* to the specialized laboratory of serotyping and subtyping, or to fill out a form to report a clinical diagnosis. The more that the participants in that process see some value to doing it, the more successful the surveillance system will be. This value can be enhanced by providing regular summary reports to all participants, and found in the actual use of the information, at local, provincial and national levels to detect and investigate outbreaks, to inform prevention decisions, and to judge their success.

Surveillance is most successful when the information is useful to those who collect it, as well as to the regional and national authorities to whom it is reported. Local authorities may respond to individual reports of illness, use surveillance to plan for local education and prevention efforts, as well as investigating local outbreaks. Regional and national authorities may detect more widespread outbreaks, track trends, and use the information to guide policy at a broader level.

Public health laboratories can add value to the reporting process by providing information feed back to providers in a medically useful timeframe. Typically this means confirming the primary laboratories' results or reporting additional information which the provider finds useful or interesting, such as serotype, susceptibility profile, or toxin type. In some countries the public health laboratory functions as the primary diagnostic laboratory, and has a direct role primary patient as well as surveillance reporting. In other countries the Public Health Laboratory serves a primary diagnostic function only for rare diseases of public health importance, such as botulism, diphtheria, measles, or plague. In either circumstance, the Public Health Laboratory needs to provide the same level of timely reporting that hospital and clinical laboratories provide.

Surveillance systems can be systematically evaluated with several performance measures, including timeliness, completeness, cost, and efficiency. However, it is difficult to justify the cost of even an efficient surveillance system year after year, if no decisions are taken as a result.

Routine Foodborne Disease Surveillance can Gather a Variety of Kinds of Information

Surveillance of foodborne disease caused by infectious and toxigenic microorganisms can be based on collecting different kinds of information, and thus can answer several different questions. This surveillance is the focus of the remainder of this article.

Individual Cases of Diagnosed Illnesses

Case-based foodborne disease surveillance relies on reports from clinicians or clinical laboratories directly to the health department. The specific conditions that are reported are

diagnosed by laboratory identification of a microbial agent, such as salmonellosis, shiga toxin-producing *E. coli* disease (STEC), or listeriosis, or are readily recognizable by distinctive clinical signs and symptoms, such as hemolytic uremic syndrome or botulism.

For some pathogens, this is increasingly supplemented by standardized subtyping of strains to detect widespread outbreaks (See The Power of Standardized Molecular Subtyping in Surveillance). For example, since 1996, multi-state outbreaks of *E. coli* O157:H7 have been identified through PulseNet, the US national foodborne disease molecular subtyping network, using a database at Centers for Disease Control and Prevention (CDC) in which all 50 US states, the United States Food and Drug Administration (FDA) and the United States Department of Agriculture (USDA) participate. Pathogens submitted for subtyping may be further characterized to ascertain changes in antimicrobial susceptibility, or virulence or antigenic profiles.

Disease Clusters and Complaints

The most common type of foodborne disease surveillance worldwide is direct reporting to health authorities of disease clusters by the public or healthcare providers. This might be a report of illnesses from a group dining event, or reports by physicians of unusual numbers of cases with a particular presentation, such as bloody diarrhea. These reports are typically made soon after the event occurred, and although a specific diagnosis is still lacking, an investigation can begin immediately, by interviewing some of the ill persons about their common exposure. Unlike surveillance that depends upon a specific pathogen subtype to link cases, clusters of disease detected in this manner are not dependent on specific agent identification, and thus can lead to the identification of new agents. For example, shiga toxin-producing *E. coli* O157:H7 was first identified as an important pathogen after a cluster of distinctive bloody diarrheal illnesses was reported by an astute clinician.

Cluster reports are most effective at detecting highly localized events, where contamination occurs close to the point of preparation, often in a single kitchen. However, more widespread events can also be detected if they are of sufficient magnitude. The 1993 Milwaukee waterborne outbreak of cryptosporidiosis which affected over 450 000 people was detected after large numbers of cases of unidentified gastroenteritis were reported to health authorities. Investigation of local clusters sometimes provides the key to explaining a more diffuse increase in a particular infection, if they can be linked by microbiological subtyping. As local event-based clusters can be reported and investigated swiftly, they have been critical for solving many large outbreaks, such as the 2008 outbreak of *Salmonella typhimurium* infections associated with peanut products, 2010 nationwide outbreak of *Salmonella enteritidis* infections associated with shell eggs in the US, and an outbreak of *Salmonella typhimurium* DT104 infections linked to imported beef carpaccio in Denmark.

Some jurisdictions gather complaints from the public about individual, sporadic cases of illness that they believe are foodborne. Though this sometimes provides useful adjunct information in an outbreak, the lack of specific agent information and confusion about the actual incubation period limit the value of this source of data for primary outbreak detection.

Summaries of Investigated Outbreaks

In addition to reporting individual cases of illness, some regional and national authorities collect standard reports of outbreak investigations. Routine reporting of investigated foodborne, waterborne or other outbreaks can track conditions for which no other standard surveillance exists such as illness caused by *Staphylococcus aureus* or *Clostridium perfringens* toxins, can help attribute the burden of a broad range of specific agents to specific food groups, and can follow trends in outbreaks related to specific pathogens or foods. These reports also provide an index of public health activity, as the jurisdiction that does not report any foodborne outbreaks is likely to have an inert food safety program, rather than extremely safe food. Codifying the variety of implicated foods and ingredients is a challenge. Capture of the methods used to diagnose the condition, and to implicate the source is important. A simple outbreak reporting system can be a useful and low cost way to begin foodborne disease surveillance in many countries.

Detailed Data Collected from Representative Sentinel Sites

For burden estimation and tracking, it can be more efficient and accurate to collect detailed data from a representative sample of sites, than from the entire population. In sentinel site systems, a group of local agencies are supported to capture case reports from defined populations, to interview the patients, and/or to characterize the pathogens more completely. The measured incidence can be combined across sites, expressed as the number of specific infections per 100 000 population, tracking infections for which reliable surveillance data are not otherwise available. Sentinel site systems have been used in the US to track the viral hepatitides in the Sentinel Counties surveillance system for acute viral hepatitis, to monitor *Pneumococcus* strains circulating in the era of multivalent vaccination in the active bacterial core sentinel sites, and to track 9 infections or conditions often transmitted through food in the FoodNet system. The ten sentinel sites in FoodNet encompass 15% of the US population. This system provides the information with which to estimate the burden of illness, conducts special studies of risk factors, and tracks trends over time. These systems can provide useful information for national decision making, with central funding from the national health authorities. The CDC program FoodNet is also supported by the food regulatory agencies USDA/Food Safety and Inspection Service and FDA.

Surveillance for antimicrobial resistance can be conducted by testing a representative sample of strains. This form of surveillance can identify the emergence of important resistance patterns, such as the general increase in fluoroquinolone resistance in *Campylobacter*, the spread of new multi-resistant strains of *Salmonella* in animal and human populations, or the appearance of highly resistant *Salmonella typhi* in travelers from other countries. As some of the resistance in bacteria that

cause human foodborne infections arises in animal populations as a result of the use of antimicrobial agents in agriculture, similar monitoring of strains detected in animals and in foods can track the flow of strains between specific animal reservoirs and humans. Using the same assays and breakpoints simplifies comparison of resistance between these groups. Linking resistance information to other clinical and epidemiological information about the cases can clarify the clinical impact of resistance, its association with foreign travel, and the role of exposure to specific foods. Selecting strains using a standard sampling frame, such as 1 of every 20 *Salmonella* strains submitted for serotyping, can limit the expense while providing a valid measure for the whole population. In the US, the tri-agency collaborative National Antimicrobial Resistance Monitoring System measures resistance to a standard panel of antimicrobial agents in *Salmonella*, *Campylobacter* and other pathogens, isolated from ill people, from meat and poultry, and from animals. In Denmark, the Danish Integrated Antimicrobial Resistance Monitoring and Research Program provides detailed resistance tracking.

Monitoring Strains from Animals and Food

Some foodborne surveillance systems encompass monitoring the food supply, and even the food animals, to identify problems early. For example, the Danish Zoonosis Center has long integrated information from all three sources into one system. In the US, the PulseNet system includes laboratories from USDA and FDA, and veterinary and meat isolates are gathered in a parallel VetNet system that uses the same method, permitting cross checking.

Foods are tested for the presence of microbes throughout the food supply as part of verification of process control, and as part of purchase contract conditions. Although such testing is beyond the scope of this article, it could provide useful warning signals to public health if reported in real time. On rare occasions, the identification of a pathogen in a specific food can reveal an ongoing outbreak, and may rapidly suggest the outbreak source, when if can be shown that the ill persons in the outbreak indeed ate that food. In 2009, the FDA began collecting mandatory reports of isolates of pathogen from ready-to-eat foods that it regulates. This 'Reportable Foods Registry' may open a new window into contamination of many foods. Systematic random sampling of foods has been used to identify the frequency of contamination, useful for risk analysis and modeling, and to track specific problems such as antimicrobial resistance.

Serosurveillance

Measuring the frequency of antibodies to specific pathogens has long been used in viral disease epidemiology, and is also being used now to measure the true incidence of other infections. For toxoplasmosis, serological studies of the population show that 11% of the US population is infected. Recent exploratory work in Denmark and the Netherlands for *Salmonella* and *Campylobacter* indicates that these infections are far more frequent that had been previously estimated. Many of these immunizing infections may be asymptomatic, so they would not be detected by surveillance that depends on clinical illness. Such estimates may simplify comparison of infection rates among countries.

Non-Specific Clinical Syndrome Events

Surveillance can be based on health indicators that are less specific than a specific microbial diagnosis. These indicators may be based on broad clinical syndromes, such as the number of visits to emergency departments for acute gastroenteritis, the number of stool specimens submitted for culture to clinical laboratories, or the number of calls to poison control centers (Henning, 2004). They can also be more general markers of illness, such as sales of antidiarrheal medications, rates of school absenteeism, or even the number of internet browser searches about diarrheal illness. Such methods have been useful in establishing the scope and size of an outbreak once it is detected, but though in theory they might signal problems with food or water earlier than other systems, but in practice have not yet usefully detected a foodborne outbreak before other standard means of surveillance. The nonspecific nature of the tracked indicators makes for an unfavorable signal-to-noise ratio, which requires that the outbreak need to be large to stand out from background illnesses. Furthermore, the potential disease clusters that are identified still need to be followed up by traditional investigation methods, further reducing potential time advantages.

Monitoring Consumer and Health Care Provider Behaviors

Periodic surveys can gather information at the consumer level on the frequency of diarrheal illness, health care seeking, of dining in restaurants, or taking an international trip, or of risky behaviors such as eating raw shell eggs, raw shellfish, or raw ground meat. Such surveys are typically done by telephone, depend on the cooperation of the survey subjects, and can provide useful information for risk assessment and to improve consumer education.

One can also survey health care providers and microbiologists about their behaviors. Physicians and nurses can describe their usual practice given a patient with gastroenteritis, though response rates are typically low. Surveys of microbiologists can track changes in diagnostic procedures, types of transport media, enrichment or culture media used, and non-culture based 'dip-stick' diagnostics they use, that may have an impact on laboratory based surveillance.

General Limitations of Surveillance

All Surveillance is Incomplete

All surveillance systems capture a fraction of the total number of illness cases that occur, so reported cases, reported outbreaks, complaints, nonspecific health indicators, or other surveillance data represent the tip of the iceberg of illnesses. That fraction is small when a surveillance system begins, and increases as more participants start reporting, so a new surveillance system matures over several years. This fraction is the result of sampling, partial utilization of healthcare or healthcare products, underdiagnosis of illnesses, and of

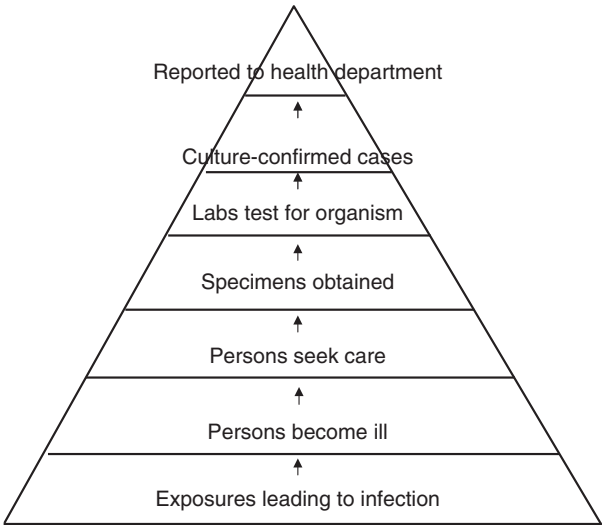

Figure 2 The pyramid of under-recognition and under-reporting in public health surveillance. The sequence of events from the sick individual to the final report to public health loses events at each step.

underreporting. To be reported as a case for surveillance based on diagnosed illness, a cascade of events must occur, which can be visualized as the 'surveillance pyramid' (See **Figure 2**). The ill person must first seek medical attention, then have a diagnostic study such as a stool culture. The diagnostic study must yield a positive result, and that result must be reported to the health authorities. The cumulative probability of these steps, from the bottom to the top of the pyramid, is the multiplier, which estimates the number of cases that actually occur for each one that is reported. For example, it was estimated using data from around the year 2000 that in the US there were 38 cases of human salmonellosis for every one that is reported. The multiplier for a given disease can vary over time. The same multiplier was estimated to be 29.3 using more recent data from around 2007. In addition to underdiagnosis, many cases may be diagnosed, but not reported. Thus, in 1995 passive voluntary case reporting of *Campylobacter* infections in the US yielded an incidence of 2.5 per 100 000 population, whereas active surveillance in FoodNet showed the actual rate for 1996–98 was 21 diagnosed infections per 100 000. In general, it is more important to understand the multiplier than to try to change it, as efforts to 'improve' a stable surveillance system by increasing the fraction of cases diagnosed can introduce artifacts that make interpretation of results more difficult. Surveillance of disease clusters (such as group events) and complaints similarly are likely to capture only a fraction of disease that might be reported by these mechanisms.

Case-Based Surveillance Depends on what can be Diagnosed Routinely

Surveillance collects reports of diagnosed clinical conditions, so conditions that are not routinely diagnosed are not trackable by this mechanism. Neither norovirus nor *Clostridium perfringens* intoxication are systematically diagnosed in clinical practice, and as a result, case-based notification does not occur for these conditions. Before 1979, though they likely were common, *Campylobacter* infection were rarely diagnosed. After new diagnostic assays were introduced routine clinical use, surveillance could begin to capture these infections. More recently, the routine diagnosis of *E. coli* O157 and other shiga toxin producing *E. coli* has made surveillance for those infections possible. The increasing use of nonculture based rapid screening tests that do not yield a microbe that can be subtyped now threatens surveillance based on molecular subtyping, unless definitive microbiological diagnosis is also pursued.

Surveillance Varies Greatly by Country

As each surveillance system is wedded to a particular health care delivery system, the results of surveillance can vary among nations because of differences in their health care systems. This complicates the comparison of those results. For example in the late 1990s, the reported annual incidence of *Campylobacter* infection was reported to be 21 per 100 000 in the US and five times higher, 110/100 000 in England. However the estimated reporting multiplier to account for underdiagnosis was 38 for the US, suggesting the estimated actual incidence 798/100 000 in the US, whereas the multiplier was only 8 for England, suggesting the estimated actual incidence there was 870/100 000, very similar to that of the US at the time. This shows how differences in the frequency of health care seeking and of obtaining cultures can greatly change the reported incidence. If in one country, health care is freely available, and microbiological diagnosis of a broad range of pathogens is routine, and in a second, the patient may often pay for a consultation, and for diagnostic microbiology, whereas in a third it is restricted to hospitalized illnesses, and to a limited range of pathogens, the results of surveillance should vary substantially. One would expect the reported rate of infections to be much higher in the first country, lower in the second and even lower in the third. That means that systematic measurement of the frequency of those key steps in health care seeking and testing is needed to make such comparisons. Reporting requirements may also vary from one state or province to another within a single country, further complicating interstate or international comparison.

Advanced surveillance can provide the detailed data needed to determine which differences are real. For example, in the US, FoodNet sentinel surveillance revealed substantial geographic variation in the annual incidence of campylobacter infections, from 7 per 100 000 at the lowest site to 34 per 100 000 at the highest. These differences were not explained by data from surveys of laboratory practices, health care seeking behavior or risk factors; they may indicate real but unexplained differences in frequency of poultry contamination with *Campylobacter*.

Surveillance Costs Money, and More Detailed Surveillance Costs More

Surveillance is rarely of direct value to the patient themselves, but rather benefits society in general. Therefore, the cost of surveillance is usually borne by the public sector, often

separated from the cost of clinical diagnosis. Even in countries with full coverage of healthcare costs, the surveillance information is assembled by a specialized group, and the laboratory subtyping is done in a specialized laboratory, both of which are separate from the clinical diagnostic staff. The true value of surveillance lies in the better decisions around prevention it leads to, and it is difficult to justify the cost of conducting surveillance year after year, when no decisions are taken on the findings.

Case-Based Surveillance is Usually Slow

Once symptoms occur, the cascade of events in the surveillance pyramid takes time, so that the report to the health department may occur several weeks later. It is thus not unusual for the initial public health interview of a case-patient to take place three or more weeks after the person was exposed to whatever it was that made them sick. Depending on human memory for details of food exposures at such a remove can be challenging. Outbreaks associated with fresh produce may be over before they are solved, which restricts our ability to directly prevent continuing transmission, although the feedback provided to industry and regulatory agencies helps in long-term prevention efforts. The time frames for testing and reporting can be accelerated, by using faster shipping and testing protocols, though this is also likely to be more expensive. Laboratory-based surveillance is too slow for botulism, which requires immediate and rapid action to ensure the risk is controlled. For this reason, botulism surveillance in the US and other countries depends on reporting the clinical suspicion of a likely case, supported by the provision of antitoxin at no cost by public health authorities. Despite these limitations, pathogen-based surveillance remains the single most effective tool for detecting and resolving widely spread, low-level events, such as outbreaks commonly associated with contamination of mass distributed food items.

Not All Outbreaks are Caused by a Single Strain of Pathogen

Outbreaks are usually presumed to be an increase in one specific type of infection, a presumption that is reinforced by the use of surveillance case definitions and subtyping methods that add specificity. It is well to remember however, that contamination is not always with a single organism. Outbreaks occur that are caused by more than one strain of a pathogen, or even by more than one pathogen. Identification of a second pathogen in the food vehicle that caused the outbreak can sometimes uncover a second unsuspected cluster of infections from the same source. Focusing on the dominant strain leads to efficient identification of the problem, but when many strains are characterized within a single outbreak it is not unusual to find some variant strains, and even sometimes other pathogens. When this occurs in a waterborne outbreak, the presumption is that the water was contaminated with sewage containing multiple kinds of pathogens. When it is recognized in foodborne outbreaks, it may indicate a similar sort of contamination has occurred.

Using Surveillance for Public Health Decision Making

Detecting and Investigating Outbreaks

The investigation of foodborne outbreaks is a key driver for pushing food safety. An increase in reports of a particular illness, or of infections with one subtype of a pathogen, above the background noise of expected cases is the signal that something has gone wrong, and that an epidemiological investigation is needed to determine whether there is a correctable food safety gap. Once a cluster of possibly related cases is detected, and an investigation begins, ongoing surveillance continues to be critical to the investigation, identifying new cases, defining the scope and dynamics of the outbreak, and showing when it is over. Detecting an outbreak is at its heart a signal-to-noise problem, and the nature of the outbreaks detected thus depends on the sensitivity of the surveillance system itself. If hundreds of cases of a severe illness occur over three days in one city, even the most informal and primitive surveillance will suffice, whereas the same number of cases distributed over two months across an entire country is likely to be missed, without sophisticated surveillance based on pathogen subtyping. Without such subtyping, the dispersed outbreaks will be invisible against the background of sporadic unrelated cases. Subtype-based surveillance can also be used to assess risk of an apparent cluster. If a group of illnesses thought to have some connection are shown to have a variety of unrelated subtypes, the likelihood that the cluster represents a common source is reduced.

Measuring Trends in Incidence

Ongoing surveillance can document changes in the incidence of an infection over many months or years (**Figure 3**). An increase can herald a new problem, and detailed analysis of trends may reveal that changes are prominent in certain age or ethnic groups, providing more clues about the drivers for the change. Once a major control measure is put in place, surveillance to track a decrease in incidence of the target pathogen is a key outcome measure. Programs that reduce the frequency of pathogens in food may be assessed by the frequency of contamination in food samples, but the ultimate measure is a decline in the incidence of disease. For this reason, in 1996 in the US, when a major pathogen reduction effort was about to be introduced for the American meat supply, the FoodNet active surveillance program was set up to provide an important measure of success. National incidence targets were set for several key infections, and reaching the goal for STEC *E. coli* O157 in 2009, was a major milestone for judging the overall success of the prevention program.

Estimating the Burden of Illness

Estimates of the overall burden a pathogen places on the health of the public can help decision makers focus prevention on the greatest threats. Surveillance provides the foundation for estimates of the burden of diseases. By multiplying the incidence of reported cases by the frequency of underreporting and under-diagnosis, the true number of illnesses can be estimated. This can be done for individual

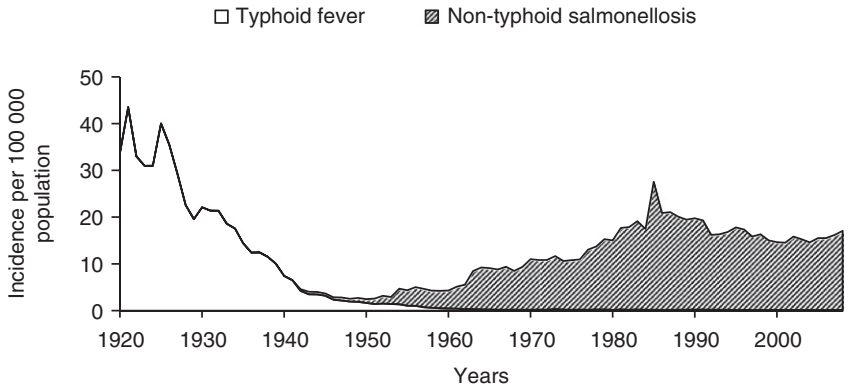

Figure 3 The fall and rise of the reported annual incidence of salmonellosis in the United States, 1920–2008. The first part of the 20th century is dominated by infections with *Salmonella typhi*, controlled by water and sewage treatment, and milk pasteurization. The second part is dominated by non-typhoid salmonellosis.

infections under notifiable disease surveillance, for the conditions that are only tracked as the result of outbreak reporting, and even by extension for illnesses of undetermined etiology. The burden can be expressed in terms of illnesses, hospitalizations, or deaths based directly on surveillance data, or in terms of the number of days of missed work or school, of days in hospitalization, or as the loss of years of life due to death or disability.

Attributing Foodborne Infections to Specific Foods or Commodities

Estimating the fraction of the burden of illness that specific foods are responsible for can help focus decisions based on risk, so that control measures are targeted at the highest risk foods. This attribution is not a simple calculation, and can be done in a variety of ways. It is complicated because food itself is complex, with many different food types and ingredients, and preparation methods. It is also complicated because the attribution may be considered at several points in the food chain, from the point of consumption, the point of processing or the ultimate reservoir. Most public health data provides information at the point of consumption. For a few infections routine case surveillance can provide this attribution. For example, home-canned vegetables cause most cases of botulism, and eating raw shellfish causes most *Vibrio* infections. For other infections, such as those caused by *Campylobacter* or *Salmonella enteritidis*, for which several important food vehicles have been identified, a case-control study of reported sporadic cases, that compares the reported exposures of diagnosed cases with those of healthy controls can provide useful information about the point of consumption attribution. In Denmark, the distribution of bacterial subtypes of *Salmonella* found in human infections is compared with that of *Salmonella* found in food or food animals has been used to estimate the fraction of salmonellosis due to different animal reservoirs. A similar approach may in the future be possible with multilocus sequence typing for *Campylobacter*. The most generally applicable strategy for attributing foodborne illness to food commodity groups is based on outbreaks reported to foodborne outbreak surveillance. Using simple attribution, which counts outbreaks linked to specific individual foods, the Health Protection Agency of England and Wales follows trends in outbreaks attributable to specific commodities. Attribution based on analyzing food identified in outbreaks by their component ingredients, or so-called complex attribution shows promise.

Managing Risk with Quantitative Risk Assessment

Quantitative risk assessment (QRA) depends on information from many sources, including public health surveillance. Where the final output of QRA models is human illness, surveillance data are often used to calibrate the entire model. For many risk assessments, surveillance data and attribution derived from them are the basis of the model. For example, a QRA that preceded new regulations enacted for eggs in the US was based on surveillance, case-control studies and information gathered during outbreak investigations. An extensive QRA of *Campylobacter* in the Netherlands used surveillance data and other information to provide attribution estimates).

The Power of Standardized Molecular Subtyping in Surveillance

Since the mid 20th century, pathogen-based case reporting has played an important role in food safety. *Salmonella* serotype-based surveillance was one of the first and most successful of such programs. Serotype-based attribution studies in Denmark allowed the development of targeted interventions that resulted in marked reductions of *Salmonella* contamination in pork, chicken, and eggs. Serotype surveillance led to the control of baby turtle-associated salmonellosis in the US during the 1970s, and the development of *Salmonella enteritidis* control programs in egg production. One of the largest outbreaks detected through routine serotype surveillance was the 1995 outbreak of *Salmonella enteritidis* associated with ice cream contaminated by raw eggs. This outbreak was responsible for an estimated 250 000 widely dispersed cases throughout the US over a several month period. The capacity of nations to conduct serotype-based foodborne disease surveillance varies greatly. Differences between nations are primarily due to the strength of the underlying surveillance

infrastructures, which in turn are owing to differences in funding, training, availability of reagents, priorities, and even cultural traditions. The World Health Organization Global Foodborne Infections Network (GFN; formerly Global Salm-Serv) seeks to improve the capacity of countries to conduct surveillance for foodborne and enteric infections by identifying and minimizing these limitations through interdisciplinary partnerships (WHO, 2010).

Molecular-based pathogen-specific surveillance has brought the power of pathogen-based surveillance to a new level in those areas of the world that have fully implemented it. In the US, PulseNet, the national and international molecular subtyping network for foodborne disease surveillance, has become one of the most sensitive tools available for detection of unrecognized problems in our food safety systems related to microbial contamination. This type of surveillance, like serotype-based surveillance, uses the underlying presumption that individuals sharing a common exposure are more likely to share a genetically matching microbe than unrelated individuals. By using a refined case definition which separates likely related from likely unrelated cases, the signal-to-noise ratio is improved, which in turn reduces the number of cases required to detect outbreaks and make meaningful associations. Using molecular subtype data as part of routine surveillance resulted in dramatic increases in recognized outbreaks of *E. coli* O157:H7 and *Salmonella typhimurium*. PulseNet has been responsible for detection of most of the high-profile foodborne disease outbreaks due to *Salmonella* species and *E. coli* O157:H7 in the US during the 2000s, resulting in the recall of hundreds of millions of pounds of contaminated products and remediation of underlying problems in multiple industries.

Local outbreaks thus identified may also be linked together to identify broader trends, such as the 1998 international shigellosis outbreak due to contaminated parsley the 2008 outbreak of *Salmonella typhimurium* infections associated with peanut products, 2010 nationwide outbreak of *Salmonella enteritidis* infections associated with shell eggs in the US. In the latter two examples involving relatively common agents and exposures, local events linked by pulsed-field gel electrophoresis (PFGE) triggered trace-back investigations, adding critical specificity to the investigations.

Standardized molecular subtype-based surveillance has become our most effective mechanism for detection of low-level widespread contamination events. Detection of these 'new scenario' outbreaks has become increasingly important as our food supplies have become centralized, and food is routinely shipped to all corners of the globe. PulseNet, and other similar surveillance programs, depend on strict standardization in laboratory methodology to make possible laboratory-to-laboratory and country-to-country strain comparisons. Rapid development in the field of molecular biology has yielded many technologies competing with PFGE, such as multi-locus variable number tandem repeat analysis, multi-locus sequence typing and subtyping systems based on single nucleotide polymorphisms. New subtyping technologies may offer significant advantages over PFGE such as greater strain discrimination, ability to evaluate strain similarity in a meaningful manner, reduced labor cost, and higher throughput. However, it can be argued that standardization with a single global method has been far more important than the particular method chosen. Because food production and food contamination have truly become a global issue, effective surveillance requires a global testing standard.

Standardization of epidemiological data and methods has the potential for increasing the effectiveness if foodborne disease outbreak detection and investigation for the same reasons that standardization has proved useful for laboratory data. Case interview forms are generally not standardized within countries or even local jurisdictions, making region-to-region or country-to-country comparisons of exposure or demographic data difficult to impossible. To a large degree these differences are necessary, as regional foods and food preparation practices are an important part of our rich global cuisine. Nevertheless, global trade has significantly increased the amount of globally common food exposures, and at each smaller jurisdictional level commonalities would be expected to increase. Standardization of common data elements is a promising new frontier of foodborne disease epidemiology. The Listeriosis Initiative and OutbreakNet projects in the US seek to address this problem by building common data elements among participants. The Council to Improve Foodborne Outbreak Response (CIFOR) has created guidelines in an effort to stimulate global adoption of common best practices (Cifor, 2009).

Finally, integration of food monitoring and animal disease monitoring data with human disease data (described above) has the potential for making all three data streams more valuable. PFGE pattern matches between case isolates and food or animal isolates are used for hypothesis generation in case cluster investigations, and the presence or absence of human disease cases matching regulatory sampling isolates helps food regulatory agencies assess risk.

The Future of Surveillance

More Rapid Diagnosis and Subtyping

Determination of subtype is a critical part of much surveillance. Faster methods for subtyping can speed up the detection of clusters. For example, molecular serotyping of *Salmonella* can be completed in one day, whereas traditional methods take three. A faster protocol for PFGE in PulseNet decreased the time required to one day rather than three. Express shipping of organisms from clinical to public health laboratories can speed up surveillance.

In the near future, a more profound change is likely for some foodborne pathogens. The move toward culture-independent diagnostic testing in clinical laboratories is likely to make surveillance more difficult, and interfere with disease tracking, burden estimation and outbreak detection and investigation. New rapid diagnostic testing technology based on lateral flow (immunochromatographic) strip and enzyme-linked immunosorbent assays can make testing faster, and more available in parts of the world where bacteriological culture is impractical. However, these methods do not yield a bacterial isolate, which means they undercut the isolate-based surveillance programs such as PulseNet. As clinical laboratory practice moves away from traditional bacteriology, it is critically important for public health to develop new subtyping

assays and platforms that do not depend on culture-derived isolates, and to integrate them with the new clinical laboratory practice. The same challenge faces regulatory agencies that depend on the isolation of an organism to take some legal actions.

Faster and More Standardized Interviews of Reported Cases

The patient interview is the other critical element of routine public health surveillance. A detailed interview with the patient provides vital exposure information that can be used to identify the food vehicle in the outbreak setting. It depends on human memory, which places a premium on conducting the interview soon after the illness begins, even before an association with other cases is identified. The initial interview is the best time to collect a detailed food history for the relevant time period preceding the illness, but finding the person and conducting that detailed interview takes time. Many jurisdictions conduct a rapid screening interview lasting no more than 5 min, and then later conduct a more detailed interview when the person's illness is found to be part of an outbreak. This means more subjects forget what they ate. A dedicated case interview team may overcome the obstacles, and obtain detailed information rapidly. As more diffuse outbreaks straddling jurisdictions are detected, it becomes more important to standardize the elements of this interview across jurisdictions, so that the information can be compared and combined rapidly

In future such information may be gathered more efficiently via the internet. An interview could be triggered on diagnosis, and might be integrated into the health care system itself. For infections often transmitted by foods, an initial screening questionnaire collected by self-report could include a standard list of foods and other exposures, as well as an opportunity to schedule a more detailed person-to-person interview.

New Pathogens will Emerge

Unpredictably but reliably new pathogens will continue to emerge around the globe as microbes evolve, changing trade and traffic transfer new foods and pathogens, and changing food production opens up new niches. Conditions that were once unrelated to food may be foodborne after all, like Chagas' disease in Brazil now transmitted by contaminated açai fruit juice. Nipah virus encephalitis in Bangladesh is transmitted from fruit bats to humans via contaminated sugar palm juice. Surveillance systems will need to change constantly to keep up. In the US, infections with the non-O157 shiga toxin producing *E. coli* are now being recognized with at least the same frequency as those due to O157, and surveillance methods need to adapt to capture them. Detailed microbiological investigation of well-investigated outbreaks of foodborne illness of undetermined etiology is a particularly efficient way to identify new or unrecognized foodborne agents. Such outbreak investigations may lead to new pathogen discovery, with methods such as DNAse sequence-independent primer amplification, high density arrays, or metagenomic methods.

The Distinction Between Sporadic Cases and Outbreaks will Diminish

Sporadic cases may often not be truly sporadic single cases, but may be part of far-flung unrecognized clusters. In the modern world, both people and foodstuffs can travel far, and as a result people affected in a single outbreak can fall ill in many different neighborhoods, counties or states. The old highly focal outbreak affecting persons attending one school or one local gathering still occur, but many outbreaks spread across multiple jurisdictions. These dispersed outbreaks are recognized by surveillance that matches up individual strains, and investigated with collaborative teams. Many dispersed low intensity outbreaks may be occurring all the time, generating the background noise of surveillance that we recognize as the status quo.

Collaborative Global Networks will Reveal Global Outbreaks

Growing global collaboration in surveillance networks and investigation is likely to reveal more truly global outbreaks. In a review of foodborne outbreaks associated with international trade over a 7-year period on three continents, two outbreaks among 30 reviewed were identified that affected at least two continents at once. Both were outbreaks causing *Salmonella* infections in Europe and Australia. One was traced to contaminated sesame seed halvah from the Middle East, and the other to dried peanuts from China. For both, detection was aided by *Salmonella* serotyping, which is a universal typing system that has fostered the detection of many outbreaks and is used globally. Use of more standard surveillance and investigative techniques around the world is a critical part of preparing the global surveillance of the future. The collaborative training network called WHO Global Foodborne Infection Network fosters successful basic methods of foodborne disease diagnosis, surveillance, outbreak investigation, and estimates of the burden of illness. Begun in 2000, this program has trained persons in nearly all countries from public health, agricultural and food safety agencies. The global molecular subtyping consortium PulseNet International has trained public health microbiologists in 70 countries, using 7 regional coordinating centers. While still in development, PulseNet International has already played a critical role in detection or investigation of several multi-country outbreaks, such as an outbreak of *Salmonella senftenberg* infections associated with prepacked basil, shigellosis outbreaks associated with catered airline meals and raw baby corn. As more countries build robust programs for surveillance and investigation of foodborne disease and join global subtyping networks, we should expect to find and investigate many more global foodborne outbreaks, which will identify more unsuspected food safety hazards, and lead to progress in reducing the global burden of foodborne diseases.

See also: Bacteria: *Campylobacter*; *Listeria monocytogenes*. Foodborne Diseases: Foodborne Diseases and Vulnerable Groups; Foodborne Diseases in Travelers; Overview of Biological Hazards and Foodborne Diseases; Overview of Chemical, Physical, and Other Significant Hazards; Overview of Emerging Food Technologies;

Prevalence of Foodborne Diseases in Africa; Prevalence of Foodborne Diseases in Australia and New Zealand; Prevalence of Foodborne Diseases in Europe; Prevalence of Foodborne Diseases in North America; Prevalence of Foodborne Diseases in South East and Central Asia; Prevalence of Foodborne Diseases in Western Pacific Region. Public Health Measures: Foodborne Disease Outbreak Investigation; Modern Approach to Food Safety Management: An Overview. Risk Analysis: Estimating the Burden of Foodborne Disease. Viruses: Norovirus

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European Centre for Disease Control – Food and Waterborne Diseases and Zoonoses Programme.

http://www.who.int/gfn/en/
WHO – Global Foodborne Infections Network.

PUBLIC HEALTH MEASURES

Foodborne Disease Outbreak Investigation

J Rocourt, Paris, France

Glossary

Carrier An individual (or animal) who harbors a pathogen but does not exhibit clinical symptoms of disease and may be a source of infection.

Case An infected person having specific clinical, laboratory, and epidemiologic characteristics.

Cluster A cluster is a small group of cases of one disease that may be linked by time and geography. In the context of foodborne disease, after an investigation of a cluster, cases may be linked by a common food or food premise and may be described as an outbreak.

Common- or point source outbreakAn outbreak of disease in which susceptible individuals are exposed simultaneously to one source of infection. Foodborne disease outbreaks are most often point source outbreaks. The epidemic curve for this type of outbreak is characterized by a sharp rise in the number of cases that slowly decrease. Most illness appears within one incubation period.

Continual source outbreak An extended outbreak of disease or illness that occurs when a source remains contaminated and individuals continue to be exposed to this source. The epidemic curve for this type of outbreak is characterized by ongoing peaks overtime (e.g., weeks and months).

Epidemiological evidence The demonstration of an association between a food and human illness through an increase in the number of cases in a population, place, or timeframe with exposure to the same food product; or a statistically significant association between illness and food consumption.

Foodborne outbreak Refers to two or more cases resulting from ingestion of a common food (epidemic is often reserved for crises or situations involving larger numbers of cases over a wide geographical area).

Propagated source or person-to-person outbreak An outbreak of disease that spreads from one person to another via the fecal–oral route. The epidemic curve for this type of outbreak is characterized by a relatively slow, progressive rise. The curve will continue for the duration of several incubation periods of the disease.

Sporadic case A case that cannot be linked epidemiologically to other cases of the same illness.

Foodborne Outbreaks

A foodborne illness (or disease) is any illness of an infectious or toxic nature caused or thought to be caused by eating or drinking a contaminated food or beverage. A foodborne illness outbreak is defined as: (1) an occurrence of two or more cases of a foodborne illness linked to a common source or event, resulting from the natural, accidental, intentional, or malicious contamination of foods by microbiological, chemical, inert physical hazards, and other similar substances; or (2) an unexplained, unexpected increase in the number of a similar illness, and food is a likely source. However, a single case for certain foodborne illnesses such as botulism or chemical poisoning, justifies an in-depth investigation.

The size and scope of a foodborne outbreak can greatly vary according to the pathogen or toxin involved, the volume of food which is contaminated, the step of the food chain where contamination occurs, where the food is consumed, and the number of people who eat it. Briefly, there are two major types of foodborne outbreaks:

- *A common source outbreak*: A small, acute, and highly local outbreak, which happens when several people get sick after eating, for example, at a classic church dinner, wedding reception, or other social event. The outbreak is immediately apparent to those in the local group who promptly contact medical and public health authorities. Attack rates are high and food contamination, with high levels, occurs just before consumption because of a terminal food-handling error in a kitchen.
- *A community exposure outbreak*: Regional, national, or international diffuse outbreak caused by a commercially distributed food product, as a result of increasingly centralized production and wide distribution of large volumes of a great diversity of raw and processed products moving across boundaries (people are ill with similar symptoms, a pathogenic microorganism is involved, but there is no known exposure that links these people together). Attack rates are low and surveillance data must be rapidly compared over increasingly broad regions to detect cases as the increase in cases may be inapparent against the illness background. Food vehicles are contaminated early in the

production process or distribution chain, with low levels of pathogens. Such outbreaks require a large investigation team to clarify the extent of the outbreak, identify a specific food, and determine the source of contamination. Controlling and preventing such outbreaks can have industry-wide implications.

The objectives and the overall structure of investigation are the same for both types of outbreaks.

The scope of this document is intended to provide a short overview of the investigations of foodborne outbreaks, which go through several steps that are described here in a certain order, but in reality investigations are dynamic and several steps may happen at the same time.

Stages in an Outbreak Investigation

The primary reason of the investigations is to protect public health by mitigating or containing the effects of a foodborne illness outbreak in a timely and effective manner, which means: (1) reducing to the minimum the number of primary cases of illness, (2) reducing to the minimum the number of secondary cases of infection, by identifying cases and taking appropriate action to prevent any subsequent spread, and (3) preventing further episodes of illness by identifying continuing hazards and eliminating or minimizing the risk they pose.

These investigations include identifying the etiologic agent, people at risk, the mode of transmission and vehicle, the source of contamination and contributing factors, determining the potential for ongoing transmission, implementing specific interventions or corrective actions that will stop the outbreak, fulfilling statutory obligations and responding to public and political concern, and evaluating existing recommendations or strategies for preventing similar outbreaks in the future.

Keys for Successful Investigations

A Multiagency, Multidisciplinary Team

There are three major components in foodborne disease outbreak investigations:

- epidemiologic investigation to characterize the disease, determine the etiology of the outbreak, identify risk factors for disease, and implicate foods and/or food handlers as transmission vehicles through descriptive and analytical studies;
- environmental assessment to determine routes of contamination and contributing factors and recommend appropriate control; and
- laboratory analysis to provide definitive etiologic diagnosis by testing human and environmental specimens.

All these steps will frequently identify applied epidemiologic and food safety research.

A proper foodborne outbreak investigation cannot be conducted without these components, which are often performed simultaneously. The different steps that include identifying and investigating an outbreak, conducting traceback and source investigations, developing and implementing control measures, and taking steps to prevent recurrence require the efforts of a team of individuals with different areas of expertise, such as epidemiology, sanitation, food control and safety, clinical medicine, clinical and food microbiology and chemistry, public health, law enforcement (in case of intentional contamination of food), risk management, and communication. This means that no single agency has the expertise, resources, or authority to complete an investigation from beginning to end; public health and food agencies in charge of foodborne illnesses must, therefore, operate together in good partnership, sometimes on several administrative levels depending on the nature of the outbreak. The identification of key stakeholders is crucial and depends on the size and scope of the outbreak (number of cases and population at risk, causative agent and severity of the disease, the location and extent of the outbreak, public concern, media impact, potential public health problem, etc.).

Communication

- *Communication within the team*: Successful investigation of an outbreak depends on the close communication, coordination, and collaboration in a consistent and timely way within the multidisciplinary team. Roles and responsibilities of each investigator and his corresponding agency should be very early and clearly defined. A comprehensive system of communication among investigators – epidemiology, environmental health, and public health laboratory – and their respective agencies is critical in ensuring concurrent activities do not interfere with each other and guide the activities of individual investigators, in controlling outbreaks and preventing additional illnesses. Especially regarding community exposure outbreaks, it is important that information is communicated to other involved agencies when the outbreak is detected and investigation is ongoing rather than waiting until it has been completed.
- *Public communication*: Ensuring that communication activities reflect general risk communication principles as well as emergency and crisis communication practices are a crucial aspect of successful outbreak management. This means early, regular, and accurate communication by experienced personnel who share relevant information with at least:
  - health, food, water, agricultural, and veterinary authorities to ensure accurate case finding and facilitate the implementation of control measures;
  - the media who are a major interface between the general public and health authorities and can play a role in outbreak investigation and control; and
  - the general public and those at greater risk (regular press releases via newspapers, radio, television, Internet, etc.): information on implicated food products and how they should be handled and advice on hygiene measures to reduce the risk of spread.

Surveillance

Beside the well-known means of detection of 'traditional' outbreaks – i.e., associated with a local event – such as

notification by citizens, clinicians, laboratories, review of passive surveillance, etc., the development and enhancement of current surveillance systems of foodborne disease play a major role in detecting clusters of cases spanning large geographical areas in terms of time, place, or patient characteristics and the linkage of seemingly unrelated cases. Early detection will maximize control and prevention efforts. Among the various surveillance methods, laboratory reporting and disease notification contribute importantly to outbreak detection (in spite of underreporting of cases and long delays in notification). Disease notification is the collection of reports of cases of illness required by law, which include information on symptoms, on demographic characteristics, and key risk factors. Laboratory-based surveillance relies on the collection of information about microorganisms that have been previously identified by laboratory testing of ill persons. The systematic and timely analysis of data can provide useful information for detecting outbreaks, particularly when cases are geographically scattered or clinical symptoms are nonspecific. Detecting widespread outbreaks is also facilitated by early typing of isolates, which may detect a surge of a particular subtype and link apparently unrelated infections. This has been frequently demonstrated by PulseNet, which is a US network of local, state, and national public health and regulatory agency laboratories that use pulsed-field gel electrophoresis (PFGE) with standardized protocols for subtyping of case isolates to rapidly identify groups of isolates that have the same PFGE pattern, which may indicate that the cases have a common origin. In addition to epidemiologic and laboratory surveillance, monitoring of food products by conducting facility inspections, sampling foods, and analysis of consumer complaints helps in identifying food processing deviations.

Standardized Documents

Planning and preparation activities are essential and investigations should be organized in a standardized approach that is critical in saving time and resources and responding in an effective manner to foodborne alerts. Any implicated agency should develop a procedure manual that includes a number of standardized documents such as guidelines, procedures, questionnaires, forms, etc., which can be adapted with minimal efforts to each outbreak hypothesis: list of staff to constitute the outbreak investigation team with their responsibilities/tasks; list of contacts who may become involved; outbreak investigation flowchart; notifiable disease form; case definition; questionnaire for epidemiologic investigation; clinical and food microbiological protocols for sampling, swabbing, labeling, storage, transport, detection, and identification of foodborne pathogens; checklist for food inspectors; general and pathogen-specific food vehicle-specific questionnaires; templates for sharing information with other agencies; communication protocols for health-care professionals, consumers, media and industry; final report, etc. This enables investigators to become proficient and ensures that pertinent information is collected; staff should be trained in the use of these items. All information received and decisions taken by the investigation team should be recorded reliably and with the appropriate level of confidentiality, and special attention should be paid to the chain of custody, the validity of sampling procedures, as well as the handling of samples being increasingly under scrutiny in legal cases. The means by which these procedures are carried out may vary depending on number and experience of staff, resources, type of outbreak (common source outbreak vs. community exposure outbreak), etc.

Epidemiologic Investigation

The main objectives of an outbreak investigation are to (The Centers for Disease Control and Prevention (CDC; Atlanta, GA, USA) developed a computer software program called Epi Info™, which can be used to prepare questionnaires and analyze data. (The software is free, a copy of which can be obtained via the Internet.)

1. collect information related to the outbreak to identify cause and the source of an outbreak;
2. limit the exposure of consumers;
3. implement corrective measures, such as recall of products, information to consumers, and diagnosis and treatment of affected persons; and
4. develop conclusions and recommendations for control and prevention of further outbreaks.

Establishing the Existence of an Outbreak

Foodborne disease outbreaks may be identified from various sources, such as complaints from private citizens, medical examination of ill individuals at hospitals, clinics or physician offices, regular surveillance of reportable foodborne disease by epidemiologists, laboratory-based pathogen-specific surveillance, laboratory subtyping surveillance, information received through the media and public information officers, reports from food safety regulators and environmental health specialists, etc.

Most reports of foodborne illness are sporadic and are often not associated with a recognized outbreak. To determine if there is an outbreak, the current number of new cases is compared with incidence data of the same disease over a similar time period. If the number is unusually large or unexpected for the given place and time, an outbreak may be occurring (it must be made sure that a rise in numbers is not due to changes in reporting procedures, case definition, diagnostic methods, increased awareness, etc.).

Following detection or reporting of a suspected outbreak, further information is collected to confirm and describe the outbreak. Confirmation of the existence of an outbreak requires verifying that the diagnosis is correct and common to all cases. Detailed information should be collected as soon as possible – basic demographic information, 3-day food history, symptoms, hospitalization, treatment and test(s) ordered and/or results, large gatherings attended (e.g., common meals, social events, information on any contacts, including those who also are ill, etc.).

The preliminary information collected during the initial report may help determine if an investigation is necessary or not, may also provide important clues about the cause and source of the outbreak and help guide the direction of the investigation.

Defining Cases and Conducting Case Finding

As the outbreaks and their corresponding investigations can quickly become complex, it is important to establish a clear understanding of the outbreak as early as possible. Organizing the preliminary information will help in the development of a case definition and may also provide clues about the pathogen, its transmission, and source.

After verifying the diagnosis by (1) obtaining appropriate clinical histories by interviewing ill persons, family members, and/or physicians to record all relevant symptoms, possible exposures, and other details that might reveal the disease in question and (2) performing laboratory analysis (patient and/or environmental samples) to confirm the etiologic agent, a case definition is developed. It is a standard set of criteria to be used in the outbreak investigation to decide who is a case and who is not and includes information about simple and objective clinical criteria, pathogen or toxin if known, laboratory results, and essential elements of person, place, time, etc. The initial case definition may be refined as new information becomes available during the course of the investigation. Separate definitions may be developed for confirmed, probable, and suspected cases.

Using the case definition and a standardized questionnaire (demographic information, occupation, date of onset and symptoms, laboratory results, medical treatment, list of foods recently consumed, travel history etc.), investigators search for additional cases related to the outbreak by reviewing surveillance reports, requesting local clinical and laboratory professionals to notify cases of the particular illness more quickly, reviewing emergency room records or nursing homes for similar illnesses, obtaining lists of attendees from social events/functions, asking health officials in surrounding areas for illnesses that might be related, and alerting the public directly through the media if needed. This allows a more accurate estimate of the magnitude of the outbreak, reduces the likelihood of bias that may occur by focusing on cases detected early, and increases the sample size (and consequently the statistical power to identify risk factors).

At this point, the investigator is searching for common associations based on time, place, and person. Two major tools are used to arrange all incoming data: an epidemic curve and maps which should be regularly updated.

An epidemic curve is a graph that depicts the association of the time of illness onset of all cases that are associated with the outbreak. Time is plotted on the horizontal axis and the number of cases is plotted on the vertical axis. An epidemic curve provides information about the extent of the outbreak, potential period of exposure, and possible mode of transmission. Its shape may suggest what kind of outbreak is occurring: a common source or point source outbreak, propagated source or person-to-person outbreak, and continual source outbreak.

Maps and pictures are helpful in showing the geographical location or layout of the place in which an outbreak has occurred. The 'spot map' is a pictorial description of the spatial distribution of cases within a specific setting or geographical area. The cluster of cases may indicate a local exposure in the community as restaurant, place of employment, school attended, or in contrast a wide geographical area in case of continual source outbreak, for example. This spatial information may be crucial to the outbreak investigation and may provide clues about the source of the agent and/or nature of exposure.

At the end of this preliminary phase, a careful decision will be needed about whether or not to continue the investigation; it may be apparent at this stage that there is no further public health risk or in contrast that further action is needed.

Developing Explanatory Hypothesis about Likely Sources

Using the information previously gathered, the next step is to consider which specific exposure(s) may be the source of the disease and develop a hypothesis (or several hypotheses) to guide the direction of the investigation and initiate appropriate control measures. A useful hypothesis is testable, sensible, and fits the full picture of what is known about the cause of the outbreak and factors that may have contributed to illness.

Analysis of data collected from the interviews with patients (symptoms, incubation and recovery periods, and food items consumed), laboratory (characteristics of pathogens), and inspection of the premises provide insights into the source and cause of illness. Formulating hypothesis about the likely source of the outbreak should begin early in an outbreak investigation to narrow the focus of the investigation (by preparing a short list of suspected food and drinks) and uses time and resources most effectively. It should be an iterative process in which possible explanations are continually refined or refuted as more information becomes available. The sooner those hypotheses are developed, the sooner public health interventions and appropriate control measures may be instituted.

Achieving a hypothesis is often difficult and may take more time for outbreaks distributed over very large geographic areas; the delay inherent in surveillance systems contributes to the time needed to recognize clusters; as a consequence, food history interviews of cases, which are highly dependent on their memories, may be imprecise and this is especially challenging given the vast variety of existing foods and their ingredients. Investigation of restaurant clusters may be particularly informative in this type of outbreaks.

Developing Epidemiological Studies to Test Hypothesis

The goal is to compare the characteristics of a group of well persons with those of ill persons to quantify the association between specific food exposures and disease under investigation; this is done using a questionnaire that is designed specifically for the common exposure. This type of evidence may also be useful to further guide the environmental and/or laboratory investigation. The most commonly used analytical methods during foodborne outbreak investigations are cohort studies and case–control studies. The study design is usually chosen according to the characteristics of the outbreak.

Retrospective cohort studies are feasible for outbreaks in small, well-defined populations in which all exposed and nonexposed persons are identifiable (e.g., a church supper). In this study, the risk of illness is compared by what was eaten and what was not eaten. The outbreaks analyzed using cohort studies are usually small; they require significant resources as all exposed and unexposed individuals are interviewed.

In many circumstances, no clearly defined group of all exposed and nonexposed persons can be identified. In such

situations (primarily when the illness is rare or not easily identified or when it is easier to select participants for the study based on the illness status), case–control studies are used (e.g., when a widely distributed, branded food commodity is implicated in a nationwide outbreak). In this study design, cases and controls (nonill individuals) are compared to determine the likelihood of having eaten specific foods. Ideally, controls should be similar to cases and represent the same population except they do not have the disease.

Using appropriate statistical tests, the investigators determine the strength of the association and whether more than one food might be involved. Additional studies to supplement the epidemiological study might also be conducted at the same time: an environmental study to help determine the source of exposure and mode of transmission; and laboratory studies to confirm that a particular microorganism or chemical agent is present in clinical and/or environmental samples.

Analyzing the Data Collected and Interpreting Results

Analyzing epidemiologic investigation include: (1) reevaluating the case definition and ensuring that persons classified as cases meet the case definition; (2) updating epidemic curves; (3) calculating frequencies and percentages, median, and ranges for the incubation period and recovery period, the attack rate, food-specific attack rates, and relative risk ratios for a cohort study or odds ratios for a case–control study.

This information should then be integrated with the findings obtained during the environmental assessment and laboratory analysis. General knowledge about foodborne illnesses should be collectively used to help explain what happened, what measures should be taken immediately, and what steps should be selected to prevent similar situations from occurring in the future.

For a causal relationship the following criteria should be met: (1) the epidemiologic investigation obtained evidence from most of the people in the study; (2) the time relationship between the consumption of the food item and onset of illness is consistent with the incubation period of the disease under investigation; (3) the probability of the association being due to chance is less than 1% ($p = .01$) if there are several food items being tested, or less than 5% ($p = .05$) if the study began with a hypothesis of an association with a single food item; and (4) no important causes of bias in the conduct of the investigation was detected. Finding no statistical association does not mean that the outbreak was not foodborne but only that the source could not be identified.

Further investigations would likely be required for the following circumstances: outbreak is still ongoing, insufficient information to implement control measures, etiological agent, source and mode of transmission unknown, new or unusual etiological agent, and high public/media interest.

Environmental and Food Assessment

The primary goals of an environmental investigation are: (1) identifying the source, mode, and extent of the food contamination; (2) assessing how the causative agent could have survived; (3) determining whether conditions were conducive for subsequent growth or toxin production by the causative agent; and (4) identifying and implementing corrective interventions. This investigation step may involve one food facility or several. It is conducted in parallel with epidemiological and laboratory investigations.

An early visit to implicated food premise(s) should be made as soon as possible as the amount of physical evidence will diminish with time. The range of information obtained during the investigation will depend on the nature and size of outbreak, the causative agent, the type of establishment involved (catering operations, food manufacturer, health-care facilities, day cares and schools, community functions, etc.), the resources available and local priorities; as much information as possible should be obtained. During investigations of diffuse outbreaks spread over wide geographical areas, traceback extends beyond the immediate preparation of the implicated food to the whole food chain and cooperation at all levels of industry is required.

To prevent future occurrences, the establishment(s) should be regularly revisited after the outbreak to assess how the recommended specific measures are implemented.

Investigation of a Food Establishment

During a foodborne disease outbreak, investigation of a food establishment will often require:

- interviewing food workers (managers and any employees who may have had a role in the processing or preparation of suspected foods);
- collecting information on menus, production schedules, food sources and supplies, water system, processes (cooking, storage, service, possible cross-contamination sites, temperature and processing records (including work schedules)) and on the overall operations and hygiene with details of food safety management systems (such as Hazard Analysis Critical Control Point (HACCP));
- producing a map of the premises, showing its layout and food flows and pathways; factors such as the location and availability of sinks and appropriate hand washing facilities, and determining whether separate areas are maintained for the preparation of raw and ready-to-eat foods may be crucial in the understanding of food contamination;
- reviewing employee sickness records and medical screening of food handlers and staff training.

To save time and resources, the sanitary investigation should be guided by what is already known about the outbreak from epidemiological and laboratory investigations and about known reservoirs for the suspected causative agent.

Investigation of a Suspected Food

When the role of a suspected food is investigated, the purpose of the traceback investigation is to identify high-risk food preparation and handling practices that contributed to transmission of a particular disease in an outbreak. Main steps are the following:

- Preparing suspected product description (list of all raw materials and ingredients used, sources of the ingredients, physical and chemical characteristics including pH and water activity, use of leftover foods in processing, intended

use, product lot numbers, expiration dates and sales records, volume prepared, preparation schedule, etc.).

- Observing process: The food inspector should determine the three hazard categories that pose the greatest potential for foodborne illness:
  - Contamination hazards: Food source (food from unsafe sources, adulterated food, etc.), cross-contamination (raw foods not well separated from ready-to-eat foods, equipment not properly cleaned and sanitized, etc.), poor personal hygiene (lack of hand washing, bare hand contact with ready-to-eat food, ill food workers, etc.), environmental contamination (improper storage, usage of chemicals, presence of insects or rodents, lack of potable water, improper sewage disposal, etc.).
  - Survival hazards: Inadequate cooking, unfit reheating temperatures, etc.
  - Bacterial growth or toxin production hazards: Improper holding, unsafe cooling, or inadequate refrigeration, improper cold/hot holding temperatures, preparation several hours before serving, etc.

When conducting a traceback investigation, additional information should be collected such as label and package information, product name, package code/lot number, expiration/sell by/use by date, date of purchase, etc.

- *Interviewing food handlers*: Food handlers are persons who directly handle or prepare food. They may work as paid employees or volunteers, serving food in a variety of settings. Food handlers have an important responsibility to follow safe food preparation and handling practices to prevent illness. Poor hygiene practices among food workers continue to be a major contributing factor to foodborne illnesses. Analysis of more than 800 foodborne outbreaks demonstrated that the most frequently reported factor associated with the involvement of the infected worker was bare hand contact with the food followed by failure to properly wash hands, inadequate cleaning of processing or preparation equipment or kitchen utensils, cross-contamination of ready-to-eat foods by contaminated raw ingredients, and (for bacterial pathogens) temperature abuse. All food handlers should be carefully interviewed about food practices of the suspected food during the relevant period (exact flow, preparation, handling, unusual circumstances, practices, etc.), recent illnesses and knowledge and training in food safety. Although workers have been implicated in outbreaks, they were not always aware of their infections, either because they were in the prodromic phase before symptoms began or because they were asymptomatic carriers. Specimens for microbial analysis should be obtained from food handlers who are ill. If the same type of pathogen is recovered from specimen of a worker and the suspected food, it is essential to determine whether he or she is a potential source of the problem or is infected because of having eaten the same food. Because of the potential for food handlers to transmit pathogens through the food they serve, work restriction and exclusion requirements have been established for infected food handlers in a number of countries.
- *Taking appropriate measurements*: To understand circumstances of food processing and contamination with a pathogenic agent, some measurements can help such as time and temperature conditions, water activity, and pH.
- *Drawing a flowchart of the operations and conducting an outbreak hazard analysis*: After getting a complete history of the suspected vehicle and its environment, all data collected should be entered on a flowchart to facilitate the assessment of factors that may have contributed to the outbreak. The flowchart should show the flow of the different operations of the suspected food, equipment used, results of measures taken, name of persons performing operations, etc. This allows to conduct a hazard analysis and identify critical control points. If practices at the time of the outbreak can no longer be reconstructed, a flowchart of current practices may be useful.

In case no source of contamination at the place of preparation is identified, the possibility that contamination may have occurred before the food or ingredient arrived at the establishment should be documented. The simultaneous occurrence of multiple outbreaks due to the same pathogen at different places is often evidence of primary contamination by raw foods that may commonly be contaminated.

Managers of the implicated establishment(s) or company/companies will be kept informed of developments by the inspection authority.

Laboratory Analysis

Both epidemiological and food safety investigations usually involve laboratory testing. The purposes are: (1) identifying or confirming the clinical diagnosis through identification of the causative agent (frequently microorganism and more rarely chemical agents) from human samples; (2) ensuring proper identification of the disease; (3) determining if the causative agent is present in the implicated food and/or its environment; and (4) tracing the sources of, and evaluating the extent of, contamination of food that may be associated with the outbreak.

At any stage of the epidemiologic investigation, advice can be provided on potential or likely microorganisms or toxins that may be causing the outbreak, the appropriate specimens to collect, and timeliness of tests that can be used for diagnosis and typing. Laboratory identification of a pathogen can quickly validate a hypothesis and allow easier implementation of control and preventive measures.

One of the most important factors in the identification of etiologic agents responsible for foodborne disease outbreaks is the collection and analysis of clinical specimens as early as possible, at the time of first contact with the patient. Most foodborne infections are diagnosed through the identification of the pathogen in stool (vomitus samples can be used in some cases; blood cultures and serology for systemic infections); other specimens such as swabs from rectum, nostrils, skin, or nasopharynx may be collected from food handlers. Because pathogens or toxins may remain in the intestinal tract for only a short period of time after illness onset, stool specimens should be collected within 48–72 h after onset of symptoms during the period of active diarrhea (and whenever possible from individuals who have not received antibiotic treatment). Once the diagnosis has been confirmed

by laboratory tests, there is usually no special need to obtain additional samples if individuals manifest characteristic symptoms.

The main objective of the food and environmental testing is to support the epidemiologic investigation in detecting the pathogen in the suspected food and understanding how and why the outbreak occurred. The value of laboratory results in food microbiology depends on the quality of the samples submitted. Sampling and laboratory analysis of foods should be oriented by epidemiologic and environmental investigations to avoid wasting time and resources. Suspected foods (raw items, ingredients, leftovers, unopened packages, foods known to be associated with the causative agent, etc.) and environmental samples (work surfaces, equipment, containers, hand-washing facilities, etc.) should be collected early in the investigation as the amount of physical evidence will diminish with time. If an implicated food has not been identified at the time of sampling, a large number of specimens may be collected and stored for subsequent laboratory testing as additional information becomes available.

Molecular methods have contributed substantially to improve foodborne disease outbreak investigation. Polymerase chain reaction technology is increasingly used for the rapid detection and identification of pathogens. Comparison of PFGE patterns of isolates from clinical and food specimens can provide additional evidence to support epidemiologic data; PFGE can also be used to include additional related cases and exclude cases that are epidemiologically unrelated to an outbreak. Genetic sequencing technology is becoming more and more useful for assessing the relatedness of various foodborne pathogens.

Enhanced laboratory testing of clinical, food, and environmental specimens is important to confirm the pathogen of a foodborne disease outbreak, and therefore should always be considered as a high priority. However, confirmed laboratory identification is not needed to start an investigation, and outcomes of an investigation may be based solely on epidemiologic evidence (food with a short shelf life no longer available, leftover foods or foods in open containers may have been contaminated after opening, pathogen difficult to detect because of low number of cells, heterogenous distribution of the pathogen in food, overgrowth by other organisms, no test to detect a new pathogen, etc.).

Control Measures

The priority should be to implement effective control measures to prevent future foodborne cases as early as possible in the course of the investigation based on the initial hypotheses. Control measures and possibly regulatory actions should be considered at any time during the investigation and implemented as soon as new evidence is available. Ideally, control measures should be based on the results of all investigations but this may delay the prevention of further cases. Public health officials may not wait for laboratory proof of contamination, especially during diffuse and widespread outbreaks, and decide on control measures on the basis of strong epidemiologic evidence on the origin, spread, and development of the disease. This practice results in earlier action to protect the public's health. However, before initiating a control measure, it is necessary to consider the effectiveness, timeliness, costs, availability of resources, personnel requirements, and possible secondary consequences of proposed actions. Specific interventions – such as recalling a food product or closing food premises – can have very important economic and legal consequences.

Control measures will depend on the mode of spread and are dictated by the particular circumstances of each outbreak. They can be implemented through three main areas: (1) the outbreak source (e.g., modification of a food production or preparation process, exclusion or restriction of food handlers who are at high risk of spreading illness, closing of food premises, prohibition of the sale or use of certain foods, etc.); (2) the vehicle of transmission (recall/seizure of contaminated product, modified handling, or cooking instructions, etc.); and (3) human transmission (exclusion of cases from school or work, protecting groups at risk, advice on personal hygiene, food safety education of food workers, etc.). Continued monitoring of the control measures is essential to ensure that the measures are effective in the long term.

Preventing reoccurrence of outbreaks requires the implementation of a well functioning and integrated food control system, with collaboration among all the components, including food law and regulations, food control management, inspection services, food monitoring, laboratory services, and education of the consumer.

Deciding the End of the Outbreak, Debriefing, and Final Report

Information collected from the epidemiologic investigation, in combination with the environmental investigation and laboratory testing results, should be regularly reviewed to determine whether the outbreak investigations can cease or whether further investigations are required. Further investigations would likely be necessary if the outbreak is still ongoing, if there is insufficient information to implement control measures, in case of unknown, new, or unusual etiological agent and/or transmission mode, and if there is a high public/media interest.

An outbreak ends when illnesses are declining and the number of new illnesses reported returns to the number usually expected. Surveillance continues even after to make sure the outbreak is over and detect possible new cases associated with an incompletely controlled source or a secondary contamination involving another food or location linked to the first outbreak.

The postoutbreak debriefing/reviews should be organized in a timely manner after the resolution of the outbreak and are useful, especially to consider any lessons that can be learned from the outbreak and its management, and to discuss dissemination of this information to prevent future outbreaks. After analysis of epidemiologic, laboratory, and environmental data, conclusions should be summarized in a report. The report should also include a root cause analysis of the incident, i.e., identifying the underlying factors which have led to the failures causing the incident, for example, weakness in inspection of food establishments, gaps in legislation, or failures in training or education of food handlers.

This report should be written in a scientific publication format, should clearly reflect the complexity of the outbreak, and should improve the quality of future investigations. It includes, at least, a description of the outbreak, data on confirmation of the outbreak source, methods to prevent a reoccurrence, difficulties met in implementing the control measures, assessment of the effectiveness of outbreak control measures, discussion of any legal issues that may have arisen and possible needs for further scientific studies. Data must be protected from public disclosure to the extent allowed by law so that they can be shared with other agencies and it may be wise to seek legal advice about production of final reports in case of potential legal proceedings. The report is distributed to all investigators, to appropriate local and national authorities, and individuals; it should also be provided to the managers of any facility involved in the outbreak for prevention measures and education of food workers. Synopses may also be used for press releases and postings on websites.

Finally, proper reporting of the investigation includes also notification to the national foodborne outbreak surveillance system whose aim is to provide data to improve understanding of the human health impact of foodborne outbreaks and identify the most frequently involved pathogens, foods, settings, and contributing factors.

Conclusion

The careful and meticulous investigation of foodborne and waterborne outbreaks is essential for disease control and prevention. However, too often, outbreaks of foodborne disease go unrecognized or unreported or are not investigated. Analysis by CDC of data on foodborne outbreaks reported in 1998 and 1999, which occurred in the US in FoodNet surveillance areas, indicates that in 71% of these outbreaks, no confirmed etiology was identified, and in 46%, no suspected food vehicle was identified. Main reasons include delay in the outbreak notification, insufficient staff or resources, no specific food hypothesis, too small number of cases for an analytical epidemiologic study, or lack of memory of what has been eaten by people, negative food testing. In resource-limited countries, outbreaks of foodborne disease are poorly investigated, if at all, mainly because appropriate skills and resources are unavailable. Developing web-based alert systems, enhancing current surveillance systems to identify large outbreaks as early as possible, establishing multiagency/multidisciplinary foodborne outbreak investigation teams with role and responsibilities clearly identified, working with specific written protocols/procedures, standardization of investigations methods and of sharing reports, training of investigators by online courses, especially for food traceback, improving communications between agencies are some developments to be considered worldwide.

Most foodborne etiologic agents were discovered in the course of outbreak investigation. An important objective of an outbreak investigation, which is a unique comprehensive source of information, is to identify and resolve unanswered scientific questions about foodborne disease and pathogens; well-conducted investigations may provide significant information on the natural history of the disease, reveal new vehicles, transmission modes, contributing factors, groups at risk or specific risk factors, and improve our understanding of food processing and how the contamination occurred at specific points in the food supply. Field investigations provide unique opportunities to address research issues, such as new detection, diagnosis, and typing/subtyping methods, studies on the behavior, resistance, or elimination of the pathogen in food, the emergence of new antibiotic resistance; case–control study of sporadic cases to identify risk factors evidenced during outbreaks. Data derived from outbreaks can be used for risk assessment, particularly for dose–response and source attribution. New knowledge on food safety may also be gained by monitoring the impact and effectiveness of control measures. Such understanding not only facilitates future investigations but is also essential for developing effective long-term control strategies. By updating regularly our scientific understanding about agent, disease, host, and environment, outbreak investigations play a key role in preventing foodborne disease, demonstrating the need for new public health or regulatory policies and/or broader education efforts – of the public, the food industry, or health-care providers. In addition, outbreak investigations can also provide valuable information to ongoing surveillance activities, may be used to evaluate and improve public health programs, and guide future strategies in these areas; epidemiologic investigations can also contribute to teaching, as an example of the systematic and logical approach an epidemiologist follows in an investigation, and a valuable on-the-job training and experience for future outbreaks.

See also: Disciplines Associated with Food Safety: Epidemiology. Food Safety Assurance Systems: Hazard Analysis and Critical Control Point System (HACCP): Principles and Practice; Investigation of Incidents in Industry; Personal Hygiene and Employee Health; Recall Systems and Disposal of Food. Public Health Measures: Alerts and Early Warning Systems; Environmental Assessment in Outbreak Investigations; Food Control and Public Health Laboratories; Modern Approach to Food Safety Management: An Overview; Surveillance of Foodborne Diseases

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PUBLIC HEALTH MEASURES

Environmental Assessment in Outbreak Investigations

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Glossary

Contributing factors These are determinants that directly or indirectly cause an outbreak. A contributing factor can be biological, behavioral, or attitudinal; or an element of the physical or social environment; or the result of policies related to the problem. Examples include retort, pasteurization, or cooking temperatures that do not destroy or reduce pathogens, poor personal hygiene of food workers, or cross-contamination. Contributing factors are what happened to cause a foodborne outbreak.

Critical control point (CCP) A step at which control can be applied and is essential to prevent or eliminate a food safety hazard or reduce it to an acceptable level.

Critical limit (CL) A criterion that separates acceptability from unacceptability.

Environmental antecedents These are supporting factors for the contamination, survival, or increase of biological or chemical agents in food. They may be related to people, equipment, food process, food type, economics, or other circumstances. In other words, antecedents are the reason why the contributing factors occur. Antecedents are sometimes referred to as root causes of foodborne outbreaks.

Environmental investigation It is a generic term used to refer to all aspects of the environmental component of an foodborne illness outbreak (FBIO) response. It encompasses the environmental assessment and/or traceback activities.

Foodborne illness outbreak (FBIO) environmental assessment (EA) The systems-based component of an FBIO response that fully describes how the environment contributed to the introduction and/or transmission of agents that cause illness or could cause illness. Environment is everything external to the host, including air, food, water, animals, plants, climate, etc., as well as people and the social and built environments. All aspects of the external environment can be listed as variables that, in relation to transmission, are neutral, conducive, or protective. From this description, contributing factors and environmental antecedents to an outbreak can be determined.

Hazard analysis critical control point (HACCP) plan A document prepared in accordance with the principles of HACCP to ensure control of hazards that are significant for food safety in the segment of the food chain under consideration.

Traceback investigation An investigation activity that is conducted to support the epidemiological investigation in determining the likely food vehicle and/or the location of the food contamination, such as the point of final service, production, food source, etc.

Introduction

For decades, public health and food regulatory agencies have been investigating foodborne illness outbreaks (FBIOs) to identify and understand their etiology. There are three critical components of an outbreak investigation: epidemiological, laboratory, and environmental. The epidemiological and laboratory investigations have typically focused on identifying the pattern of illness, the pathogen, and the food associated with the outbreak. There are accepted epidemiological and laboratory procedures and methods for conducting these components of FBIO investigations. Some organizations have compiled and published the find ings from these investigations. Data from these reports are sometimes used to measure the burden of foodborne dise ase within a population, to characterize the agents involved, and to suggest food safety priorities. The epidemiological and laboratory components of a foodborne outbreak investigation are discussed in more detail elsewhere within this encyclopedia.

Investigations of FBIOs are historically conducted by local and state/provincial public health agencies. FBIO response requires a multidisciplinary approach involving laboratorians, epidemiologists/communicable disease control authorities, and environmental health/food regulatory personnel. This multidisciplinary team's focus is to:

- stop an outbreak quickly if it is ongoing;
- understand what happened to cause the outbreak (i.e., contributing factors);
- implement immediate measures to prevent the ongoing contamination of food;
- understand why the outbreak occurred (i.e., environmental antecedents);
- implement long-term measures to prevent future outbreaks in the establishment; and

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- use the information from the outbreak investigation to inform public policy on preventing future outbreaks.

The team may include – in addition to epidemiologists, clinical and environmental laboratorians, and environmental public health professionals – microbiologists, food technologists, engineers, hydrologists, geologists, veterinarians, and others, as outbreak circumstances warrant.

Detection of FBIOs is accomplished through laboratory-based surveillance for reportable diseases and through consumer complaint follow-ups. In the USA, more FBIOs are identified through consumer complaints than through laboratory surveillance. In either instance, once an outbreak has been detected, epidemiological investigations with laboratory support are conducted to verify that an outbreak has occurred and to determine the outbreak agent and vehicle. Other investigation activities, such as environmental or traceback investigations or other environmental activities, may follow.

Environmental assessments (root cause analysis) determine the contributing factors and environmental antecedents that led to the outbreak and/or to support the epidemiological investigation as needed. Traceback investigations support the epidemiological investigation when the source of contamination, such as the point of final service, production, etc., has not been determined or when there is a need to assist epidemiologists in determining the likely vehicle. Traceback investigations may follow the vehicle back through the farm-to-fork continuum to determine the source of contamination; when the food vehicle has not been identified; such investigations involve detailed food ingredient menu item reviews at the point of final service.

Other activities, such as regulatory/enforcement actions, can involve recalls, public alerts, and legal actions when indicated. Prevention/research activities may be initiated on the basis of investigation findings to permit a further understanding of the agent, the mode of transmission, and contributing factors, as well as to permit identification of ways to prevent similar outbreaks from occurring in the future. Research addressing data gaps may also be conducted by industry, academia, and government agencies.

FBIO Environmental Investigations

The processes and or methods of all aspects of a foodborne outbreak investigation have evolved over decades. A routine inspection using the current regulations as a guide provided the first approach to FBIO environmental investigations. This often produced a list of violations of the regulations, but often missed the true causes. During the late 1970s and early 1980s, published articles by Dr Frank Bryan and others called for a focus on the factors that caused outbreaks rather than regulation violations during FBIO environmental investigations. In more recent years, hazard analysis critical control point (HACCP) principles, such as identifying hazards, critical limits (CLs), and critical control points (CCPs), are employed in FBIO environmental investigations. The addition of systems theory as an additional tool to understand why outbreaks occur represents the latest step in the evolution of the FBIO environmental investigation process.

Unfortunately, the FBIO environmental investigation is carried out less frequently and with less insight than other activities during an outbreak investigation. In many instances, these investigations are conducted by food safety regulatory agencies that often conduct regulatory inspections in response to outbreaks, rather than making epidemiologically, laboratory data-driven/systems-based environmental assessments to identify both contributing factors and environmental antecedents.

The findings from FBIO environmental investigations are also less frequently compiled and shared. As a result, there is often a lack of data from the environmental component of an investigation. In addition, there are doubts regarding the quality of the data that are reported, as those data relate to actual contributing factors. An example of such findings is the suggestion that improper food holding temperatures are a possible contributing factor for norovirus illness outbreaks. This is an unlikely relationship for an agent that does not reproduce outside a host cell. Improper food holding temperatures are a common violation in food inspection work; yet, such food holding temperatures are not an appropriate contributing factor to report in a viral FBIO.

To identify and understand the environmental causes of FBIOs, an investigator must conduct a systems-based environmental assessment, informed by available epidemiological and laboratory data, seeking to identify both contributing factors and their environmental antecedents. The FBIO environmental assessments will be covered in the remainder of this article. Developing an understanding of FBIO environmental assessments requires a basic understanding of a few general systems theory concepts, and an understanding of the food chain and its corresponding systems.

General Systems Theory

Systems theory is not new. It was first proposed in the 1940s by biologist Ludwig von Bertalanffy. He is recognized as one of the founders of general systems theory. His theory has been applied to a number of fields and served as the source of inspiration for those working to understand and influence complex systems.

Some of the basic concepts Bertalanffy proposed are especially helpful in framing the complexity of food facilities in such a way that food safety managers and foodborne outbreak investigators can become more effective in managing risk and understanding why outbreak events unfold, thus providing a basis for improved risk management. Those concepts include:

- understanding that the deep underlying interactions of all forces that make up a system is key to influencing or changing that system;
- all systems have 'set points' or set outcomes that are predetermined by the nature of these underlying interactions;
- changes to complex systems require a great deal of information about the nature of these underlying interactions; and
- unless this deeper understanding is achieved, efforts to change the system will ultimately fail and the system will return to its 'set point.'

Whether a facility food safety manager, regulatory inspector, or outbreak investigator, all have experienced either the cycle of making a food safety correction, only to see it occur repeatedly or having the same food facility involved in more than one or two foodborne outbreak investigations. Using Bertalanffy's concepts can help move foodborne outbreak investigation and food safety programs away from these cycles that do not support food safety.

Farm-to-Fork Continuum and Food Systems

The FBIO environmental assessment occurs within the context of the farm-to-fork continuum, sometimes referred to as the food chain. The farm-to-fork continuum represents how food flows from its source through processing or manufacturing, distribution, and finally to the point of final service, which may be a retail establishment such as a restaurant or a consumer's home. The source is where the food originates. It may include a farm where produce is grown or a sea where fish are harvested. Processing or manufacturing includes all the steps along the continuum that prepare the food for distribution. This point in the continuum may be as simple as washing whole produce or as complex as pasteurization or low-acid canning. Distribution includes everything from storage and warehousing to repacking, reprocessing, and transporting to the next point in the continuum. Sometimes distribution involves multiple points along the farm-to-fork continuum. Finally, the concept of point of final service includes any points where foods are purchased and/or consumed, such as grocery stores, restaurants, and delis, or the home.

Each point in the farm-to-fork continuum represents its own unique system (Figure 1). Although the systems themselves are unique, they all consist of the same components:

- Inputs – items that enter the system.
- Processes, steps, and methods to which the inputs are subjected.
- Internal system variables – factors that exert positive, negative, and neutral effects on all other aspects of the system.
- Outputs – immediate results of the system.
- Outcomes – what happens as a result of the outputs.
- Feedback to that particular system on the basis of the outcomes (Figure 2).

Food Systems

Inputs and Processes

At the source, inputs might include the weather, soil conditions, and the hydrology of the watershed as it relates to irrigation source water, type or breed of animal, organisms, and/or chemicals inherent to the product or from the environment. Inputs at subsequent points along the farm-to-fork continuum will include much more than circumstances directly related to food safety such as ingredients for the final product that include organisms or chemicals that may or may not be harmful if consumed. They could include infusions of financial and human capital or other elements that are less obvious, such as management structures that do not support a food safety culture within the establishment.

The flow of food through such processes as storing, cooking, etc. to which inputs are subjected at different points along the farm-to-fork continuum provides a road map for the environmental assessment at that particular point in the continuum. It is essential to describe the related processes step by step, from receipt of ingredients through disposition of the final product or output, whether that is shipping the product to the next stop on the farm-to-fork continuum or to final service or consumption. There may be a few processes involved or many, depending on the complexity of the food product. Using the establishment's HACCP plan, if there is one, also helps identify potential or real hazards, CCPs, and CLs along the way. Mapping the flow of food through processes is necessary for identification of contributing factors (Figure 3). This basic mapping of the flow of food through the establishment's food processes is the total extent of many foodborne outbreak environmental assessments. However, to understand the environmental antecedents, the internal system variables must be examined.

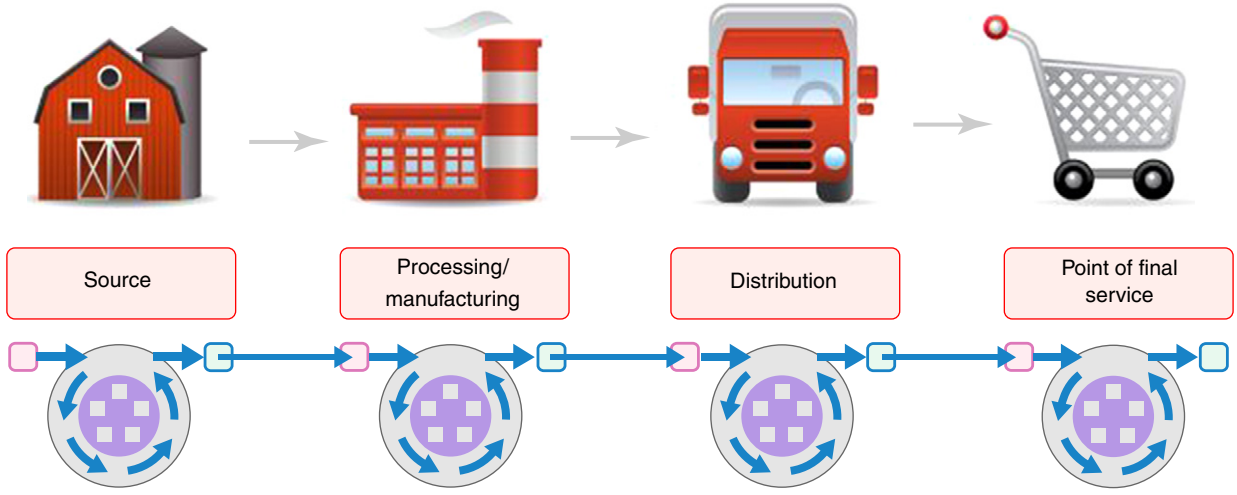

Figure 1 Farm-to-fork continuum.

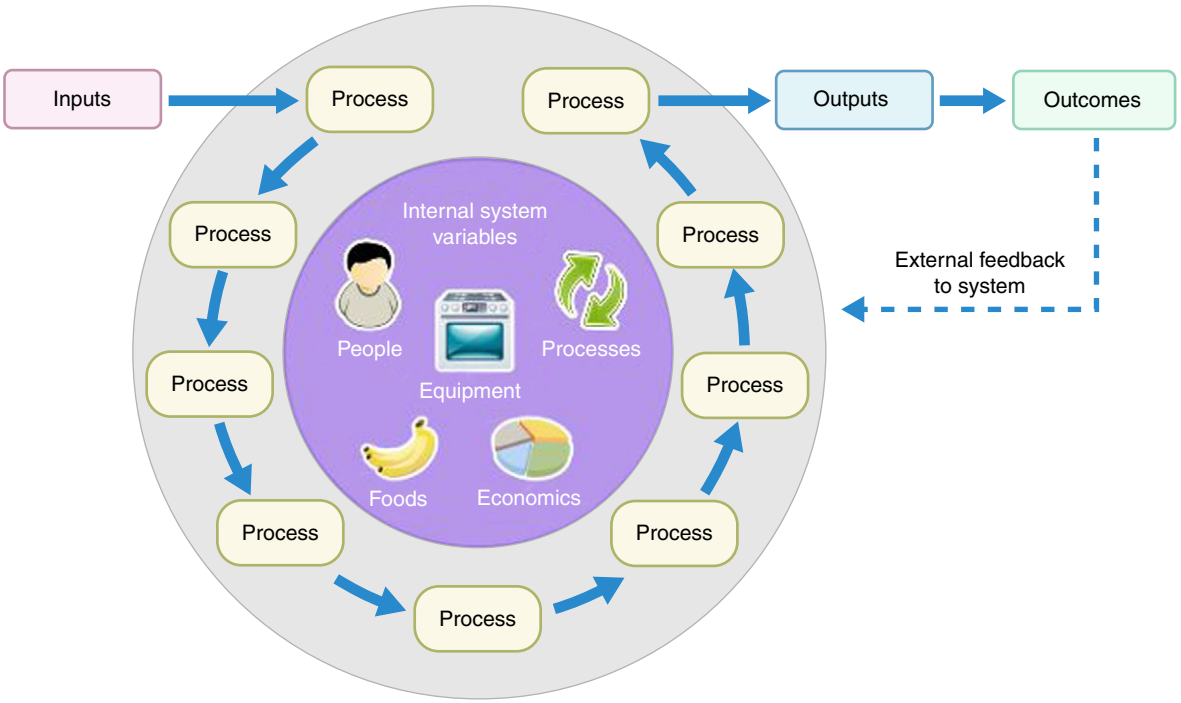

Figure 2 System components.

Internal System Variables

Understanding internal system variables and especially their interactions with each other is important to understand how the system operates and why it operates the way it does. Knowledge of these variables and their interactions can also lead to an informed analysis of the degree of control exerted over critical food safety hazards. It can also explain how, in various circumstances, that degree of control may change. Although no system is completely safe or unsafe, properly managing internal system variables helps to influence the system's outcome toward a safe product.

Internal system variables include:

- People
- Equipment
- Processes
- Foods
- Economics (Figure 2).

These are variables currently recognized as having the greatest influence on food safety. Understanding these variables and their influences helps in determining why the outbreaks occurred. The internal system variables and their potential influences on food safety are complex; on any given day, they may have a positive, negative, or neutral influence on food safety within a particular system in the farm-to-fork continuum. The environmental assessment team must determine the role of these variables during its investigation.

The people, as an internal variable, exert the greatest influence on all aspects of the system at any point in the farm-to-fork continuum, from inputs through outcomes. This internal system variable refers to the individuals working at any point in the continuum and the food safety culture within which they work. The food safety culture at any point in the farm-to-fork continuum is reflected by such things as the owner's/manager's/supervisor's knowledge and commitment to food safety, the existing written standard operating procedures, HACCP plans that include monitoring, the recordkeeping and corrective actions, the supervision of employees, etc. The people variable also refers to how an individual is inclined to behave and how an individual interprets standard operating procedures. For example, some food workers are not inclined to view diarrhea as an illness, and therefore they do not report it to management.

The internal system variable equipment refers to the physical layout of the facility and the equipment appropriate to that point in the farm-to-fork continuum. To support safe food practices, the equipment must be properly designed and constructed for its intended purpose. It must be properly located, not only for its proper operation but also for its facilitation of the most efficient work flow for the processes involved. Proper installation also requires adequate space in the facility to accommodate both the equipment and the work flow in the facility. In addition, equipment that is not properly maintained can potentially exert a negative influence on food practices and CLs. Finally, poorly located or maintained equipment may also influence workers to develop procedures independently to work around the problems caused, thus negatively influencing CLs (Figure 4).

The internal system variable processes refer to the inherent qualities of processes but not to such actual food-processing steps as cooking, holding, storing, etc., as depicted in the flow of food at any point along the farm-to-fork continuum. Although a process may be capable of delivering a safe end result, the inherent nature of the process may pose circumstances that negatively influence the safety of the system. An example is the complexity of the process. A

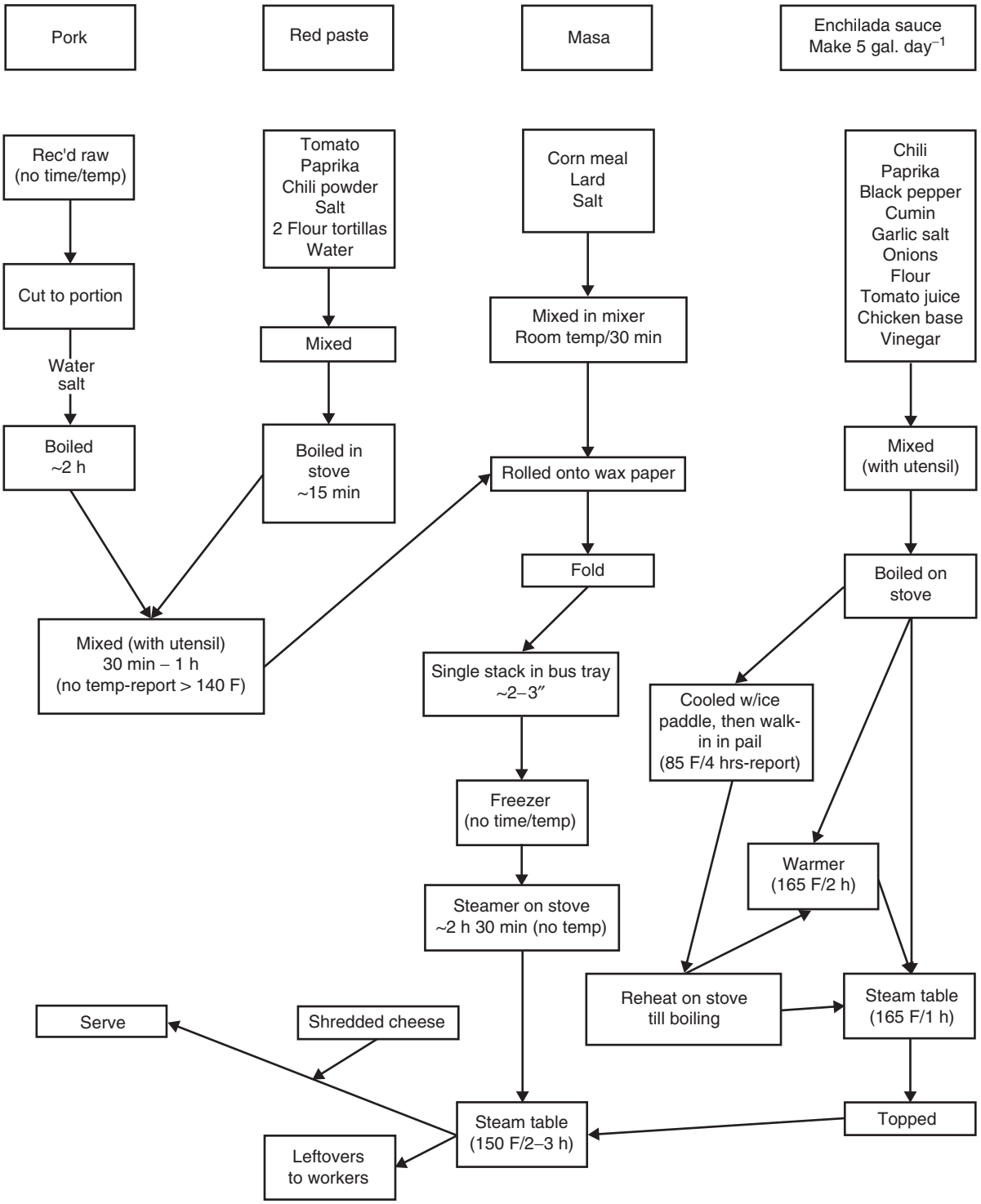

Figure 3 Food flow.

process that is highly complex may pose many risks for contamination or for survival or proliferation of the agents that cause foodborne illness. Industry efforts to reduce the complexity of processes can be found at many points along the farm-to-fork continuum. For example, a manufacturer or restaurant manager may remove steps from a complex process to make it as simple as possible. By changing the inherent nature of the process from complex to simple, a manufacturer or restaurant manager reduces opportunities for contamination or for survival or proliferation of agents. However, complexity of a process is more obvious than other examples, such as the influence of traditions in food

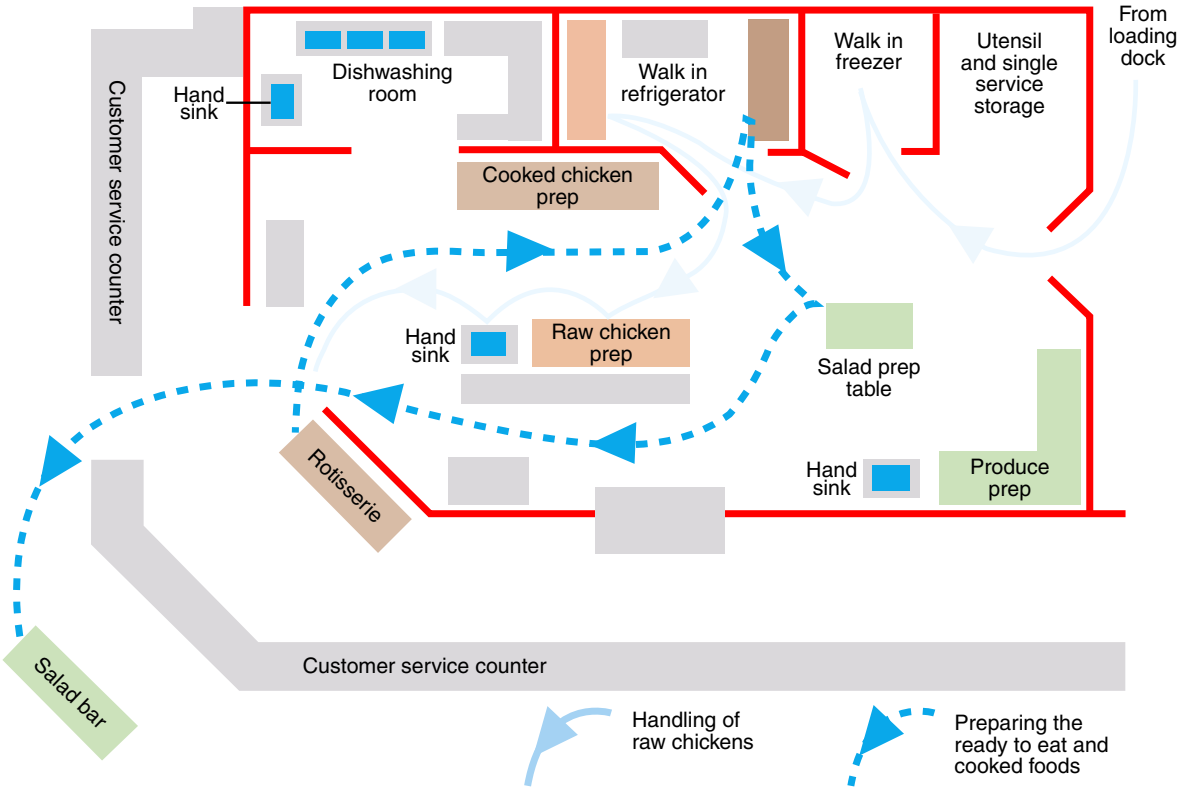

Figure 4 Physical facility layout.

processing or when pathogens change rendering the inherent properties of a process from safe to unsafe. A good example is the ability of *Salmonella* to develop a protective barrier that renders low water activity of a process to be ineffective in reducing pathogens.

The internal system variable food refers to the inherent qualities of food that may positively or negatively influence food safety. These qualities include pH, water activity, texture, and viscosity. For example, the texture of leafy greens renders them difficult to clean; the thickness of such a food as refried beans may require specialized cooling practices.

The internal system variable economics refers to issues affecting the costs and profit margins at any point along the farm-to-fork continuum. For example, a restaurant may be required to change its menus to accommodate changes in food trends, and doing so can potentially affect costs and profit margins. Adequate profit margins may allow any point along the farm-to-fork continuum to maintain itself in a way that promotes safe food practices, whereas poor profit margins may contribute to inadequate staffing, training, or maintenance of equipment, all of which can negatively influence food safety.

People, equipment, processes, foods, and economics are the internal system variables currently recognized as being related to food safety. Overtime well-conducted FBIO environmental assessments will identify other important variables.

Outputs, Outcomes, Feedback

The last three system components along the farm-to-fork continuum are outputs, outcomes, and feedback. Outputs are represented by the final food item that moves from one point in the continuum to the next, ultimately including the final product that is consumed by customers. Outcomes include such elements as customer satisfaction, profit, and customer health. These are only a few of the outcomes of the individual systems represented along the farm-to-fork continuum, but they may be most closely related to public health.

Outcomes spark results that are fed back into each system in one way or another. The feedback may prompt changes within an individual system or in the larger farm-to-fork continuum. For example, feedback on customer satisfaction may trigger a change in menu at a restaurant, or a change in the production of a particular food at manufacturing; feedback on profits may trigger a change in practices or processes at any point along the continuum. Finally, feedback from large foodborne outbreak events can result in system changes at each point in the farm-to-fork continuum.

Although the individual food systems along the farm-to-fork continuum are unique, they are interrelated. Each point affects food safety of any subsequent points along the continuum. A breakdown in food safety at any point along the continuum can contribute to an outbreak of foodborne illness (Figure 1).

Internal System Variable Interaction

It is important to understand all the components within an individual system, whether that system resides at the farm, the manufacturing plant, in the distribution, or at the point of final service. But it is especially important to understand the

internal system variables, because these variables exert such strong influences on every aspect of the individual food system, from inputs through outcomes, including customer health. Specifically, the internal variables can pull the system toward a safe or an unsafe outcome – or sometimes, in varying degrees, toward both a safe and an unsafe outcome. Understanding these variables, and especially their interactions with each other, is pivotal to understanding how an individual system operates and why it operates the way it does. Knowledge of these variables and their interactions can also lead to an informed analysis of the degree of control that system managers have over food safety hazards. It can also facilitate an analysis of how, in various circumstances, that degree of control may change. Properly managing the underlying variables helps to pull the individual system's outcome toward a safe product. But it is important to understand that no individual food system is ever completely safe or unsafe. Instead, the system 'tends' to be safe or unsafe. A simple analogy can clarify this point.

If a room's thermostat is set at 70 F, then 70° is the system's set point, the point the system will attempt to maintain. The heating and cooling mechanisms will work to keep the temperature at 70°. It will try to maintain this temperature in spite of the negative effects of some of the system's internal variables, such as insufficient attic or window insulation or improperly sealed doors.

If it is cold outside and a door is opened, the temperature may temporarily drop to 65°. That is because opening the door overwhelms the set point of the system. However, once the door is closed, the system works to regain the set point in spite of its internal variables' negative influences.

Understanding a system and how it reacts to internal variables is important. In the case of a room with a heating and cooling system, an open window will not change the thermostat setting. The thermostat will try to help the room regain its set point, which is what all systems do. It is important to understand that the system's set point is one condition that, together with internal variables, is influenced by positive and negative factors. Successfully changing the system's set point, if that is necessary, requires understanding these variables and their influences.

The internal variables influence the individual food systems described along the farm-to-fork continuum. Each of these variables may have a positive, negative, or neutral influence on food safety at any given point in time, and yet the food system works to remain at its set point. However, if the negative effects of one or more variables overwhelm the other variables' positive or neutral influences, the result can be contamination and/or survival or proliferation of an etiologic agent in food, to such an extent that an FBIO occurs.

For one to determine contributing factors and environmental antecedents, the interactions of these variables with each other and within the individual food system itself must be understood. Such an understanding provides the information required to strengthen internal system variables and if necessary change the food safety system's set points, thus reducing the opportunity for a similar outbreak to occur. Over time, data from foodborne outbreak environmental assessments can be compiled, analyzed, and trends determined and provided to decision makers to inform food safety policy development.

Environmental Assessment

Environmental assessment, as a part of an FBIO investigation, is different from other environmental/food safety inspection activities at food establishments. An FBIO environmental assessment reconstructs past events. It is triggered by an outbreak of foodborne illness. It describes the outbreak influences of people, equipment, processes, food, and economics on variables that may have contributed to the outbreak. This assessment identifies contributing factors and environmental antecedents to the outbreak. It is a forensic process that looks at clues and data to develop an hypothesis regarding the cause of the outbreak and to implement appropriate controls to prevent future outbreaks.

By contrast, routine regulatory inspections involve the present – what an inspector can observe or measure at the time of the visit and what violations of regulations can be cited. Routine regulatory inspections involve documenting current conditions at the establishment to provide snapshots of observable conditions at the time of the inspection. It may be risk based – that is, focused on risks that are most likely to cause foodborne illness – and it is conducted when specific information is not available to suggest that any process is out of control. This activity should also be based on an environmental assessment, one that is conducted in the present.

A plan review/HACCP development inspection focuses on future operations at a facility. It identifies potential problems before they lead to a foodborne illness, and it identifies control points for preventing foodborne illness in the future. It allows the facility to evaluate plans and procedures. This activity should also be based on an environmental assessment, one that is conducted on the basis of expectations of the future.

FBIO Environmental Assessment

An FBIO environmental assessment is an in-depth, multidisciplinary, systems-based approach to determine how the environment contributed to the introduction and/or transmission of the agent that caused illness. The environment can include everything external to the host, including air, food, water, animals, plants, climate, etc., as well as people and social and built environments.

The objectives of an assessment are to identify contributing factors and environmental antecedents, as well as to generate recommendations for informed interventions. Contributing factors are divided into three categories:

- Contamination
- Survival
- Proliferation/amplification

Contamination factors refer to how an etiologic agent got onto or into the food vehicle. Examples of contamination factors include a contaminated ingredient or bare-hand contact by a food handler/worker/preparer suspected to be infectious.

Survival factors refer to processes or steps that would have eliminated or reduced an etiologic agent if conducted properly. Although survival factors primarily relate to bacterial outbreaks, under limited circumstances they may be appropriately cited in viral outbreaks as well. For example, although norovirus is more heat resistant than most bacteria, it can be inactivated by cooking processes (185 °F/85 °C for 5 min or boiling for 1 min). As a result, depending on the cooking processes involved, citing survival of the agent as a contributing factor in an outbreak can be appropriate. Examples of survival factors include insufficient time and/or temperature during cooking/heat processing or insufficient time and/or temperature during reheating.

Proliferation/amplification factors identify how an etiologic agent was able to increase in number and/or produce toxic products before the vehicle's being ingested. These factors relate only to bacterial outbreaks. Examples of proliferation/amplification factors include improper cold or hot holding of foods or inadequate processing, such as acidification, water activity, or fermentation.

Environmental antecedents are directly related to contributing factors. They explain why the outbreak occurred and are often referred to as the root causes of outbreaks. For example, a worker who cooks food may not speak the native or primary language of food managers or supervisors. This language barrier may limit the worker's ability to understand food safety training properly, thus resulting in improper cooking of food.

FBIO environmental assessments are best accomplished with a team approach. Team members will vary depending on the setting and the expertise needed, but the team can include such specialists as microbiologists, epidemiologists, water experts, environmental health specialists, food technologists, and veterinarians. The team must understand the farm-to-fork continuum, the systems that make up each point along the continuum, and the interrelationship between different points on the continuum. They must be able to think critically as they filter through information once it evolves over the course of the outbreak investigation, describe each system relevant to the outbreak event, determine the most likely contributing factors and environmental antecedents, and provide recommendations for informed interventions.

Recommendations for informed intervention are based on the findings of the FBIO environmental assessment. These recommendations may be implemented during the environmental assessment in order to stop the outbreak and prevent the further spread of the agent, and/or they may result in the development of longer term strategies to reduce the likelihood of future outbreaks. Immediate steps taken might include destroying food or taking steps to stop its distribution, excluding food workers who are ill, or closing the facility. Longer term strategies might include development and implementation of an HACCP plan or updating and implementing policies regarding identifying and managing food workers who are ill with such symptoms as diarrhea, vomiting, and/or fever.

Challenges Encountered in an FBIO Environmental Assessment

There are four important challenges sometimes encountered in the course of conducting an environmental assessment: the ability to think critically; timing; distinguishing between regulatory violations and factors and antecedents that contribute to foodborne outbreaks; and the seasonal nature of growing foods.

The foodborne outbreak environmental assessment is complex, requiring a high level of critical thinking skill among team members to determine the most likely contributing factors and environmental antecedents in an outbreak event. Each point along the farm-to-fork continuum represents its own unique individual food system, but the systems are interrelated. Therefore, an outbreak of foodborne illness that appears related to one part of the continuum can actually be the result of a food safety breakdown in another part of the continuum. The team must continuously assess an outbreak event, not only in terms of the immediate system under scrutiny but also in terms of the other systems in the farm-to-fork continuum. Such an assessment requires a continuous and iterative analysis and assessment of current thinking within the team as information is gathered and premises are revisited until the team is convinced that the best possible conclusions have been reached. Objectively and continuously evaluating a hypothesis based on information gathered at a point in time during a foodborne outbreak event is one of the most challenging aspects of critical thinking during an environmental assessment. Developing such an hypothesis requires giving equal weight to information that weakens a favored hypothesis and information that supports it. After all, human nature renders it difficult to prove yourself wrong. Therefore, evidence that conflicts with assumptions tends to be discounted. During an environmental assessment, it is human nature to seek information that confirms a hypothesis, rather than information that refutes it. There is a tendency to believe what is expected to be believed. Such a tendency can lead to flawed or inaccurate conclusions.

Another important challenge is timing. Agencies may become aware of an FBIO when it is over or nearly over. Affected persons may no longer be sick, and they may have a hard time remembering what they ate, thereby making it difficult for epidemiologists to identify the vehicle. Food preparation may have been days or weeks earlier, and management and food workers may have a difficult time remembering what actions they took. Investigators try to reconstruct what happened and why food workers acted the way they did, but the workers may not be able to give investigators a complete picture. Therefore, agencies must act quickly when they receive a report that an outbreak may have occurred.

Differentiating between violations of regulations and variables that may have led to an outbreak represents another significant challenge. A food establishment system has its own set point at which some level of regulatory compliance occurs on a day-to-day basis. At any point in time, violations of regulations may be present, but such violations may not result in an FBIO. An FBIO is usually the result of a convergence of factors, and investigators must focus on determining the specific system variables that led to the outbreak. These variables are often associated with a regulatory requirement, but some variables may not be. At the same time, there may be multiple regulatory violations that are unrelated to the FBIO event. For example, floors, walls, and ceilings of a food establishment

that are in poor repair or are dirty are likely violations of food regulatory requirements and are likely to be noted during a routine inspection. However, during an environmental assessment, these things may be noted as part of a general description of the establishment, but they are not at all likely, for instance, to be related to a *Clostridium perfringens* or a *Staphylococcus aureus* outbreak.

Finally, the growing, harvesting, and processing activities for implicated commodities may have ceased for seasonal operations by the time an environmental assessment is being started. Such a cessation means that investigators cannot observe operations or conditions that might have contributed to the outbreak.

Conclusion

Although there have been improvements in FBIO investigation and reporting, the reports still leave many questions regarding environmental causes of outbreaks. Environmental assessments are a critical part of the FBIO response. Unfortunately, information from FBIO environmental assessments, if they are conducted, is sometimes missing or minimally supplied in subsequent publications on specific FBIOs or in outbreak surveillance systems. A compilation and analysis of environmental causes of FBIOs provides the data needed to inform public policies, procedures, and training at the point(s) involved along the farm-to-fork continuum; such a compilation also identifies research gaps and evaluates the impact of food safety programs in reducing the risks of FBIOs. All these benefits, however, depend on a standard method or approach to conducting an FBIO environmental assessment and to reporting and analyzing the data from FBIOs.

In 1999, the US Centers for Disease Control and Prevention (CDC) began to explore using a systems approach, as described in this article, during FBIO environmental assessments. This approach was further explored by state and local food safety programs participating in the CDC Environmental Health Specialists Network and by the US Food and Drug Administration (FDA). As a result, the US FDA began conducting systems-based environmental assessments in 2011 during that agency's foodborne outbreak responses. In 2002, the US National Park Service Public Health Program began its exploration of a systems approach for its field assessments of foodservice establishments. The Park Service continues to work with this approach to determine if it can help regulators and food managers gain additional control over food safety issues.

In 2013, CDC will launch an e-learning program on how to conduct an FBIO environmental assessment. This training program will be required of participants who report assessment data to the National Voluntary Environmental Assessment Information System (NVEAIS), an expansion of the existing CDC FBIO reporting system. This new reporting system is expected to help identify factors that can be routinely monitored to prevent or reduce the risk for FBIOs. By building on the foundation of a standard method for FBIO environmental assessments, NVEAIS provides a basis for understanding and preventing future outbreaks, as opposed to simply responding to them.

See also: Food Safety Assurance Systems: Essentials of Crisis Management; Investigation of Incidents in Industry; Root Cause Analysis of Incidents. Public Health Measures: Foodborne Disease Outbreak Investigation; Surveillance of Foodborne Diseases

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Safe Use of Wastewater for Agricultural Production

KO Medlicott and R Bos, World Health Organization, Geneva, Switzerland

Glossary

Health-based target A defined level of health protection for a given exposure. This can be based on a measure of disease, for example, 10^{-6} DALYs per person per year, or the absence of a specific disease related to that exposure.

Log reduction Organism removal efficiencies: 1 log unit = 90%; 2 log units = 99%; 3 log units = 99.9%; and so on.

Multiple barriers Use of more than one preventive measure as a barrier against hazards.

Operational monitoring The act of conducting a planned sequence of observations or measurements of control parameters to assess whether a control measure is operating within design specifications (e.g., for wastewater treatment turbidity). Emphasis is given to monitoring parameters that can be measured quickly and easily and which can indicate if a process is functioning properly. Operational monitoring data should help managers to make corrections that can prevent hazard breakthrough.

Restricted irrigation The use of wastewater to grow crops that are not eaten raw by humans.

Risk The likelihood of a hazard causing harm in exposed populations in a specified timeframe, including the magnitude of that harm.

Unrestricted irrigation The use of treated wastewater to grow crops that are normally eaten raw.

Validation Testing the system and its individual components to prove that it is capable of meeting the specified targets (i.e., microbial reduction targets). Should take place when a new system is developed or new processes are added.

Verification monitoring The application of methods, procedures, tests, and other evaluations, in addition to those used in operational monitoring, to determine compliance with the system design parameters and/or whether the system meets specified requirements (e.g., microbial water quality testing for *Escherichia coli* or helminth eggs, microbial, or chemical analysis of irrigated crops).

Introduction

Domestic wastewater is increasingly used for agriculture in both developing and industrialized countries. In developed nations, wastewater is typically used under controlled conditions after secondary and, most often, tertiary treatment. However, in developing countries the greatest proportion of wastewater use is with raw or partially treated wastewater applied either directly or indirectly in diluted form from polluted rivers and streams.

The value of wastewater has long been recognized by farmers worldwide. It is less affected by seasonal variations than rivers and streams and can provide a reliable year-round source of water. It also contains the nutrients necessary for plant growth. The use of wastewater in agriculture is a form of nutrient and water recycling, which can reduce downstream environmental impacts on soil and water resources.

However, wastewater also contains waterborne pathogens found in the local population and can promote the spread of water-related vectorborne diseases, if not managed carefully. In addition, if the wastewater contains a substantial amount of industrial component chemicals, it may affect soil conditions and plant growth as well as having potential impacts on human health.

Growing competition between the agricultural and urban uses of high-quality freshwater supplies, particularly in arid, semiarid, and densely populated regions, will increase the pressure on this ever-scarce resource. The concept of 'water fit for purpose' is increasingly important in this context: Wastewater use for agriculture will substitute high-quality freshwater, which can instead be used for urban piped water consumers. Within the next 50 years, more than 40% of the world's population will live in countries facing water stress or water scarcity.

In Africa and Asia, the urban population is predicted to double between 2000 and 2030. As these urban and periurban populations grow, the amount of wastewater produced and demand for food, fiber, and freshwater will grow proportionately. This presents urban planners with the challenge to make cost-effective use of resources and provide sufficient quantities of safe food and water to cities. These combined factors are leading to a growing understanding of the value of wastewater as a resource for agricultural production rather than as a waste product.

The third edition of the World Health Organization (WHO) Guidelines for the Safe Use of Wastewater, Excreta and Greywater in Agriculture and Aquaculture provides a summary of the evidence of health impacts from use of

waste-water, excreta, and greywater, and put forward a health risk assessment and management approach. The approach is a departure from the earlier concept of fixed guideline value and norms for wastewater quality: it incorporates multiple barriers along the sanitation chain rather than rigid treatment quality thresholds, and starts with the establishment of viable health-based targets. This article provides a summary of Volume II of the Guidelines: Safe Use of Wastewater in Agriculture.

Health Risks Associated with Wastewater Use

Wastewater is likely to contain a variety of pathogens, many of which are capable of survival in the environment (in the wastewater, on the crops, or in the soil) long enough to be transmitted to humans.

Health risks may manifest directly as outbreaks of food-, water-, and vectorborne diseases or less visible yet persistent diseases such as helminth infections, and noncommunicable diseases resulting from exposure to industrial waste containing heavy metals. Indirect health effects are also possible through contamination of drinking water sources with nitrates or by causing the production of toxic cyanobacteria. However, there are also significant potentially positive health effects from improved food supply and nutrition in arid and food-insecure areas.

The greatest health risks are associated with crops that are cultivated in close proximity to the soil and are eaten raw, such as salad crops, onions, and radishes. Intestinal helminths are the most likely infection in places where wastewater is used without adequate treatment due to the long survival time of their eggs, up to several years in water and soil. Studied viruses, bacteria, and protozoa have shorter survival times in water of usually less than 10–70 days. Factors that affect survival of pathogens in the environment include humidity, temperature, soil content, pH, ultraviolet radiation levels, plant and foliage type, and competition with other native flora and fauna.

Information available from epidemiological studies of infectious disease transmission related to wastewater use in agriculture is summarized in Table 1.

The Stockholm Framework and Health-Based Targets

The Stockholm Framework is an integrated approach that combines risk assessment and risk management to control water-related diseases. The approach allows countries to develop health-based guidelines and standards for water- and sanitation-related microbial hazards within a harmonized framework.

The framework includes health risk assessment followed by setting of health-based targets and the development of national guideline values, best practice, and agreed control approaches together with evaluation of the impact of these combined controls on the stated public health objectives. A

Table 1 Summary of health risks associated with the use of wastewater for irrigation

Group exposed	*Health threats*		
	Nematode	*Bacteria/viruses*	*Protozoa*
Consumers	Significant risk of *Ascaris* infection for both adults and children with untreated wastewater	Cholera, typhoid, and shigellosis outbreaks reported from use of untreated wastewater; seropositive responses for *Helicobacter pylori* (untreated); and increase in nonspecific diarrhea when water quality exceeds 10^4 thermotolerant coliforms per 100 ml	Evidence of parasitic protozoa found on wastewater-irrigated vegetable surfaces, but no direct evidence of disease transmission
Farm workers and their families	Significant risk of *Ascaris* infection for both adults and children in contact with untreated wastewater; risk remains, especially for children, when wastewater treated to <1 nematode egg per liter; and increased risk of hookworm infection in workers	Increased risk of diarrheal disease in young children with wastewater contact if water quality exceeds 10^4 thermotolerant coliforms per 100 ml; elevated risk of *Salmonella* infection in children exposed to untreated wastewater; and elevated seroresponse to norovirus in adults exposed to partially treated wastewater	Risk of *Giardia intestinalis* infection was insignificant for contact with both untreated and treated wastewater; increased risk of amebiasis observed with contact with untreated wastewater
Nearby communities	*Ascaris* transmission not studied for sprinkler irrigation, but same as above for flood or furrow irrigation with heavy contact	Sprinkler irrigation with poor water quality (10^6–10^8 total coliforms per 100 ml) and high aerosol exposure associated with increased rates of infection; use of partially treated water (10^4–10^5 thermotolerant coliforms per 100 ml or less) in sprinkler irrigation is not associated with increased viral infection rates	No data on transmission of protozoan infections during sprinkler irrigation with wastewater

Source: Reproduced with permission from WHO (2006b).

health-based target can be based on a standard metric of disease, such as a disability-adjusted life year (DALY) (e.g., 10^{-6} DALYs lost), or it can be based on an appropriate health outcome, such as the prevention of the transmission of vectorborne diseases resulting from exposures to wastewater used in agricultural practices.

The Stockholm Framework provides the conceptual framework for the WHO Guidelines for the Safe Use of Wastewater, Excreta and Greywater and other WHO water quality-related guidelines. The WHO Guidelines for Drinking Water Quality recommend a target of 10^{-6} DALYs lost, and this is also adopted within the Safe Use of Wastewater Guidelines on the premise that consumers should reasonably expect food to be as safe as drinking water. However, countries may opt for a lower target of 10^{-4} or 10^{-5} DALYs lost in situations where the disease burden is high and other water, sanitation and hygiene interventions may be more effective.

The Multiple-Barrier Approach to Achieve Health-Based Targets

To achieve a health-based target, a range of health protection measures need to be developed along the wastewater treatment and use chain. The target may be achieved entirely in the treatment step, but in situations where treatment is poor or nonexistent the 2006 WHO Guidelines allow flexibility for the target to be met through a combination of subsequent health protection measures. This is the essence of what is commonly referred to as the multiple-barrier approach.

A health-based target of 10^{-6} DALYs is equivalent to a total of 6–7 log reductions of pathogens from the point of waste generation to the point of contact with humans. Figure 1 illustrates different combinations of health protection measures that can be used to achieve the 10^{-6} DALYs or 6–7 log reductions for excreta-related diseases.

As shown in Figure 1, a variety of health protection measures can be used to reduce health risks to consumers, workers and their families, and local communities to meet the health-based target.

The following health protection measures have an impact on product consumers:

- *Wastewater treatment*: Waste treatment methods include primary treatment such as septic tanks, primary and secondary sedimentation tanks, anaerobic sludge blanket reactors, aerated lagoons, or low-rate processes such as constructed wetlands. In some cases, tertiary treatment steps for disinfection and nutrient removal are included. Wastewater treatment can reduce exposure to pathogens by 1 to >6 log units depending on the treatment methods selected and efficiency of operation.
- *Crop restriction*: It requires that wastewater is not used for crops that are eaten raw or nonfood crops such as cotton. Food consumers are protected through subsequent food processing or cooking. Crop restriction can only be used in circumstances where there is a strong enforcement and management of restrictions and profitable restricted crop options exist in the local market conditions.

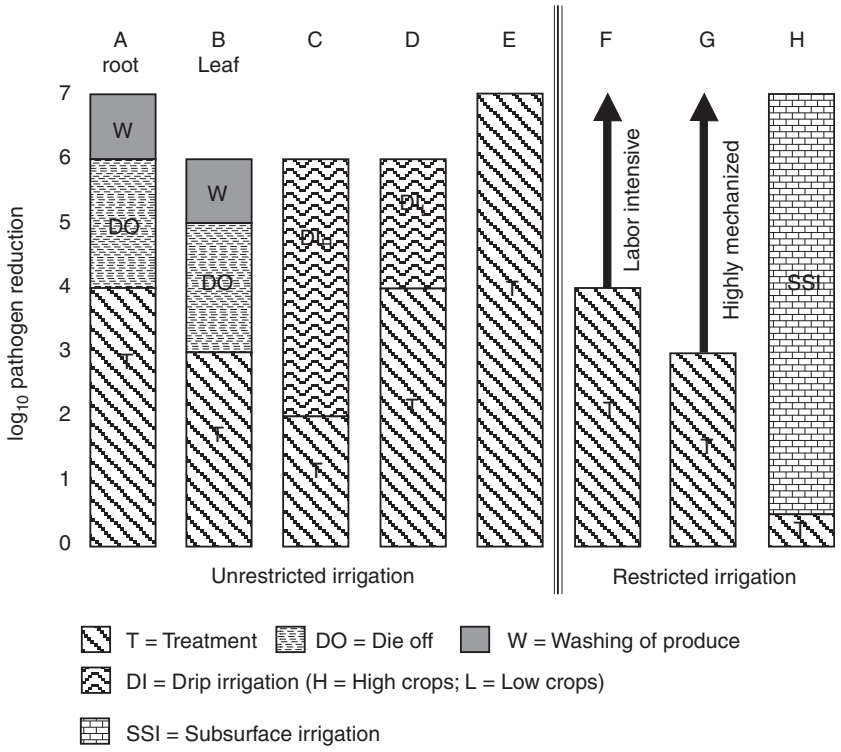

Figure 1 Examples of options for the reduction of pathogens by different combinations of health protection measures that achieve the health-based target of $\leq 10^{-6}$ DALYs per person per year. Reproduced with permission from WHO (2006b).

- *Wastewater application techniques that minimize contamination*: Techniques such as drip irrigation, subsurface irrigation, spray drift control, and enforcement of spray buffer zones can greatly decrease the contact of wastewater with edible parts of the crop, workers, and surrounding communities. Localized irrigation systems and subsurface irrigation can substantially reduce exposure to pathogens by 2–6 log units.
- *Withholding periods to allow pathogen die-off after the last wastewater application*: Allowing 1–2 weeks between the last application of wastewater and harvesting effectively reduces crop contamination by providing time for pathogen die-off naturally. The interval between final irrigation and consumption reliably reduces pathogens by approximately 1 log unit per day. Freshwater can be used for irrigation during the withholding period if needed.
- *Hygienic practices at food markets*: Hygienic practice at market is needed to prevent recontamination of produce. Safe water should be provided at markets for washing and freshening produce and regular inspections to be conducted by food safety authorities to ensure that proper procedures are being used at markets or restaurants where products are prepared.
- *Health and hygiene promotion*: Targeted hygiene promotion programs are required to inform food handlers in their homes, restaurants, and markets on how and why they should wash, disinfect, peel, or cook wastewater-irrigated produce effectively and prevent recontamination.
- *Produce washing, disinfection, and cooking*: Washing of rough-surfaced crops such as lettuce and parsley and vegetables eaten raw in safe water reduces bacteria by at least 1 log unit. For smooth-surfaced crops such as tomatoes, the reduction is approximately 2 log units. Washing in a disinfectant solution and rinsing in safe water can reduce pathogens by 1–2 log units. Peeling fruits and root vegetables reduces pathogens by at least 2 log units. Immersion in boiling or close to boiling water until the food is cooked ensure pathogen destruction.
- *Chemotherapy and immunization*: Immunization for most water-related diseases is currently not possible. However, immunization programs for poliovirus and typhoid and chemotherapy treatment of intestinal helminths and schistosomiasis can complement other control measures above in providing palliative health protection, provided they are applied at the appropriate frequency.

Although not directly related to the consumers of wastewater-irrigated food products, the measures for the protection of farm workers and their families are also important in addressing the range of public health threats posed by wastewater use. Additional controls that can be effective in protecting farm workers and their families and local communities include:

- use of personal protective equipment;
- access to safe drinking water and sanitation facilities at farms and in local communities;
- disease vector and intermediate host control;
- reduced vector contact;
- restricted access to irrigated fields and hydraulic structures; and
- access to safe recreational water, especially for adolescents.

Risk Assessment and Risk Management Plans

The key concept put forward by the 2006 Guidelines for the Safe Use of Wastewater, Excreta and Greywater and the Stockholm Framework is an integrated risk assessment and risk management approach as the most effective means of consistently ensuring safety in the agricultural application of wastewater.

The approach includes the three components of system and exposure assessment, system management, and system

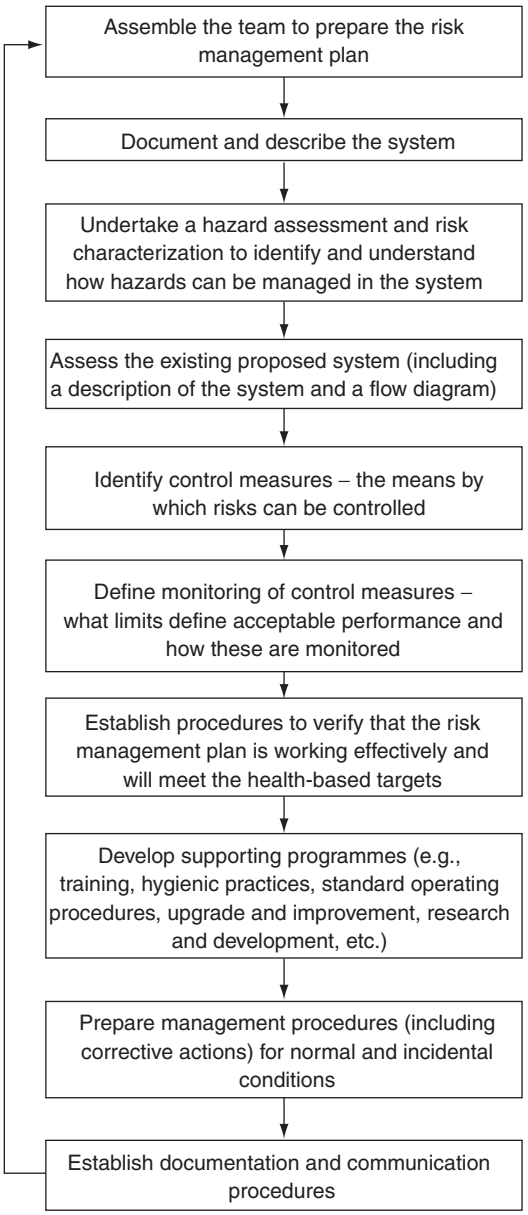

Figure 2 Development of a risk management plan. Reproduced with permission from WHO (2006b).

monitoring, and goes beyond monitoring of effluent quality against the primary management tool.

The approach can be tackled by a multisectoral team representing the wastewater use system from the point of generation to the point of produce consumption using the stepwise process outlined in Figure 2.

The first step in the risk assessment and risk management approach is to establish a system boundary for study and assemble a team of institutional and stakeholder representatives which are responsible for the various elements in the wastewater use chain from generation to use.

The team can then describe the system by generating a flow diagram or geographic map of the system, identifying and describing the path of all fractions of the wastewater through the system as well as the various groups of people at risk of exposure or disease transmission at critical points along the system path. The team should also gather data on the chemical and microbial quality of the wastewater to be used as well as existing surveillance data on waterborne and water-related vectorborne diseases in the area as part of the system description. The system description should also include other relevant contextual information such as water quality standards to be met, certification and auditing requirements, and information on local demographics and land use patterns as well as known weather or seasonal variations that may affect the safe management of the system. All data collected should be validated through onsite investigations.

The team can then assess the hazards, hazardous events, and associated risks at each stage of the system and make an assessment whether the hazard/risk is already adequately managed or not. The prioritization of hazards can then be done using a matrix of likelihood and severity to rate the risk posed by each hazard.

The prioritized risk assessment allows the team to identify new control measures to improve the system in order of the greatest health risk. Ideally, improvements should be targeted as near to the source of the hazard as possible and provide reliable long-term control. However, it may be quicker and more cost-effective to plan for interim controls that are less costly until more comprehensive controls implemented. The improvement plan identifying new and improved control measures is a key output of the risk assessment and risk management process.

The other key output is a monitoring plan. Even if no new controls are identified in the improvement plan, a clear monitoring plan is needed with agreed operational and verification monitoring parameters at critical points in the system. Each monitoring parameter needs to have identified limits and action to be taken in the event these are exceeded.

Operational monitoring parameters mainly include simple measurements that can be carried out on a day-to-day basis to indicate that processes are working as expected. Monitoring of this type relies on simple measurements that can be read quickly so that decisions can be made in time to remedy a problem. Examples of operational monitoring parameters include flow rates and retention times, turbidity, pH, monitoring of types of crops grown, length of withholding periods observed, and adherence to market hygiene and food preparation advice.

Verification monitoring is used to show that the end product (e.g., treated wastewater, crops) meets treatment targets and ultimately the health-based targets. Information from verification monitoring is collected periodically and thus would arrive too late to allow managers to make decisions to prevent a hazard breakthrough. However, verification monitoring can indicate trends over time such as improvements or deterioration of the efficiency of a specific process. Examples of verification monitoring include *Escherichia coli* and helminth egg counts and chemical levels as shown in Tables 2–4 as well as monitoring measures such as analysis of plant contamination levels, hygiene of markets and food preparation areas, and prevalence and intensity of infections in potentially affected populations.

Table 2 Verification monitoring[a] (*Escherichia coli* numbers per 100 ml of treated wastewater) for the various levels of wastewater treatment in Options A–G presented in Figure 1

Type of irrigation	*Option (Figure 1)*	*Required pathogen reduction by treatment (log units)*	*Verification monitoring level (*E. coli *per 100 ml)*	*Notes*
Unrestricted	A	4	$\leq 10^3$	Root crops
	B	3	$\leq 10^4$	Leaf crops
	C	2	$\leq 10^5$	Drip irrigation of high-growing crops
	D	4	$\leq 10^3$	Drip irrigation of low-growing crops
	E	6 or 7	$\leq 10^1$ or $\leq 10^\circ$	Verification level depends on the requirements of the local regulatory agency[b]
Restricted	F	3	$\leq 10^4$	Labor-intensive agriculture (protective of adults and children under 15 years of age)
	G	2	$\leq 10^5$	Highly mechanized agriculture
	H	0.5	$\leq 10^6$	Pathogen removal in a septic tank

[a]Verification monitoring level refers to what has previously been referred to as effluent standards or effluent guideline levels.

[b]For example, for secondary treatment, filtration, and disinfection: 5-day biochemical oxygen demand, <10 mg l^{-1}; turbidity, <2 nephelometric turbidity units; chlorine residual, 1 mg l^{-1}; pH 6–9; and fecal coliforms, not detectable in 100 ml (State of California, 2001).

Source: Reproduced with permission from WHO (2006b).

Table 3 Maximum tolerable soil concentrations of various toxic chemicals based on human health protection

Chemical	*Soil concentration (mg kg^{-1})*
Element	
Antimony	36
Arsenic	8
Barium[a]	302
Beryllium[a]	0.2
Boron[a]	1.7
Cadmium	4
Fluorine	635
Lead	84
Mercury	7
Molybdenum[a]	0.6
Nickel	107
Selenium	6
Silver	3
Thallium[a]	0.3
Vanadium[a]	47
Organic compound	
Aldrin	0.48
Benzene	0.14
Chlordane	3
Chlorobenzene	211
Chloroform	0.47
2,4-D	0.25
DDT	1.54
Dichlorobenzene	15
Dieldrin	0.17
Dioxins	0.000 12
Heptachlor	0.18
Hexachlorobenzene	1.40
Lindane	12
Methoxychlor	4.27
PAHs (as benzo[*a*]pyrene)	16
PCBs	0.89
Pentachlorophenol	14
Phthalate	13 733
Pyrene	41
Styrene	0.68
2,4,5-T	3.82
Tetrachloroethane	1.25
Tetrachloroethylene	0.54
Toluene	12
Toxaphene	0.0013
Trichloroethane	0.68

[a]The computed numerical limits for these elements are within the ranges that are typical for soils.

Abbreviations: DDT, dichlorodiphenyltrichloroethane; PAHs, poly-aromatic hydrocarbons; PCB, polychlorinated biphenyl.

Source: Reproduced with permission from WHO (2006b).

To ensure the plan is consistently applied the team may need to develop a range of supporting program and management procedures. This may include actions such as operator training programs, development of standardized data collection and reporting formats, development of standard operating procedures taking into account normal and critical incident situations, and public communications and feedback mechanisms.

Crucially the risk management plan should be periodically reviewed and updated as new information becomes available and especially after system upgrades or incidents, or changes in system performance requirements.

Verification Monitoring Levels

The verification monitoring levels shown below and in Tables 2–4 summarize the verification monitoring levels presented in the 2006 WHO Guidelines for the Safe Use of Wastewater, Excreta and Greywater in Agriculture and Aquiculture, Volume II.

For helminth infections, no excess helminth infections for both product consumers and farmers could be measured when wastewater quality of ≤ 1 helminth egg per liter was used for irrigation with the exception of children less than 15 years of age being exposed in the fields.

The 2006 Guidelines for the Safe Use of Wastewater, Excreta and Greywater also generally validated the 1989 WHO Guideline value of 1000 *E. coli* per 100 ml of unrestricted use of wastewater in agriculture but puts forward various monitoring levels based on the type of irrigation used and other control measures in place after the treatment step with reference to the options shown in Figure 1.

The level of chemical pollutants in wastewater is dependent more on crop tolerances than health concerns. Table 3 on good agricultural practice outlines concentrations of chemicals in irrigation water that impact agricultural productivity.

However, over time, application of wastewater with chemical concentrations tolerable to plants may accumulate to levels of soil contamination unacceptable for human health. For chemical exposure, numerical limits for the maximum tolerable pollutant concentration in wastewater-irrigated soils are derived by establishing the acceptable daily human intake for a pollutant. Limits shown in Table 2 are derived from a wastewater → soil → plant → human route.

Sociocultural Considerations

Public perceptions about wastewater use are generally negative and often accompanied by disproportionate fears about chemical exposure over microbial exposure. Well-planned and technically sound projects can fail if managing public perception is not adequately accounted for in the project design.

The multiple-barrier approach requires a greater degree of human behavior change to implement control measures after the treatment step compared to enforcing wastewater treatment thresholds. Health protection measures such as changing irrigation methods, enforcing crop restriction or die-off periods, using personal protective equipment, or market and food hygiene measure all require behavior change that may be more or less easily accepted in differing sociocultural contexts.

Behavioral patterns and the social feasibility of behavior change in order to introduce wastewater use schemes or to reduce disease transmission in existing schemes need to be assessed on an individual project basis. It cannot be assumed

Table 4 Threshold levels of trace elements in irrigation water for crop production

Element		*Recommended maximum concentration*[a] *(mg l^{-1})*	*Remarks*
Al	Aluminum	5	Can cause nonproductivity in acid soils (pH <5.5), but more alkaline soils at pH >7 will precipitate the ion and eliminate any toxicity
As	Arsenic	0.10	Toxicity to plants varies widely, ranging from 12 mg l^{-1} for Sudan grass to less than 0.05 mg l^{-1} for rice
Be	Beryllium	0.10	Toxicity to plants varies widely, ranging from 5 mg l^{-1} for kale to 0.5 mg l^{-1} for bush beans.
Cd	Cadmium	0.01	Toxic to beans, beets, and turnips at concentrations as low as 0.1 mg l^{-1} in nutrient solutions Conservative limits recommended due to its potential for accumulation in plants and soils to concentrations that may be harmful to humans
Co	Cobalt	0.05	Toxic to tomato plants at 0.1 mg l^{-1} in nutrient solution Tends to be inactivated by neutral and alkaline soils
Cr	Chromium	0.10	Not generally recognized as an essential growth element. Conservative limits recommended due to lack of knowledge on its toxicity to plants.
Cu[b]	Copper	0.20	Toxic to a number of plants at 0.1–1.0 mg l^{-1} in nutrient solutions
F	Fluoride	1	Inactivated by neutral and alkaline soils
Fe[b]	Iron	5	Not toxic to plants in aerated soils, but can contribute to soil acidification and loss of availability of essential phosphorus and molybdenum Overhead sprinkling may result in unsightly deposits on plants, equipments, and buildings
Li	Lithium	2.5	Tolerated by most crops up to 5 mg l^{-1}; mobile in soil. Toxic to citrus at low concentrations (<0.075 mg l^{-1}) Acts similarly to boron
Mn[b]	Manganese	0.20	Toxic to a number of crops at a few-tenths to a few mg l^{-1}, but usually only in acidic soils
Mo	Molybdenum	0.01	Not toxic to plants at normal concentrations in soil and water. Can be toxic to livestock if forage is grown in soils with high concentrations of available molybdenum
Ni	Nickel	0.20	Toxic to a number of plants at 0.5–1.0 mg l^{-1}; reduced toxicity at neutral or alkaline pH
Pd	Lead	5	Can inhibit plant cell growth at very high concentrations
Se	Selenium	0.02	Toxic to plants at concentrations as low as 0.025 mg l^{-1}, and toxic to livestock if forage is grown in soils with relatively high levels of added selenium. Essential element to animals, but in very low concentrations
V	Vanadium	0.10	Toxic to many plants at relatively low concentrations
Zn[b]	Zinc	2	Toxic to many plants at widely varying concentrations; reduced toxicity at pH >6 and in fine textured or organic soils

[a]The maximum concentration is based on a water application rate that is consistent with good irrigation practices (5000–10 000 m^3 ha^{-1} $year^{-1}$). If the water application rate greatly exceeds this, the maximum concentrations should be adjusted downward accordingly. No adjustment should be made for application rates less than 10 000 m^3 ha^{-1} $year^{-1}$. The values given are for water used on a continuous basis at one site.

[b]Synergistic action of Cu and Zn and antagonistic action of Fe and Mn have been reported in certain plant species' absorption and tolerance of metals after wastewater irrigation. If the irrigation water contains high concentrations of Cu and Zn, Cu concentrations in the tissue may increase greatly. In plants irrigated with water containing a high concentration of Mn, Mn uptake in the plants may increase, and, consequently, the concentration of Fe in the plant tissue may be reduced considerably. Generally, metal concentrations in plant tissue increase with concentrations in the irrigation water. Concentrations in the roots are usually higher than in the leaves.

Source: Adapted from Ayers and Westcot (1985); Pescod (1992); and WHO (2006b).

that controls that are effective in one part of the world would necessarily be effective in another.

Environmental Aspects

The use of wastewater in agriculture has the potential for both positive and negative environmental impacts. With careful planning and management, the use of wastewater in agriculture can be beneficial to the environment.

Where wastewater treatment services are not provided, the use of wastewater in agriculture actually acts as a low-cost treatment method, taking advantage of the soil's capacity to naturally remove contamination. In addition, the nutrients contained in wastewater can offset or replace the need for chemical fertilizers that can damage the soil structure. Therefore, the use of wastewater in irrigation can help to reduce downstream health and environmental impacts that would otherwise result if the wastewater were discharged directly into surface water bodies.

However, there are potential negative environmental impacts of wastewater use such as soil salination and heavy metal contamination, and these vary according to the composition of the wastewater used and properties of the land used and

duration of the application of wastewater. Wastewaters with industrial effluents included are generally more harmful that domestic effluent.

Efforts should be made to reduce or eliminate practices that entail the mixing of industrial and domestic wastewater, particularly where wastewater is used for agriculture. Many environmental impacts can be reduced by good agricultural practices.

See also: Bacteria: *Salmonella* Typhi and *Salmonella* Paratyphi; Shiga Toxin-Producing *Escherichia coli* and Other Pathogenic *Escherichia coli*; *Shigella*; *Vibrio cholerae*. Food Safety Assurance Systems: Good Practices in Fisheries and Aquaculture. Protozoa: *Cryptosporidium* spp. Safety of Food and Beverages: Safety Consideration in Developing Functional Foods; Water (Bottled Water, Drinking Water) and Ice. Viruses: Hepatitis A Virus; Hepatitis E Virus

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World Health Organization (2006b) *Guidelines for Safe Use of Wastewater, Excreta and Greywater in Agriculture and Aquaculture, Vol. II: Wastewater Use in Agriculture*. Geneva, Switzerland: WHO.

Further Reading

World Health Organization (2006a) *Guidelines for the Safe Use of Wastewater, Excreta and Greywater in Agriculture and Aquaculture, Vol. I: Policy and Regulatory Aspects*. Geneva, Switzerland: WHO.

World Health Organization (2006c) *Guidelines for Safe Use of Wastewater, Excreta and Greywater in Agriculture and Aquaculture, Vol. III: Wastewater and Excreta Use in Aquaculture*. Geneva, Switzerland: WHO.

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Relevant Websites

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PUBLIC HEALTH MEASURES

Food Control and Public Health Laboratories

MFG Jermini, Cantonal Laboratory, Ticino, Switzerland

Introduction

The principal objectives of national food control systems are:

- Protecting public health by reducing the risk of foodborne illness;
- Protecting consumers from unsanitary, unwholesome, mislabeled, or adulterated food; and
- Contributing to economic development by maintaining consumer confidence in the food system and providing a sound regulatory foundation for domestic and international trade in food.

Food control systems should cover all food produced, processed, and marketed within the country, including imported food. Such systems should have a statutory basis and be mandatory in nature. Although the components and priorities of a food control system will vary from country to country, most systems will typically comprise the following components:

- Food law and regulations
- Food control management
- Inspection services
- Laboratory services: food monitoring and epidemiological data
- Information, education, communication, and training

An important element of a national food control system is its integration in a national food safety system so that links between food contamination and foodborne diseases can be established and analyzed. Access to reliable and current intelligence on the incidence of foodborne illness is critical. The laboratory facilities for this type of activity are generally situated outside the food control agencies. It is essential, however, that effective linkages are established between food control agencies and the overall public health system including epidemiologists and microbiologists as well as the food inspection services. In this way, information on foodborne diseases may be linked with inspection data such as degree of implementation of hazard analysis and critical control point (HACCP), results of analytical own-check programs, official food monitoring and surveillance data, and lead to appropriate risk-based food control policies. This information includes annual incidence trends, identification of susceptible population groups, identification of hazardous foods and economic sectors, identification of high-risk food businesses, identification and tracing of causes of foodborne diseases, and the development of early-warning systems for outbreaks and food contamination. Only in this way risk-based inspections and risk-based food control programs can be planned as stated in many national legislations. The European Union (EU) regulation 854/2004 states indeed that 'the nature and intensity of the official controls should be based on an assessment of public health risks, animal health and welfare, where appropriate, the type and throughput of the processes carried out, and the food business operator concerned.'

Therefore, the present contribution considers that the work of a public health food laboratory should not be dissociated from considerations regarding inspection services; they both are elements of the risk management section within the framework of the risk analysis process. The entities should indeed work in close collaboration, taking into consideration important interactions that should exist between them and other governmental bodies or societal institutions.

What is Public Health?

The United Nations' World Health Organization (WHO) defines health as 'a state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity.' Public health is typically divided into epidemiology, biostatistics, and health services. Environmental (e.g., food, water, waste, and noise), social, behavioral, and occupational health are other important subfields. Public health is 'the science and art of preventing disease, prolonging life, and promoting health through the organized efforts and informed choices of society, organizations, public and private, communities, and individuals.'

Investing in state and territorial public health systems is a direct investment in the health and wellness of a nation's population. The goal of public health is to improve lives through the prevention and treatment of disease and foster a nation of healthy people who will help strengthen the economy by creating a more productive workforce and lessen the burden on medical costs by lowering the need for intensive treatment for chronic diseases.

Among others, this is achieved by the following strategies:

- Incorporating positive opportunities for health, including access to healthy food choices and protection of people from largely preventable factors – causes of death and disability – such as microbial agents, toxins, and contaminants;
- Developing and implementing national, state, and local policies using science-based evidence that promotes prevention and wellness.

Food and Health – The 'Farm-to-Fork Life Cycle Approach'

A comprehensive food and nutrition policy comprises three strategies: on nutrition, food safety, and food security (sustainable food supply). This framework provides a starting point to address the question of how to promote public health through food. The strategies are interrelated because the food supply influences both the safety and the composition of food. Usually a number of government departments or agencies are concerned about food, including health, agriculture, fisheries, trade, tourism, education, environment, planning, and finance.

A comprehensive and integrated approach 'from farm-to-fork' is therefore required to ensure effective food safety policy.

Although the predominant concern during the seventies and for most of the eighties related to the risks of chemical pollution of the environment and food chain (due to pesticides, ground water pollution by animal waste, natural toxins, and drug residues in food), the later period has seen zoonotic disease emergencies of global public health significance. In both developing and industrialized countries, where nutritional issues and/or chemical contamination are still of concern, microbial contamination of food and the emergence and reemergence of zoonotic diseases continues to be a major problem along with concerns caused by the rising antimicrobial resistance and its possible links with the use of antimicrobial agents in food-producing animals. Despite recent activities and initiatives, the incidence of some foodborne and zoonotic diseases continues to increase, and it is a consequence of the influence of different factors, often interrelated and complex. These factors include changes in:

- Food supply system (see Figure 1), resulting in mass production and distribution leading not only to opportunities for contamination but also to larger outbreaks; in intensive agriculture and animal husbandry practices leading to increased contamination of the raw foodstuffs, increased use of pesticides and veterinary drugs; in international trade and importation of potentially contaminated food and infected animals; in a longer food chain as a result of urbanization and globalization with greater opportunities for contamination, growth, and survival; in the rapid increase in the number of food service establishments where food handlers do not necessarily have any training in food hygiene.
- Health and demographic situation, including population growth; increase in the number of vulnerable people such as the elderly and immunocompromised individuals, malnourished persons; rapid urbanization and ruralization of cities, particularly in developing regions with poor infrastructures.
- Social situation, behavior, and lifestyle entailing increased consumption of food outside of home; closer association between animals and humans, for example, pet animals; increased travel and exposure to unsafe food; change in food preparation habits; poverty and lack of education; lack of time and strive to increase economic profit; lack of training and education of animal keepers and food handlers.
- Health systems and infrastructure meaning a decrease in resources with simultaneous increase in the number of businesses which require supervision, guidance, and control; weaknesses in the investigation and surveillance of zoonotic and foodborne diseases and monitoring contaminants leading to incapacity to evaluate impact of these diseases; cutting resources for preventive public health and veterinary program and privatization of veterinary services.
- Environmental conditions such as pollution; climatic conditions and its changes; and changes in microbial as well as ecological systems.

Nutritional challenges vary as we progress through the human life cycle. Good nutrition during the first few years (maternal nutrition, breastfeeding, and introduction of safe and adequate complementary food) pays dividends throughout

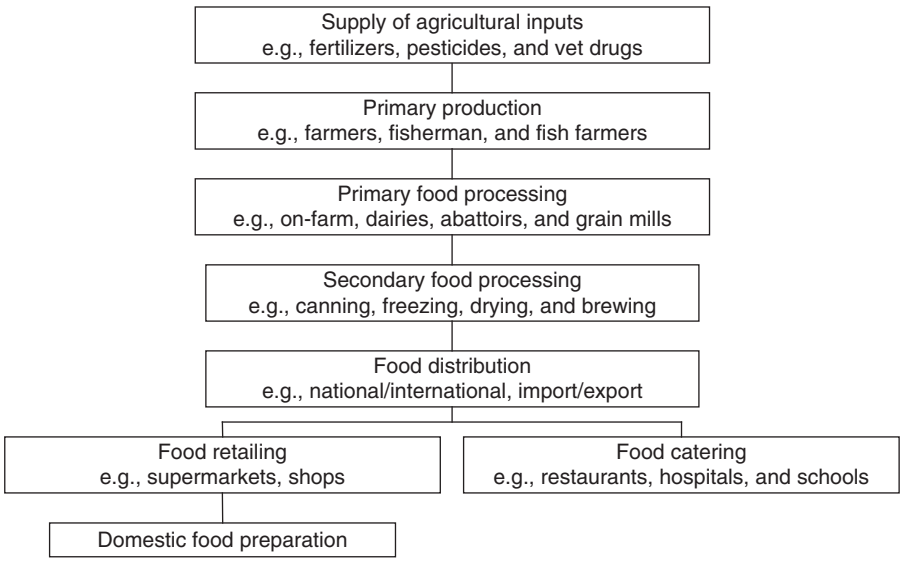

Figure 1 Principal stages of the food supply chain.

life. In adolescence, a period of rapid growth, the health impact of nutrition is pronounced. In adulthood, the main challenge is to avoid premature death from cardiovascular diseases and cancer. The issue of healthy ageing is also of major concern.

Contradictory opinions may be held by various parties involved; to be effective, food and nutrition policy will need to harmonize these opinions as far as possible. A 'farm-to-fork life cycle approach' taking into consideration food safety and nutritional aspects helps to find a consensus among potentially conflicting interests and strengthening partnership between sectors.

What is Food Control?

The terms food safety and food quality can sometimes be confusing. Food safety refers to all those hazards, whether chronic or acute, that may make food injurious to the health of the consumer. It is not negotiable. Quality includes all other attributes that influence a product's value to the consumer. This includes negative attributes such as spoilage, contamination with filth, discoloration, and off-odors and positive attributes such as the origin, color, flavor, texture, and processing method of food. This distinction between safety and quality has implications for public policy and influences the nature and content of the food control system most suited to meet predetermined national objectives.

Food control is defined as:

> ….a mandatory regulatory activity of enforcement by national or local authorities to provide consumer protection and ensure that all foods during production, handling, storage, processing, and distribution are safe, wholesome, and fit for human consumption; conform to safety and quality requirements; and are honestly and accurately labeled as prescribed by law.

The foremost responsibility of food control is to enforce the food law(s) protecting the consumer against unsafe, impure, and fraudulently presented food by prohibiting the sale of food not of the nature, substance, or quality demanded by the purchaser. It is again obvious that it would be inappropriate to dissociate analytical from inspectional food safety control activities.

Risk-Based Approach for Food Safety Control

Modern food safety control systems should include risk-based approaches in both testing and inspection.

As far as inspectional activities are concerned in identifying the level of risk, most of the systems presently in place in the EU have three levels, for example, high, medium, and low. Risk-analysis scores are used to determine classification of the establishment, which in turn determines the frequency of inspection or other controls. Allocation of responsibility for planning controls is often split between central planning and local application. Generic risk is normally determined at central level, based on inherent hazards, with a focus on the local level for targeting noncompliances. The approach of risk categorization might be very varied and there is no correct answer for risk categorization. A number of challenges have been identified:

- Ensuring an appropriate balance between risk to the consumers and political and economic risk.
- Identifying a cost-effective means of providing reasonable levels of assurance and increasing compliance distortion of categorization by media interest groups and political intervention.
- The system should be easy and not too costly in term of resources.
- The system should be documented.
- The system should be flexible to allow changes as necessary, especially for continuous improvement.

The EU Regulation 854/2004 states that the frequency of official controls should be regular and proportionate to the risk, taking into account the results of the checks carried out by feed and food business operators under HACCP-based control programs or quality assurance programs, where such programs are designed to meet requirements of feed and food law, animal health, and animal welfare rules. *Ad hoc* controls should be carried out in case of suspicion of noncompliance. Additionally, *ad hoc* controls could be carried out at any time, even where there is no suspicion of noncompliance.

As far as analytical activities are concerned, several elements are used to identify and rank food according to risk as well as to determine frequency of sampling and testing. Some national regulations lay down specific requirements for food sampling. In Germany, for example, these require 5 food samples and 0.5 samples of food commodities and cosmetics per 1000 people per year. Risk oriented sampling systems also consider food product risk, product compliance data over a defined period, business compliance over a defined period, and emerging risk and changing consumption patterns. In line with the farm-to-fork approach, sampling program also consider feed. In Austria, the population size, percentage of noncompliancy within different food types, and type of noncompliancy (leading to health problems related only to fraudulent activity, labeling, etc.) are used by the Central Authority, Agentur für Gesundheit und Ernährungssicherheit (AGES), to define type and number of samples which have to be taken officially by the different regions (Länder) of the country. In Ticino, Southern Switzerland, cantonal guidelines approved by the regional parliament laid down performance requirements for food control services of 1 food sample per 300 people per year and 1 water sample per 1000 people per year together with yearly inspections in minimum 25–33% of the registered business to be achieved by an overall expenditure of maximum 10 Swiss francs per habitant per year. Efficiency of the services are monitored by safety indicators related with the percentage of noncomplying inspected food businesses (should always decrease) and the incidence of foodborne diseases (appropriate level of protection) for *Salmonella* spp. and *Listeria monocytogenes*, which should be decreasing year after year and be below the national average.

Regulation 882/2004 of the EU requires each Member State to prepare a single integrated multiannual national control plan. This plan must contain general information on the structure and organization of the systems of feed

and food control and of animal health and animal welfare control in the Member State concerned among others on:

- The strategic objectives of the plan and on how the prioritization of controls and allocation of resources reflect these objectives;
- The risk categorization of the activities concerned;
- The designation of competent authorities and their tasks at central, regional, and local level, and on resources available to these authorities;
- The general organization and management of official controls at national, regional, and local level, including official controls in individual establishments;
- Control systems applied to different sectors, and coordination among the different services of competent authorities responsible for official controls in these sectors.

Similarly other nonEU countries have introduced multiannual programs, for example, Switzerland.

Organization of a Food Control Laboratory and Inspectorate

In many governmental structures there are staff and facilities located in different agencies that are involved in some or all aspects of food control, but their efforts are often not highly effective because of the lack of facilities, expertise, or an adequate legal or administrative framework. Food control laboratories and food inspectorates, as complementary elements within risk management, should be located within the same institution (ideally a public health institution) or alternatively have strong means for coordination.

Staffing of the Laboratory

Besides a head of the laboratory, analytical, administrative, and support staff should be active. The head of the laboratory may include a director and a deputy if the staff is sufficiently large. The head of the laboratory should be a graduate chemist or microbiologist trained in food safety. In some countries, conditions for assuming the position of a chief executive officer (or food inspector/ controller) within the food safety system are legally set; as a consequence, special university curricula to train official food safety officers are organized. Head of an official food control laboratory should receive a broad spectrum education including all food chemistry, microbiology and technology, elements of nutrition, food legislation at national as well as international level, food toxicology, strategies for risk-based food control, hygiene and HACCP, quality assurance, epidemiology, risk analysis (assessment, management, and communication), and elements of business and administration. Supervisors, or on-site managers of the laboratory, are also expected to be involved in analytical work; therefore, they also should be graduated in the specific area of work and have considerable experience in food analysis. A reasonable maximum number of analysts or lab technicians to be supervised by one person is 10–12. A team leader may also be appointed to do specific tasks or type of analysis. The main job of the analytical staff is to analyze the samples received. It can be drawn from three levels: university graduates, trained technicians, or unqualified staff who have received on-the-job training. The support staff of a laboratory are all those persons working in and for the laboratory who are not conducting analysis or are not involved in administrative duties. Some examples include washing, cleaning, and housekeeping. Laboratory administration includes staff responsible for sampling (if not assigned to official inspectorates), receiving and assignment of samples, storage and disposal of samples, budgeting, purchasing, supply management, equipment maintenance, housekeeping, training, and so on. Most of these activities, in line with the certification scheme ISO17025, are, however, assigned to scientific staff dealing with testing.

Analytical Activities

Normal activities of food control laboratories encompass tests on composition and residues of contaminants of different nature (chemical, microbiological, or radiological) using different techniques such as wet chemistry, titration, potentiometry, atomic absorption or emission, ionic, liquid, or gas chromatography, with different detection systems, gamma spectrometry, immunological tests, commercial rapid tests, and so on, for the chemistry and radiology or culture-based respectively, or molecular biological methods for microbiology. Accurate laboratory testing is essential to public health. Yet, no laboratory test can be guaranteed to be accurate 100 percent of the time. To assure that test results are acceptable and reliable, a laboratory must incorporate both quality assurance and quality control procedures into its daily laboratory routine. Laboratory design and features must be in line with the quality requirements set by international standards, such as the basic structure, safety features, ventilation and air conditioning, space utilization, and utilities.

Selection of the Contaminant-Commodities to be Investigated

Different strategies can be adopted to identify commodities and contaminant worthy of investigation. Ideally contaminants' choice should be related to epidemiological data; where data on foodborne diseases show high incidence of a particular pathogen among the population, and the frequency of sampling and testing of suitable commodities known to be potentially contaminated should be increased. Therefore, trend analysis of foodborne disease including typing patterns (e.g., different serotypes of *Salmonella enterica* sub. *enterica* or different biovars of *L. monocytogenes*) is essential to be able to identify and adopt correct risk-management options when dealing with important outbreaks or increased epidemiological figures. However, literature data or reports from other countries or international organizations such as WHO or Food and Agriculture Organization of the United Nations (FAO) regarding presence of pathogens or contaminants in certain food types should immediately foster specific testing at national or local level. The list of microbiological or chemical contaminants to be tested could be indeed drawn from trend

analysis from international alert systems such as the EU Rapid Alert System for Food and Feed or from other databanks such as the International Food Safety Authorities Network or from Food Global Environment Monitoring System – Food Contamination Monitoring and Assessment Program. Multiannual analytical programs from other countries, for example, for pesticide residues in the EU might also be suitable tools to identify appropriate commodities-contaminants to be investigated. In this context, analysis of food to assess compliance with nutritional composition standards should also be taken into consideration; content of vitamins or folic acid, presence or absence of allergens, fatty acid composition, and more should also be addressed within a national risk-based analytical plan.

Strategy and Aim of Sampling: The Link between Food Inspectors and the Food Laboratory

A sampling strategy means a planned procedure for selecting samples from a population and conducting the sampling to obtain the information needed. Terminology for selecting samples from a population is under development in EUROSTAT/Food Safety statistics. The competent authority should establish a sampling strategy. The following definitions for sampling strategies in the context of control and monitoring activities have been proposed by EUROSTAT:

- Objective sampling: A planned strategy based on the selection of a random sample, which is statistically representative of the population to be analyzed. Each unit, within the framework population, has a specified probability of being selected. This strategy provides data from which statistical inference can be implemented. It means that the results inferred are comparable. This approach could be applied to a convenient selection of food retailers for hygiene controls where the retailers are grouped according to predefined characteristics or a stratified regional selection of food producers for HACCP controls. It would give results that could be extended to all the population and breakdown by region. For example, sampling of soft cheese at the retail level for detection of *L. monocytogenes* to determine the prevalence in soft cheese at a specific point of the food chain or other monitoring schemes or surveys on the prevalence of foodborne pathogens in certain food categories.
- Selective sampling: A planned strategy where the selection of the sample is from previously defined 'high-risk' population groups. Samples are normally selected to either illustrate or document unsatisfactory conditions or suspected adulteration of a product. The sampling is deliberately biased and is directed at the particular products or manufacturers. For example, sampling of high-risk products, for instance, vacuum-packed cold-smoked fish product supporting the growth of *L. monocytogenes* during its shelf-life for detection and/or enumeration of *L. monocytogenes* to determine the rate of contamination and/or the level of contamination.
- Suspect sampling: A selection of samples where the units are selected on the basis of the judgment and experience regarding the population, lot, or sampling frame. The samples obtained from this procedure are not randomly extracted. For example, sampling carried out as a part of a foodborne-outbreak investigation or where an inspection indicates that there may be a food safety problem or where a HACCP plan review results in concerns regarding potential food safety problems.

The aim of sampling might be different, but usually sampling is carried out for monitoring and surveillance purpose as well as to assess food safety management plans. Monitoring is the performance of routine analysis aimed at detecting contamination of foodstuffs from which useful prevalence data may emerge. Surveillance is the performance of routine analysis aimed at detecting contamination of foodstuffs for the purpose of applying appropriate control measures. Such control measures are normally determined in advance. One of the main objectives of surveillance is to follow-up unsatisfactory results with an investigation and possible enforcement action. When assessing a food business operator's food safety management plan based on HACCP, good hygienic practices (GHP), and good manufacturing practice (GMP), the food inspector might find it necessary to take additional samples for official control if it has concerns about the food safety management systems or verification of the system. The extent of such official sampling is dependent on the food business operator's analyses' results and the competent authority's assessment of a food business operator's food safety management plans. However, the focus of the activities of the competent authority should be on both the assessment of the activities of the food business operator and on requesting them to correct their food safety management plans to ensure that the food business operator remedies weaknesses in their food safety control.

Staffing of the Inspectorates

Food control inspectorates should also be managed under quality control schemes, in particular ISO17020 (quality assurance scheme for inspection bodies). Food inspectors not only should possess a set of techniques and apply procedures to verify compliance with food safety and quality regulations, but also should foster food safety partnerships with food processors. This approach is derived from many experiences in countries where such partnerships have contributed greatly to the active involvement of inspectors in the improvement of existing quality and safety management systems. The approach requires a change of philosophy regarding the inspector's traditional regulatory role, which is generally limited to verifying that regulations are complied, to a vision of him or herself as a food safety professional actively contributing to improving the system through incremental changes that are meaningful with regard to enhancing the safety of food products. Thus, the philosophy relies on placing the inspector's emphasis on factors that are likely to lead to foodborne disease. The traditional regulatory inspection seeks only to obtain correction of food safety concerns that already exist rather than to prevent future violations from occurring. Although this approach may have helped to improve sanitation in the past, it emphasizes reactive rather than preventive measures. In contrast, the future should be on prioritizing inspections using a risk-based approach. This new approach

has proven effective in changing the attitude of the regulation to a new level of respect toward the inspector. In addition to a new philosophy and approach to food inspection, the focus should be replaced from the inspector's attention from environmental aspects and end-product testing to a risk-based process. That change of focus is from the simple (and often unfounded) 'verification' of the compliance of a product or premises with dated-prescribed regulations to an assessment of the controls put in place in the operation to address foodborne disease risk factors that could put the processor's products at risk (i.e., cause disease). Food inspectors should, therefore, have similar professional profile to the graduated staff working in the testing facilities, however, with a broad-documented experience in auditing techniques, especially in HACCP and GHP. Ability to confirm and verify whether or not the HACCP system of the inspected company is correctly working (all hazards identified, risk properly evaluated, CCP correctly defined and critical limits correctly set, monitoring procedures and corrective action in place, and verification and documentation implemented) are essential tools of a modern food inspector. Independency between inspectors and controlled institutions must also be granted to avoid conflicts of interest.

Inspectional Activities

The activities of a food inspector are mainly the following:

- Prepare for an inspection. If a full HACCP system is in place, then an audit (assessment/evaluation) should be performed. Preparation includes consulting the food control authority's records to gain an insight into the background of the operation to be inspected, its history of compliance and the product(s) it handles.
- Prepare a list of potential foodborne disease risk factors identified for the type of product and facility to be inspected.
- Prepare the necessary inspection wear (unless it is provided by the establishment), tools, and equipment.
- Make an adequate time allocation for the inspection based on the size and complexity of the operation.
- Obtain information about the registration status, the identification number of the facility, and, hopefully, the name(s) of the person(s) to communicate with before, during, and, if necessary for follow-up, after the inspection.
- Take care of personal aspects such as obtaining protective clothing (unless provided by the inspected establishment) and preparing the necessary equipment (e.g., flashlight, thermometer), sampling tools, note-taking materials, and official forms.
- Consider the task at hand and organize each inspection. Unless the inspection is a follow-up to a complaint or known violation, in which case a nonannounced visit is in order, establishments should be notified of inspections in advance so that management will be available to accompany the inspector during the inspection, and the necessary records will be available. The notion that this will result in preinspection-fixing of problems in the inspected establishment may be countered with the argument that if the inspection serves to fix something that is wrong, it is worth it. After all, the sooner non-compliances and violations are corrected, the better.
- Plan an opening meeting to get to know the management, explain the objective and scope of the inspection and the procedure to be followed, go over the relevant regulations, review existing records, discuss the quality and safety management system, and ask pertinent questions.
- Evaluate paper work regarding prerequisite programs, HACCP system, traceability, and recall procedures.
- Walk-through should be done to assess hazards and the associated controls, to observe the performance of the members of staff and floor personnel, and to talk to them.
- Take GHP or HACCP verification samples.
- Carry out an exit meeting, where noncompliance findings and ways of improving food safety and quality in the future are discussed.
- Record the observations and document them as needed.
- Have management sign the original and keep a copy of the inspection form.
- File the original of the inspection form with the food control authority.
- File a copy of the inspection form in a 'pending' file if follow-up is necessary, and, if the case, conduct an unannounced visit to ensure corrective actions have been taken.

As far as the prerequisite plan is concerned, the inspection must cover all the following aspects:

1. Plant construction and equipment program, i.e., the physical characteristics of the facility;
2. Standard Operating Procedures, the established procedures for conducting specific processing operations;
3. Sanitary Standard Operating Procedures, the plant and equipment sanitation schedules and procedures;
4. Pest control program;
5. Management review, the training received by management and the knowledge that managers have about food safety;
6. Personnel hygiene;
7. Training program;
8. Customer complaints and handling;
9. Suppliers' specifications and control;
10. Record keeping.

If the facility follows the HACCP system and has a HACCP plan in place, it should be available to the inspector. If a HACCP is in place, an audit will be undertaken, not an inspection. The traceability and recall program comprises all the techniques and procedures in place at the facility to (a) maintain records of incoming materials, indicating date, lot number, supplier, carrier, amount and condition, and (b) implement a recall program that allows products to be traced to retail in case they need to be withdrawn from the marketplace. This requires coding of all products, keeping records of lot and/or batch numbers, and keeping distribution records.

To trace back activity and justify the quality of the performance delivered, documentation and record keeping should be in line with the internationally recognized quality norms ISO17020 for inspectional bodies and ISO17025 for testing facilities.

Food inspectors as well as supervisors of the analytical laboratories should deliver inspection and testing report with exhaustive conclusion in order to facilitate interpretation of data and further action by responsible managers.

Interactions between Food Control Services and Other Public Health Authorities, Other Governmental Bodies or Societal Institutions

An effective, integrated national food control system is essential to protect the health and safety of domestic consumers.

As depicted in Figure 2, effective food control systems should work in close contact with other elements of the public health system as well as with other important socio-economical elements of our society. In general:

Food control services, consisting of food microbiology, chemistry, and radioactivity laboratories as well as food inspectorates should be element of an important network of institutions and liaise – as risk-management instrument – with risk assessors and other risk managers at all level (national, regional, and local). They should

a. Provide data on food contamination to risk assessors for their exposure and dose-response assessment as well as technological parameters of food for risk management;
b. Provide data of inspectional noncompliance, especially on pre-requisite programs and HACCP systems, to risk managers to define risk-based analytical programs as well as inspection frequency;
c. Careful evaluation, based on local epidemiology and environmental patterns, of the tests to be carried out on specific commodities;

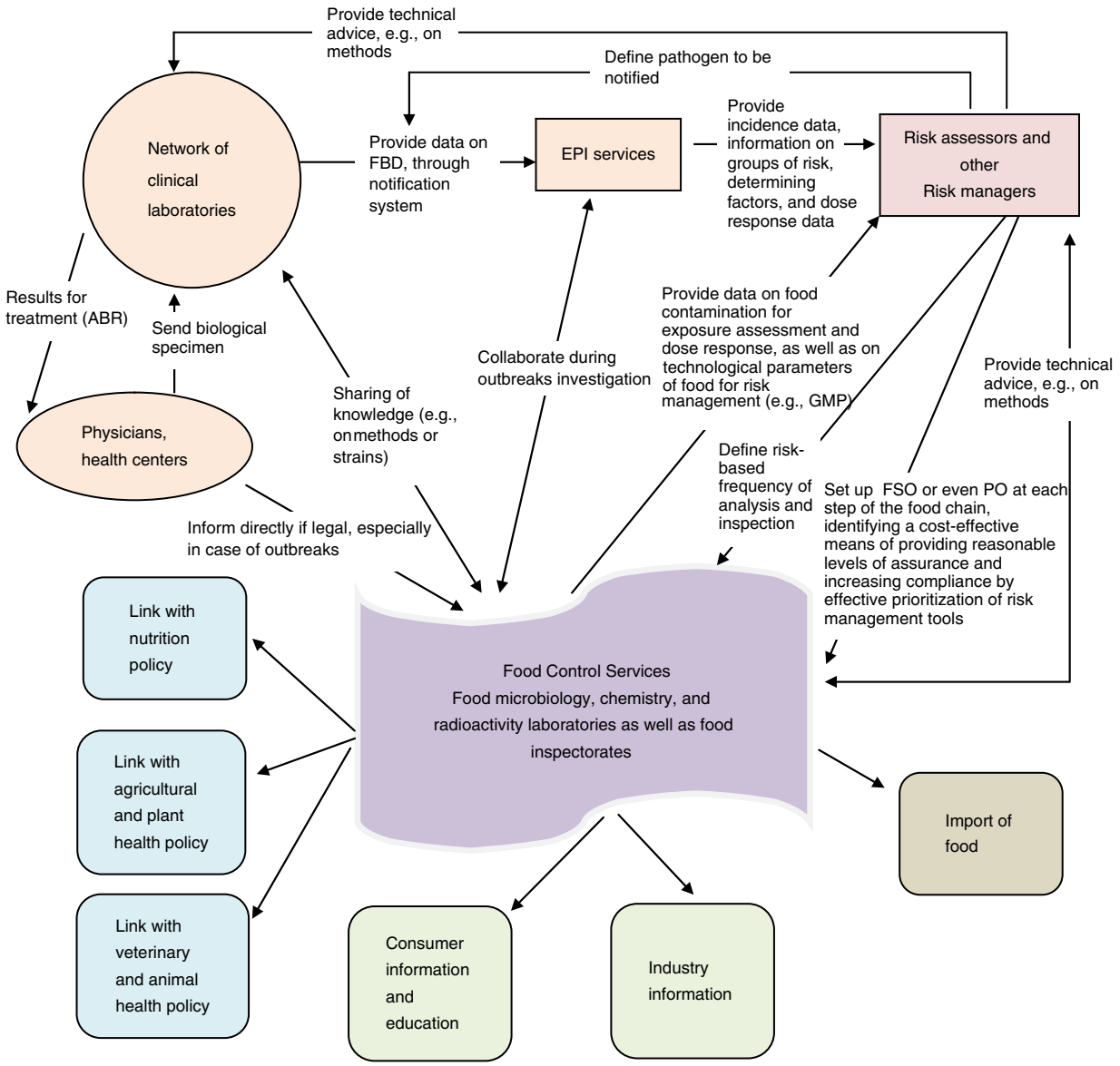

Figure 2 Interactions between food control and other public health authorities, other governmental bodies, or societal institutions.

d. Receive from risk managers (in principle from legislators) essential information on Food Safety Objectives in form of maximum residue limits or even Performance Objective for each step of the food chain, to be implemented as risk management tools;
e. Provide or receive, depending on their nature and capacity, important technical information or advice, for example, on analytical methods;
f. Liaise with custom services to provide adequate protection at importation level;
g. Interact with industry and other elements of the private sector to disseminate relevant information not only on legislation, but also on other risk-management tools such as sectorial GMP;
h. Keep in touch with consumer associations to disseminate relevant information necessary to implement food safety at home;
i. Communicate not only with the agricultural sector, thus primary producers, but also with the agricultural and economic institutions as well as veterinary services within the governmental administration to harmonize the approach and minimize conflicting situation between the economic and the health sector. Responsibility for food control in most countries is shared among different agencies. Further the roles and responsibilities of these agencies may be quite different and duplication of regulatory activity, fragmented surveillance, and a lack of coordination are common. There may also be wide variations in expertise and resources among the different agencies, and the responsibility for protecting public health may conflict with obligations to facilitate trade or develop an industry or sector;
j. Be in close contact with public health elements of the governmental administration dealing with nutrition in order to harmonize the approach and minimize conflicting situation between the safety and nutritional aspects of the food supply;
k. Work together with the physicians, epidemiological services, and network of clinical laboratories to collaborate during outbreak investigations as well as to share important information on agents and their antibiotic resistance, symptoms, methods of analysis and typing, patient patterns, data relevant for dose-response assessment, type of food involved and their technologically important parameters (such as pH, water activity, and redox potential), and so on. Close liaison must exist among food scientists and nutritionists, veterinarians, medical and other health professionals including epidemiology, surveillance, occupational health, and environmental control and laboratory services.

See also: Food Safety Assurance Systems: Food Safety and Quality Management Systems. Public Health Measures: Alerts and Early Warning Systems; Challenges of Developing Countries in Management of Food Safety; Challenges of Industrialized Countries in Food Safety Management; Evaluation of the Efficacy of National Food Control Programs; Food Inspections and Enforcement Systems; Foodborne Disease Outbreak Investigation; Fundamentals of Food Legislation; Modern Approach to Food Safety Management: An Overview; Monitoring of Contaminants; Surveillance of Foodborne Diseases

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PUBLIC HEALTH MEASURES

Health Education, Information, and Risk Communication

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Glossary

Case-control study Observational study in which subjects are enrolled based on the presence (cases) or absence (controls) of the disease of interest. Information is collected about earlier exposures and compared between cases and controls.

Fecal–oral transmission A means of spreading pathogenic microorganisms from feces produced by an infected host to another host, usually via the mouth, e.g. contact between contaminated hands or objects and the mouth.

Foodborne disease Any disease of an infectious or toxic nature caused by or thought to be caused by consumption of contaminated food, including drink. Frequently, the term food poisoning is used to mean foodborne disease. However, this use is misleading and is discouraged.

Foodborne pathogens Disease-causing microorganisms that are transmitted to humans via foods.

Food handler Any person who handles, prepares, or serves food, be they domestic food handlers, such as preparing family food, or professional food handlers, such as those working in food service establishments (cooks, waiters), retail stores, supermarkets, etc. (See also food worker.)

Food worker Individuals who harvest, process, prepare and serve food, i.e., across the whole food chain to retail/foodservice; it is a broader term than that of food handler, who typically works in foodservice establishments; however, the two terms are used interchangeably in the literature. (See also food handler.)

Outbreak A group of at least two cases of a single illness that are demonstrated by epidemiological investigation to have a single common exposure or source.

Introduction

There is no better introduction to such an article than the visionary speech of President John F. Kennedy, who in 1982 introduced The Consumer Bill of Rights, inscribing four rights:

1. The Right to Safety.
2. The Right to Be Informed.
3. The Right to Choose.
4. The Right to Be Heard.

Later, in 1985, the concept of consumer rights was endorsed by the United Nations through the United Nations Guidelines for Consumer Protection, which expanded these rights to eight basic rights, among which is The Right to Consumer Education.

The Consumer Bill of Rights was the forgoer of developments in food safety, which started in the 1980s, i.e., the recognition of

1. the importance of food safety,
2. the role of health education and consumer information, and
3. risk communication as an integral part of risk analysis, which in itself was to become the new approach to the decision-making process and management of food safety at national level.

From the above, it is clear that education and information of consumers in food safety or, more generally speaking, health education of consumers and risk communication are key responsibilities for any government. This should, however, not undermine the fact that other sectors such as academia and industry also have a role to play. For instance, through clear and validated labeling, industry is responsible for providing consumers with safety information, for example, declaration of food allergens, instructions on safe preparation or storage conditions, appropriate age or conditions of consumption, and/or any other safety warnings. However, the focus of this article is on the education and information of consumers as a governmental activity.

Health education in food safety goes beyond the simple exchange of information or communication of risks, although these constitute an integral part of it. Education per se can have many meanings, but it is generally defined as the process of learning and acquiring information. It is carried for different purposes such as imparting or acquiring particular knowledge or skills for a profession, a university degree, or more generally for developing the power of reasoning and judgment.

For the purpose of this article, health education in food safety refers to a process where the educator is providing necessary knowledge to empower and motivate the subject to a change of behavior, to adopting hygienic practice and/or making an informed and reasoned choice.

Education is also to be differentiated from training. Training is the process of teaching a person (or an animal) a particular skill or type of behavior, whereas education in food safety aims at influencing the way of life and empowering people to make

a reasonable and informed choice without imposing preconceived values. A key difference between training and education is that in training, the subject may learn to practice a behavior without knowing the reason or the science behind it. In education, the subject receives the knowledge and motivations to make informed decisions and choices.

The subjects of risk communication and crisis communication are addressed in other articles. Here, the subject of risk communication is addressed as it relates to health education and information.

Despite many international policies calling for increased education in food safety, and laudable efforts made during the past decades, in most countries of the world, the subject of health education in food safety has still not received the attention that it deserves, and a systematic education in food safety is yet to be established in the national public health and educational programs. For instance, to date, in spite of news on alarming outbreaks, few countries have introduced food safety in the curricula of their school health programs. Therefore, at the start of this article, it is important to write on the importance of the subject.

Importance of Health Education and Information

Although safety of food has probably been a cause of concern since the beginning of the existence of human beings, and foodborne diseases, be they microbial or intoxication, have prevailed since the dawn of history of humankind, it was in the mid 1980s that the subject of 'foodborne diseases' and its corollary 'food safety' have received increased and specific attention in public health.

A major milestone in the recent history of foodborne diseases was when a renowned group of experts of the World Health Organization recognized the gravity of the situation and in 1983 declared that "illness from contaminated food was perhaps the most widespread health problem in the contemporary world and an important cause of reduced economic productivity."

This declaration came at a time when several foodborne diseases such as salmonellosis and campylobacteriosis had started to increase, and new illnesses such as listeriosis and *Escherichia coli* O157 infections were emerging in the industrialized countries. Experience from these countries showed that with improvements in personal hygiene, safe water supply and basic sanitation, and the application of food technologies, such as pasteurization, a number of foodborne diseases, for example, shigellosis, typhoid and paratyphoid fevers, cholera, or brucellosis had decreased or been effectively prevented. Nevertheless, in spite of their extensive food control system (including legislation and enforcement mechanisms), industrialized countries continued to experience an impressive number of episodes of foodborne illnesses and these too encountered a major public health problem.

For instance, even to date, data from the US show that of the 178 million estimated acute gastroenteritis occurring each year in the USA, 48 million (27%) are believed to be foodborne in origin, resulting in 128 000 hospitalizations and 3000 deaths. This percentage is consistent with that found by a similar type of estimate made in the Netherlands, where 30% of the 1.8 million gastroenteritis cases are probably foodborne.

Additionally, an analysis of reported foodborne disease incidents and outbreaks in these countries has shown that a great proportion of these illnesses occur as a result of faulty handling of food during preparation and storage in homes or in food service establishments. Not undermining the fact that raw agricultural products can also be contaminated and are partly a source of these outbreaks; domestic or professional food handlers could render food safe, or prevent their contamination, if they are better educated in food safety (see Section Methods and Approaches for Selecting Key Behaviors) for the predominant errors in food handling that lead to foodborne illnesses.

From the experience of industrialized countries, it is very clear that a regulatory system, no matter how comprehensive it may be, cannot alone prevent the occurrence of foodborne illnesses.

Experience from industrialized countries has shown that when regulatory and educational measures have been combined, the measures have been effective in reducing foodborne diseases. A case in point is the measures taken in the UK and the USA to prevent listeriosis. Combined regulatory and educational measures have been successful in significantly reducing the incidence of this disease.

In developing countries, where foodborne illnesses are more widespread, the situation is alarming. These countries are affected by a range of foodborne illnesses: amebiasis, brucellosis, cholera, campylobacteriosis, *E. coli* gastroenteritis, poliomyelitis, and salmonellosis are only a few examples. With poor or nonexistent reporting systems in most countries, reliable statistics on these diseases are not available and their magnitude is, therefore, difficult to estimate. The gravity of the situation can, however, be appreciated by the high prevalence of diarrheal diseases, particularly in infants and children. Globally, some 1.3–1.5 million deaths of children aged less than five, i.e., nearly one in five child deaths is associated with diarrhea. Although this is a remarkable decrease from the 4.6 million of the 1980s, diarrheal diseases remain the second leading cause of death in children, after respiratory diseases. According to the 2004 estimates, an estimated 4.6 billion episodes of diarrhea occur worldwide per year for all ages and 2.2 million die as a result.

In spite of the reduced mortality, the morbidity has not declined in the past 30 years. In the developing regions of the world, on average, children less than the age of five continue to suffer 3.2 episodes of diarrhea per year. In a great proportion of these cases – possibly up to 70% – these are due to contaminated food and water used for drinking or for preparation of food. A plethora of pathogens, i.e., bacteria, viruses, and parasites are associated with infectious diarrhea. These include: pathogenic *E. coli*, *Shigella* spp., *Salmonella* spp., *Vibrio cholerae*, *Campylobacter*; protozoa such as *Giardia lamblia*, *Entamoeba histolytica*, *Cryptosporidium* spp.; and viruses such as rotavirus. In this context, it is to be noted that infections with *E. coli* are probably the most common cause of diarrhea in developing countries. The frequent contamination of foods with *E. coli* and pathogens of fecal origin signifies the contamination of food with fecal matters. Consequently, any pathogen that can be transmitted through the fecal–oral transmission of pathogens

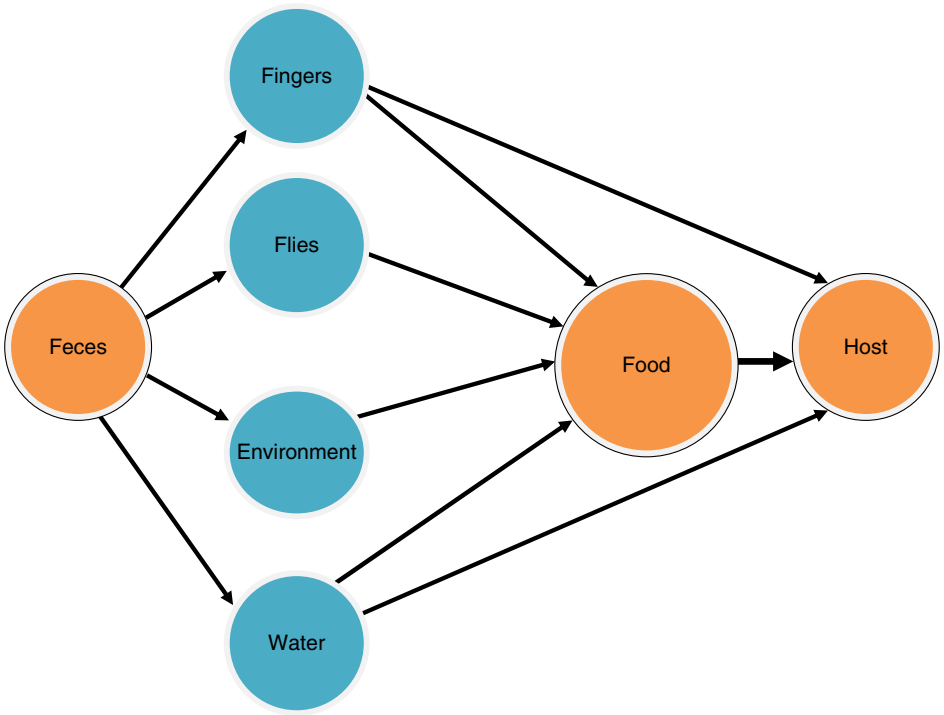

Figure 1 Role of food in the fecal–oral transmission of pathogens.

can be transmitted through food. The role of food in the fecal–oral transmission of pathogens is shown in Figure 1. Therefore, in developing countries, where subsistence farming and agriculture is more prevalent and food production and/or preparation are even less industrialized, education of the population in food safety, or guidance on how they should manage food safety risks in a contaminated environment, becomes even more important. Poverty, rudimentary sanitary conditions, and lack of food preparation facilities in the poorest countries are all aggravating factors and urge for a nonregulatory approach to food safety.

Over and above health aspects, education in food safety is also important because it contributes to the reduction of health care costs, as the cost of foodborne illnesses and their sequelae can by itself be a significant source of economic loss. An improved food safety situation also promotes trade and tourism, which are all contributing factors to prosperity and economic development.

There are also other reasons for which education in food safety should receive particular attention.

As alluded above, preparation is the last stage of the food chain before consumption and hence it is a critical control point (CCP) of the food chain. In this last stage, any foodborne pathogens that may have contaminated food at an earlier stage or during food preparation itself, or have found conditions favorable for growth to disease-causing levels, if not inhibited at this stage, are likely to cause infection in the target host. Also, as a result of urbanization, the food chain has become longer and more complex; thus, opportunities for contamination or growth of microorganisms are increased. Additionally, mass production has contributed to the spread of infections among food animals and contamination of derived foodstuff. All these factors underline the increasing role of food handlers and consumers and the importance of their awareness and compliance with good hygienic practices.

Certain groups of the population are subject to a greater risk of contracting foodborne infections and intoxication than others. These people need to be educated to protect themselves. Two high-risk groups can be distinguished:

1. Travelers, because they are more likely to be exposed to contaminated food and lack immunity to the microbiological flora of the country they visit.
2. Vulnerable people, because, for physiological or other reasons, they are more susceptible to foodborne infections. They include: infants and children, the elderly, pregnant women, persons who are undernourished, those with underlying illness, for example, liver disease or diabetes, and those who are immunocompromised due to an infection, for example, acquired immunodeficiency syndrome (AIDS), the malignancy of their disease, or their immunosuppressive treatment. Poor health and nutritional status caused by war, famine, and natural disasters make the population more vulnerable. In these latter conditions, the risk of epidemics is greater. Thus, all the populations more vulnerable to foodborne illnesses should be educated to protect themselves.

Tourism, migration, as well as international trade in food also lead to change in the dietary habits and expose the population to foods and pathogens that may be new to them. For instance, an increased immigration in Denmark in the 1990s led to increased consumption of red kidney beans among the local population. As the indigenous population was not familiar with the proper preparation of the beans, the

number of cases of intoxication due to hemagglutinin increased. Subsequently, the health authorities in Denmark launched a campaign to educate consumers in the proper cooking of various beans. Incidence of salmonellosis also increased in Japan after a change in dietary habits and increased consumption of foods of animal origin.

Advances of new food technologies in the society also justify the need for information and education of the population on the new technologies and the handling of the derived products. For instance, so often people think that a dried product is sterile as they can store the product at room temperature without it spoiling. A case in point is infant formula that after its reconstitution and storage at ambient temperature led to various illnesses in infants, such as salmonellosis and *Cronobacter* infection (previously referred to as *Enterobacter sakazakii*). Also, frozen prepared meals improperly warmed in microwaves have led to outbreaks.

Finally, consumers can make an informed decision which is right for them only if they are properly informed of the risks and benefits of the foods they eat and of the technologies that are used for their production or processing.

Frequently, the question is raised about the evidence for the efficacy of education for the prevention of foodborne diseases, in particular foodborne diarrhea. The problem in answering this type of request is that often education alone is not sufficient to prevent foodborne illnesses and as mentioned above about listeriosis, a combination of efforts is necessary. Also, when foodborne illnesses are prevented and an adverse event does not occur, it is still not possible to attribute the outcome to the efficacy of a specific preventive measure. Nevertheless, there are some studies and report which provide evidence for the positive impact of training and education in food safety. A case is the report of Seville Expo in 1992, where reportedly a 15 h accredited training course of some 8000 food handlers (including reserve staff) before opening of the Expo was effective in providing safe meals and foods for the visitors. Similar initiative were taken in case of 2010 International Federation of Association Football World Cup in South Africa. However, an experience from Latin America provides an insight to factors which may negatively influence the outcome of an educational and training activity. During 1995–96, the Pan American Health Organization conducted a study to evaluate the microbial contamination of street food sold in eight Latin American cities. Over and above the fact that a significant proportion of the foods were contaminated, it was also found that much of the effort made in training of street food vendors did not produce significant changes in the contamination of the foods prepared by them. Most of the hygienic procedures taught to food handlers, like hand washing, using dispensable utensils, clean clothing, etc. created additional costing that ultimately impacted on the price for consumers. Because consumers were not educated to appreciate the importance of improved hygiene, they preferred food with lower price. Subsequently, the discouraged street food vendors returned to their old habits.

Selection of Key Behaviors

Experience from the various fields of health has shown that change of behavior in the population, in particular the adult population, is often very difficult. Therefore, it is necessary to focus the educational program on a selected number of behaviors that are likely to have the highest impact on health and effectively prevent foodborne diseases. These behaviors are referred to here as key behaviors.

The selection of such behaviors is a fundamental question in any health education program. As opposed to some other areas of health where one hazard or behavior is targeted, such as the prevention of tobacco, the selection of key behaviors in food safety is particularly complex. These reasons are:

1. Education in food safety is aimed at preventing a wide range of diseases caused by totally different etiological agents: bacteria, viruses, parasites, naturally occurring toxins, and other hazardous chemical agents and antinutritional factors.
2. The etiological agents differ considerably in their behavior and ecology. Some, like most bacteria, grow on food, whereas others such as viruses and parasites do not. Bacteria alone show a wealth of variation. The range of temperature for bacterial growth varies widely. Some agents such as salmonellae are mesophilic and their growth is slowed down or stopped at refrigeration temperatures (below 10 °C); others such as *Listeria monocytogenes* or *Yersinia enterocolitica* can grow at these temperatures. Although, most parasites are killed by thorough freezing (-18 °C for at least 24 h), bacteria and viruses survive. Some agents like *V. cholerae* are sensitive to acids and may not survive an acidic food environment or even gastric acid, whereas others such as *E. coli* O 157 are acid resistant. Some bacteria grow or produce toxin only under anaerobic conditions, whereas others require oxygen. Some bacteria are hazardous if ingested (e.g., *Campylobacter*), whereas others are hazardous only if they have the opportunity to produce toxins in food (*Clostridium botulinum*). The toxin of some bacteria (*C. botulinum*) is thermolabile and is destroyed by adequate heating, whereas the toxin of some others (e.g., *Staphylococcus aureus*) is heat resistant. There may be variations even within one species. For instance, depending on the type of strain (diarrheal or emetic), *Bacillus cereus* may produce heat-labile or heat-stable toxins.
3. Food preparation is a complex procedure that involves a multitude of actions, some influenced by socioeconomic conditions or cultural habits. Not only the actions themselves may be hazardous (e.g. undercooking, storing food at room temperature, and touching with contaminated hands) but also the order in which they are carried out may constitute a risk factor.
4. The likelihood of contamination, i.e., the risk that a certain food or behavior will cause disease varies according to environmental conditions and source of food. For instance, manure or wastewater may present a health hazard, but if vegetables are grown according to good agriculture practice, they are generally safe. In times of algal bloom or red tide, in the sea, the consumption of shellfish or some species of fish may present greater health risk than that in normal circumstances. Thus, the type of food or method of preparation may be safer at one time or in one area, but unsafe at other times or in other environmental conditions.

5. The infective or toxic dose varies according to pathogens, toxic substances, individuals, and the food matrix. Some pathogens, such as *Shigella* spp., have a low infective dose, and even a few cells can cause disease. Others such as salmonellae may require a large number of cells to cause infection, and healthy adults are at risk only if the food is grossly contaminated or has been subject to time/temperature abuse. Even pathogens with a generally high infective dose may sometimes present a danger to health in low numbers if the composition or structure of the food is likely to protect from gastric acid. For example, some *Salmonella* serotypes in a chocolate matrix have been found to be infective at a low number, possibly due to the protective effect of the lipid component.
6. The risk posed by foodborne diseases is not the same for all individuals. Certain individuals are more vulnerable to some pathogens than other individuals are. Thus, a food or behavior may constitute a risk factor for one person but not for another. In addition, some persons may have allergic reactions to some foods or to ingredients, whereas others are not affected.
7. The likelihood of an adverse food safety event is related not only to the above factors but also to a range of other factors that find their root in the socioeconomical cultural conditions.

Therefore, for an effective health education intervention that requires a considerable amount of resources, time, and energy to be successful, it is important to:

1. base the health education intervention on the changes of behavior which have proven to lead to illness or are of relevance to food safety and
2. consider the sociocultural and economic factors underlying the risky behavior.

Methods and Approaches for Selecting Key Behaviors

Depending on the situation and nature of hazards, different methods can be used.

Toxicological or Epidemiological Evidence of Hazards Inherent in Certain Foods

Toxicological or epidemiological studies can provide evidence of hazards associated with certain foods, such as toxicants in mushrooms or wild green plants, marine biotoxins in fishery products, and hemagglutinin (lectin) in red kidney beans.

Monitoring Contaminants

It is a prospective approach in which information on the extent and level of hazardous contaminants and their health risk is obtained through monitoring of foodstuffs. Information collected can be used for regulatory action or education of the population. For instance, results of monitoring of methylmercury in fish have led some countries to advise pregnant women to limit consumption of some species of fish in order to keep the dietary intake of methylmercury within safe levels. In times of disasters (e.g., Chernobyl and Fukushima nuclear disasters), monitoring data have been used to advise the population to restrict consumption of affected foods.

Foodborne Disease Outbreaks and Epidemiological Investigation and Surveillance

Surveillance of investigated foodborne disease outbreaks and the root cause analysis of outbreaks provide information on major illnesses and their trend, the principal factors (mishandling) leading to foodborne diseases, places where food is likely to be contaminated, and the type of food frequently implicated. For instance, reviews of foodborne disease outbreaks in several countries have shown that major risk factors for salmonellosis and *S. aureus* intoxication is time/temperature abuses, whereas for shigellosis and typhoid fever it is the handling of food by an infected food handler.

The limitation of this approach is that in most countries, particularly in developing countries, the infrastructure and trained personnel for investigation of foodborne disease outbreaks are poor or nonexistent. Also, usually only outbreaks involving large numbers of people are investigated. Thus, statistics may not adequately reflect the incorrect handling leading to sporadic cases of foodborne diseases.

Worldwide investigation of outbreaks have shown that factors that are the predominant cause of foodborne disease outbreaks and should be the subject of health education measures are:

1. preparation of food several hours before consumption, combined with storage at a temperature that favors growth of pathogenic bacteria and/or formation of toxins;
2. insufficient cooking or reheating of food to reduce or eliminate pathogens;
3. use of contaminated water or raw food material;
4. cross-contamination in the premises where food is prepared; and
5. infected or colonized persons in charge of the preparation of the meals.

Case-Control Studies of Sporadic Cases

Epidemiological case control of studies of sporadic cases is widely used to study risk factors and thus identify behaviors that need to be changed or promoted. Case-control studies can be simple and economical to carry out. However, the validity of the factors identified and the success in targeting the relevant risk factors depend very much on the design of the study. As the studies become more comprehensive, the validity of the results improves, but so do the expenses and the complexity of the study. Unfortunately, many studies omit factors related to food safety or do not reflect them properly. As a result, conclusions may be misleading. For instance, for the prevention of diarrheal diseases, scientists have investigated the impact of toys, baby bottles, and dirty diapers strangely without even questioning the role of food contamination.

The Hazard Analysis and CCP (HACCP) System

The HACCP system is a scientific method for food safety assurance. It consists of a systematic identification and assessment of hazards and the determination of effective control measures and monitoring of those that are critical for safety. The steps identified as critical are referred to as CCPs and are the key behavior that should be the target of educational interventions.

The system was originally developed for ensuring the safety of foods for astronauts. Today, it is applied worldwide, across the entire food chain from primary production, processing, and manufacturing to final preparation and consumption of foods. It is intended to focus the attention, efforts, and resources on the measures critical for the safety of food. The full system as applied in the food industry has seven principles, including documentation and record keeping. In health education in food safety, the HACCP system has two applications. First, the system can be used as training of professional food handlers. In this case, food handlers are taught to think along the first five principles of the HACCP system, i.e., to evaluate the potential risks with their raw materials and operations and how to use the various control measures (cooking, refrigeration, washing, etc.) to manage the safety of prepared foods.

The second is the application of the system for analyzing the food preparation practices in homes, street food vending operations, cottage industry, etc., and for identifying the steps and practices that are critical for safety or are CCPs. As mentioned above, these are the 'key behavior' which should be subject of educational interventions. The application of the HACCP system has been particularly important for the education of caregivers in the preparation of infant food or of street food vendors. Another application, outside of the scope of this article, is in the training of food inspectors so that they focus their attention, in priority, on CCPs of the food operations and confirm that these points are well managed.

In this context, it is to be noted that the risks at each step of food preparation, i.e., the decision whether the step is critical for safety or not depend on the general hygienic conditions and an accurate evaluation of these. In other words, the focus on critical steps should not undermine the importance of underlying hygienic conditions that are a premise for the validity of the hazard analysis.

Factors Influencing Behavior

In relation to food safety, behavior is influenced by a number of cultural, socioeconomic, and environmental factors. Experience has repeatedly demonstrated that knowledge of risks alone will not bring a change in behavior. There are cases where people will adopt risky practices or eat a risky food, knowing the dangers that are involved.

There may be different reasons for this; for instance, it may be due to their perception of the risk and their acceptability of the risk, their bias and overoptimism about the likelihood of occurrence of the illness, or the severity of it. There may also be economical or social reasons. Therefore, social scientists have classified the conditions necessary to change behavior into three groups:

1. Predisposing factors: These are the antecedents to the behavior that motivate or provide the person with the rationale to change behavior. Knowledge of food safety hazards and their risks for health are among these. Other such factors are beliefs, values, attitudes, confidence, and existing skills.
2. Enabling factors: These are the conditions in the environment that enable the motivation to be realized. Examples of enabling factors are availability or accessibility to facilities for food preparation (e.g., easy access to water and facilities for hand washing, means for cooking, or refrigeration) and supporting policies (e.g., legal framework such as paid sick leave for professional food handlers or maternity leave enabling mothers to breast feed and care for their children).
3. Reinforcing factors: These are the factors that follow the behavior. They provide the continuing reward or incentive for the behavior and contribute to its persistence and/or repetition. Among the reinforcing factors is the reward or approbation of parents, a teacher, or the manager in the case of a food service establishment for hygienic practices or reprimand by them in case of poor practices. Peer pressure is also an important reinforcing factor that can encourage the individual to adopt certain positive or negative behavior.

Therefore, over and above identifying the factors that lead to illness from a pure scientific perspective, it is important to also understand the underlying factors that motivate individuals to adopt or reject good practices, in particular their perception of risk and their belief, and build the education program accordingly. **Box 1** presents examples of risk perception of people or professionals on food safety or on the causes of foodborne illness. These lead to errors in their practices or influence their acceptance of risk.

To better understand these social and cultural factors, sociologists have different methods such as Knowledge,

Box 1 Common misconceptions in food safety, among both general public and health professionals

1. An unsafe food will look, smell, or taste bad
2. What is visibly clean is safe (free from bacteria)
3. Cooked food can be stored at ambient temperature for extended time as the bacteria are killed
4. A dried food, for example, powdered milk and infant formula, is sterile
5. Warming or cooking food is for improving palatability and digestibility (its importance for safety is often overlooked)
6. Food additives are a health hazard
7. Food irradiation makes food radioactive
8. Unprocessed foods are generally safer
9. Diarrhea is a benign and self-limiting health problem with no long-term health impact
10. Contamination with a few bacteria has no major health consequence, ignoring some bacteria have a low infective dose
11. Diarrhea is caused by imbalance of heat and cold associated with food (general public)
12. Diarrheal illnesses are mainly waterborne (health professionals)
13. Outbreak data represent the magnitude of foodborne diseases (health professionals)
14. Microbial safety of products can be prevented by testing
15. All hazardous chemicals in food present a risk
16. Naturally occurring chemicals are not hazardous

Attitude, and Practice studies; surveys; focus groups; or root cause analysis in case of an incident.

Implementation of Health Education in Food Safety: Strategy, Plan of Action, and Evaluation

Target Audience

In principle, health education in food safety should target almost the entire population from the policy makers to food producers, food processors, professional food handlers, and consumers, as all have a role in food safety and all are also potential food handlers. However, for the purpose of this article, the target groups considered here are specifically those who have a direct and key role in food preparation, such as professional food handlers or consumers, or those that, due to their susceptibility (e.g. elderly, pregnant women), need to receive a greater emphasis in the health education programmes. These include:

1. Domestic food handlers, particularly mothers of small children.
2. Professional food handlers including those who handle, prepare, and serve food in cottage industries, food and catering establishments, retail outlets, supermarkets, and street food vendors.
3. High-risk groups, including travelers.

Partners

Governments, in particular the health sector, have the leading role in health education in food safety. They are responsible for initiating, coordinating, and implementing food safety educational programs and related measures. However, the impact of their effort is strengthened if the educational program is developed and implemented in partnership and collaboration with other sectors or community groups. Examples of partners who are in a position to influence food handlers are presented in Table 1.

Plan of Action

Problems, sociocultural features of the population, and available resources vary from country to country. It is, therefore, not possible to devise a plan of action or provide a curriculum for a food safety education program that would be globally applicable. Each country or region should develop a plan of action that is relevant to its needs, population characteristics, and infrastructure.

However, here few recommendations are given on the subject. They are valid for educational programs, whether they are applied at national level are more specific or limited in scope or time (Figure 2).

Recognition, Commitment, and Resources

Political recognition and endorsement of the aims and objectives of a food safety education and support from prominent community leaders and institutions are essential to the success of a program. Thus, the first steps toward health education in food safety are to raise the awareness of policy makers on the importance of foodborne disease and the role of health education in food safety, ascertaining commitment for integrating education in food safety into national food, nutrition, and related public health policies. Resources for the implementation of the program also need to be identified and made available.

Coordination

Education in food safety has a greater chance of success if all concerned sectors are appropriately involved. The public health sector has the leading and coordinating role. However,

Table 1 Partners in education in food safety by target population, as applicable to the system of the country

Partners for implementation	*Target population*
Public health services, primary health care centers, clinics, hospitals, and physicians	Parents of small children High-risk groups (elderly, children, immunocompromised individuals, pregnant women, and travelers) and general public
Maternal and child health centers	Pregnant or lactating mothers of infants and young children
Academia (universities and research institutes)	Food workers, public health professionals, food scientists, food inspectors, and policy makers
Secondary and primary schools	Children and adolescents, teachers, and parents
Professional schools	Professional food handlers (cooks and waiters/waitresses) and managers of hotels and restaurants
Food industry, including food service and catering establishments	Professional food handlers or food workers, supermarkets, retailers, and consumers
Supermarkets and retailers	Professional and domestic food handlers
Mass media	Policy makers and the general public
Food inspectors (consumer affair bureau)	Consumers, food industry, food service and catering establishments, and street food vendors
Consumer groups	Consumers and policy makers
Religious and social institutions	Consumers, particularly those disadvantaged
Tourism sector (travel agencies and tour operators)	Travelers and hotel management personnel
Local police and municipalities	Street food vendors, retailers, and food service establishments

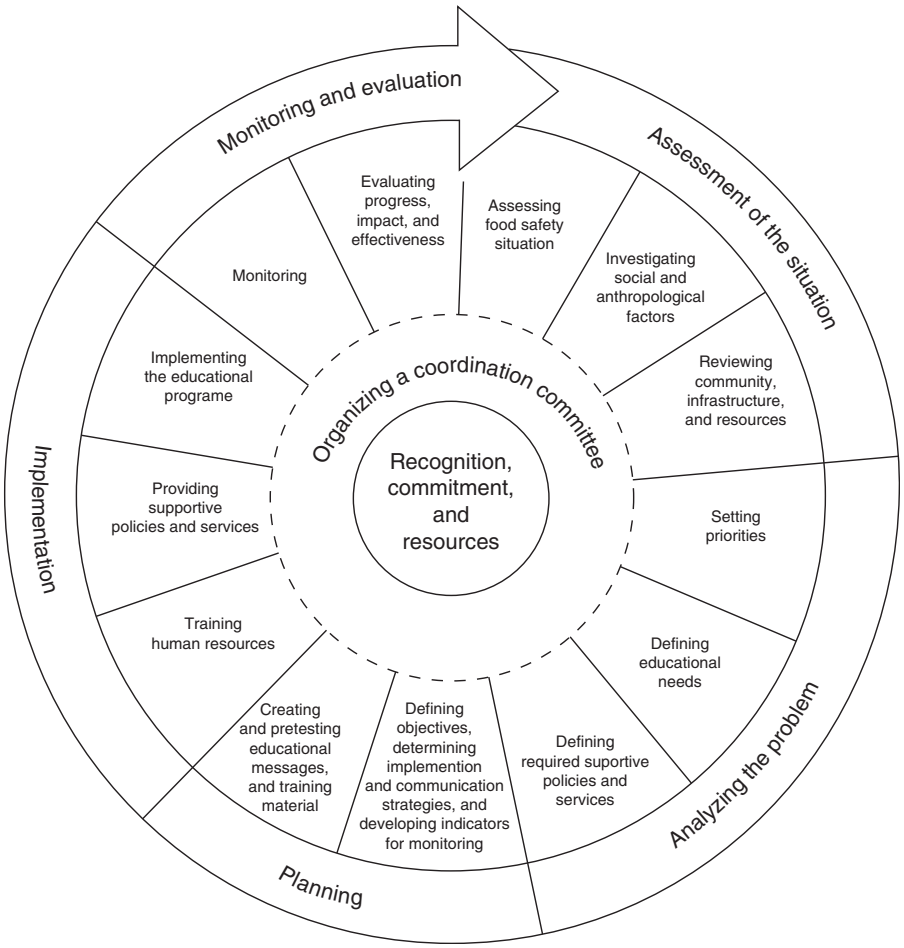

Figure 2 Key elements of a plan for health education in food safety.

through an interdisciplinary committee, or a similar mechanism, it must also involve representatives of other sectors, be they governmental or nongovernmental (e.g., universities, research, and consumer groups).

Assessment of the Situation

Health education in food safety should be culture specific. It must respond to the health, technological, social, and economical situation that prevails in a particular society or in particular cultural or social groups. The educational program or interventions should take into account problems and needs that are specific to the target groups. As mentioned before, it should be based on a combination of two types of information: (1) technical information on food safety and practices that leads to foodborne illnesses and (2) underlying social and economic factors that influence food safety. It should also take into account available resources and the characteristics of the target population. The latter will influence the strategy and partners for implementing the program.

Where such data are not available, research studies may need to be initiated before planning and implementing the program. Universities and other health and educational institutes can facilitate this kind of study. The assessment of the situation and the identification of the problems would also require an interdisciplinary team including epidemiologists, food scientists, and anthropologists.

Analysis of Problems and Decision Making

The results of the assessment should lead to the analysis and identification of problems and priorities, selecting appropriate strategies, defining educational needs, and decisions on the supportive policies and services.

Planning and Implementation

Following the identification of problems and educational needs, a plan should be developed. The plan should specify priorities, define objectives, and determine strategies for communication and for the training and education of various target groups or population groups. The plan should determine the need for qualified and trained human resources, supportive policies, and other services as well as the means to achieve them.

The selection of strategies and partners for implementation should take into consideration the characteristics of the population and the existing infrastructure. As part of the planning and implementation process, training material or educational messages need to be developed for the different

Table 2 Criteria for evaluating food safety education programs

Criterion	*Definition*	*Example of application*
Effectiveness	The degree of attainment of predetermined objectives	Have food handlers' practices improved? Have the levels of knowledge increased and behavior changed? What percentage of food handlers has adopted the desired behavior?
Impact	The overall effect on health and related socioeconomic development	What is the overall effect on health and related socioeconomic development? Has there been a decrease in foodborne diseases or related economic costs?
Efficiency	The relationship between the results obtained and resources spent	What is the relationship between the results obtained (decrease in incidence of foodborne diseases or number of food handlers trained) and resources spent?
Progress	The comparison of actual with scheduled activities to ensure that operations are proceeding as planned	Did the program go as planned? How many food handlers have been trained compared with the number originally planned? How many households have been covered compared with the planned number?
Adequacy	Whether sufficient attention has been paid to certain previously determined courses of action	Has the program adequately covered all the target audiences? Has sufficient attention been paid to vulnerable groups (e.g., infants, pregnant women, and the elderly)?
Relevance	The rationale for selecting behavior in terms of relevance to foodborne diseases as well as the social and economic consequences	Is the behavior being changed in relevance to the foodborne diseases in question (e.g., hand washing will not be relevant to prevention of botulism)?

target groups. This involves translating the technical and scientific information into training programs and educational messages that are readily understood and accepted by the target populations, taking into account their culture and socioeconomic situation.

Before launching a long-term program, it may be necessary to carry out a pilot study to test its efficacy. Pretesting of educational material is important to ensure that the message is understood correctly. The message or material can be tested for clarity and accuracy on a small representative group of the general audience. Pretesting of the educational material is particularly important when the material is intended for persons of different cultures.

At the planning and implementation stages, it is also important to consider indicators that will be used in monitoring and evaluation. This will enable constraints to be identified and remedial actions to be taken if necessary.

Monitoring and Evaluation

Even when educational programs are well designed and implemented, monitoring and evaluation can significantly contribute to their improvement. They should be considered as integral parts of each program and should take place as the program proceeds as well as when it ends. The purpose of evaluation is to ascertain whether the intervention has been successful. Evaluation also helps to identify changes that may be desirable or necessary to improve the program. Pretesting of the education material and the research studies mentioned above are themselves forms of evaluation.

Depending on the results of the evaluation, or other findings and changes (e.g., change in the epidemiology of foodborne diseases, levels of food contaminants, food production practices, and emerging hazards), it may be necessary to bring changes to the educational plan.

Evaluation can be carried out according to criteria listed in Table 2.

Conclusion

To date, foodborne diseases remain a major public health problem. Health education in food safety is the cornerstone of strategies for the prevention of foodborne disease, keeping the population abreast with technological developments and enabling them to make informed choices. In principle, the entire population should receive education in food safety, as everyone has a role in food safety and everyone is a potential food handler or a consumer. To this end, a systematic, effective, and long-lasting approach is needed. Public health authorities must play the leading role, particularly as the medical community is often the most trusted source of information.

Many health education initiatives have failed because the fundamental conditions were not met. Some among these are as follows:

- Understanding that transfer of knowledge is necessary but not a sufficient condition for an effective program. Health education interventions based on the sole transfer of knowledge are not effective in changing behavior. There is a need for complementary measures such as policies, services and infrastructure, and social–cultural promotion. For instance, health education programs in the industrialized countries have for years provided knowledge on simple hygiene processes such as the need to wash one's hands after using the toilet. However, repeatedly, surveys show that deficient habits persist among food handlers. Similar observations have also been made in the medical community where physicians, both in the industrialized

and developing countries, neglect to wash their hands before examining their patients, in spite of the knowledge. Transfer of knowledge should go hand in hand with understanding and addressing factors that are a constraint or that can motivate the person to change behavior.

- Failures in the planning process. Health education should not be a shopping list of actions but should be based on scientific evidence of factors leading to illness and understanding the underlying sociocultural factors. Modifying behavior should have a significant impact on health. Many resources have been wasted promoting behavior that has little or no relevance to health problems.

In conclusion, health education in food safety should be considered as an essential public health function; together with regulatory measures, it is a pillar for an effective national food safety program. A rigorous scientific approach is essential as a poor or erroneous education is likely to do more harm than good.

Disclaimer

This text is based on, and is an update of, an earlier publication by the author, a book entitled *Foodborne Disease: A Focus for Health Education*, published by the WHO in 2000.

See also: Food Safety Assurance Systems: Essentials of Crisis Management; Hazard Analysis and Critical Control Point System (HACCP): Principles and Practice; Personal Hygiene and Employee Health. Foodborne Diseases: Foodborne Diseases and Vulnerable Groups; Foodborne Diseases in Travelers; Overview of Biological Hazards and Foodborne Diseases; Overview of Chemical, Physical, and Other Significant Hazards; Overview of Emerging Food Technologies. Public Health Measures: Management of Food Safety in Food Service Sector. Risk Analysis: Food Safety Training and Health Education: Principles and Methods; Risk Communication: Biological Hazards; Risk Communication: Chemical Hazards; Risk Communication: Novel Foods and Novel Technologies; Risk Communication

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Management of Food Safety in Food Service Sector

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Glossary

Catering The preparation, storage and, where appropriate, delivery of food for consumption by the consumer at the place of preparation or at a satellite unit.
Cook-chill A specialized food production and distribution system for prolonging the life of prepared and cooked food by rapid chilling, storage at refrigeration temperatures, and reheating at the time of service.
Cook-freeze A specialized food production and distribution system for prolonging the life of prepared and cooked food by rapid freezing, storage at frozen temperatures, and reheating at the time of service.
Institutional catering Providing a mass catering service to people in an enclosed community such as a hospital, school, old people's home, or prison.
Mass catering The preparation, storage, and/or delivery and serving of food to a large number of people.
Open-air catering Providing a mass catering service to people out of doors such as in a disaster or civil emergency or attending an open-air sporting event or festival.
Sous-vide A specialized food production and distribution system for prolonging the life of food by cooking it in a vacuum pack, then rapidly chilling it for storage at refrigeration temperatures, and reheating at the time of service.
Travel catering Providing a mass catering service to people in transit such as on an aeroplane or ship or on holiday staying in a hotel or holiday camp.

Introduction

Mass catering technologies include the cook-freeze, cook-chill, and sous-vide systems. The situations where mass catering is used can present additional risks, for example, catering in hospitals or in schools can expose vulnerable groups to risks that might not affect the population at large.

Mass catering requires sophisticated technology, fully trained staff, intensive monitoring, and a considerable capital outlay. If these are not available then simpler, more labor intensive traditional systems need to be employed.

Mass Catering

Traditional catering is the professional supply of food to order for individuals or small groups. Consequently, a relatively small amount of food is prepared when ordered and served with little delay. In contrast, mass catering involves the preparation of large volumes of food to be stored in advance for a set period of time and then served not only to individuals and small groups but also often to a large number of people at the same time. In the current economic climate, there is an increasing demand for mass catering with its associated convenience and economy of scale. Preparing food in advance for serving at different times of the day can also be referred to as 'time-shift catering'. Mass catered food may also be prepared centrally and then distributed to a number of outlets or consumers that may be geographically situated over a wide area. Therefore, in order to be practical, economical, and safe, mass catering can necessitate the production, storage, distribution, and serving of food in bulk quantities. Traditional catering premises, or domestic kitchens for that matter, are not geared up for this type of operation. Bulk food preparation is predicated on the use of new technologies and specialized equipment and processing. These can introduce risks not typically seen with traditional small scale catering. As a result, the management of food safety in this food service sector has to develop novel food management techniques to account for these highly specialized production systems.

In short, mass catering requires sophisticated technology, fully trained staff, intensive monitoring, and a considerable capital outlay. If these are not available then simpler, more labor intensive traditional systems may need to be employed. Alternatively, less complicated meals or smaller quantities of food should be produced if circumstances allow this.

Management of Food Safety in Mass Catering

Although many of the principles of hygiene, food safety, and nutrition are common to all forms of catering, many systems of mass catering and the situations in which mass catering is employed present their own particular challenges. Many of the hazards are common to all forms of catering but the mass catering food environment is different. For example, when cooking bulk quantities of food, it is much more difficult

to deliver sufficient heat to kill vegetative pathogenic microorganisms than with smaller quantities of food. Similarly, it is much more of a challenge to subsequently cool bulk quantities of food and then chill or freeze them to prevent germination of spores and subsequent increase of vegetative cells to numbers of concern. Following this, reheating bulk quantities of food is much more difficult than for small-scale catering. Finally, serving bulk quantities of food at the same time presents many logistical challenges with numerous associated opportunities for cross-contamination from food handlers or other sources.

Risk assessment of the use of mass catering in the food service sector reveals an additional factor that demands major consideration. The hazard identification step requires an examination of the susceptibility of the exposed population to the potential hazards being assessed. As will be seen later in this article, mass catering food service techniques are sometimes used in hospitals, schools, care homes, and even in disaster situations where vulnerable groups that will have reduced resistance to foodborne illness will be present.

By definition, an outbreak associated with mass catering is likely to involve large numbers of people in some cases in the hundreds or thousands. An outbreak associated with mass catering also has the potential to be geographically widespread, either through people dispersing after consuming the contaminated food, for example, air travel or people coming from long distances for an event, whether a wedding reception or the summer Olympic Games.

It is not easy to find reliable statistics on the number of cases of foodborne illness caused by mass catering. Often the general term 'catering' is used, so it is difficult to discriminate between traditional and mass catering. Table 1 shows the verified outbreaks of foodborne illness in the European Union in 2009 by reported setting. The largest outbreak was caused by *Shigella sonnei*, involving 58 cases and took place in a workplace canteen. Todd et al. reviewed outbreaks where food workers have been implicated and reported that some of the outbreaks were very large; 11 involved more than 1000 persons, 4 with more than 3000 ill. The larger outbreaks tended to be extended over several days with a continuing source of infections, such as at festivals, resorts, and community events, or the contaminated product had been shipped to a large number of customers, for example, icing on cakes or exported raspberries. During an outbreak of salmonellosis caused by *Salmonella indiana* during the European Summit Conference in Maastricht in 1981, it is estimated that 600–700 of the 1000–1200 exposed persons became ill. The cause of the outbreak was probably a salad base, which was used in various snacks and cold dishes. Serious errors were made during preparation of this salad base which allowed the *S. indiana* present in the product to multiply on a large scale.

Table 1 Verified outbreaks of foodborne illness in the EU in 2009 by setting

	Percentage (N = 977)
Household	36.4
Restaurant, café, pub, hotel	20.6
Unknown	15.0
Temporary mass catering (fairs, festivals)	2.8
Hospital or medical care facility	4.8
Canteen or workplace catering	4.9
School, kindergarten	5.5
Other setting (nursing home, prison, boarding school, aircraft, ship, train)	9.9

Source: Reproduced with permission from European Food Safety Authority and European Centre for Disease Prevention and Control (2011) EU summary report on trends and sources of zoonoses and zoonotic agents and food-borne outbreaks 2009. *EFSA Journal* 9(3): 2090. [378 pp.]. doi:10.2903/j.efsa.2011.2090. Available online: www.efsa.europa.eu/efsajournal

Risks and Hazards Associated with Mass Catering

The risks and hazards associated with mass catering are more or less the same as with any other food production system and can be classified as biological, chemical, or physical. Biological hazards include: infectious bacteria, for example, *Salmonella* and *Listeria monocytogenes*; toxin-producing organisms, for example, *Clostridium botulinum*; and viruses, for example, norovirus. Biological hazards are controlled by following the general rules of food hygiene set out in the WHO Five Keys to Safer Food, namely: keep clean; separate cooked and raw; cook thoroughly; keep food at safe temperatures; and use safe water and raw materials. Generally speaking, foods should be cooked to an internal temperature of 70 °C. Chilled foods should be held at as low a temperature as possible, generally <5 °C depending on the food, whereas frozen foods are generally held below -18 °C. In any case, every effort should be made to avoid holding foods in the 'danger zone' of 5–60 °C for more than 2 h. Of course, the technical and practical challenges of achieving these requirements with high volumes of food are significantly more demanding for mass catering than for traditional catering and are discussed later in this article, along with control measures relating to more specialized products, for example, sous-vide foods.

Chemical hazards include some food additives and a range of food contaminants such as heavy metals, dioxins, radionuclides, veterinary drug residues, and pesticide residues as well as contaminants from processing and packaging or other environmental contaminants; allergens such as peanuts and naturally occurring toxins such as those from poisonous mushrooms or toxic algae in seafood. It is difficult, if not impossible, to eliminate chemical hazards from foods therefore controls focus on using uncontaminated raw materials and preventing contamination during processing, storage, and distribution. In terms of food allergens, all staff, including servers, should appreciate the importance of this subject and be sensitive to this issue when serving clients.

Physical hazards are myriad and include: metal filings, glass, jewellery, stones, and bone chips. Control measures include source control, for example, vendor certification and raw-material testing; or production control, for example, metal detectors and visual inspection.

General Risk Management

The Codex Alimentarius Commission has produced a code of hygienic practice for precooked and cooked foods in mass catering based on the principles of the hazard analysis critical control point (HACCP) system. It covers many of the general principles of food hygiene including and prerequisites to HACCP. Key points specific to mass catering are:

- Establishments should be located in areas, which are free from objectionable odors, smoke, dust, or other contaminants and are not subject to flooding. However, in order to allow for safe storage and processing of bulk quantities of food, mass catering really should take place only in specifically designed premises. Simply scaling up a hotel or restaurant kitchen is plainly not good enough. The equipment needs to be properly designed for the storage, chilling, freezing, cooking, and handling bulk quantities of food. Furthermore, it needs to be designed to operate hygienically and be easy to clean.
- As for all food premises, mass catering facilities not only need to be sited near a good infrastructure with potable water and a reliable power supply but must also avoid areas of heavy industry where environmental pollution might contaminate the food.
- Managers should arrange for adequate and continuing training of every food handler in hygienic handling of food and in personal hygiene so that they understand the precautions necessary to prevent contamination of the food. Training should initially cover the rules of general hygiene but then progress to be specific to the type of operation under consideration. However, many countries lack sufficient suitable skilled workers required for some of the more complicated modern specialized equipment and processes designed for mass catering. In addition, this industry sector is well known for its rapid turnover of staff. All of these factors can make it difficult to guarantee a workforce well trained in mass catering equipment and techniques. In some countries, the competent authorities will require records of any training undertaken to be kept for inspection.
- No raw materials or ingredient should be accepted by the establishment if known to contain parasites, microorganisms, or other hazards that will not be reduced to acceptable levels by normal plant procedures of sorting and/or preparation or processing. Catering for large numbers of people demands an adequate supply of raw materials of satisfactory safety and quality. This can place a significant strain on local food supplies, especially in developing countries or temporary sites such as festivals or disaster relief. Linked to the raw materials is a coincident need for trained staff and management as well as sufficient QA and laboratory analysis resources.
- Raw foods of animal origin should be stored between 1° and 4 °C. Other raw foods which require refrigeration should be stored at as low a temperature as quality permits.
- Frozen foods and raw materials should be stored at or below −18 °C.
- Reheating the food should be carried out rapidly: a temperature of at least 75 °C should be reached in the center of the food within one hour of removing it from refrigeration. The reheated food should reach the consumer as soon as possible and at a temperature of at least 60 °C.
- In self-service establishments, the foods should be protected from contamination and held at either below 4 °C or above 60 °C.
- Care needs to be taken with transportation of cooked meals so that they remain hot not only in the vehicle but also during distribution to the patrons who may be hospitalized patients or passengers in an aircraft.

New Technologies

Cook-Freeze and Cook-Chill

Cook-freeze is a specialized food production and distribution system for prolonging the life of prepared and cooked food by rapid freezing, storage at frozen temperatures, and reheating at the time of service. Meals are cooked at a central factory and then rapidly frozen and stored at temperatures of at least −18 °C and preferably as low as −30 °C. They are then distributed in refrigerated transport to where the food is to be reheated and served when needed. The length of storage depends on the food, but typically it can be stored for months. For longer storage, the food may be subjected to pasteurization after cooking.

Cook chill is similar to cook freeze except that the food is cooked but then stored and distributed at chill temperatures of 5 °C, or preferably, below. Organisms that survive the cooking process or contaminate the food after cooking can grow, albeit slowly, at chill temperatures, so these products have a much shorter shelf life than Cook-freeze products, typically 5 days or less. Hence, more attention needs to be applied to stock control for safety and quality reasons. The advantage for these foods over Cook-freeze relate mainly to the lower energy usage and the potential for a better quality final product. In contrast, cook-freeze requires more energy but is more flexible because of the longer shelf life of the product.

Cook-chill foods should be stored at below 5 °C. Because of this the organisms of most concern are those that have the potential to grow below this temperature after having survived the cooking process or re-contaminated the food after cooking. The main organism of concern is *L. monocytogenes*, especially for cook-chill foods with a shelf life of more than 5 days. The risk is managed by hygienic design of the production process and ensuring that every part of the dish receives a heat treatment of 70 °C for 2 min, or equivalent, on reheating. Risk management for psychrotrophic *C. botulinum* is discussed in the sous-vide section below.

For operations on the scale of mass catering, both systems require dedicated cooking equipment and design of the product to maximize its freezing or chilling and reheating properties. In particular, they demand modern equipment in order to facilitate rapid chilling or freezing of the product to limit or prevent the growth of pathogens. The key feature of blast chillers and blast freezers is that they rapidly reduce the temperature of hot foods to low, safe temperatures. Such equipment is not suitable for use in domestic kitchens because they can produce large amounts of condensate and so may require specially designed premises to pipe the condensate away from the food. They can be difficult to maintain and

repair and clean. Indeed, if not cleaned properly, the equipment itself can be a source of contamination, for example, by *L. monocytogenes*. In addition, they need a continuous supply of power as well as instructions for what to do if they fail or there is an interruption to the power supply.

Cook-chill and cook-freeze foods can require specialist reheating equipment because pathogenic organisms can still potentially be present. The main target group for cook-chill and cook-freeze foods is people who have no time or facilities to spend cooking. Typical categories would be schools, pensioners, and hospitals.

Sous-Vide

Sous-vide involves cooking a dish that has been vacuum packed before cooking. This has two food safety benefits compared to cook-chill. First, the exclusion of air from the vacuum bag greatly reduces the growth of aerobic bacteria and thus delays spoilage of the contents. Second, cooking in pack prevents recontamination by organisms such as *L. monocytogenes*. Pasteurizing sous-vide foods will kill vegetative pathogens but not spores. This includes spores of psychrotrophic *C. botulinum* (Types B, E, and F), an organism that can grow and produce potentially fatal neurotoxins under refrigeration (down to 3.3 °C) under anaerobic conditions: conditions that can apply to sous-vide foods. The risk of botulism is compounded by the absence of vegetative spoilage organisms that would otherwise out-compete the *C. botulinum* or spoil the product so that it would not be consumed. However, to date, there have been no reports of sous-vide foods held under refrigeration having caused any cases of botulism.

The following controlling factors can be used singly or in combination to prevent growth and toxin production by nonproteolytic *C. botulinum* in chilled foods with a shelf-life of greater than 10 days, the shelf-life beginning as soon as the controlling factors have been first applied:

- a heat treatment of 90 °C for 10 min or equivalent lethality,
- a pH of 5 or less throughout the food and throughout all components of complex foods,
- a minimum salt level of 3.5% in the aqueous phase throughout the food and throughout all components of complex foods,
- a water activity of 0.97 or less throughout the food and throughout all components of complex foods, and
- a combination of heat and preservative factors, which can be shown consistently to prevent growth and toxin production by nonproteolytic *C. botulinum*.

Microwave Reheating

Microwave ovens reheat foods by dielectric heating: electromagnetic radiation vibrates water molecules in the food to the extent that they generate heat, thereby cooking or reheating the food. It is well recognized that microwave equipment can suffer from uneven reheating, resulting in cold and hot spots. For this reason, the general advice is to use microwaves to reheat foods not cook them and ensure that the food reaches 70 °C for 2 min or equivalent or is 'piping hot throughout'. Domestic scale microwave ovens are not suitable for mass catering. Industrial scale microwave ovens should have fewer problems with uneven heating and use thermocouples to ensure that the target time/temperature is met.

Institutional Catering

Hospitals

Hospital catering presents a unique challenge to food safety because of the presence of various types of vulnerable groups: be they very young, elderly, pregnant, immunocompromised, or generally ill. Such consumers are potentially susceptible to hazards or potential hazards that might not have an effect on more robust 'healthy' people. Hence, food safety systems need to be applied even more scrupulously than for the general population at large. In particular, some susceptible groups such as immunocompromised patients will be at risk from organisms that would be considered to be opportunistic pathogens, for example, some strains of *L. monocytogenes*. Unfortunately, *L. monocytogenes* has been previously associated with cook-chill meals, so extra care needs to be taken when using such systems in hospitals.

It needs to be remembered that catering in hospitals is used for both patients and staff. Other factors that need to be borne in mind are the use of catering at off-peak times, such as night shifts, and the need to train staff on wards how to handle the foods safely. A number of outbreaks have been associated with catering in hospitals. In the UK, in 1984, an outbreak of *Salmonella* food poisoning at the Stanley Royd Hospital resulted in 19 elderly patients dying and another 300 patients and staff being taken ill. Numerous instances of basic food hygiene failures were found in the subsequent public inquiry.

Schools, Homes for the Aged and Meals on Wheels

As with hospitals, these tend to be populated by vulnerable groups – either the very young or the elderly. A peak demand can arise in the middle of the day, requiring the reheating or large amounts of chilled or frozen food at the same time. Meals on wheels are home delivered meal programs that deliver meals to elderly or disabled individuals at home who are unable to purchase or prepare their own meals.

In some cases the food is transported hot to the point of consumption. This means that the food will spend some time in the danger zone and care needs to be taken to ensure that the food is held hot for a short time and certainly never more than 2 h unless guaranteed to be above 60 °C. Failure to do this can result in food poisoning from either *Clostridium perfringens* or *Bacillus cereus* where the spores can germinate resulting in massive outgrowth of the vegetative cells.

In the UK, an outbreak of food poisoning after a meals-on-wheels lunch delivery affected 49 persons, one of whom died. *Bacillus cereus* and *Bacillus licheniformis* were isolated in large numbers from many of the patients, including the deceased and from the remains of the meal. Food kept warm during distribution was thought to have provided an ideal environment for bacterial multiplication.

The growth of the aging population is likely to make this form of food service more common in the future, reinforcing the need for good food safety management.

Open Air Catering

Disasters and Civil Emergencies

In the event of a disaster, bulk preparation of food is often the best use of resources. In addition, if circumstances permit, the food can be prepared in a facility where it can be protected from pests, vermin, and the weather.

Mass catering in the event of a disaster is particularly hampered by a number of factors. The first is siting: in some cases the refugees will have left the disaster area, and in other cases the refugees are still in the area and so food will need to be brought into them. In these situations there may be some choice available when siting the mass catering operation in which case appropriate consideration can be given to issues such as availability of dry ground, potable water, and an adequate power supply. In other cases, there may be little choice available for siting the operation.

It is important to remember the cultural aspects of food. For example, if the food is prepared by people of one cultural background, but the recipients are of a different cultural background, the recipients might not know whether the food was prepared properly and safely. There may be religious differences to consider as well.

Almost by definition, the population will be vulnerable to disease due to malnutrition; exhaustion; and lack of shelter, hygiene, and medical facilities. In such circumstance, even mild foodborne illness can be extremely serious. Climate change and the resulting extreme weather events, such as flooding, droughts, and hurricanes, are likely to increase the necessity for well managed mass catering disaster relief.

Festivals

In many ways, a large festival event can share the logistical problems of a disaster in terms of the need to feed a large number of people at site where catering is not normally performed. Of course, the recipients are more likely to be healthier and fitter than refugees.

Festivals tend to take place outdoors in the summer, making temperature control potentially difficult. Therefore, it is best if only small quantities of food are on display and these are monitored to ensure they are on display for as short a time as possible. Outdoor festivals will necessitate an adequate supply of clean potable water for drinking, cooking, washing, and other hygiene requirements.

Festival promoters should use only reputable experienced caterers, including smaller sub-contractors, for example, hot dog stands and outlets run by voluntary organizations. Many problems can be avoided if promoters liaise with the relevant competent food inspection authorities well in advance of the festival commencing. In 1988, attendees at an outdoor music festival that was held in Michigan became ill with *Shigella sonnei*. The onset of the illnesses peaked two days after the festival ended, after attendees had returned home. An uncooked tofu salad served on the last day was associated with risk of illness. A smaller outbreak of *S. sonnei* occurred among festival volunteers before, and during, the festival. There was limited access to soap and running water for handwashing.

Large Scale Sporting Events

Large scale sporting events such as the World Cup and the Olympic Games tend to be held in one place and at a specified time. They do involve large numbers of people congregating in one place for a limited time. Hence, facilities often need to be purpose built and are generally well designed if there is enough time to prepare for the event. However, the need to employ large numbers of catering staff for a limited time can impose limitations on the training available. This should not be skimped. In addition, commercial sponsorship tends to limit the catering activities to only those agreed by the organizers on behalf of the sponsors, but there can be large number of more opportunistic traders operating outside the zone of strict control. These need to be properly organized and inspected. Unfortunately, large scale sporting events such as the Olympic Games have proved to be a target for terrorism in the past, so there is an increased need for vigilance in these circumstances to protect against bioterrorism in the foods on offer at them. In the 2012 London Olympics particular care was taken with food supplies and thankfully no issues arose of which we are aware.

Travel Catering

Tourist Hotels and Holiday Camps

Catering at tourist hotels and holiday camps is vulnerable to food safety risks for a number of reasons. The tourist season is often seasonal meaning that there will be an increased demand for people to process and handle the food but for only a limited period. This can lead to difficulty in sourcing and training suitable staff with a consequential increased risk, from breaking basic food hygiene rules or from contamination via food handlers.

There has been an increase in the number of tourists seeking compensation if their holiday is affected. Payouts of millions of dollars are not uncommon, and there are now a plethora of websites advising people how to seek compensation. In addition to paying compensation, the hotel can suffer because of bad publicity either in the media or in the many holiday advisor and rating websites that have also sprung up.

Developing countries in particular rely on the tourist trade to generate employment and foreign currency. They can be hampered by lack of local amenities or suitably trained staff. In addition, there may cultural differences such that hygiene habits may differ or visitors from abroad may be exposed to new foods and thereby potentially to organisms they have not met before, resulting in illness. Travellers are generally advised to avoid raw milk and raw meats, unwashed fresh fruit and vegetables, and ice from untreated water. Even if the main food dishes are properly prepared and served using good hygienic practices, garnishes and side dishes that form the displays of elaborately decorated buffets may be a source of

contamination, not least if they are displayed for extended periods of time at high ambient temperatures. Although not exclusively foodborne, an outbreak of norovirus can shut down an entire hotel and the organism can be difficult to subsequently eradicate.

Air Travel

Aircraft have very limited space for sanitary facilities and food storage and processing materials. Hence, mass catering systems are used almost exclusively. The size can vary from a small kitchen to a large catering establishment producing many tens of thousands of meals per day, including provisions for long-haul flights. A single unit may have contracts with tens of airlines.

Serious problems can arise if a major food-poisoning outbreak occurs on board and the aircraft is far away from an airport and from adequate medical services. Foodborne illness can be extremely serious, especially during a prolonged international flight. In 1992, 75 passengers went down with cholera (from contaminated shrimp) on a flight from Lima, Peru, to Los Angeles. Ten of them were hospitalized and one died. Such emergencies can present significant problems because of the limited medical resources available on board.

The identification and investigation of an outbreak caused by a meal served on an aircraft is complicated if the causative agent has a longer incubation period than the flight takes and the passengers become ill after disembarkation. Those affected can travel to widely different destinations, making it impossible to recognize a common origin to their illness.

Some flight catering companies freeze samples from each batch of each meal so that they can later be analyzed if they are suspected of having caused illness. Cabin staff need to be trained in how to reheat and handle foods safely.

The safety of meals for technical crew such as pilots and flight engineers is even more critical than for passengers. In some instances, specific ingredients can be banned completely from flight crew meals, including all egg products and dairy products that have not been sterilized, for example, by ultra heat treatment. Individual meals can be labeled with the position of the crew member for whom they are intended, and the service designed such that no flight crew member eats any of the same products as their colleagues. This ensures that each pilot eats a different meal to minimize the risk of all the pilots on board being ill while the plane is in flight.

Great care needs to be taken when taking on drinking water. It is not unknown for aircraft to pick up untreated water at airports where the hygiene provisions are suspect and resulting in illness in the passengers when they get home. As many flights are associated with holidays abroad, there is also a significant industry of potential litigation associated with air travel. Todd *et al.* reviewed outbreaks where food workers have been implicated and reported 18 outbreaks associated with commercial travel in air flights, trains, and cruise ships over several decades.

Ships

In many ways cruise ships are like floating hotels with similar food safety risks and controls including displays of elaborately decorated buffets that can be a source of contamination if they are on display for extended periods of time at high ambient temperatures. Although not exclusively foodborne, an outbreak of norovirus can shut down the entire ship for many days as the organism can be difficult to subsequently eradicate. As for hotels and flights, there can be substantial litigation associated with cruise ship foodborne illness.

In 2001, the World Health Organization carried out a search of reports of an outbreak or incident of infection associated with a ship since 1970. They found more than100 outbreaks of food and waterborne diseases have been associated with ships. One-third of the outbreaks were foodborne and one-fifth waterborne. A wide range of pathogenic bacteria and parasites were implicated as well as viruses such as norovirus.

Factors contributing to the foodborne outbreaks included deficiencies in food hygiene and infected food handlers.

Rooney et al. reviewed data on 50 outbreaks of foodborne disease associated with passenger ships. The majority of reported outbreaks were associated with cruise ships and affected that almost 10 000 people. *Salmonella* spp. were most frequently associated with outbreaks. Factors associated with the outbreaks reviewed include inadequate temperature control, infected food handlers, contaminated raw ingredients, cross-contamination, inadequate heat treatment, and onshore excursions. Seafood was the most common food vehicle implicated in outbreaks.

Other Catering for Travellers

In a similar fashion to aircraft, food handling facilities on trains and coaches can be very limited. Often food will be available only at the train or coach station. If vending machines are used in these circumstances, then great attention has to be paid to cleanliness, temperature control, and stock rotation.

Banqueting

A banquet is a formal meal for a large number of people on a social occasion such as a wedding. The UK Health Protection Agency (HPA) revealed that more than 90% of outbreaks of *Campylobacter* food poisoning at catering venues in 2011 were linked to consumption of undercooked chicken liver pate. Fourteen outbreaks occurred in catering venues of which seven were linked to wedding receptions at hotels, banqueting venues, or public houses.

Banquets can be at risk because large numbers of people arrive needing to be served at the same time. Often foods more exotic than those normally eaten may served, introducing new organisms. Another risk factor is the need to employ large numbers of catering staff who may lack training in good hygiene.

Often the banquet may be held outside making the provision of potable water and other hygiene requirements difficult. Sometimes, for example, Christmas, a small business may take on a job bigger than its facilities can safely handle. Banquets can also use displays of elaborately decorated buffets that can be a source of contamination if they are on display for extended periods of time at high ambient temperatures.

Well-documented problems can occur when nonspecialized people such as a family or small caterer undertake to supply more people/food than they are used to with no training or facilities for bulk handling of food. This can be a recipe for disaster.

See also: Food Safety Assurance Systems: Building Design; Personal Hygiene and Employee Health. Foodborne Diseases: Foodborne Diseases in Travelers; Overview of Biological Hazards and Foodborne Diseases. Public Health Measures: Modern Approach to Food Safety Management: An Overview

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Food Safety in Hospitals and Other Healthcare Settings

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Glossary

Immunocompromised Having the immune response attenuated as a result of disease, malnutrition, administration of immunosuppressive drugs, age, or pregnancy.

Macrophage A tissue-based cell that can engulf and kill microorganisms and that secretes proteins that have an important role in the immune response.

Neutrophil The most abundant circulating white blood cell, which is recruited to inflammatory sites and can engulf and enzymically digest microorganisms.

Tumor necrosis factor alpha A secreted protein that functions as a mediator of immune and inflammatory reactions.

Vulnerability of People in Healthcare Settings

In healthcare settings a high proportion of people are liable, through age and/or illness, to be especially susceptible to foodborne and waterborne infection. In the USA vulnerable groups have been estimated to comprise almost 20% of the population. In the UK people aged over 65, who tend to show increased susceptibility to infection, form approximately 16% of the population; many of these and also younger people may suffer from conditions that increase susceptibility. Factors that increase susceptibility to infection, in particular to foodborne and waterborne disease, are shown in Table 1.

Supply of Meals in Healthcare Settings

Hospitals

In hospitals the meals may be freshly cooked onsite, or they may be cooked in advance using cook-chill or cook-freeze systems, either onsite or prepared offsite by a commercial organization. Cook-chill and cook-freeze systems afford flexibility in the preparation and serving of meals. Guidelines on their use for catering operations were published by the Department of Health (UK) (1989), the essential features of these systems are shown in Boxes 1 and 2.

Where the supply of meals is outsourced the supplier should be audited. In England and Wales the National Health Service (NHS) Supply Chain organizes central tendering for contracts to supply food to many hospitals within the NHS. The NHS Supply Chain ensures that their food products are sourced from approved suppliers who are audited to a standard that provides a due diligence defense for NHS trusts.

Many hospitals have canteens serving visitors, but inpatients, outpatients and staff can often also purchase food and drinks. The food from these outlets must also be safe for patients and staff.

Care Homes

In care homes meals may be freshly prepared onsite, but many also obtain meals prepared by commercial caterers.

Meals-On-Wheels

Hot meals or frozen meals are delivered to vulnerable people in their homes by a variety of providers.

Main Foodborne Microbiological Hazards

Improvements in sanitation and water supplies and the control of food production in industrialized countries mean that the major foodborne, including waterborne, pathogens responsible for illness differ from those in developing countries. For example, cholera is rare in industrialized countries but is common in parts of the world including regions of India and subSaharan Africa. It is associated particularly with a lack of safe water supply and with consumption of raw or undercooked shellfish. In hospitals in developing countries infections are a major problem. These infections can be attributed to lack of knowledge and training in infection control and inadequate infrastructure, resources, and systems of care.

In the USA between 2000 and 2008 eight pathogens were reported to cause the great majority of cases of foodborne illness, hospitalizations, and death due to known pathogens. Whereas norovirus caused the greatest number of reported cases, (Table 2) nontyphoidal *Salmonella* (NTS) caused the

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Table 1 Host factors that increase the risk of foodborne and waterborne infection or the severity of the disease

Host factor	*Reason for increase in risk or severity*
Primary immunodeficiency	Immune system inadequate to combat infections
Secondary immunodeficiency: resulting from leukemias, treatment with cytotoxic drugs during organ transplantation or for cancers or autoimmune disease, irradiation treatment, treatment with corticosteroids, infection with human immunodeficiency virus (HIV) removal of the spleen, malnutrition involving protein, calories, vitamins or trace minerals.	Immune system inadequate to combat infections
Excessive iron in the blood	High levels of iron increase growth of certain pathogens
Cirrhosis and other liver disease, kidney function (alcoholism)	Iron overload, immune system dysfunction
Stress, e.g., as a result of surgery	Changes in metabolism reduce resistance to infection
Diabetes	Poor glycaemic control is associated with impaired neutrophil functions
Pregnancy	Altered immunity
Age <1 year	Gut microflora allows colonization by spores of *Clostridium botulinum*
Age <5 years	Lack of developed immune system, smaller infective dose required for infection
Age >65 years	Immune system deteriorating, also affected by chronic ailments
Consumption of antacids, particularly proton pump inhibitors	Increased pH in the stomach, increasing survival of pathogens
Consumption of large volumes of liquids, including water	Dilution of acids in the stomach
Ingestion of fatty foods (e.g., chocolate, cheese, hamburger) containing pathogens	Fat protects pathogens from acid in the stomach

Box 1 Features of cook-chill meals for catering operations

1. Full cooking of food. The temperature throughout the food should be held above 70 °C for not less than 2 min
2. Fast chilling
3. Maintain at 0–3 °C during storage and distribution for upto 5 days including the day of cooking
4. Immediately after removal from chill storage reheat to an internal temperature of at least 70 °C and maintain at not less than 70 °C for at least 2 min
5. Service of food should commence within 15 min of reheating. The temperature must not fall below 63 °C
6. Foods intended to be eaten cold or at room temperature should be consumed, preferably, within 30 min of removal from chilled storage

Box 2 Features of cook-freeze meals for catering operations

1. Full cooking of food. The temperature throughout the food should be held above 70 °C for not less than 2 min
2. Fast freezing
3. Storage at -18 °C or below
4. Thaw food and hold at or below 3 °C throughout storage and distribution
5. For hot meals, reheat to an internal temperature of at least 70 °C and maintain at not less than 70 °C for at least 2 min
6. Service of food should commence within 15 min of reheating. The temperature must not fall below 63 °C
7. Foods intended to be eaten cold or at room temperature should be consumed, preferably, within 30 min of removal from chilled storage

greatest number of hospitalizations (Table 3) and NTS, *Toxoplasma gondii*, and *Listeria monocytogenes* caused the greatest number of deaths (Table 4).

In Australia between 2001 and 2008, 5.9% of all reported food- and water-borne outbreaks occurred in long-term-care facilities for elderly residents. *Salmonella* caused 17 outbreaks, *Clostridium perfringens* 14 outbreaks, *Campylobacter* 8 outbreaks, and norovirus 1 outbreak; of 15 outbreaks of unknown etiology 11 were suspected to be due to *C. perfringens*. Puréed foods were implicated in several outbreaks.

Infection with Norovirus generally causes relatively mild and short-term symptoms, but in the elderly, chronically ill, or immunocompromised patients it can result in death.

Table 2 Top five pathogens causing foodborne illness acquired in the USA, 2000–08

Pathogen	*%*
Norovirus	58
Salmonella, nontyphoidal	11
Clostridium perfringens	10
Campylobacter spp.	9
Staphylococcus aureus	3
Subtotal	91

Source: Data from Scallan E, Hoekstra RM, Angulo FJ, *et al.* (2011) Foodborne illness acquired in the United States – Major pathogens. *Emerging Infectious Diseases* 17: 7–15.

Table 3 Top five pathogens causing foodborne illness resulting in hospitalization in the USA, 2000–08

Pathogen	*%*
Salmonella, nontyphoidal	35
Norovirus	26
Campylobacter spp.	15
Toxoplasma gondii	8
Escherichia coli (STEC) O157	4
Subtotal	88

Source: Data from Scallan E, Hoekstra RM, Angulo FJ, *et al.* (2011) Foodborne illness acquired in the United States – Major pathogens. *Emerging Infectious Diseases* 17: 7–15.

Table 4 Top five pathogens causing foodborne illness resulting in death in the USA, 2000–08

Pathogen	*%*
Salmonella, nontyphoidal	28
Toxoplasma gondii	24
Listeria monocytogenes	19
Norovirus	11
Campylobacter spp.	6
Subtotal	88

Source: Data from Scallan E, Hoekstra RM, Angulo FJ, *et al.* (2011) Foodborne illness acquired in the United States – Major pathogens. *Emerging Infectious Diseases* 17: 7–15.

Table 5 Relative susceptibilities of different subpopulations to listeriosis, based on the incidence of listeriosis cases (outbreak and sporadic) in France in 1992

Condition	*Relative susceptibility*
Transplant	2584
Cancer – blood	1384
Acquired immunodeficiency syndrome (AIDS)	865
Dialysis	476
Cancer – pulmonary	229
Cancer – gastrointestinal/liver	211
Noncancer liver disease	143
Cancer – bladder and prostrate	112
Cancer – gynecological	66
Diabetes – insulin-dependent	30
Diabetes – noninsulin-dependent	25
Alcoholism	18
Perinatals[a]	14
Aged >65 years	7.5
Less than 65 years old, no other medical condition (reference population)	1

[a]Information from the USA.

Source: Reproduced from Food and Agriculture Organization of the United Nations (2004) Buchanan R, Lindqvist R, Ross T, Smith M, Todd E, and Whiting R. Risk Assessment of *Listeria monocytogenes* in ready-to-eat foods. Microbiological Risk Assessment Series 5. Rome: FAO/WHO. Available at: http://www.fao.org/docrep/010/y5394e/y5394e00.htm (accessed on June 2013).

NTS and *Campylobacter* infections have the most severe effects in elderly persons, particularly in those with a comorbidity. In infants, the elderly, and immunocompromised people NTS bacteraemia may occur and be life-threatening. Younger people with HIV infection, autoimmune diseases, and/or immunosuppressive therapy are at an increased risk of NTS bacteraemia; elderly people, often with underlying conditions, are liable to NTS bacteraemia and extraintestinal infections.

Toxoplasma gondii is a rare cause of illness after transplant and can result in encephalitis, myocarditis, or disseminated infections, and cause high mortality. A high proportion of the population shows evidence of latent infection with *Toxoplasma* and reactivation of infection can occur as a result of immunosuppression. Prophylactic use of trimethoprim-sulphamethoxazole (TMP-SMX) against *Pneumocystis jirovecii* pneumonia generally inhibits *T. gondii*, but cases of toxoplasmosis tend to occur when TMP-SMX prophylaxis is discontinued because of ill effects. Toxoplasmosis is also a risk for cancer patients and has been reported in patients treated with tumor necrosis factor alpha (TNF-α) inhibitors for rheumatoid arthritis or Crohn's disease.

Toxoplasmosis can result from congenital infection, primary infection, allograft infection, or reactivation of latent infection as a result of immunosuppression. In women who are infected with *Toxoplasma* during pregnancy there is a risk of transmission to the fetus. Infection during early pregnancy may cause fetal death and abortion or severe damage including chorioretinitis, intracranial calcification, hydrocephalus, or microcephalus. Infection during late pregnancy is often subclinical in the newborn with manifestations such as retinochoroiditis or, rarely, neurological disorders.

Listeria monocytogenes is of particular concern in vulnerable people because it causes septicemia and meningitis and has a case fatality of 20–30%. The elderly and those with preexisting medical conditions are particularly susceptible (**Table 5**). In pregnant women infection usually causes a mild, flu-like illness, but the bacterium can cross the placenta and infect the fetus, leading to abortion, stillbirth, or delivery of an acutely ill baby.

Immunocompromised patients are at particular risk of infection by *Pseudomonas aeruginosa*. A range of reservoirs exist in hospitals, including potable water.

These examples illustrate the increased risk of infection and of serious illness in vulnerable groups of the population resulting from exposure to foodborne pathogens.

Examples of Outbreaks and Cases of Foodborne Disease in Healthcare Settings

Foodborne outbreaks have been reported in different types of healthcare settings (**Table 6**).

It is probable that only a proportion of cases and outbreaks in healthcare settings are reported and described in scientific publications. Several themes emerge from reports of outbreaks:

1. Outbreaks in healthcare settings can result in a high mortality. For example, the outbreak due to *Escherichia coli* O157 in a nursing home in Canada in 1985 resulted in the death of 17 of the 55 residents affected (31%) and the outbreak due to *L. monocytogenes* in hospitals and nursing

Table 6 Selected outbreaks of foodborne illness in healthcare settings

Place, date,	*Causative organism*	*Setting*	*Cases (deaths)*	*Food implicated*	*Factors leading to outbreak*	*References*
Austria, 2006	*Campylobacter jejuni/coli*	Tertiary care hospital	Patients, 7 (0), staff, 14 (0)	Poultry dishes	Food prepared in hospital kitchen with recently established cook-chill process and no Hazard Analysis Critical Control Point system	Jelovcan *et al.* (2008)
UK, 1995	*Clostridium perfringens*	Hospital	17 (0)	Precooked, vacuum sealed pork	Faults at company that supplied ready-cooked joints; cooked meat had only cooled to 28 °C after 50 h	Regan *et al.* (1995)
Australia, 1997	*C. perfringens*	Nursing home	Residents, 25 (1)	Pureed food	Not reported	Tallis *et al.* (1999)
Japan, 2001	*C. perfringens*	Home for senior citizens	90 (0)	Boiled beans	Beans cooked in large quantities, cooked slowly, not reheated adequately before serving	Tanaka *et al.* (2003)
Belgium, 1998	*Cronobacter (Enterobacter) sakazakii*	Neonatal intensive care unit	12 (2)	Powdered milk formula	Powder contaminated	Van Acker *et al.* (2001)
Mexico, 2010	*Cronobacter (Enterobacter) sakazakii*	Maternity hospital	2 (0)	Powdered infant formula	Powder rehydrated at 45 °C instead of recommended 70 °C	Flores *et al.* (2011)
Canada, 1985	*Escherichia coli* O157	Nursing home	Residents, 55 (17), staff, 18 (0)	Ham or cheese sandwiches	Food handler who prepared sandwiches had recently recovered from diarrhea	Carter *et al.* (1987)
USA, 2003	*E. coli* O157	Nursing home	Residents, 32 (2), staff, 14 (0)	Raw spinach	Not reported	Reiss *et al.* (2006)
Finland, 1998–1999	*Listeria monocytogenes*	Tertiary care hospital	25 (6)	Soured cream butter	Outbreak strain in dairy environment and in butter	Lyytikäinen *et al.* (2000)
Germany, 2006–07	*Listeria monocytogenes*	Hospital	11 (5)	Ready-to-eat, – presliced, scalded sausages	Low number of *L. monocytogenes* found in sausages and in environment of factory	Winter *et al.* (2009)
Norway, 2007	*L. monocytogenes*	Cancer and transplantation hospital	15 (3)	Camembert cheese made with pasteurized milk	Contamination in factory after pasteurization of the milk	Johnsen *et al.* (2010)
Canada, 2008	*L. monocytogenes*	Hospitals, nursing homes	57 (21)	Delicatessen meats from external supplier	Contamination from meat slicer, inadequately cleaned, with meat residue deep inside slicing mechanism	Weatherill (2009)
Northern Ireland, 2008	*L. monocytogenes*	Two hospitals	7 (3)	4 cases linked to sandwiches	*L. monocytogenes* found in sandwiches at <20 cfu g^{-1}. Temperature control during distribution and service unreliable	Irvine (2009)
UK, 2008	*L. monocytogenes*	Hospital	3 (0)	Probably sandwiches	*L. monocytogenes* found in sandwiches, prepared by external supplier, at <20 cfu g^{-1} and also on cutter blade in production environment. Temperature control and storage in hospital defective	Health Protection Agency (2008)
USA, 2008–09	*L. monocytogenes*	Hospital	5 (3)	Tuna salad	Contamination of tuna from the environment; tuna salad held at 5 °C for up to 4 days	Cokes *et al.* (2011)
Denmark, 2009	*L. monocytogenes*	Meals-on-wheels delivery	7 (2)	Sliced, cooked beef served with sauces and vegetables; intended for microwave cooking by consumer	Beef had been cooked by the supplier at a lower temperature than usual. Four patients had cancer, one had systemic lupus erythematosus, three were aged >80	Smith *et al.* (2011)

(*Continued*)

Table 6 Continued

Place, date,	*Causative organism*	*Setting*	*Cases (deaths)*	*Food implicated*	*Factors leading to outbreak*	*References*
Germany, reported 2008	*Pseudomonas aeruginosa*	University hospital, intensive care units	19 (4)	Bottled still water (BSW)	Unopened bottles of BSW contained outbreak strain. German legal requirement specified absence in 250 ml	Eckmanns *et al.* (2008)
UK, 1993	*Salmonella* Enteritidis	Two hospitals with shared catering	Patients, 22, staff, 7 (0)	Association with meals prepared by one asymptomatic carrier	Three chefs were asymptomatic carriers	Dryden *et al.* (1994)
USA, 1993–94	*Salmonella* Senftenberg	Hospital	Patients, 18 (0), staff, 4 (0)	Suspected contamination from turkey	Deficient processes in kitchen, some patients immunosuppressed Possibly >200 persons affected	L'Ecuyer *et al.* (1996)
Brazil, 1999–2000	*S.* Enteritidis	Tertiary care hospital	Patients, 8 (3)	Commercial, enteral diet	Contamination of egg albumin, lyohilized and used in enteral diet	Matsuoka *et al.* (2004)
Netherlands, 2001	*S.* Enteritidis	Hospital and nursing home served by same hospital kitchen	82 (5)	Bavaroise	Raw eggs used, underheated, no temperature checks	Bruins *et al.* (2003)
Australia, 2001	*Salmonella* Typhimurium	Aged care facility	Residents, 16 staff, 2 (0)	Rice pudding, meat-based potato pie	Raw shell eggs whisked into rice pudding immediately before serving, and added to potato topping of pie before lightly browning	Tribe *et al.* (2002)
UK, 2002	*S.* Enteritidis	Hospital	29 (0)	Imported eggs	Eggs contaminated with outbreak strain and undercooked	Public Health Laboratory Services (PHLS) (2003)
Greece, 2005	*S.* Enteritidis	Hospital	Patients, 86 (2), visitors, 31, staff, 16	Association with roast chicken, spaghetti, cheese	Cross-contamination of food, inadequate temperature control and/or inadequate cooking	Gikas *et al.* (2007)
Germany, 2006	*S.* Enteritidis	Nursing home	Patients, 94 (1), staff, 17	Cakes supplied by outside baker	Cream cakes contaminated and not adequately refrigerated	Frank *et al.* (2007)
Australia, 2008	*S.* Typhimurium	Aged care facility	10 (0)	Chocolate mousse dessert	Dessert made with raw eggs. Free-range, second-grade eggs, some cracked and dirty, were used	Roberts-Witteveen *et al.* (2009)
Australia, 2010	*Salmonella* Infantis	Aged care facility	22 (2)	Thickened fluid added to food to promote safe swallowing, or used for taking medication	Probable contamination of thickened fluid from chicken mince in kitchen	Najjar *et al.* (2012)
Sweden, 1999	Norovirus	30 day-care centers served by the same caterer	400 including secondary spread (0)	Pumpkin salad	Contamination by food handler	Götz *et al.* (2002)
Denmark, 2005	Norovirus	Hospital, 2 nursing homes, meals-on-wheels service	Patients, ~970 staff, (0)	Imported, frozen raspberry pieces	Contamination during growth/harvesting on several small-scale farms.	Falkenhorst *et al.* (2005)
Austria, 2009	Norovirus	Hospital and rehabilitation center	114 (0)	Sliced, cold sausage; meat dish with salad; spinach pancake	Contamination by one of five asymptomatic excretors among the kitchen staff who prepared the food	Schmid *et al.* (2011)

homes in Canada in 2008 resulted in death of 21 of 57 residents affected (37%).
2. Asymptomatic chefs or other food handlers have been linked to several outbreaks. For example, that due to *E. coli* O157 in a nursing home in Canada in 1985, two outbreaks linked to *Salmonella* in the UK in 1993 and in Greece in 2005, and two linked to norovirus in Sweden in 1999 and in Austria in 2009.
3. Food handlers may be the victims of an outbreak rather than the cause and epidemiological analysis must distinguish between these two possibilities.
4. Serving high-risk foods to vulnerable patients has caused several outbreaks. For example, in several of the outbreaks due to *Salmonella* spp. raw eggs were used and were undercooked, despite the known risk of contamination of eggs with *Salmonella* particularly if the eggs are from stocks not immunized against *S.* Enteritidis. A further example is the supply to patients of sandwiches, sausages, or delicatessen meat containing low numbers of *L. monocytogenes*.

Factors Leading to Foodborne Disease in Healthcare Settings

Some of the main factors leading to foodborne disease in these settings are:

1. Food from unsafe sources;
2. Inadequate cooking;
3. Improper holding temperatures;
4. Contaminated equipment and cross contamination;
5. Poor personal hygiene.

Food from Unsafe Sources

It is a particular problem in the case of foods that are not subjected to a processing that will inactivate foodborne pathogens. For example, the series of outbreaks of norovirus infection in Denmark in 2005 was associated with one large batch of frozen raspberries, composed of fruit grown on several small-scale farms in Poland (Table 6).

Inadequate Cooking

This may have contributed to the outbreak of listeriosis associated with a meals-on-wheels delivery in Denmark (Table 6). UK advice is that foods should be cooked so that the temperature throughout the food is held at 70 °C or higher for at least 2 min, or equivalent heat treatment, to give sufficient inactivation of nonspore-forming microorganisms. The US Food and Drug Administration (FDA) Food Code specifies temperatures and holding times for cooking specific foods, for example, a temperature of 74 °C throughout the food for 15 s for poultry.

Inadequate Cooling

Cooked foods should either be eaten immediately, or kept for a short time at a temperature higher than 63 °C, or cooled rapidly and kept below 7–8 °C (ideally below 4 °C) to control clostridia, particularly *C. perfringens*, and other bacteria, and reheated to at least 72 °C before consumption. Spores of *C. perfringens* can survive cooking and give growth of the bacteria at temperatures between 12 °C and 52 °C. In the UK and the USA guidelines for cooling cooked foods are designed to ensure that no more than a tenfold increase in numbers of *C. perfringens* can occur during cooling. A computer program (perfringens predictor) is available that makes it possible to determine whether any proposed rate of cooling will comply with the UK and the US guidelines. Two of the three outbreaks of infection with *C. perfringens* in Table 6 were attributed to inadequate cooling of cooked food.

Contaminated Equipment and Cross Contamination

This led to the outbreak of listeriosis in Canada in 2008, which contributed to the deaths of 21 people (Table 6). It required considerable time to take meat-slicing machines apart, thoroughly sanitize, and reassemble. This limited effective cleaning. Meat residue was found deep inside slicers, providing a niche where *Listeria* could grow and contaminate meat during slicing.

Poor Personal Hygiene

Asymptomatic carriers and food handlers continuing to work while infected led to outbreaks of *E. coli* O157 in Canada in 1985, *S.* Enteritidis in the UK in 1993 and in Greece in 2005, and norovirus in Sweden in 1999 and Austria in 2009 (Table 6).

Outbreak investigations refer frequently to inadequate levels of staffing in the kitchen of hospitals and care homes, poor training, low wages, and lack of support from managers.

Prevention of Foodborne Disease in Vulnerable People in Healthcare Settings and in the Community

Control of Food Provision

The Food Hygiene (England) Regulations 2006 and similar Regulations in Scotland and Wales, gave effect to European Community Regulations that include a requirement that food businesses put in place, implement, and maintain procedures based on the principles of Hazard Analysis Critical Control Point (HACCP). The HACCP system is designed to ensure that hazards are prevented, eliminated, or reduced to an acceptable level before a food reaches the consumer, and involves seven principles.

In order for a HACCP system to be effective, programs to control basic operational conditions and sanitation (prerequisite programs) must be in place. These should include, for example, good design of premises, cleaning and training, good hygiene practices, use of approved suppliers and supplier auditing schemes, and other programs.

People like the consultant in communicable disease control, infection control team, and local environmental health officers should advise the catering management team in healthcare facilities on all aspects of food hygiene.

Hospitals and other healthcare facilities should provide adequate staffing for catering service, provide suitable training and pay rates, and allow paid leave if staff are infected. In the UK it is advised that, in general, food handlers suffering from gastroenteritis should be excluded from work for at least a period of 48 h after symptoms stop of their own accord or from the end of treatment with medication such as antidiarrhoeal drugs. In the case of illness caused by *Salmonella* Typhi or *S*. Paratyphi, verocytotoxin-producing *E. coli*, and Hepatitis A virus, longer periods of exclusion are required and medical advice is necessary. Anyone who has household contact with someone infected with *E. coli* O157 should be excluded from direct handling or serving of open, ready-to-eat foods and, if they are a parent, excluded from all food handling duties and areas until the patient has been given microbiological clearance. The FDA Food Code stipulates exclusion of food handlers showing symptoms of these infections and conditions for reinstatement. It also prescribes that a food employee who is diagnosed with symptoms of infection with norovirus, *Shigella*, enterohaemorrhagic *E. coli*, or Shiga toxin-producing *E. coli* but is asymptomatic, or has a sore throat with fever, should be excluded if they work in a food establishment serving a highly susceptible population, or restricted if they work in an establishment not serving a highly susceptible population. If staff are not paid when they are excluded from work this can lead to them working while ill and can result in food safety problems.

A survey in the USA found that 4.7% of food workers in the retail industry (approximately 900 000 people) continued working while they were ill with diarrhea or vomiting.

Types of Food Served to Vulnerable People, and Advice for These People

A low microbial diet is one from which certain foods are excluded and safer foods are substituted in order to reduce the risk of foodborne infection. A low microbial diet is recommended for hematopoietic stem cell transplant and solid organ transplant patients.

The American Cancer Society advises that "a low microbial diet (neutropenic diet) may be suggested if a patient's absolute neutrophil count (ANC) is low." An ANC of $<1000\ \mu l^{-1}$ means that the immune system is weak, an ANC of $<500\ \mu l^{-1}$ for a few days results in a high risk of infection, and an ANC of $<100\ \mu l^{-1}$ for more than a week means that the risk of infection is extremely high.

Many hospitals recommend low microbial diets for high-risk patients.

Guidelines in Scotland advise that a Grade 1 Neutropenia diet is used for hospital patients with a neutrophil count of $500–2000\ \mu l^{-1}$ and a more restricted, Grade 2 Neutropenia diet is used for patients with a neutrophil count of $<500\ \mu l^{-1}$.

There are differences between the components of low microbial diets specified by different organizations, and agreement on the components of such a diet would be valuable.

In Germany low microbial diets were only considered for patients with severe neutropenia (neutrophils $<500\ \mu l^{-1}$); in the outbreak of listeriosis in a hospital in Germany in 2006–08 (Table 6) none of the patients met this criterion, and had received regular food during their hospital stay. But the patients were being treated with corticosteroids and proton pump inhibitors; corticosteroids suppress activity of macrophages, and association has been reported between intake of corticosteroids and listeriosis. It was suggested that food items served in hospitals could be classified as 'food intended for special medical purposes,' and should include the criterion of absence of *L. monocytogenes* from 25 g.

It has been argued that evidence for the effectiveness of low microbial diets is lacking, but it is clear that certain foods carry a risk of infection with foodborne pathogens. It is not acceptable to subject severely ill patients, who are receiving expensive and complex treatments, to foods that may contain pathogenic microorganisms, particularly because equally nutritious and safer foods are available.

Following the outbreak of listeriosis in the USA associated with tuna salad (Table 6), a survey was made of food policy in 54 New York City hospitals – 9 public and 45 private. 29 (54%) of the hospitals reported contracting their food service to an outside provider. Of the 54 hospitals, 72% and 52%, respectively, had an obstetrics or an oncology unit, 19% had a solid organ transplant unit, and 20% had a bone marrow transplant unit. Of 53 responding hospitals 81% reported serving ready-to-eat deli meat to any patient in their hospital and only 25% of 16 responding hospitals reported having a policy that specified that ready-to-eat deli meats must be heated until steaming hot before they were served in patient meals. This is despite the fact that ready-to-eat deli meats are liable to contamination with *L. monocytogenes* and have been associated with outbreaks of listeriosis (e.g,. Table 6).

The FDA (2012) has published the following brochures or fact sheets, which give advice to vulnerable people:

1. Food Safety for People with Cancer;
2. Food Safety for Transplant Recipients;
3. Food Safety for People with HIV/AIDS;
4. Food Safety for Pregnant Women;
5. Food Safety for Older Adults;
6. Food Safety for People with Diabetes.

Each of these brochures contains guidelines on selecting lower risk foods and advice on purchasing, storing, and cooking foods.

Several groups of workers have recommended that patients being treated with TNF-α inhibitors should be advised about specific high-risk foods to avoid.

An enteral diet was the cause of one nosocomial outbreak in Brazil (Table 6). In developed countries commercial, sterile, enteral feeds are available, but in some countries hospital-prepared enteral feeds may still be used. Some of these feeds contained high numbers of bacteria, and the use of HACCP during reconstitution, supplementation, or preparation, or use of commercial, sterile products is advocated. In England and Wales it is recommended that, in order to reduce the risk of contamination, prepackaged, ready-to-use feeds should be used in preference to feeds requiring decanting, reconstitution, or dilution.

Drinking Water and Ice

Some outbreaks of nosocomial infection, particularly those due to *P. aeruginosa*, have been linked to tap water; a few have been attributed to tap water used for consumption. To prevent such infections, recommendations were made to prevent contamination of jugs used for drinking water for patients and for limiting the time for which jugs of drinking water are allowed to stand before use.

Guidelines in the UK and the USA advise certain immunocompromised patients to boil all drinking water to avoid the risk of *Cryptosporidium* infection. UK guidelines state that persons with certain T-cell deficiencies (including patients with HIV infection) should boil and cool their drinking water, from whatever source, and that ice cubes should be prepared from boiled and cooled water. It has been suggested that the use of end-line water filtration was the best way to provide drinking water for immunocompromised patients, provided that there are arrangements to ensure that filter cartridges are changed at appropriate times.

Bottled, still water caused an outbreak of infection with *P. aeruginosa* in a tertiary care hospital in Germany, which resulted in pneumonia in many patients (Table 6). European Community standards for bottled water specify the absence of *P. aeruginosa* in any sample of 250 ml, but increase in numbers can occur after bottling and this bacterium has been found in bottled, noncarbonated water from various countries.

Ice and ice-making machines have also been the cause of nosocomial infections and recommendations regarding the purchase, use, and maintenance of such machines have been made as part of Infection control. It was advised that ice made in automated ice machines should not be given to immunocompromised patients.

Powdered Infant Formula (PIF)

PIF has been responsible for infection of infants with *Cronobacter sakazakii* (*Enterobacter sakazakii*) and related organisms, and *Salmonella*. Measures to prevent contamination during commercial production have been described. PIF is not sterile, and if these bacteria are present they can survive for long periods in the dry powder. Hygiene during reconstitution and storage at low temperature is important for maintaining safety. For high-risk infants (preterm, low birth weight, immunocompromised) the use of sterile, ready-to-feed, liquid formula is recommended. Precautions required when reconstituting PIF include treating bottles with boiling water, reconstituting in water that has been boiled and cooled to not less than 70 °C, and storage of reconstituted formula below 5 °C.

Antimicrobial Prophylaxis

Antimicrobial prophylaxis is used widely during treatment of many groups of vulnerable people, particularly those affected by neutropenia. Recommendations have been made that this prophylaxis should be limited to patients with severe neutropenia.

TMP-SMX is used widely in transplant centers, primarily to prevent *Pneumocystis* pneumonia. This antimicrobial is also effective in preventing infection with *L. monocytogenes* and *T. gondii* and may reduce the incidence of *Salmonella* infections, but some resistance has been reported in *Salmonella*. Prophylactic use of TMP-SMX has also been recommended during treatment of certain cancers and this, or other antibacterials, for prophylaxis in persons receiving TNF-α inhibitors for treatment of rheumatoid arthritis or Crohn's disease.

The development of antibiotic-resistance in microorganisms and the need to control the use of antibiotics are liable to limit their use in these situations. This emphasizes the fact that environmental precautions, including avoidance of provision of high-risk foods to vulnerable people, are essential in preventing infection in these people in healthcare settings.

Conclusion

A high proportion of people in hospitals and other healthcare settings is particularly susceptible to infection as a result of their disease, of medication, or because their immune system is compromised as a result of age or pregnancy. It is particularly important to ensure the microbiological safety of food supplied to people in these settings. Suppliers of food in these settings are required to have in place a HACCP system, whether the food is prepared in a hospital, in an institution, or by an external supplier. For immunocompromised patients a low microbial diet, which excludes higher risk foods and substitutes safer foods, is important for avoidance of infection. Guidance on the choice of foods to avoid infection is also important for vulnerable people in the community.

See also: Bacteria: *Listeria monocytogenes*. Food Safety Assurance Systems: Hazard Analysis and Critical Control Point System (HACCP): Principles and Practice. Foodborne Diseases: Foodborne Diseases and Vulnerable Groups. Protozoa: *Toxoplasma gondii*. Public Health Measures: Health Education, Information, and Risk Communication; Management of Food Safety in Food Service Sector. Risk Analysis: Food Safety Training and Health Education: Principles and Methods

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FOOD SAFETY ASSURANCE SYSTEMS

Contents

Food Safety and Quality Management Systems

DL Newslow, D. L. Newslow & Associates, Inc., Orlando, FL, USA

Glossary

Audit An evaluation to determine if activities and results comply with defined requirements.

Benchmarking Benchmarking is a methodology that is used to sea'rch for best practices. It can be applied to strategies, policies, populations, processes, products, and organizational structures. The global food safety initiative (GFSI) applies the benchmarking process for its approval of food safety management system schemes comparing these to the most current version of the GFSI Guidance Document. (ISO 9000, 9001, and 9004, Quality Management Definitions.)

Corrective action An action or step taken to eliminate the causes of an existing nonconformance. This is a formalized process that requires root cause analysis, identified actions to be taken to correct the situation in a timely manner based on the risk of the situation, actions required to minimize or prevent its recurrence, and the evaluation of effectiveness to ensure that the action or actions applied are effective. Corrective actions address actual problems thus the corrective action process may be considered a problem-solving process. (ISO 9000, 9001, and 9004, Quality Management Definitions.) When related specifically to the Hazard Analysis Critical Control Point (HACCP)/food safety program, a corrective action is defined as 'any action to be taken when the results of monitoring at the CCP indicate a loss of control.'

Food safety management Food safety management includes all the activities that organizations use to direct, control, and coordinate food safety. These activities include formulating a food safety policy and setting food safety-related objectives.

Food safety management system A food safety management system is a set of interrelated or interacting elements that organizations use to direct and control how food safety-related policies are implemented and food

safety-related objectives are achieved. Food safety management systems are usually developed based on the requirements of a food safety management system standard and also on requirements defined by Codex Alimentarius (www.codexalimentarius.org).

Hazard Analysis Critical Control Point Codex Alimentarius (www.codexalimentarius.org) defines 'HACCP' as the process that is used to identify, evaluate, and control (or prevent) hazards that are significant for food safety. Considered a process tool to identify and assess existing and potential food safety hazards to ensure the production of a safe product.

Internal audit Activity designed to apply a systematic, disciplined approach assessing compliance and performance of an organization's policies and procedures to a specific quality and/or food safety standard, procedure, and/or guideline(s). 'Internal Audits' are performed within an organization to measure its own performance, strengths, and weaknesses against its own established procedures and systems.

Nonconforming Situation Also referred to as a 'nonconformity' is considered a failure (major) or potential failure (minor) to meet a specified requirement. A requirement is a need, expectation, or obligation. It can be stated or implied by an organization, its customers, or other interested parties. (ISO 9000, 9001, and 9004, Quality Management Definitions.)

Preventive action A preventive action is a step taken to remove the cause of a potential nonconformance and the action put in place to correct the situation preventing the nonconformance. The preventive action process is designed to prevent occurrence of nonconformities or situations that do not yet exist. Preventive actions address potential problems, problems that have not occurred thus the preventive action process may be considered a risk analysis process. (ISO 9000, 9001, and 9004, Quality Management Definitions.)

Second party audit Also known as an external audit that is usually performed by a company representative from an outside source, such as a customer.

Third party registration audit This is performed by an independent, accredited registrar independent of any involvement with the company business. Note: the registrar has been accredited to perform the registration audit to the specific standard of choice.

Understanding the Management System Standards

ISO 9000 Series – Quality Systems Requirements

Because the requirements of ISO 9001 truly provide the foundation for most, if not all, management system-based standards, it is important to initially understand the basis and foundation of this standard. '*Quality Management System*' is a term that has evolved over the years with the inception of the ISO 9000 standards in 1987. ISO 9001 is an international standard directed at the quality management process of an organization. However, this statement can be misleading because this standard focuses on an organization's complete system. The system is made of processes within processes that result in a total 'system.' Although the title is 'quality' management system, these requirements focus on the complete system and its ability to meet customer requirements while continuously improving. Quality management includes all the activities that organizations use to direct, control, and coordinate quality. These activities include formulating a quality policy and setting quality objectives. They also include quality planning, quality control, quality assurance, and quality improvements. A quality management system is a set of interrelated or interacting elements that organizations use to direct and control how quality policies are implemented and quality objectives are achieved. A Food Safety Management System is the overall defined organizational structure, procedures, processes, and resources put into place to define, implement, and maintain compliance to a specific quality and/or food safety management standard. (ISO 9000, 9001, and 9004, Quality Management Definitions.)

A brief background of how ISO 9001 came about focuses on the need for consistency and harmonization in international trade dating back as early as NATO documents AQAP-1 in 1968. In 1983, the International Organization for Standardization established Technical Committee 176 to develop an international standard that would focus on quality and management. Quality was again the focus of the organization's consistent ability to meet defined requirements, which ensure that customer requirements are clearly understood, defined, and met. It was the work of Technical Committee 176 that resulted in the publication of the original ISO 9000 series in 1987.

ISO 9000 is a term that refers to a group of international standards that are directed at the management process of an organization. 'ISO' means 'equal' in Greek. The International Organization for Standardization was founded in 1946 and is located in Geneva, Switzerland. This organization is made up of approximately 90 countries, including the USA.

To date, the ISO 9000 series of standards has been revised in 1994, 2000, and 2008. Normally, the goal is for revision every 5 years; however, due to the required extensive review process, this time frame usually exceeds a 5-year period. The original ISO 9000 certification series consisted of the following conformance standards:

ISO 9001: Quality Systems – Model for Quality Assurance in Design, Development, Production, Installation, and Servicing
ISO 9002: Quality Systems – Model for Quality Assurance in Production, Installation, and Servicing
ISO 9003: Quality Systems – Model for Quality Assurance in Final Inspection and Test

Manufacturing companies that performed design control (i.e., R&D) could choose to omit this process by becoming certified to ISO 9002; however, all choices were excluded with the elimination of ISO 9002 and 9003 with the release of ISO

9001:2000. As of this writing, the current version is ISO 9001:2008 and will be referred to as ISO 9001 from this point forward. Note: it is recommended that the reader review the current requirements per the ISO website.

The ISO 9001 quality management standard focuses on the existence, implementation, and effectiveness of a quality management system, not the individual product. The standard is generic in text and can be implemented by manufacturing and service organizations regardless of the type of product or service.

The first registration to an ISO 9000 standard in the USA happened in 1991. It was 1994 before a food manufacturing company actually achieved registration. In the years that followed, much of the food industry remained skeptical of the true advantage of developing systems compliant with this standard. Some of that concern related to the perceived extensive documentation requirements that were linked to the original standards and frankly, to ISO overall.

Related to additional system development history, it is interesting to note that basically, in the early years of compliance, those 'food' companies that chose to develop ISO 9000 compliant systems (ISO 9001, ISO 9002, ISO 9003) were in one of the following categories:

- Owned by an overseas company that required all of its sites to be registered.
- Registration was required to do business overseas. (Note: this did not affect food companies as much as it did other commodity type companies (i.e., electronics, automotive, etc.)).
- Top management identified and wanted to take advantage of the internal benefits experienced through compliance and registration.
- Top management identified and wanted to take advantage of the internal benefits of compliance applying these requirements to an internal program rather than to seek registration. In this instance, management understood the advantage of applying the requirements, but not the advantage of actually being registered.

Much of the initial concern from food industry-related sectors was related to the perceived extensive, unreasonable amounts of documentation requirements linked to the original ISO standards. However, this was not necessarily true and was reflected in the ISO 9001:2000 revision. The ISO 9001:2000 totally reorganized its requirements providing a stronger focus on defining, implementing, and managing a quality management system and focused more on measureable objectives, process application, and continuous improvement.

In discussing documentation, a good rule of thumb is 'as little as possible, but as much as necessary.' ISO 9001 actually only requires six documented procedures, which are *Internal Audits*, *Control of Nonconforming Product*, *Document Control*, *Record Control*, *Corrective Actions*, and *Preventive Actions*. Also, these requirements may be combined into one procedure that defines requirements for both such as combining corrective action and preventive action. The standard does state that these documents are required along with whatever other documents the organization feels are necessary for the structure and success of its system. Common sense and logic are very important when evaluating and deciding what actually must be defined in a documented procedure.

As previously stated, with the inception of ISO 9001:2000, food companies began to demonstrate more interest in developing a compliant system. ISO 9001 requirements were totally re-organized providing a stronger focus on defining, implementing, and managing a quality management system that focused on measurable objectives, process application, and continuous improvement.

The Management Responsibility section of ISO 9001:2000 provided a clear directive for top management of the organization to become involved and make decisions based on the effectiveness and suitability of the system, required resources, continuous improvement, and the system's ability to meet its defined 'measureable objectives.' Keep in mind that improvements are very difficult to track without structured 'measurements.'

Many food companies translated the requirements of ISO 9001:2000 into their own internal management system. Systems were defined, implemented, and monitored internally rather than seeking formal registration from a third party registrar. It was stated that meeting this structure and discipline through internal compliance resulted in considerable internal benefits to the company. The benefits from certification were many fold. The following information has been compiled from personal discussions with approved companies and also from independent market research surveys regarding the impact that ISO 9001 certification has had on doing business both internally and externally:

External benefits:

- External appraisal helped system.
- Enhanced marketing/advertising.
- Improved market share.
- Reduced customer audits.
- Increased competitiveness in international markets.

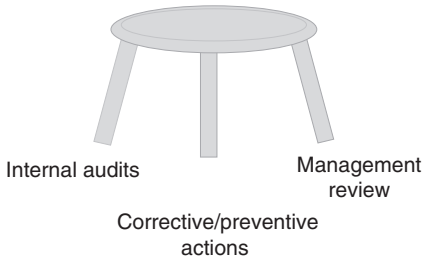
Internal benefits:

- Improved management control.
- Greater consistency.
- Cost reduction through reduced scrap and rework.
- Improved customer satisfaction.
- Identified 'nonvalue' activities.
- Improved efficiency and shortened time to market.
- Improved documentation and defined requirements.
- Improved staff motivation.
- Increased level of internal ownership and roles in the process.
- Structured communication between all levels.
- Enhanced teamwork and understanding of the 'internal customer'.
- Enhanced understanding of the 'team' approach.
- A system is a collection of processes within processes.

Whereas realizing external benefits depend heavily on the specific business and focused market, the internal benefits just make good business sense when applied effectively to an organization's complete system.

ISO 9001 is a management tool ,which, when integrated into a process, provides the foundation and structure through documentation and objective evidence that promotes consistency throughout the entire operation while focusing on

continuous improvement and meeting customer's needs and expectations. A well defined and formalized system clarifies management responsibility, policy statement, measureable objectives, and expected performance as the basis for its effective management system. Continuous improvement is inherent in the system, especially through effective processes from top management review meetings, internal audits, and corrective and preventive actions. This is demonstrated in the following diagram:



Effective processes for internal audits, corrective/preventive actions, and management review are essential for the development, maintenance and sustainability of every management system. If one leg of the above stool falters then the effectiveness of the whole management system is at risk.

As the reader reviews this initial section on ISO 9001, it is imperative to remain focused on the fact that this standard is the basis and foundation for almost every standard that has followed since its inception. Food Safety Management Standards, including a sampling of those Global Food Safety Initiative (GFSI)-recognized schemes, are discussed in subsequent sections of this article. It is important to remember that although focus may be on food safety, ISO 9001 'as the foundation' should always be recognized as the basic premise.

As previously stated, it is not necessary to reinvent processes to be compliant with the requirements of a management standard. Compliance is not a replacement! Existing Hazard Analysis Critical Control Point (HACCP), Good Manufacturing Practices (GMP), sanitation, pest control, and food hygiene programs must be integrated into the structured management system. This promotes adherence to defined procedures while enhancing programs through continuous improvement. Keep in mind that combining the focus of HACCP-based systems and management systems overall makes good sense. The HACCP program focuses on product safety whereas the management system focuses on the total system and those processes included in that system. A compliant structured management system such as one that meets the requirements of ISO 9001 provides the structure and discipline for which the HACCP program can be incorporated.

The requirements of an effective management system can be summarized in three major categories:

Plan the business: The organization's 'measureable' objectives and how the organization is structured to achieve them must be defined in the 'Quality Manual.' Specific elements define the implementation policy and the documented system required to control system activities.

Control the process: Specific elements focus the greatest emphasis on activities and controls related to providing a final product or service that meets defined requirements. These relate to both internal and external customer requirements.

Maintain the system: Once the system is implemented, specific element requirements address the maintenance of the system to ensure that it continues to operate effectively and is in compliance. The system is maintained for effectiveness through compliance with all elements and as stated previously, specifically through the processes for corrective/preventive actions, internal quality audits, and top management review. The overall functionality and status of the management system along with results from specific process activities must be evaluated by top management at the Management review meetings.

Management review meeting activities are planned and recorded to ensure and confirm that the policy statement, measurable system objectives, resources, and the system itself continue to be suitable and effective to the overall organization and its customers. Management review is the defined process for top management to manage, track, and ensure compliance and improvement of the management system. The overall purpose of a management review meeting is to evaluate the suitability, adequacy, and effectiveness of an organization's management system and to look for improvement opportunities. Management review meetings are performed at defined intervals in compliance with the specific quality and/or food safety standard. (ISO 9000, 9001, and 9004, Quality Management Definitions.)

The actual amount of resources (i.e., time, cost, etc.) and degree of difficulty necessary to bring a system into compliance depend on many elements including the size of the organization and its existing processes. Before beginning the project, it is important that those individuals responsible for guiding the organization through the implementation process receive competent education and training in the requirements. Earlier, it was stated that the standards are generic in nature and can be applied to any manufacturing process or service. This is true. It is essential that system leaders have an accurate understanding of how these requirements relate to their industry and more specifically, to their operation. Research, benchmarking, and acquiring the assistance of qualified individuals (i.e., consultants with credentials and experience in their industry) pay tremendous dividends in total cost, time, and employee motivation through all stages of system definition, implementation, and maintenance.

It is essential to evaluate existing processes and documentation to determine the extent to which these meet the requirements. System gaps can then be identified and addressed. Remember that processes must not be recreated or reengineered around the requirements; instead, the requirements should be integrated into the current system.

The question as to whether or not ISO 9001, as a management standard, fits the current world of food safety has been alluded to in the previous text. This is a question that is continually asked by top management of food companies of all sizes. Admittedly, as the food safety management system (FSMS) standards have evolved over the recent years, some companies that were ISO 9001 chose to drop their certification and replace it with a management system that focused only on food safety. Fortunately, at the time of this writing, these

companies are in the minority. The majority of ISO 9001-approved food companies have chosen to integrate the requirements for food safety into their current programs. Actually, most had already done this long before the FSMS concept was identified. After all, it is truly not possible to have quality without food safety. Actually, it has been found that it is quite difficult to define, implement, and maintain a FSMS that does not include 'quality' parameters. Some of the food safety management standards such as the current versions of Safe Quality Food (SQF) and British Retail Consortium (BRC) integrate food safety requirements with quality requirements. Many companies becoming certified to ISO 22000 and Food Safety System Certification (FSSC) 22000 have chosen to expand their management systems to include not only ISO 9001, but also ISO 14001 and OHSAS ISO 18001. 'ISO 14001 – Environmental Management' uses the same format as ISO 9001 and ISO 22000 in defining and ensuring compliance with environmental requirements. 'OHSAS 18001 – Occupational Health and Safety' applies the same format with focus on health and safety requirements.

Borrowing from the text *The ISO 9000 Quality System Applications in Food and Technology* (Newslow, Wiley C2001 US), the following applies to an effective management system:

- (Certification) promotes a system that anticipates and prevents problems rather than one that simply reacts.
- Builds quality in rather than inspects it in.
- Creates a platform by which a company can continuously evaluate and improve the business.
- Without a disciplined structure, a company may not have the complete tool set required to ensure proper resolution of business opportunities; without this commitment, many companies were basically departmentalized and primarily became a quality control department.
- Required system elements are exactly what many food companies in the US and abroad seek along with effective FSMSs such as HACCP, GMPs, sanitation programs, and other appropriate PRP programs.

FSMS Standards

When discussing food safety, it is critical to understand the terminology. The term 'food safety' is defined as the process of producing a food product that does not contain a food hazard when used according to its intended use when consumed. A 'food hazard' is defined as a biological, chemical, or physical agent either in the food or as a condition of the food that either results in or has the potential to cause an adverse health effect. Literature has many different versions of the above definitions, but most importantly, a food safety hazard is understood as those elements that could occur within the product or in the process; whereas, food security is an intentional act of sabotage focusing on causing harm. A 'safe' product is manufactured free of biological, chemical, or physical hazards that either have the potential to cause illness or other harmful outcomes on consumption of the product. Food safety hazards may occur at any stage in the food chain; thus, it is essential that sufficient controls are in place from 'farm to fork.' An effective food safety program of any kind must include effective controls and food safety commitment throughout the entire food chain.

Historically, it all began at the beginning of the twentieth century with the passage of the Federal Meat Inspection Act (FMIA) in 1906. Upton Sinclair's book *The Jungle*, which detailed significant food safety issues in meat packing facilities, had a direct impact on the passage of the FMIA. The Pure Food and Drug Act, which later became the Federal Food, Drug and Cosmetic Act, was enacted in the same era in US history.

The 'HACCP' concept was first introduced in the early 1960s by Dr. Howard Bauman when Pillsbury and NASA joined forces to produce a 'safe' product for the astronauts. HACCP guidelines are defined in Codex Alimentarius (2003) *Hazard analysis and critical control point (HACCP) system and guidelines for its application*. Monitor the Codex Alimentarius website for current requirements.

At the turn of the twenty-first century, we saw an emphasis on food safety in which regulatory agencies were requiring (or proposing) the HACCP process for what was considered high risk segments of the food industry (e.g., meats and poultry, seafood, 100% juice products).

On 4 January 2011, President Obama signed into law the FDA Food Safety Modernization Act (FSMA), which is the most encompassing reform of food safety laws in more than 70 years. This Act focuses its aim on ensuring that the US food supply is safe, applying prevention controls rather than responding to a contamination outbreak. The time has passed for the food industry to learn from its mistakes, now we must be proactive, not reactive. Effectiveness must be measured by evaluating our processes, products, ingredients, packaging materials, processing aids, and product contact surfaces using the eyes of prevention to identify existing and potential hazards, then putting actions in place that either eliminate or correct the situation. We must have confidence that we are producing a product that is safe for consumption. Every associate has the responsibility to ensure the production of a safe product taking every measure necessary to ensure this safety. It is time that top management seriously evaluates its processing system to define a proactive food safety program that identifies and ensures the control of existing and potential food safety hazards, based on current scientific principles, standards, and concerns.

As this article goes to press, because of the complexity of the FDA FSMA, the food industry in the USA is waiting for some rules to be clarified prior to the full scope of the law taking effect. In the meantime, consumers (above and beyond government laws) are demanding safe products. Food manufacturers are receiving extreme pressure from their customers to develop a proactive program that forces management its products, processes and suppliers to ensure that products are free of hazards and safe for consumption. In today's world of food safety, we define the word 'safe as a food that is free of any food hazards. 'Food safety' is defined as a finished product that does not contain a food hazard when used according to its intended use.

When one mentions the term 'HACCP', it reflects the process that is used to identify, prevent, evaluate, and control hazards, which are significant for food safety as defined in Codex Alimentarius. HACCP is a management tool designed to identify existing and potential hazards: biological pathogenic,

chemical, and physical in the ingredients and the process of the manufacturing of consumable goods from the farm to the table, establishing controls that reduce or eliminate risk for public health. HACCP provides the framework to produce foods safely and to prove they were produced safely. An effective HACCP program is built on the foundation of effective prerequisite programs.

Subsequent text describes many examples of currently accepted food safety management standards, also referred to as food safety management programs, but it is important to focus on the fact that an effective FSMS applies the concept of a structured management system as discussed earlier with the requirements of effective HACCP programs including requirements for specific prerequisite programs. Prerequisite programs (PRP) are defined as the, 'basic conditions and activities that are necessary to maintain a hygienic environment through the food chain suitable for the production, handling, and provision of safe end products and safe food for human consumption.' (ISO 22000:2005.) PRPs provide the foundation for the HACCP Program and address the basic conditions and activities required for food safety that are necessary to either control or eliminate a potential hazard, address a quality parameter, and/or maintain a hygienic environment throughout the operation.

In the past, some organizations have chosen to develop and maintain a system compliant with a third party food safety standard but not verified by an independent third party. It is recommended and, in many instances, required by the customers of the organizations, to have compliance confirmed and monitored by independent third party companies. This format adds additional discipline and removes some of the 'variances' that could be issued internally. It also builds consistency within the food chain sectors.

While food safety standards do have some differences and preferences for specific standards (in many instances, food sector specific), all focus on applying applicable FSMS requirements and evaluation of the system. The following sections provide an overview on GFSI and a brief description of each standard or program that has been benchmarked as an approved schemes meeting the current defined GFSI guidelines. Although specific websites are not referenced for the Individual FSMS, it is very important that the reader review these websites to receive current information. All the standards are revised on a defined schedule (some may even become obsolete through the years) making the individual websites the best source for current information and requirements.

GFSI is a nonprofit foundation established in May 2000, managed by The Consumer Goods Forum, and was launched in May 2000. The GFSI Foundation Board of Directors includes representatives from major global retailers, manufacturers, and food service operators that oversee basic management and direction.

This GFSI Foundation Board of Directors has developed a Guidance Document that outlines requirements that must be met by the Food Safety Management Standards that it recognizes. The GFSI Guidance Document is not a food safety management standard, nor does GFSI get involved in certification or accreditation activities. The GFSI Guidance document is reviewed by the Board at a minimum of once every 4 years; however, this may be more frequent based on current trends and issues as determined pertinent by the GFSI Board. Membership to the GFSI Board of Directors is by invitation only.

GFSI defines its vision to drive continuous improvement in food safety while strengthening consumer confidence worldwide with the primary focus of its objectives to:

- Reduce food safety risks by delivering equivalence and convergence between effective FSMSs.
- Manage cost in the global food system by eliminating redundancy and improving operational efficiency.
- Develop competencies and capacity building in food safety to create consistent and effective global food systems.
- Provide a unique international stakeholder platform for collaboration, knowledge exchange, and networking. (Quoted from http://www.mygfsi.com)

Through continuous improvement, GFSI provides a recognition process that can be used by food industry stakeholders; however, it is clearly stated in its documentation that it is each organization's choice as to which GFSI benchmarked scheme(s) is chosen. This decision may be based on the industry sector, company policy, customer requirements, customer needs, regulatory requirements, organization's preferences, and/or product liability.

The process for GFSI to review a FSMS standard confirming it meets the requirements of the most current version of the GFSI Guidelines is known as 'benchmarking.' In other words, 'benchmarking' is defined as a "procedure by which a food safety-related scheme is compared to the GFSI Guidance Document."

As previously mentioned, the GFSI board does not get involved in the actual certification process. Certification is achieved through a third party audit by an approved registration body against one of the recognized GFSI approved schemes.

Originally, four food safety standards (SQF, BRC, Dutch HACCP, and International Food Standard (IFS)) were recognized as GFSI schemes. In 2009, ISO 22000 in combination with PAS 220 (now known as ISO 22002-1) was benchmarked becoming the fifth recognized scheme. The standard representing the combination of ISO 22000 and PAS 220 became known as FSSC 22000. In subsequent years, additional food safety standards were benchmarked and confirmed compliant.

On 5 January 2011, GFSI released the Sixth Edition of its Guidance Document. On release of the Sixth Edition, all previously recognized standards were required to submit a request to be benchmarked in compliance with the GFSI Guidance Document Sixth Edition by 31 December 2011. The revised version of this guidance document provides more prescriptive and detail for identifying and providing clearer definitions required for the production of safe food in different industry sectors.

Industry sectors are divided into the following categories:

- Farming of Animals;
- All Farming of fish;
- B1 Farming of plants;
- B11 Farming of grains and pulses;

- C Animal conversion;
- D Pre-processing handling of plant products;
- E1 Processing of animal perishable products;
- E11 Processing of plant perishable products;
- E111 Processing of animal and plant perishable products (mixed products);
- EIV Processing of ambient stable products;
- L Production of (bio) chemicals;
- M Production of food packaging.

The revised version of this Guidance Document also provides an improved definition of the benchmarking process. This process is a scientifically based method that the GFSI board applies to identify competent food safety schemes. This approach allows food organizations to select the FSMS that best fits its needs and industry sector.

A brief description of the recognized schemes (May 2012) along with a brief description of the standard quoted from each website is provided in the text that follows; however, because this list may change at any time, it is recommended that the astute reader monitor the GFSI website for the most current information.

BRC Global Standard for Food Safety (sixth edition) is the current version at the time of publishing this article. The BRC Global Standards are widely used by suppliers and global retailers. They facilitate standardization of quality, safety, operational criteria, and manufacturers' fulfillment of legal obligations. This standard is designed to provide protection to the consumer. The BRC Global Standards are a leading global safety and quality certification program used throughout the world by over 17 000 certificated suppliers in 90 countries through a network of over 80 accredited and BRC-recognized Certification Bodies. (http://www.brcglobalstandards.com/GlobalStandards/Home.aspx)

CanadaGAP (Canadian Horticultural Council (CHC) On-Farm Food Safety (OFFS) Program): 'CanadaGAP (Good Agricultural Practices) is the name of the CHC's OFFS Program. The program consists of national food safety standards and a certification system for the safe production, storage, and packing of fresh fruits and vegetables.' (http://www.canadagap.ca/)

FSSC "FSSC 22000 (Food Safety Standard Certification) contains a complete certification scheme for FSMS based on existing standards for certification (ISO 22000, and ISO 22002-1 with latter technical specifications for sector PRPs)." Manufacturers that are already certified against ISO 22000 will only need an additional review against technical specifications for sector PRPs to meet this certification scheme. Organizations that want to integrate quality in their management systems follow the requirements of ISO 9001. FSSC 22000 is developed for the certification of food safety systems of manufacturers in the food chain that process or manufacture animal products, perishable vegetable products, those products with a long shelf life, (other) food ingredients like additives, vitamins, bio-cultures, and food packaging material manufacturing (quoted from http://www.fssc22000.com/en/).

Global Aquaculture Alliance "Seafood Processing Standard is an international, nonprofit trade association dedicated to advancing environmentally and socially responsible aquaculture. GAA recognizes that aquaculture is the only sustainable means of increasing seafood supply to meet the food needs of the world's growing population. Through the development of its Best Aquaculture Practices certification standards, GAA has become the leading standards-setting organization for aquaculture seafood." (Quoted from http://www.gaalliance.org/)

The GLOBALG.A.P. is a "private sector body that sets voluntary standards for the certification of production processes of agricultural (including aquaculture) products around the globe. (This standard) is primarily designed to reassure consumers about how food is produced on the farm by minimizing detrimental environmental impacts of farming operations, reducing the use of chemical inputs, and ensuring a responsible approach to worker health and safety as well as animal welfare. GLOBALG.A.P. serves as a practical manual for GAP anywhere in the world. The basis is an equal partnership of agricultural producers and retailers who wish to establish efficient certification standards and procedures." (Quoted from http://www.globalgap.org/)

Global Red Meat Standard (GRMS) is a scheme specifically developed for the meat industry. "Its cornerstone is product safety, focusing on critical areas affecting the maintenance of high meat safety requirements. The fact that GRMS has been specifically developed for the meat industry provides its customers with an invaluable tool for measuring a supplier's performance." (Quoted from http://www.grms.org/)

IFS Food Version 5 (International Featured Standard) includes "associated members of the German retail federation – Hauptverband des Deutschen Einzelhandels – and of its French counterpart – Fédération des Entreprises du Commerce et de la Distribution – drew up a quality and food safety standard for retailer branded food products, named the IFS Food, which is intended to allow the assessment of suppliers' food safety and quality systems, in accordance with a uniform approach. This Standard applies to all the postfarm gate stages of food processing." (Quoted from http://www.ifs-certification.com/)

PrimusGFS "is a private scheme that establishes requirements for the certification of products of the Agricultural sector in a voluntary manner at a world-wide level. The scope of PrimusGFS is focused on the food safety of those products of the agricultural sector designated to human consumption in their fresh or minimum processed way. PrimusGFS establishes a series of requirements for managing the production, handling, processing and storing operations, which should be met for consumer safety." (Quoted from http://www.primusgfs.com/)

SQF Edition 7 is a program that "provides two standards based on the type of food supplier: SQF 1000 for primary producers and SQF 2000 for manufacturers and distributors. Within these two standards, SQF helps make certification more attainable for smaller companies by dividing the process into three steps: from Level 1, which incorporates fundamental food safety controls appropriate for low-risk products all the way to Level 3, indicating a comprehensive implementation of food safety and quality management systems development." (Quoted from http://www.sqfi.com/)

Related to Dutch HACCP, The Foundation for Food Safety Certification, which also owns this standard, has stated that although the Dutch HACCP Standard was previously recognized against the GFSI Guidance Fifth Edition, it will not be resubmitted to GFSI for benchmarking to the GFSI Guidance

Document Sixth Edition. The stated intent is for the Foundation to focus on the management of the FSSC 22000 scheme, which they also own and which has been resubmitted for benchmarking. Dutch HACCP Option B certificates that were still in circulation in 2012 will be accepted as valid against the GFSI Guidance Document Fifth Edition during that year. However, any certificates issued after 1 January 2012 will not be considered to have been issued against a GFSI-recognized scheme.

Related to Synergy 22000, on 30 January 2012, the management of FSSC 22000 and of Synergy 22000 reached an agreement on a joint future strategy regarding the use of ISO 22000 as the basis for certification of FSMS in food supply chains worldwide. The cooperation will solve the user-unfriendly and confusing situation of having two equivalent schemes in the marketplace and generate synergies involving all available forces and competences behind a common objective within the FSSC framework.

The Food Safety and Quality Management System

This text has provided a comprehensive overview of both the quality management system standard from the ISO 9001 prospective and also the GFSI-approved FSMS schemes. The next step is for top management of the organization to make some critical decisions. One of the most important 'critical decisions,' if not the most important, focuses on management commitment and leadership and whether or not the organization is ready to make this journey. In many situations, organizations are finding that ready or not, their customers are making it a requirement for doing business.

Frank Yiannas, VP – Food Safety for Walmart in his book *Food Safety Culture Creating a Behavior-Based Food Safety Management System* (Springer, c2010, page 16) revisits the culture change or culture focus that must be in place in order for food safety programs to be the most effective. Mr. Yiannas states that "a food safety culture starts at the top and flows downward. It does not flow from the bottom up. It is a leadership function to create a food safety vision, set expectations, and inspire others to follow." Every standard mentioned previously addresses top management responsibility and commitment. Management commitment must also include food safety leadership. Mr. Yiannas points out that leadership and management are different. "The main difference between the two is that leadership is about influencing people to follow, while management focuses on maintaining systems and processes. Leading companies with strong safety cultures not only have strong FSMSs in place, they also have strong leaders committed to food safety who are able to influence others and lead the way to safer performance."

The development, implementation, and maintenance of a food safety and quality management system requires a serious commitment for success by the organization's management and entire associate team. Effective implementation requires resources. Resources may include adding additional staff and hiring an experienced consultant in both the standards of choice and in the organization's food sector. The initial focus must be on creating a system that is best for the organization, integrating the requirements into the existing operation in a manner that is not only compliant but adds value to the organization.

It is very important and cost effective long term to engage the assistance of a knowledgeable industry expert to assist the implementation team in training, identifying, and closing the gaps an organization may have in meeting the food safety management standard of choice. Once the implementation team is established, the most effective next step must be the performance of a gap analysis. It is recommended that this be done by an independent external consultant, or if you are part of a larger company with corporate resources, then possibly by a trained (in the standard of choice) company associate that is external to the operation. In many instances, the organization does not make decisions on standards or time tables for completion until after the gap analysis is completed. A favorite example is that a gap analysis is like taking an X-ray. It is absolutely foolish and ineffective to set a broken leg until an X-ray is taken. This is the same with the gap analysis. Evaluate the existing organization and its activities, identify the gaps, and assist in closing these gaps to achieve compliance. A gap analysis can be performed by the registrar of choice, but the auditor representing the registrar is limited on how much assistance he or she can provide in closing the gaps. This can be considered a conflict of interest because an auditor must not audit his/her own work. When choosing a consultant or auditor, be sure to confirm his/her experience in the standard of choice and also, if possible, in the organization's food sector.

The next critical discussion point is the resource issue. Resources must be appropriate for the organization's certification project plan. Based on experience, the absolute best and most effective process to address the resource issue is to empower the entire associate team. Many times, management has stated that they just did not have the resources to do what was needed, but some of these organizations had 200 plus associates. If every one of those associates spent 1 h a week assisting the implementation teams in writing, reviewing, and implementing the process, progress within the journey would be noted, the associates would feel part of the team, learning and enhancing individual knowledge on the system and its requirements. Documentation and other activities may move forward more efficiently because more individuals who have first-hand knowledge of the processes would be assisting the process and much more.

An effective, compliant food safety and quality management system is a system developed and implemented that is suitable and effective for the organization. As it matures, an effective system continues to add value while continually improving. Compliance and continuous improvement become a way of life. Most often, those who resist the most in the beginning become the biggest supporters. However, all that said, if the primary focus is just to get the certificate and to pass the audits then maintaining a compliant system becomes very difficult, draining resources without adding value. The confidence of producing a safe product becomes limited and from the quality aspect, meeting customer requirements also deteriorate. An effective food safety and quality management system is worth the effort and makes good business sense for every organization. A system patched together for a certificate or a quick fix to make the customer happy is like patching a life boat and trying not to sink. Eventually, the patches just quit keeping the boat afloat.

Conclusion

Personal experience has shown me that adhering to a structured management system such as ISO 9001, ISO 22000, ISO 14000, OHSAS 18001, SQF, BRC, or FSSC 22000 provides the structure and discipline necessary for a strong and efficient management system. A mature system provides the foundation for continuous improvement, improved quality performance, and increased profits while meeting the customer's needs and expectations. This structure and discipline makes good business sense. Jon Porter, a consultant specializing in food safety, summed it up perfectly by stating that "ISO is the envelope, and everything fits inside." This statement applies to compliance to a structured quality and/or FSMS such as those identified in this article.

Frequently asked questions are, 'How difficult is it to achieve registration?' and 'How much will this cost?' The answers are not simple. Each system is different. The initial step must be to identify and document existing processes, evaluating how each process meets the requirements, identifying the gaps, and then addressing them. It is critical that an operation does not reengineer its processes to meet the requirements, but as much as possible, integrates these requirements into its system. Related to cost, it is going to depend on the current resources and the defined project plan for the certification process. Cost can be measured strictly in dollars and cents or this plus overtime plus extended fixed costs. Based on experience, one of the most difficult questions to answer is how much will it cost. Personally, I have seen organizations spend ridiculous amounts of money and at the end have one of the worst systems ever developed, and on the flip side, management teams that provided leadership and structure defining a realistic project and time table with added cost measurable but justified based on the benefits and resulting value added system.

The point of this article has been to provide a brief description of ISO 9001, ISO 22000, and selection of benchmarked GFSI-recognized schemes related to food safety; however, it is important that the reader understands that these same concepts can and should apply to other management system standards and recognized schemes even those that may be company owned, defined, and audited for compliance internally. No matter which standard is chosen, most, if not all, have been developed and the requirements defined within the shadow of ISO 9001.

There is an abundance of information related to these standards in many different locations. It is truly up to the organization to determine which standard best fits its operation, food sector, and overall scope. Whichever standard is chosen, it remains critical that top management is committed to developing, implementing, and maintaining a management system that is not only effective and compliant, but also adds overall value to the organization. It is just as critical that resources are applied effectively and timely, based on the system's policy statement and its measureable objectives. Top management must understand, communicate, and support the development of the system including the assignment of resources in a manner that provides adequate opportunity for the development of an effective system. One of the greatest challenges may be trying to do too much too fast with inefficient resources. Even if success is achieved, sustainability often becomes difficult without continued strong and effective focus; without adequate support, such a system could self-destruct. Planning, assessing gaps, and whenever possible, integrating the requirements into the current system rather than reengineering will help to realize success along with promoting team work and communicating management support.

It is critical to focus on the positive through top management's leadership and commitment applying common sense to integrating the management system requirements into the operation. Remember the Deming PDCA (Plan, Do, Check, Act) concept. Set realistic, achievable objectives; identify the gaps, develop a plan, integrate, monitor, and apply resources effectively and efficiently. The road to compliance is not candy coated, but it is worth the effort. Russ Marchiando, production manager for Wixon, St. Francis, Wisconsin, described the certification process as "a journey, not a destination."

See also: Food Safety Assurance Systems: Audits of Food Safety Management Systems; Documentation and Record Keeping; Hazard Analysis and Critical Control Point System (HACCP): Principles and Practice. Institutions Involved in Food Safety: International Organization for Standardization (ISO). Public Health Measures: Evaluation of the Efficacy of National Food Control Programs; Modern Approach to Food Safety Management: An Overview

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Good Practices in Fisheries and Aquaculture

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Glossary

Aquaculture The farming during part of the life cycle of finfish, crustaceans, and mollusks.

Capture fisheries It refers to all kinds of harvesting of naturally occurring living resources in both marine and freshwater environments.

Good aquaculture practices Those practices of the aquaculture sector that are necessary to produce quality and safe food products conforming to food laws and regulations.

Good hygienic practices All conditions and measures necessary to ensure the safety and suitability of food at all stages of the food chain.

Hazard analysis and critical control point (HACCP) A system that identifies, evaluates, and controls hazards that are significant for food safety.

Prerequisite program A program that is required before the application of the HACCP system to ensure that a fish- and shellfish-processing facility is operating according to the Codex Principles of Food Hygiene, the appropriate Code of Practice, and appropriate food safety legislation.

Introduction

World fish production from fisheries and aquaculture contributes significantly to global food security and food trade, with an average per capita supply of 18.6 kg (live weight equivalent) in 2010. World fish and seafood supply amounted to approximately 148 million tons of fish in 2010 (with a total value at harvest of US$217.5 billion), of which approximately 128 million tons were utilized for human consumption (Table 1).

Although production from wild fisheries has stagnated over the years at approximately 91–95 million tons, the increasing demand has been steadily met by a robust growth in aquaculture, estimated at an average 8.8% yearly growth in volume during the period 1980–2010, bringing the contribution of aquaculture to fish food supply to 47% in 2010 from a mere 9% in 1980. This trend is projected to continue, with the contribution of aquaculture to fish food supply estimated to reach 60% by 2020, if not before.

Table 1 World fisheries and aquaculture production and utilization

	2006	*2007*	*2008*	*2009*	*2010*	*2011*
Production (in million tons)						
Capture fisheries						
Inland	9.8	10	10.2	10.4	11.2	11.5
Marine	80.2	80.4	79.5	79.2	77.4	78.9
Total capture fisheries	90	90.3	89.7	89.6	88.6	90.4
Aquaculture						
Inland	31.3	33.4	36	38.1	41.7	44.3
Marine	16	16.6	16.9	17.6	18.1	19.3
Total aquaculture	47.3	49.9	52.9	55.7	59.9	63.6
Total fish production	137.3	140.2	142.6	145.3	148.5	154.0
Utilization (in million tons)						
Human consumption	114.3	117.3	119.7	1263.6	128.3	130.8
Nonfood uses	23	23	22.9	21.8	20.2	23.2
Population (billions)	6.6	6.7	6.7	6.8	6.9	7
Per capita food fish supply (kg)	17.4	17.6	17.8	18.1	18.6	18.8

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Fish and seafood are highly perishable commodities. To prolong their shelf life and preserve their nutritional attributes, they are processed in a variety of ways. They are distributed either live, fresh, chilled, frozen, heat treated, fermented, dried, smoked, salted, canned, or as a combination of two or more of these (Figure 1). Utilization of live/fresh fish and seafood has increased over the years more significantly than the other forms of preservation.

Likewise, fish and fishery products are among the most traded food commodity worldwide. The total world fish trade increased significantly from US$8 billion in 1976 to a record export value of US$109 billion in 2010. A specific feature of fish trade is the wide range of product types and participants. In 2010, 205 countries reported exports of fish and fishery products, of which 89 were net exporters, with a significant contribution of the sector to their national economies. More than 67% of the traded seafood is imported by three major markets: the European Union (EU), Japan, and the USA. These three markets are characterized by stringent and exacting requirements for consumer protection and food safety.

This article provides an update on the major fish and seafood safety issues and how to address them by developing and implementing reliable food safety and quality systems. Many academic and industry publications have been devoted to this area during the past 20 years. These works have been extensively used by Food and Agriculture Organization of the United Nations (FAO), World Health Organization, and Codex to develop an international guidance that is practical and adaptable to both developed and developing countries. This article draws on this extensive international work, in particular on the FAO flagship publication on the subject and other international initiatives by the Codex and national food control authorities to provide the reader with a practical guidance on fish and seafood safety issues and how to manage them from harvest to distribution.

Fish- and Seafoodborne Illnesses and Causative Agents

When harvested in clean environments and handled hygienically, fish and seafood are very safe. Unfortunately, water pollution, unhygienic practices, and insufficient or delayed icing or refrigeration have been the cause for many outbreaks of fish- and seafoodborne illnesses (Table 2).

Many of these causative agents are also responsible for illnesses caused by other types of food, especially food of animal origin. They are described elsewhere in this encyclopedia, including their ecology and association with foods. Following is a brief description of these agents.

Bacteria

Apart from *Vibrio* spp., *Listeria monocytogenes*, and *Clostridium botulinum*, which are part of the indigenous fish flora, the other bacteria that can contaminate fish and cause the majority of fishborne illnesses may come from the environment, the handlers, water used for washing the fish, or ice. Bacteria that are indigenous to the aquatic and the general environment may be associated with fish at the primary production stage (aquaculture or fish harvesting) and those derived from the general environment or from animal/human reservoir may be introduced as a result of contamination of the water or during handling and processing of fish. In either case, the initial levels of the bacteria are generally low and multiplication of the organism in fish to reach an infective dose or to produce toxin in fish precedes fishborne illnesses. Therefore, for management of the risk due to these pathogens, preventing their growth using refrigeration, freezing, or other preservation techniques is very important.

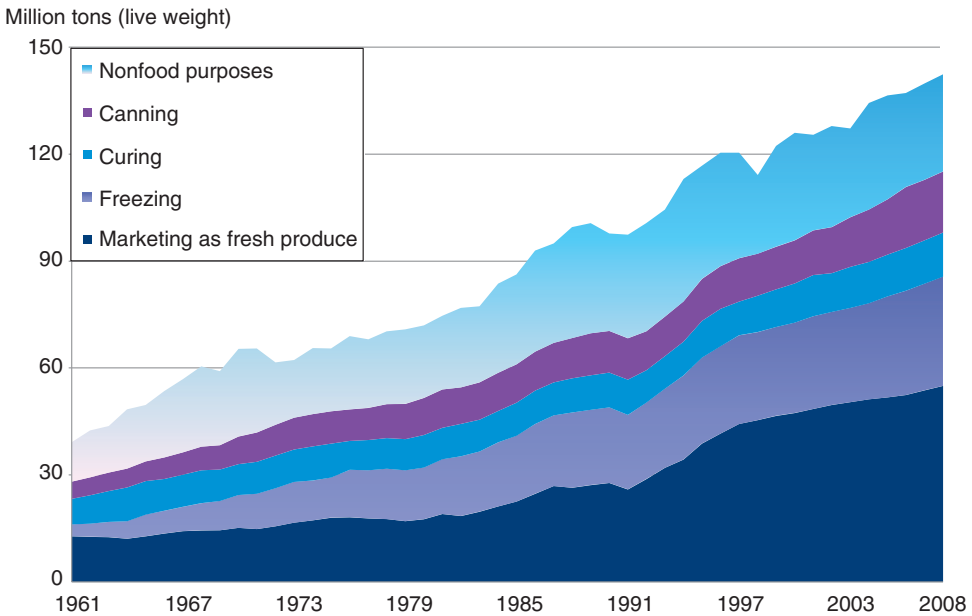

Figure 1 Fish and seafood utilization (1961–2008), in volume.

Table 2 Types of fish- and seafoodborne illnesses

Types of illness		*Causative agent*
Infections	Bacterial infections	*Listeria monocytogenes*, *Salmonella* spp., *Escherichia coli*, *Vibrio vulnificus*, *Shigella* spp., and *Vibrio cholerae*
	Viral infections	Hepatitis A, norovirus, and hepatitis E
	Parasitic infections	Nematodes (roundworms), cestodes (tapeworms), and trematodes (flukes)
Intoxications	Microbial	*Staphylococcus aureus*, *Clostridium botulinum*, and histamine
	Biotoxins	Ciguatera, paralytic shellfish poisoning, diarrheic shellfish poisoning, amnesic shellfish poisoning, and neurotoxic shellfish poisoning
	Chemical	Heavy metals: mercury, cadmium, and lead
		Dioxins and polychlorinated biphenyls
		Additives: nitrites and sulfites

Biotoxins

Biotoxins or phycotoxins are marine toxins that accumulate in fish or bivalve mollusks (mussels, oysters, scallops, clams, etc.). Most of these toxins are produced by species of naturally occurring marine algae (phytoplankton). There are approximately 5000 species of marine algae, but only 70–80 species are known to produce toxins. A proportion of the toxic phytoplankton has a red-brown pigmentation, the reason why the algal blooms are named 'red tides.' However, not all colored algae are toxic and incidences of poisoning have occurred in the absence of red tides. Bivalve mollusks are filter feeders and continually pump water through their gills for feeding by removing and ingesting particulate matter. During a bloom, bivalves can accumulate sufficient toxin to cause human illness after filter feeding for only 24 h. Biotoxins have been responsible for incidents of large-scale death of sea life and are increasingly responsible for human intoxication. Seafood poisoning syndromes associated with toxic marine algae are paralytic shellfish poisoning, amnesic shellfish poisoning, diarrheic shellfish poisoning, neurotoxic shellfish poisoning, and azaspiracid shellfish poisoning. Other types of biotoxins associated with finfish include ciguatera fish poisoning and puffer fish poisoning.

Histamine Fish Poisoning (HFP)

HFP is an intoxication that can be caused by consumption of many different types of marine finfish that contain toxic levels of histamine. HFP commonly occurs worldwide. Many species of marine finfish have caused HFP, and the intoxication is often referred to as scombroid or scombrotoxin poisoning because of the frequent association of the illness with the consumption of scombroid fish such as tuna, skipjack, saury, and mackerel. However, nonscombroid fish such as anchovies, bluefish, herring, mahi-mahi, marlin, sardines, and swordfish have also been implicated in outbreaks of this illness. These fish species have significant amounts of histidine in their muscle tissues where it serves as a substrate for bacterial histidine decarboxylase and formation of histamine. Consequently, to reduce HFP, preservation techniques to reduce growth and activity of histamine-producing bacteria should be applied.

Viruses

Foodborne viruses are derived from the human gastrointestinal tract, and their presence in water and food is a result of contamination with sewage, poor hygiene, or contamination by food handlers. Viral diseases are associated mainly with bivalves because they feed by filtering large amount of water, which causes the viruses to concentrate when the harvesting water is contaminated. Though a number of viral groups have been detected in bivalves, clear epidemiological links with seafood exist only for norovirus and hepatitis A virus. Astrovirus has also been reported as an etiological agent in a limited number of shellfish-associated outbreaks.

Chemical Contaminants

The main chemical contaminants of concern in fisheries and aquaculture are heavy metals (mercury, cadmium, and lead); organic pollutants such as dioxins and polychlorinated biphenyls (PCBs); and antimicrobial substances including veterinary drugs and additives such as metabisulfites. Some of these substances have maximum regulatory limits (MRLs), whereas others are banned or should have no residues. MRLs are defined for authorized veterinary drugs, antibiotics, additives, and certain contaminants that are already part of the environmental background.

Many organic pollutants were considered useful products before their negative impact on the environment and biota was noticed. These include herbicides and pesticides for agriculture, and PCBs, which have been used as additives and fire retardants in a range of consumer and commercial products such as electronics or textiles. Some other compounds like dioxins are by-products of certain industrial processes (e.g., metallurgical industry) and combustion processes like waste incineration, or during natural processes like forest fires or volcanic eruptions.

Heavy metals are naturally present in the aquatic environment and in its biota due to volcanoes, geological anomalies, and geothermal events, but anthropogenic pollution results from various industrial activities. The distribution between the natural background concentration of heavy metals and anthropogenic heavy metals in fish varies, depending on the element, the species, and the area of

capture. In open seas, which are much less affected by pollution, fish carry mostly the natural burden of heavy metals. In moderate or heavily polluted areas such as those that do not have sufficient exchange with the world oceans (e.g., Baltic sea and Mediterranean sea), in estuaries, in rivers and lakes, and especially in places in close vicinity to industrial activities, heavy metal concentrations actually found in seafood can exceed the natural concentrations.

There is evidence that some antibacterials are used as prophylactics and growth promoters in aquaculture. This can result in

- the presence of high levels of residues of approved antibiotics and/or residues of unapproved or banned antibiotics and
- the development of resistance to antibiotics in microbial pathogens in the environment.

Of major concern in aquaculture are residues of chloramphenicol, nitrofurans, and malachite green. Their use in food production is banned by many countries.

Proper selection of aquaculture sites and periodic monitoring of fishing and aquaculture water for potential chemical contaminants should assure appropriate consumer protection. The presence of residues of antimicrobials over approved MRLs could be eliminated through applying good management practices, hazard analysis and critical control point (HACCP) procedures, education, and awareness building.

Parasites

Fishborne zoonotic parasites are prevalent in many regions of the world and are among the most important of all zoonotic parasites infecting humans. The number of people currently infected with these parasites may exceed 20–30 million, with the number of people at risk worldwide estimated at more than half a billion. Fishborne parasites include species of nematodes (roundworms), cestodes (tapeworms), and trematodes (flukes). They are found in both marine/brackish and freshwater wild and cultured fish. In all the important species, a larval stage present in the fish host is transmitted to a suitable final host, in which full development to the reproducing adult stage occurs. For some parasite species, however, the larval stage does not mature in the human host (i.e., the nematodes *Anisakis* and *Gnathostoma*), but remain in a larval stage that can migrate through the host's tissues causing pathological damage. Importantly, fishborne parasite infections in people often exist as a multiple species complex, because they have common transmission modes that are favored by well-entrenched cultural traits, particularly fondness for raw or lightly cooked, cured, or pickled fish and fish products.

Physical Contaminants

Finally, physical contaminants such as glass, metal and wood pieces, nails, bones in fish fillets, hooks, etc. have also been at the origin of consumer health distress and need to be considered when designing a seafood safety assurance program.

Fish and Seafood Safety and Quality Systems

Robust safety and quality assurance systems that build on good practices are necessary to prevent and control fish- and seafoodborne illnesses. These systems and practices aim at preventing the contamination of fish, crustaceans, and shellfish during harvesting, landing, handling, processing, and distribution and preventing microbial growth after harvesting. Figure 2 describes the main hazards and the main elements of an assurance system for the three major fish and seafood value chains.

Depending on the fish or seafood species, the prevention requires monitoring the harvesting grounds, implementing GAPs and good hygienic, and handling and manufacturing practices during the postharvest stages. Government authorities have the primary responsibility for implementing robust monitoring programs, whereas the industry has the primary responsibility for implementing good practices in aquaculture and during the postharvest stages, using HACCP-based programs where applicable. In the latter, government authorities are responsible for certifying that good practices are adhered to on board fishing vessels, in fish farms, and during processing and distribution.

Monitoring of the Harvesting Grounds

Fishing should be carried out only in clean waters. Regular monitoring of the water quality is key in assessing whether the area is suitable for harvesting fish, crustaceans, or bivalves for human consumption.

Open seas are unaffected by pollution, and the finfish and crustaceans harvested in these areas are generally clean and fit for human consumption. Monitoring programs are required for some freshwater, estuaries, and coastal waters contaminated by sewage, or where shoreside industries are located or intensive agriculture using pesticides or other agrochemicals is practiced. These monitoring programs are generally enacted through regulations that define responsibilities and resources to food control authorities that will manage the monitoring programs, although research and industry are also involved. Environmental monitoring can identify species susceptible to contamination, magnitude of contamination, and spatial distribution of contamination. For example, the US Environmental Protection Agency has developed guidance for assessing chemical contaminant data for use in fish advisories. This would be a very useful guide for the development of a national fish contaminant-monitoring plan in other countries. Likewise, in the EU regulations, monitoring fishing and aquaculture areas for environmental contaminants has been included as a part of the regulatory food safety management. A guide for the establishment of environmental- and residue-monitoring plans for compliance with EU regulations has been developed for use by countries exporting fish to the EU. The development of a sampling and analysis plan by a team consisting of the food control authority, research, and industry should be based on the knowledge of the fishery and the likely sources of contamination. When contaminants above permissible limits are found, it is necessary to

- trace the source of contamination;

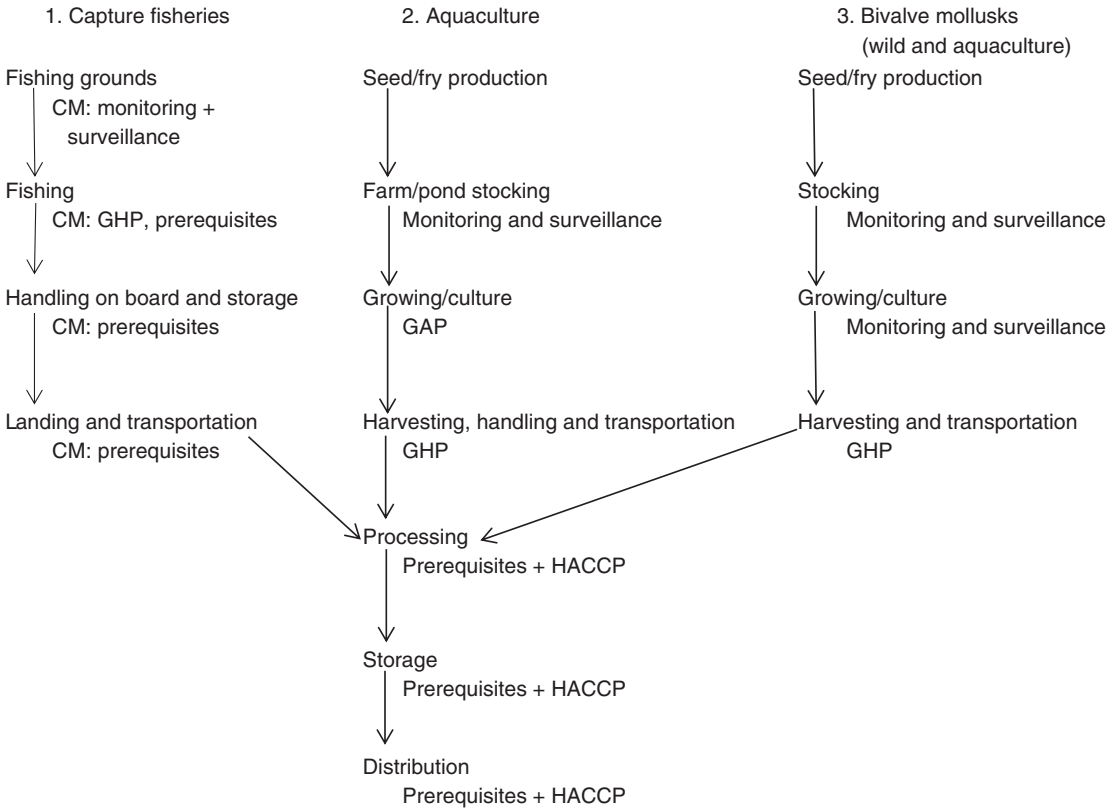

Figure 2 Fish and seafood safety management in fisheries and aquaculture. CM, control measures; GAPs, good aquaculture practices; GHPs, good hygienic practices.

- define the affected area and map the boundaries;
- suspend fishing in affected areas; and
- review the status with further sampling and analysis.

Monitoring and surveillance programs are also required for areas where bivalve mollusks are grown. The main hazards associated with the production of bivalve mollusks are contamination by bacteria, viruses, or biotoxins from the harvesting waters.

The identification, classification, and monitoring of these areas is a responsibility for the competent authority (CA) having jurisdiction, in cooperation with fishermen and primary producers. *Escherichia coli*/fecal coliforms or total coliforms may be used as an indicator for the possibility of fecal contamination. The results of the microbiological analysis would enable the CA to classify the growing areas as either

- suitable for harvesting bivalves for direct human consumption;
- relaying the harvested bivalves in acceptable water (relaying is the removal of bivalve mollusks from a microbiologically contaminated growing area to an acceptable growing or holding area under the supervision of the CA and holding them there for the time necessary for the reduction of contamination to an acceptable level for human consumption);
- depuration of the harvested bivalves in an approved depuration center (depuration is the reduction of microorganisms to a level acceptable for human consumption by the process of holding live bivalve mollusks for a period of time under approved, controlled conditions in natural or artificial seawater suitable for the process, which may be treated or untreated);
- approved processing to reduce microbial contamination to acceptable level; or
- unsuitable for growing or harvesting bivalve mollusks.

When sampling shellfish meats for classification purposes, if the limits of any biological or chemical hazard set in the end product specification are exceeded, appropriate measures must be taken under the responsibility of the CA. If biotoxins are found in the bivalve mollusks flesh in hazardous amounts, the growing area must be closed for harvesting bivalve mollusks until toxicological investigation shows that the bivalve mollusk's meat is free from hazardous amounts of biotoxins. Harmful chemical substances should not be present in the edible part in such amounts that the calculated dietary intake exceeds the permissible daily intake.

GAPs

Assurance of fish and seafood safety and quality in aquaculture requires the adoption and implementation of GAPs as prerequisites for the implementation of the HACCP system. The following good practices apply to the various aquaculture systems of finfish and crustaceans. The main stages of aquaculture production covered are site selection; growing water

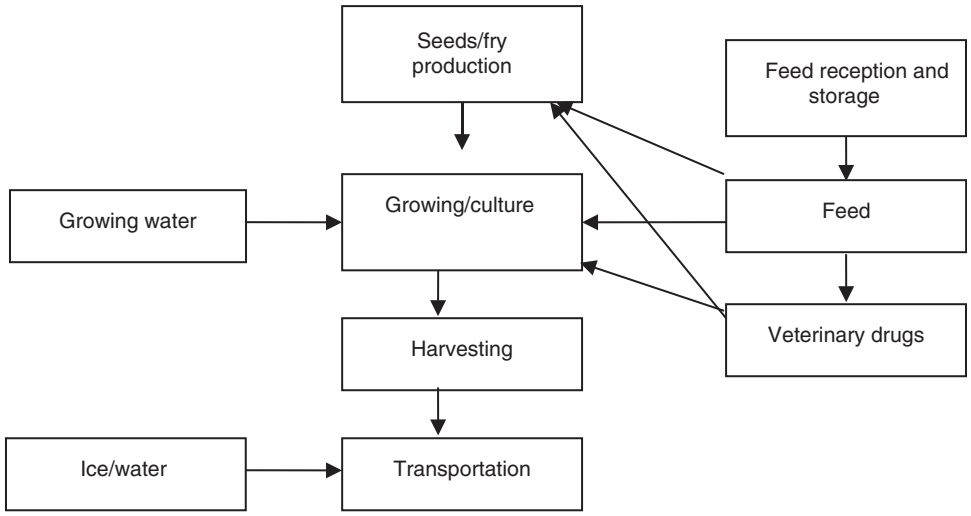

Figure 3 Example of a flow chart for aquaculture (only for illustrative purpose; Codex Alimentarius Commission (CAC) (2009) *Guidelines for the Design and Implementation of National Regulatory Food Safety Assurance Programmes Associated with the use of Veterinary Drugs in Food Producing Animals (CAC/GL 71-2009)*, 32 pp. Rome: FAO).

quality; source of fry and fingerlings; feeding; growing; and harvesting and transport (Figure 3).

Site Selection

Food safety hazards can arise from the location of the fish farm as a result of its surroundings, through the water supply, direct contact with livestock or wild animals, or airborne contamination (i.e., chemical sprays). Nearby agricultural lands that use pesticides and heavy fertilization on a regular basis could be a potential source of contamination. Fish farms should be located in areas where the risk of contamination by biological, chemical, or physical food safety hazards is minimal and where sources of pollution can be controlled. All potential sources of contamination from the environment should be considered. In particular, fish farming should not be carried out in areas where the presence of potentially harmful substances would lead to unacceptable levels of such substances in fish.

Growing Water

The water in which fish is raised should be suitable for the production of food that is safe for human consumption. Fish farms should not be sited where there is a risk of contamination of the water in which fish are reared by chemical and biological hazards. Water sources should be protected from contamination by wild (birds, lizards, snakes, turtles, and rats) and domestic (cattle, pig, chicken, ducks, cats, and dogs) animals, effluents, and runoffs. Fish farms should be designed and constructed to ensure control of hazards and prevention of water contamination. Water inlets and outlets to ponds should be screened to prevent the entrance of unwanted species. Water quality should be monitored regularly to prevent fish contamination during production.

Source of Fry and Fingerlings

The source of postlarvae, fries and fingerlings should be controlled in order to avoid the carryover of potential hazards into the growing stocks. In endemic fishborne parasitic areas, the source of fries and fingerlings should be controlled to assure that seeds are free from parasitic infection. Contaminated sources are common in endemic trematodiasis areas.

Feed Supply

Feeds can transmit harmful agents directly or by attracting pests. Feed ingredients should not contain unsafe levels of pesticides, chemical contaminants, microbial toxins, or other adulterated substances. Feed should contain only such additives, growth-promoting substances, fish flesh-coloring agents, antioxidizing agents, caking agents, or veterinary drugs that are permitted for fish by the CA. Industrially produced feeds and feed ingredients should be properly labeled. Their composition must fit the declaration on the label.

Medicated feeds should be clearly identified in the package and stored separately, in order to avoid errors. Dry fish feeds should be stored in cool and protected dry areas to prevent contamination, mold growth, and spoilage. Moist feed or feed ingredients should be properly refrigerated and should reach the fish farm in an adequate state of freshness. Fish silage, trash fish, and offal from fish, if used, and where necessary, should be properly cooked or treated to eliminate potential hazards to human health.

Veterinary Drugs

All veterinary drugs for use in fish farming should comply with national regulations and international guidelines, in accordance with the Codex guidelines on the use of veterinary drugs in food-producing animals (CAC/GL 71-2009). Drugs used on the farm should be registered with the appropriate national authority.

Control of diseases with drugs should be carried out only on the basis of an accurate diagnosis. Drugs should be prescribed or distributed only by personnel authorized under national regulations and should be used according to the manufacturer's instructions, with particular attention to

withdrawal periods. Records should be maintained when veterinary drugs are used.

Growing

The growing phase includes various activities that can significantly affect the safety and quality of farmed fish. There is a need to control the growing water quality, the design and cleaning of equipment and holding facilities, the maintenance of pond grounds, the workers' hygienic practices, and pests.

Good maintenance of the farm grounds and GHPs in the growing area and surroundings should be applied to minimize or eliminate facal contamination of pond water. A major concern is the contamination by pathogenic bacteria or parasites from waste materials or facal matter from animals or workers. Fish farms should institute a pest-control program. Good water quality should be maintained by using stocking and feeding rates that do not exceed the carrying capacity of the aquaculture system. Stocking densities should be based on culture techniques, fish species, size and age, carrying capacity of the fish farm, anticipated survival, and desired size at harvesting. Diseased fish should be quarantined when necessary, and appropriate and dead fish should be disposed of immediately in a sanitary manner.

Harvesting, Holding, and Transportation

Appropriate harvesting techniques should be applied to minimize spoilage, physical damage, and stress in the case of live fish. Harvesting should be rapid so that fish are not exposed unduly to high temperatures. In tropical areas, harvesting should be done at a time when temperature is lowest (e.g., at night). Soon after harvest, fish should be washed using clean seawater or freshwater under suitable pressure to remove excessive mud and weed and be iced or immersed in ice slurry to bring and maintain its temperature to approximately 0 °C. Equipment and utensils such as nets, bags, pumps, baskets, tubs, bins, and boxes should be designed and constructed to ensure minimum physical damage of the fish during harvesting. All equipment and utensils used during harvesting should be easy to clean and to disinfect and should be cleaned and disinfected regularly and as appropriate. Ice should be made from clean potable water.

Holding and transportation should be rapid so that fish are not exposed unduly to undesirable high temperatures. Fish should be packed in ice or immersed in ice slush aiming at keeping the temperature as closer as possible to 0 °C. All equipment for fish holding and transportation should be easy to clean and to disinfect and should be done so regularly as appropriate. Live fish should be handled in such a way as to avoid unnecessary stress. Records for transport of fish should be maintained to ensure full product tracing.

Good Practices and HACCP in Postharvest Operations

Prerequisite Programs

As stated in the Section Fish- and Seafoodborne Illnesses and Causative Agents, several pathogens and spoilage bacteria can contaminate fish and seafood during handling, processing, or distribution, either from handlers, equipment, surrounding environment, or other sources such as cleaning water or ice. To prevent this contamination from taking place, GHPs should be applied at all stages of harvesting, processing, storage, and distribution. The requirements for hygienic practices constitute the prerequisite programs that are essential for any fish and seafood operation before the implementation of HACCP.

The basis for developing and implementing GHPs are the 'Codex CoP on food hygiene (CAC/RCP 1-1969, Revision 2003)' and the 'CoP for fish and fishery products (CAC/RCP 52-2003, Revision 2009).' The provisions of these two CoPs have been used by most countries as the basis for their fish and seafood hygiene regulations, and by most fish and seafood trade associations and companies worldwide for drafting their food safety and quality policy.

A prerequisite program should include hygienic requirements for

- fishing vessel design and construction,
- processing facility design and construction,
- design and construction of equipment and utensils,
- hygiene control program,
- personal hygiene and health,
- transportation,
- product tracing and recall procedures, and
- training.

For example, the Food and Drug Administration (FDA) regulations of 2011 requires processors to have key sanitary conditions written into sanitation standard operating procedures, to monitor these conditions and practices, to correct unsanitary conditions and practices in a timely manner, and to maintain sanitation control records.

Likewise, the European Commission's 'hygiene package' addresses the prerequisite requirements both in 'horizontal' legislation and 'vertical' or commodity-specific legislation laying down specific hygiene rules for food of animal origin, including fish and fishery products.

HACCP Principles and Their Application

The HACCP is a system that identifies, evaluates, and controls hazards that are significant for food safety. It is a science-based and systematic tool that assesses hazards and establishes control systems that focus on prevention rather than relying mainly on end product testing. It not only has the advantage of enhancing the safety of the product but, because of the means of documentation and control, also provides a way for demonstrating competence to customers and compliance with legislative requirements to the food control authorities.

HACCP has been in a constant state of evolution for the past 40 years. Implementation by the fish industry has been slow and at times painful – a process that is still in progress. Application guidelines, prerequisite programs, decision trees, and training programs have been developed and implemented. Coalition of fish and seafood industries, such as the US Seafood HACCP Alliance or the Seafood Services Australia have been formed to train and certify HACCP trainers, and develop hazard analysis and generic HACCP guidance and plans. The author has also described through examples from the fish industry how an HACCP plan can be developed and implemented. The interested reader is encouraged to study

these examples to learn and practice the development of HACCP plans.

Currently, most national food control agencies and international institutions have adopted regulations, guidelines, codes, and procedures for the development and implementation of HACCP plans by the fish industry. As a consequence of HACCP becoming the food safety regulatory system of choice, policy issues have been shaping its evolution, sometimes more than science. For the future, it is important to ensure that food safety policy frameworks maintain the science basis at the heart of HACCP development to embrace future technological developments and the food safety challenges they will bring along.

HACCP can be used to deal with both safety and quality issues, although some regulatory agencies, such as the FDA, have confined it only to safety aspects. Experts in food microbiology argue that given that many control measures (e.g., hygiene, refrigeration, use of ice, thermal treatment, etc.) actually prevent the growth of microorganisms of concern to both safety and quality, it is advisable to use HACCP to address both aspects. The additional burden is related to further recordkeeping and documentation to address both safety and quality, and consequently the additional time and human power needed to verify and audit these records by the food control authorities. The Codex Code of Practice for Fish and Fishery Products in chap. 5 recommends and addresses both safety CCPs and quality defect action points.

In aquaculture, the application of GAP is effective for preventing and controlling most, if not all, food safety and quality hazards at the farm. That is why many regulatory authorities emphasize that mandatory implementation of GAP is sufficient for operating fish farms to supply safe and quality fish. However, many experts and the Codex stress that integration of GAP into HACCP-based systems at the farm level leads to improved cost effectiveness and real-time prevention and control of hazards. Although most control measures and critical limits are well specified in regulatory GAP, additional requirements such as hazard analysis, identification of corrective actions, monitoring, and HACCP verification allow the aquaculture farm to take ownership of its fish safety and quality program, respond in real time to safety challenges, and develop recordkeeping and traceability trails necessary for government or private audit and certification. In addition to being doable and cost effective, the application of HACCP in aquaculture complements effectively biosecurity measures taken to prevent fish diseases. Currently, in several countries around the world, an increasing number of aquaculture farms are applying HACCP-based concepts to control food safety issues. The challenge for small-scale farmers is being tackled in many countries such as India, Thailand, Vietnam, and Indonesia, by organizing the farmers into clusters or self-help group, which enables farmers/farms in the group/cluster to reach a size suitable for the application of GAP and HACCP with technical support from specialized extension institutions.

Training

Practical training in prerequisites, GHP, GAP, and HACCP is fundamental for operating good safety and quality assurance programs in fisheries and aquaculture. All personnel should be aware of their role and responsibility in protecting fish and seafood from contamination and deterioration. Handlers should have the necessary knowledge and skill to enable them to handle fish hygienically. Those who handle strong cleaning chemicals or other potentially hazardous chemicals should be instructed in safe handling techniques.

Each fish and seafood facility should ensure that individuals have received adequate and appropriate training in the design and proper application of an HACCP system and process control. Training of personnel in the use of HACCP is fundamental to the successful implementation and delivery of the program in fish or seafood production, handling, processing, and distribution. Managers should also arrange for adequate and periodic training of relevant employees in the facility so that they understand the principles involved in GHP, GAP, prerequisites, and HACCP. Periodic assessment of the effectiveness of training and instruction programs should be made, as well as routine supervision and checks to ensure that procedures are being carried out effectively.

Traceability

The fundamental and practical aspects of the role of traceability in food safety is discussed elsewhere in this encyclopedia, other publications and in the food legislation of countries that are major traders in fish and seafood. It is worth highlighting that traceability in the complex fisheries and aquaculture chains must be an essential component of a fish and seafood safety management system to ensure that each stakeholder in the value chain is well informed of the origin of the fish (harvesting area, aquaculture farm); its handling, processing, and distribution chain; and the control measures it has undergone.

Conclusion

The globalization of world fish trade offers many benefits and opportunities to countries, companies, and consumers. At the same time, it presents emerging safety and quality challenges. Major improvements have been achieved in this area, especially with the wide use of GHP, GAP, and HACCP. However, improved scientific tools must be adopted and novel approaches to fish safety must be sought so that regulatory actions can reflect the most current scientific evidence and that responsibility for safety is effectively shared among stakeholders along the fish and seafood value chain.

See also: Public Health Measures: Modern Approach to Food Safety Management: An Overview

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FOOD SAFETY ASSURANCE SYSTEMS

Good Animal Husbandry Practice

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Glossary

Feed Any single or multiple materials, whether processed, semiprocessed or raw, which are intended to be fed directly to food-producing animals.

Food safety Assurance that food will not cause harm to the consumer when it is prepared and/or eaten according to its intended use.

Good animal husbandry practices Good animal husbandry practices (GAHP) are guidelines that need to be adopted at the level of the primary producer to ensure production of food of animal origin that is safe for human consumption and acceptable for trade in the world market.

Hazards A biological, chemical, or physical agents in, or a condition of, an animal or animal product with the potential to cause an adverse health effect.

Incidence The number of new cases or outbreaks of a disease that occurs in a population at risk in a particular geographical area within a defined time interval.

Maximum residual limit for veterinary drugs (MRLVD) The maximum concentration of residue resulting from the use of a veterinary drug (expressed in mg kg^{-1} or μg kg^{-1} on a fresh weight basis) that is recommended by the Codex Alimentarius Commission to be legally permitted or recognized as acceptable in or on a food.

Residue The parent compounds and/or their metabolites in any edible portion of the animal product, and include veterinary drug, pesticides, or heavy metals.

Traceability The ability to trace and follow a food, feed, food-producing animal, or substance intended to be, or expected to be, incorporated into a food or feed, through all stages of production, processing and distribution.

Withdrawal period or withholding time Is the period of time between the last administration of a drug and the collection of edible tissue or products from a treated animal that ensures the contents of residues in food comply with the maximum residue limit for the veterinary drug (MRLVD).

Zoonoses Any disease or infection that is naturally transmitted from animal to man and *vice versa*.

Introduction

Animal food products such as meat, milk, and eggs, which form important component of human diet, are susceptible to contamination by potentially harmful microorganisms or chemical hazards such as residues of drugs, pesticides, and heavy metals along the food chain right from production at farm to the final consumption. This has lead to many food-borne outbreaks leading to great economic losses due to food recall and destruction of large number of livestock in addition to human illness. For instance during the bovine spongiform encephalopathy (BSE) scare in the UK and the highly pathogenic avian influenza (HPAI) outbreak in the Asian subcontinent, mass culling was adopted to contain the infection leading to economic losses running in billions of dollars.

The need today is for food that is not only safe but also perceived to be safe by consumers. The most important place to control any hazardous substance from entering the food chain is at the level of the primary producer. Animals may become a reservoir of potentially harmful chemicals like drug residues, pesticides and microorganisms due to disease contracted in their life or due to feed or water that is ingested at the farm. These harmful substances in turn enter animal food products and pose a threat to human health. A single infected animal can also contaminate the milk and meat of other healthy animals at the time of processing, thus the most important control in animal production can be exercised at the initial farm level. The increase in demand of the consumer for safe and morally acceptable food and stringent norms adopted by the Codex Alimentarius Commission have lead the World Organisation for Animal Health (OIE) and Food and Agriculture Organization of the United Nations (FAO) to frame guidelines that would help the livestock farmers to raise animals that are healthy and fit for trade in the international market. In the wake of globalization of the animal food product industry, pressure is on the primary producers to raise animals on the basis of risk analysis and control of these risks through the adoption of the good animal husbandry practices (GAHP). The present approach is based on control not by means of meat inspection but prevention by risk analysis and control throughout the production cycle, especially at the farm level. GAHP are guidelines that need to be adopted at the level of the primary producer to ensure production of food of animal origin that is safe for human consumption and acceptable for trade in the world market.

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GAHP encompass all the measures adopted at the farm level starting from rearing of healthy animals. The general measures that form a part of GAHP in relation to food safety issues are discussed under following sections in the article.

General GAHP

Animal Health

The starting point for implementation of GAHP at the farm level involves maintaining a healthy herd that is free of not only any clinical or apparent disease but also from all subclinical infections. The two most important hazards associated with animal health are the biological agents like bacteria, viruses, parasites, prions, fungus, etc., and chemicals like drug residues, pesticides, heavy metals, environmental pollutants, etc. The animal producers should protect their animals from these agents.

Prevention of Biological Hazards

The primary check to biological hazards should be exercised at the level of entry or exit of any animal from the farm and also monitoring the re-entry of a previously healthy animal. A newly introduced animal should be checked thoroughly for the presence of infection and its vaccination status. The animal should be tested for diseases prevalent in the previously inhabited area and kept separate for the appropriate quarantine period. The entry of personnel should be restricted and no new person from unknown area or endemic area be allowed to enter the farm premises. Entry of vehicles should also be restricted with a separate area of disembarking. Semen, vaccine, and other biological products should be properly screened so as not to introduce an infection to a farm.

Besides adopting measures for preventing the entry of infections, a proper health management program should be instituted so as to maintain animals in good health condition. Animals should be regularly screened for the early detection of disease. Newborn livestock should be administered colostrum to boost their immune system. Proper vaccination and deworming should be done and a record maintained for each animal. Insect population should be kept in check as they may transmit disease to animals and also lead to production loss due to stress. Sick animals should be isolated from the flock, so as to limit the disease at its initial stage. In case of any disease outbreak vaccination should be performed in adjoining farms or surrounding area to prevent spread of disease. In the event of highly infectious diseases like H5N1 avian influenza, mass culling should be done to prevent spread.

Prevention of Chemical Hazards

An emerging problem of major public health concern is the development of antibiotic resistance in pathogens whereby previously treatable diseases of minor importance are fast becoming untreatable. Development of resistance to antimicrobials due to overuse, misuse and underuse during prophylactic and metaphylactic administration to farm animals intended for dairy or meat purpose is well documented today. Use of antibiotics as growth promoters in feed has been cited as one of the reasons for the emergence of resistant pathogens. The resistant zoonotic pathogens may enter the food chain and cause disease in humans. Another food safety issue related to use of antimicrobials and/or veterinary drugs in food animals is veterinary drug residues, which may remain in animal products intended for human consumption and act as a health hazard by causing a variety of health problems in humans. These residues can cause allergic reactions (e.g., penicillin), direct toxic effect like kidney or liver damage, cancer (e.g., inorganic arsenicals), reproductive disorders, etc. Less well known is that antibiotic residues can delay or stop the normal lactic fermentation of products (e.g., salami, pepperoni) resulting in significant economic loss due to poor quality and the risk of excessive growth of antibiotic resistant pathogens.

Realizing the importance of this the World Health Organization (WHO) formulated principles for the containment of antimicrobial resistance in animals intended for food and recommended the reduction in use of critically important antimicrobials, although stopping the use of antimicrobials as growth promoters. The national authorities should monitor the level of antimicrobials and other drug residues in food of animal origin and their comparison with the acceptable standards must be done periodically. Veterinarians must ensure maximizing the efficacy of treatment, whereas minimizing the misuse and overuse of the drugs. Drugs should be administered only after thorough examination and diagnosis by a veterinarian and should only be made available on prescription. The drugs should be administered as per the dose and route prescribed for a particular species. Proper records should be maintained as to the source of the drugs, the animal(s) in which the drug is administered, etc. An important aspect for food safety is to maintain adequate withdrawal or withholding times before harvesting the animal products (e.g., milk) or permitting the slaughter of animals so as to minimize these residues. Records on the administration of drugs should be maintained for every animal or flock.

Pesticide residues and heavy metal residues in animal products are other chemical hazards compromising food safety, which can be controlled by GAHP. Pesticides and heavy metals usually gain entry in the animals through feed and water. Application of pesticides for external parasite control may also lead to presence of pesticides in animal tissue. Animals should not be fed feed that contains high concentration of heavy metals (fodder grown along road sides) or pesticides nor should the farm be close to an industrial area where there is high level of such metals in air, water and soil. The pastures should not be grazed upon immediately after application of any pesticide. The water supply should also be protected from contamination by industrial and agricultural waste.

Feeding and Watering

Feeding is important with regard to the growth and performance of the animals. In addition, it also has impact on human health and food safety. Animal feed can be contaminated with a variety of biological (bacteria, viruses, prions and molds), chemical (dioxin, polychlorinated biphenyl, mycotoxins, pesticides, and drug residues) and physical (metals and glass pieces) hazards. These hazards are excreted as such or

their metabolites may be retained and when slaughtered they may enter the human food chain.

Some recent events highlighting the importance of safe animal feed that shook the economy of their respective countries include BSE crisis in the UK and the dioxin crisis in Belgium. BSE was first observed in cattle in 1984 in a farm in West Sussex and its origin was traced back to feed containing offal (brain) of sheep having scrapie. This lead to disease in 179 000 cattle in the UK alone with precautionary culling of another 4.4 million and a loss amounting to nearly £5 billion. Another crisis occurred in Belgium in 1999 due to contamination of animal feed with dioxin, a carcinogenic compound. The contamination occurred as the fat used for making animal feed was stored in tanks previously used for storing mineral oil. The tainted feed was not only supplied to nearly 1000 farms in Belgium but also to France, Holland, and Germany. This led to a recall of all animal products like eggs, pork, chicken, and beef, and a ban on all Belgian animal products by the EU and the US.

The Codex Alimentarius Commission has developed a code for good animal feeding practices (CAC/RCP 54-2004) to ensure safety of animal feed through adoption of good manufacturing practices throughout the process of procurement, handling, distribution, and storage of feed. The feed should be of good quality and meet the acceptable standards for the presence of pathogens, toxins, and chemical residues to avoid any detrimental effect on animal and subsequently through the food chain on human health. The Codex maximum residue limits and extraneous maximum residue levels set for feed should be applied. Feed stuffs should be protected from any form of contamination and pests. Animal feed that could be a source of BSE (ruminant protein) should be avoided in the diet of ruminants. Chemicals should be kept far away from feed and water and separate equipments/containers should be used to handle feed and chemicals to avoid contamination of the feed with any undesirable residue. Feed additives and antibiotics should be administered judiciously to prevent development of resistance and to avoid presence of residues in the animal products. Water used for making feed should be of acceptable quality. Proper records should be maintained about the source of the feed and all the feed ingredients to maintain traceability. Presence of any undesirable substance in the feed that could compromise food safety should be reported promptly to the concerned authorities.

When animals are reared on pastures adequate assessment should be made about the parasitic load of the area and, if necessary, animals must be given antiparasitic drugs regularly. The pastures should be left for adequate withdrawal period after application of chemicals to prevent any hazard to the animals. Animals should be discouraged from grazing on pastures exposed to contaminated water containing industrial residues or effluents. Normal pasture rotation should also be followed wherever possible.

Water is as important as animal feed in terms of influencing animal health and ultimately the quality and safety of animal food products. Water acts as a vehicle for many hazards of a bacterial (e.g., *Salmonella*, enteropathogenic *Escherichia coli*, *Campylobacter*), viral and parasitic nature that are of concern to the animals and/or to consumers of animal food products. Therefore, care should be taken to avoid contamination of water from sources like animal excreta, human excreta, or any other farm waste. Drinking water used for the animals should be free of pathogens and hazardous chemical residues that might compromise the animal health. The water supply to the farm should be protected from contamination by pesticides and other chemicals applied to the pastures. Water troughs and dispensers should also be cleaned regularly.

Animal Welfare

Animal welfare is linked directly to animal health and involves an animal who is free of any physical, biological, or mental stress. As per the International Dairy Federation (IDF) *Guide to Good Animal Welfare in Dairy Production*, animal welfare involves following five freedoms, which should also be applicable for animals reared for meat production:

- Freedom from hunger, thirst, and malnutrition: The feeding and watering of the animals is very important and has already been discussed earlier. The farmers should follow the code of good feeding practices to ensure optimum nutrition for the herd with emphasis on the physiological state of animal. Young livestock should be fed colostrum at birth and calves should be kept on liquid diet till the full development of rumen.
- Freedom from pain, injury, or disease: The animals should also be kept in good body condition by regular vaccination and timely treatment. A veterinarian should check the animals regularly for sign of any disease. Disbudding, castration, calving, etc. should be conducted under the supervision of a veterinarian and under hygienic conditions. Restraint and milking equipment should avoid causing any injury to the animals.
- Freedom from discomfort: The animals should be housed appropriately as per the need for space, and overcrowding should be avoided at all costs. Housing should be such as to prevent animal from physical injury due to slipping and to allow proper disposal of animal excreta. Animals should be provided with bedding that is clean and of good quality. The physical environment of the animal should be clean and free of any chemical pollutant that might affect them. During transportation animals should be free from discomfort and prescribed standards with adequate space, food, and water should be followed.
- Freedom from fear and distress: General management practices should avoid any fear and distress especially while handling and rounding up the animals. Special precaution should be taken while handling pregnant livestock. They should be handled gently and left for calving or farrowing in a quiet place.
- Freedom to engage in normal physiological pattern and behavior: The animals should be allowed to move freely as per their normal physiological pattern of behavior and should not be exposed to undue stress by interfering in their normal behavior.

An animal allowed freedom as per the above mentioned points will generally be in a state of good health thereby increasing the quality of the animal product. The backbone of

animal welfare is good stockmanship. A good stockman should be able to identify any change in the normal behavior of the animals, any sign of disease or distress. He should be well trained and experienced in the good farming practices.

Building and Housing

Before setting up a farm, importance should also be given to the site, which should be far away from any industry or a previous dumping ground. The aim should be to prevent exposure of the animals to any potentially hazardous substance due to the location of the farm. It is also important that the buildings used for the storing of chemicals should be made at a distance from the animal shed, the milking room and the feed storage building to prevent chemical hazards from entering the food chain. The farm should be properly fenced in order to discourage stray or wild animals and unauthorized people from entering into the farm premises. The farm should be kept clean so as to prevent establishment of breeding ground for rodents and insects.

The shelter and housing facilities are meant for the comfort of the animals and to protect them from the inclement weather. Animal sheds should ensure free movement and total comfort with adequate space for every animal. Overcrowding should be avoided and buildings constructed according to the space requirement of each particular species or stage of animal development and prevalent legislation in that area. The building should allow efficient cleaning, disinfection and disposal of excreta. Floors should be nonslippery and the design and material of the building should not cause any injury to the animals. There should be proper ventilation and drainage to prevent the internal environment from becoming potentially harmful. In tropical countries during warm and humid months the temperature is above 40 °C, so care should be taken while constructing the animal houses to prevent heat stress to the animals. The shed should be kept clean, dry and free of animal excreta and pests. The milking parlor should be kept free of animal excreta at all times. There should be separate farrowing pens or calving sheds to provide calm, quiet and isolated place for the pregnant animals. Similarly a quarantine shed and sick animal shed should also be present in the farm preferably at some distance from the sheds for healthy livestock.

General farm Management and Environment

- Identification of animals: All the animals reared in a farm should be identified primarily for the purpose of ownership and secondarily for the purpose of traceability of an animal or its product. The commonly used methods today include branding (hot/cold), tattooing, tagging using visual code, bar code or transponders, and micro-transponders as implants or bolus. The main purpose of these methods should be to identify the animals from a distance of 1–2 m in large animals and 0.5–1 m in case of small animals. The identification marks should be easy to apply, have good visibility, and cause minimal pain to the animals, although being durable and tamper resistant. They should not contaminate the meat of the animals under any condition. The best method for developing countries where farmers have limited resources is tagging using the visual code. The animals should be tagged at an early age and records be maintained.
- Disposal of waste: The farm waste should be disposed in such a way so as not to cause environmental pollution or act as breeding ground for flies. The animal shed should be regularly cleaned and the excreta removed at the earliest to discourage flies from disturbing the animals. Farm effluents should not be allowed to run into the pastures as they may be a source of pathogens and parasite eggs. If possible the effluents should be treated before disposal on land or water. The dead animals should also be disposed at the earliest at a proper site. The dead animals should either be burned or buried, taking all precautions to prevent digging out of the carcass by dogs. Expired medicines should similarly be disposed efficiently to prevent them from finding a way into the animals' body.
- Cleaning and disinfection of the premises: Following the disposal of waste the disinfection of the farm is important especially in places where an 'all-in' and 'all-out' policy is adopted (e.g., poultry flocks). The animal sheds and houses should be regularly disinfected to prevent outbreak of diseases. Livestock sheds should also be treated with acaricides to control the tick population. The farm equipment like milking machine should be regularly cleaned and disinfected as they may harbor harmful pathogens and may act as continuous source of contamination by the pathogen. In 1999 during Nipah virus outbreak in pig farms in Malaysia, the farm equipment used to carry dead animals acted as a source of infection for other healthy animals. This highlights the importance of regular cleaning and disinfection on the farm premises for safety of food animals.
- Restrict entry to farm: The entry of stray animals and human should be restricted by means of fencing. This also helps in preventing the contact of livestock with wild animals thereby preventing the transmission of diseases from one to another especially those of public health significance. There should be a good pest management plan to control the rodent and insect population at a farm.

Records and Traceability

Traceability is the ability of an animal or an animal product to be linked back to the primary producer. Maintaining of records is an integral part of the good farm practices. Regularly maintained records of every animal or flock from placement on the farm until delivery for slaughter or sale is important in the world market especially to trace back the origin of the animal product in case of any emergencies. This helps in the timely containment of any outbreak due to accurate knowledge about all the hazards encountered by the animals at the farm. Also, the commitment of the farmer to production of safe food animals can be ascertained. Dated records should be kept of every treatment given and drug administered so as to allow proper withdrawal period before utilization of animal products or permitting the animal for slaughter. Records should also be kept of people entering or working at the farm, purchase or sale of any animal, feed procured, pest control adopted and any chemical treatment of shed, pasture, farm premises. In case

of BSE, the tracing back of the feed helped in investigation of outbreak, where the disease was attributed to animal proteins fed to cattle derived from brain of scrapie infected sheep.

Milking Hygiene

Milk is very susceptible to contamination by various pathogenic and spoilage microbes. These harmful organisms may gain entry both when the milk is in the udder and during milking. The following aspects should be considered to ensure milking hygiene at farm level:

- Animal hygiene: The health of the animal is of utmost importance while determining the quality of the milk produced. Animals that are sick especially those having brucellosis, tuberculosis, listeriosis or mastitis should not be used for milking as they may shed the organisms in their milk. Their milk should instead be discarded and they be milked at the last to avoid transfer of pathogenic organisms to the udder of healthy animals. Also important is the keeping clean the coat and udder of the animals as dust and environmental contaminants may enter milk during the milking process.
- Environmental hygiene: A clean milking parlor is equally important in milk hygiene and should be thoroughly cleaned before milking. The milking equipments should also be clean and not cause injury to the animals. In case of manual milking such practices that may injure the udder should be avoided. The milker should use basic hygienic practices and should not be suffering from any infectious disease especially with lesions in the palm region. The utensils used to collect and store the milk should be properly cleaned. Plenty of clean water should be made available on the farm.
- Storage of milk: Raw milk contains some amount of microorganisms, which multiply many-fold under the ambient temperature particularly in tropical countries. It is therefore necessary to cool the raw milk to a temperature below 5 °C immediately after collection. Also the milk storage containers and room should be clean and free of any taint or smell.

Preparation of Animals for Slaughter

Any animal that is intended for slaughter should be in the best of health. All sick or stressed animals should not be sent to the slaughter house till their recovery. Also important in such animals is maintaining the appropriate withdrawal period after treatment so to allow the clearance of drugs from the animal body and making it free of drug residues. A considerable amount of pathogenic bacteria such as *Salmonella*, enteropathogenic *E. coli* and *Camplyobacter* are housed by the animals and birds in their digestive tracts and body surfaces. Under conditions of stress there is increased excretion of these organisms in the feces, thereby increasing the chances of contamination of meat during processing. It is therefore imperative that the animals be free of disease, stress and have a clean body coat. The animals should be fed with the feed, which is high in dry matter content 48 h before slaughter. The feed should be withdrawn 24 h before slaughter, while maintaining an *ad lib* water supply. The animals kept under extensive farming conditions should be brought back to their farms well in advance in order to prevent stress just before slaughter. The animals should also carry proper identification mark before leaving the farm.

Future Changes in GAHP through the Use of Risk Analysis and Risk Management

A great deal of research is being conducted in developed countries to control specific pathogens at the farm level. The data are analyzed to identify and determine the relative value of various control measures. Risk management strategies are then identified and implemented at the national or regional level for pathogen control. Some of the notable examples worth mentioning include the control of *Salmonella* in poultry and pigs in Denmark. The main highlights of control in poultry were extensive testing of the flock and destruction of infected breeder stock, thorough cleaning and disinfection of the poultry house with a rest period of 10–14 days between flocks, although abstaining from use of culture exclusion, antibiotics and vaccines.

Similarly, in the Danish pig population, preharvest control with new feeding strategies (coarseness of feed and wet feeding), improved management with an all-in and all-out strategy, continuous monitoring of the herd and hygienic practices have greatly reduced *Salmonella*.

The proportion of layer flocks infected with *Salmonella*, especially *Salmonella* Enteritidis, was reduced from $>7\%$ in 1998 to $<2\%$ in 2001 through this program. Since then, significant progress has been made throughout much of the EU with the use of an effective vaccine.

In the case of *Escherichia coli* O157:H7 in beef cattle, various practices, like use of probiotics and bacteriophage vaccines – that have been approved by the US Food and Drug Administration (FDA) – have contributed to the decrease of shedding, whereas management practices, such as clean bedding, pest control, and sanitation have reduced the spread, but not the fecal excretion of the organism. Despite improvements in preventive measures such as GAHP and the application of combined good hygienic practice and hazard analysis and critical control points (HACCP) during slaughter, testing of trimmings and ground beef continues to be used in certain countries as an additional measure to prevent ground meat contaminated with *E. coli* O157:H7 reaching the market.

Control of listeriosis in France is another example of modifying traditional GAHP for on farm control and reduce the presence of *L. monocytogenes* in soft cheese made from raw milk. Collectively, these examples demonstrate that specific microbial pathogens of concern to humans can be minimized by making specific adjustments to the traditional application of GAHP. The continuing research efforts will definitely lead to future case specific/target specific modifications in the GAHPs as mentioned in this article.

Conclusion

With the increase in global trade and advent of the Agreement on Technical Barriers to Trade (TBT) and Agreement on the Application of Sanitary and Phytosanitary (SPS) Measures

food safety has become an important issue and various world organizations such as FAO, OIE, WHO, Codex, International Commission on Microbiological Specification for Food (ICMSF) and IDF are coming up with various guidelines and standards for food safety management. The above-mentioned GAHPs are a set of simple everyday practices that can be implemented at farm level by the primary producer to ensure that the animal products that he produces are safe for human consumption. In the case of developing countries where animal rearing is not a well organized enterprise and where animal farmers usually have limited resources, co-operatives or private meat companies can help farmers to raise livestock and poultry to produce animal food products though practices such as contract farming and backward integration. Under these schemes quality stock, feed and veterinary services are made available by the companies, thus ensuring quality animal products.

See also: Environmental Contaminants: Dioxins, Furans and Dioxin-like Polychlorinated Biphenyls. Foodborne Diseases: Overview of Biological Hazards and Foodborne Diseases. Institutions Involved in Food Safety: FAO/WHO Codex Alimentarius Commission (CAC); Food and Agriculture Organization of the United Nations (FAO); World Health Organization (WHO). Prions and Agents of TSEs: Bovine Spongiform Encephalopathy in Cattle. Public Health Measures: Modern Approach to Food Safety Management: An Overview. Safety of Food and Beverages: Meat and Meat Products; Milk and Dairy Products; Poultry and Eggs

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FOOD SAFETY ASSURANCE SYSTEMS

Building Design

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Glossary

HEPA filter High-efficiency particulate air filter, an air filter that removes at least 99.97% of all particles larger than 0.3 μm from the air that passes through the filter.

Listeriosis A disease caused by ingestion of food contaminated with the bacterium *Listeria monocytogenes*, which can be harmful and sometimes fatal for babies, elderly, and immunocompromised people.

Mycotoxins Substances produced by species of molds that can be toxic for humans at a very low concentrations, μg/kg (micrograms per kg).

Rodent resistant Designed to withstand ingress of rodents (relates to food factories in particular).

Introduction

The integrity of a building influences the access of pests (rodents and other small crawling animals, birds, and insects), microorganisms, dust, and polluted air to the products that are produced. The chances of such contamination depends on the environment of the factory and, therefore, it is important to pay attention to the site. The higher the concentration of any type of contamination in the environment, the more difficult it will be to ensure that the production area will be suitable for the production of safe food products and consequently, the more expensive it will be to meet the food safety requirements.

Reconstruction and maintenance works often are done while production is continued in other areas of the same building. At such times, the safety of the food processing operation may be severely challenged. Adequate measures to prevent loss of integrity of the operation must be taken before such works start or otherwise, the food operations must be interrupted until these works are finished and inspection shows that the plant is clean and ready for resuming production.

Regulatory Requirements

To protect the consumer, many countries currently have strict requirements with respect to food safety. However, the enforcements may be insufficient because in many countries legislation is weak or there is insufficient inspection capacity to ensure that regulatory requirements are met. Traditionally, this has to do with governmental budgets and priorities. After well-publicized food scares, the budget will temporarily be higher. Although the food safety requirements may be fairly, but certainly not completely, similar among countries, it may be obvious that there are differences in requirements with respect to the environment and buildings. This is because the burden of hazards varies between regions. Factors, such as local climate (particularly temperature and humidity), domestic pests, husbandry (use of manure), degree of air and soil pollution, and geological conditions may lead to differences in the concentration of undesirable chemicals and microorganisms in the environment of the factory. Some countries are prone to earthquakes, others to flooding, and some to both. Consequently, risks of food safety incidents may also differ. Although in many countries food safety regulations are in place, the situation is rather different for environmental regulations. In some countries, such regulations are nonexistent. The consequence is that the environment of a factory may unexpectedly change and cause tremendous problems. Authorities may decide that a certain location is the best one to dump municipal wastes because if it is nearer the residential area, it will affect the opinion of the electorate. Nevertheless, in many countries, the law requires that the premises (buildings) for food handling and processing are hygienic. These laws, however, do not specify how this must be done and hold the company fully responsible for ensuring that the premises are hygienic. An organization that does provide guidance on meeting the hygiene requirement is the European Hygienic Engineering and Design Group (EHEDG), see www.ehedg.org.

Retailer's Requirements

Retailers are the first line in dealing with the complaints of consumers; they need to care about their reputation, in particular when they have their own labels. Therefore, retailers have good reasons to have their own requirements with respect to the hygienic condition of the factories from where they obtain their products. Even in countries where food safety regulations are adequate, retailers increasingly inspect and certify their suppliers for the simple reasons that the regulators usually either fail to do so or are not effective.

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Site Selection

The site influences the design of the building in order to cope with the local conditions that may influence food safety. Examples are the quality of water and air, and the presence of local pests (insects, birds, farms, water treatment plants, etc.).

If the site is in an area with a more than an average concentration of airborne microorganisms, insects, and birds, there is also an above average chance of contamination of unprotected raw materials (such as often the case with fresh produce and meat) during off-loading. Sites near waste treatment plants and farms, in particular if downwind from them, may have to cope with severe problems because untreated wastewater and manure are likely to contain high concentrations of pathogenic bacteria, including *Vibrio*, *Salmonella*, *Escherichia coli*, *Campylobacter*, and *Yersinia* sp., and *Shigella* in addition to protozoa and viruses. These microbes may become airborne depending on the design of the wastewater treatment system and at times when farmers spread manure over the land to fertilize it. They will challenge the factory's air system. In addition, every time an entrance is open, anything flying may successfully attempt to get into the factory. The building must then be provided with loading and off-loading bays that reduce this risk to an acceptable minimum. For the same reasons, any entrance for people, materials, and air will need additional measures to keep the contamination risks low enough. This is an important aspect in the selection of a suitable site. It is better not to have the factory near a sewage treatment plant but also to make certain that such a plant will not be situated near the factory in the future. The same applies to legal waste disposal facilities and landfills. This requires checking the local zoning plans and obtaining, in writing, that within a certain distance from the factory, such plants will not be built. Nearby chemical industries may produce potentially toxic substances, which may contaminate not only the air but also the soil (with e.g., heavy metals or chlorinated hydrocarbons), which is particularly important if well water is used, as discussed below. One should be aware if the site under consideration has been polluted, for example, as a result of mining activities, chemical industries, or (legal or illegal) waste disposal. The presence of pollution in the soil should best be carefully checked.

Another requirement is the quality of water that is and will be available at the site. There are large areas in the world where safe water is readily available, but there are also large areas where it is not, not constantly or not of a constant quality. If available, but of unacceptable quality, an in-house water treatment facility must be installed and maintained. If the availability of water cannot be guaranteed, a well may have to be drilled. Such measures will add to the costs of the final product.

To operate a factory, energy is required. The energy supply may be unreliable, in particular with respect to electricity. This may severely undermine food safety management and cause conditions prone to incidents because electricity is essential to maintain conditions that prevent the ingress of contaminated air, such as maintaining pressure differences among the various zones in the factory. The same holds for cooling and freezing and for the operation of measuring, controlling, and registration equipment. If the electricity fails, so will the control of processing temperatures and flow rates through pipelines. Hence, if interruptions are likely to take place, adequate back-up systems (e.g., oil-powered electricity generators) must be installed, the capacity depending on how long the interruptions may last. It is important to obtain a guarantee from the local electricity supplier and to be certain also to ask advice from a reliable local consultant.

Site Layout

For the same reasons as discussed Section 'Site Selection', the layout of the site must prevent access of pests into the factory. To keep the animals at bay, there must be fences that are high enough to prevent dogs and cats from entering the area, but at the same time be deep enough to prevent burrowing animals (rats and rabbits) to gain access. The fences must be such that they do not allow animals (including monkeys) to climb over it. Any unpaved surface must be covered with grass that is kept short to avoid breeding of small animals. For similar reasons, there should be no shrubs or trees or they should be far away from the factory wall and particularly its entrances and air inlets. Because they provide places for microbes and insects to breed, there should be no ponds or any other possibilities for stagnant water or mud. For these reasons, pavement should be horizontal or slightly sloping toward drain pits.

External lighting should always be away from the factory walls and entries, luring the insects away from the building instead of attracting them to it.

It is all about preventing insects, small animals, and microbes from multiplying and worsening the environment of the factory: Waste disposal areas too must be such that they do not allow ingress of insects and animals. Moreover, to limit undue growth of molds and bacteria, dry solid waste should be kept dry. It means that, although the disposal area must be outside the factory, it should nevertheless be covered to cope with precipitation. Doors to the area must be rodent resistant and be tight against insects. The doors should preferably be self-closing, otherwise an alarm should go off when the door has been open for a longer time (minutes, rather than hours).

Access to Production Areas

The entrance of the production area must be equipped with hand-washing facilities, such that everybody entering the area must pass these facilities. Restrooms (toilets, washrooms, lavatories) must not be directly connected to the production area and must be easy to clean. Depending on the type of products handled in a certain area, it may be necessary to minimize the risk of transfer of contamination from the outside into that area by using a change room, where garments can be exchanged for special production room garments. It should have a step-over barrier to leave shoes and boots that are worn outside, on one side, and on the other side to put on footwear, to be used exclusively in the production area. The step-over must be sealed to the floor to prevent contamination on the floor from moving to the production area. There must be means for cleaning, disinfecting, and drying of hands.

Processing and packaging areas should not be used as a passage to canteens or other amenities. Therefore, the layout of the building should take into account that the cafeteria, kitchens, offices, laboratories, workshops, chemical stores, etc. are not connected to the production and packaging areas. Anybody, including laboratory staff, directors, and important visitors, if they need to be in the production area, should pass the change room or hand-washing facilities, as applicable, and use them as per the personal hygiene instructions. Similarly, any vehicle that is needed to transport raw materials, ingredients, or finished products should have designated routes that should not be used for anything else.

Building Design

Supporting Structure, Foundation, External Walls, and Roofs

The supporting structure of the factory should ensure that the floor is at an elevated level, so that in case of rain or other precipitation, there is no risk of water or mud entering the factory. Preferably, in areas with potentially heavy rainfall, the level of the supporting structure should be higher than any area in the surrounding to avoid the factory being flooded with the heavy rainfall.

Apart from their function to protect the factory from bad weather conditions and sometimes sun, the external walls, roofs, and the foundation are the first and most important barriers to ingress of pests, in particular rodents, geckos, birds, and insects. Hence, the design must be such that there are no openings that allow animals and insects to enter the building. In addition, it must be ensured that the structure is such that rodents, and termites in some areas in the world, cannot create access into the factory. Any area between the ceiling and the roof must be entirely closed off to prevent these areas from being used for nesting by birds, mice, or rats. Bird droppings contain high concentrations of microorganisms, including pathogens such as *Salmonella*. Special attention should be paid to effectively seal the connections between the roof and walls. Similarly, the connection between the foundation and the wall must be rodent proof.

To avoid attraction of vermin to the roof, the roof should be kept dry and, therefore, slope. External walls and roofs should be easy to clean and maintain. The outer walls should not have ridges or other protrusions that allow birds to settle and breed. Where they are unavoidable, adequate measures should be introduced to discourage birds from settling.

Entry and Exit Points

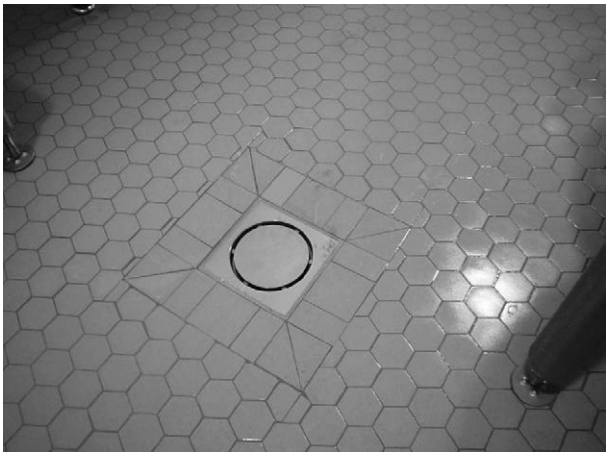
Entry points should be designed such that they allow passage of personnel and goods but prevent the entry of pests as effectively as possible. This requires automatically closing doors that are rodent resistant and in some locations also termite resistant. The doors should close such that even small insects, such as ants, cannot gain access.

It is important to build the factory in such a way that the openings are downwind, at least as much as possible, so that wind is not blowing any undesirable matter into the factory. Doors and windows that normally are not used are places where insects may breed in the narrow space between the frame and the door/window. Hence, care must be taken that such crevices are very small or, if indeed only to be used in emergencies, be sealed with a good quality of tape that is resistant to cleaning chemicals and conditions, but do not hinder opening of that exit in case of emergencies.

For security reasons, visitors should always report to the main entrance and not gain unauthorized access at any other entrance. Therefore, just after the main entrance, there should be an area where visitors can be met and kept under control until cleared and collected by a company staff member.

Internal Walls, Floors, and Ceilings

Walls must be nonabsorbent and well cleanable, and therefore not have recesses or cracks that can harbor insects. Where the wall can be hit, it must be able to withstand damage that may result in product contamination. For the same reason, any paint used should be of a quality that does not flake off. The use of strong, slightly elastic wall coatings is recommended. Corners may have to be protected by metal reinforcements. The same applies to the lower part of the walls, where bumper constructions should prevent damage when fork trucks may incidentally hit the wall.



The floor often plays an important role in product contamination incidents. Improperly designed floors may accumulate moisture and nutrients for insects and bacteria. Moving (of e.g., people or vehicles) over such floors produces aerosols that carry microorganisms. Insects full of microbes may crawl out and enter the product, visibly or not. Hence, floors unless in areas that will never be wet must be watertight and slope toward drains, so that any spilt liquid can be easily removed. The floor must also withstand cleaning chemicals and the temperature of hot water that may be needed for cleaning. Floors must be able to withstand damage by moving around of personnel and by moving of equipment. Since the 1970s, composite floor materials (epoxy, meta-acrylate, polyester, and polyurethane) have become popular, in particular, in new or refurbished factories, but often with disappointing results. The materials are not as strong mechanically, as good quality tiles. Forklift trucks, containers with raw materials or

intermediate products and waste containers, can easily damage such floors. The floors must also withstand the installation of machinery. If machines are installed on an intact epoxy floor, the integrity of that floor may be affected and moisture may penetrate to the supporting structure. The microorganisms and insects developing under these floors cannot be removed without removing the affected area of the floor. If the floor is to be subjected to heavy loads and to the movement of forklift trucks or similar equipment, tiles are to be recommended. Care must be taken, however, that the tiles are grouted such that no moisture is absorbed, creating undesirable circumstances. Hence, the grouting must be resilient and water repellent. It is also easier to repair a damaged tiled floor than a damaged composite floor. Although floors should be easy to clean and hence be smooth, they should not be so smooth that they will be too slippery to walk on. Despite the drawbacks, there may be applications where composite floors provide the best solution, taking into account the prevailing operation conditions.

To allow effective cleaning, the transition between floor and walls must be rounded. For tiled floors, special tiles are available.

To avoid dust falling down on the exposed product or product contact surfaces, ceilings must be tight and, hence, false ceilings should not be used in food processing plants. Suspended service ceilings, however, are fully acceptable and provide the advantage of mounting cables, service ducts, etc. above the process area from where they can be extended downward, vertically, to where they are needed. This way, the risk of contamination of the product by dust that accumulates on these provisions is drastically reduced. The construction of these service floors must self-evidently be such that it does not allow any dust to pass from the area above the ceiling to the production area, which means that the passages of the ducts, etc., must be effectively sealed.

Lighting

There must be enough light in the factory firstly for the personnel to do their job properly and efficiently and secondly to be able to notice dust, dirt, and detect vermin or their traces. To avoid the risk of contamination of the product with glass, generally glass windows are avoided, but modern types of glass are very strong and are available in shatterproof quality. If well mounted, they will not easily break and if they do, they do not splinter into small pieces, even if accidentally struck with a fairly great force.

Lamps for artificial illumination must be covered by shatterproof covers that are tight, so that an exploding lamp cannot contaminate the product. To avoid accumulation of insects and dust, lighting should preferably be mounted in the suspended service ceiling, with the underside flush with that ceiling. Besides preventing the collection of dust and insects, it also enables the replacement of the lamps from the top, without interruption of the production. In the absence of a suspended ceiling or if that ceiling is too high above the surfaces to be illuminated, the top of the housing of the lamps must slope down at an angle of approximately 45° ($\pi/4$), to prevent anything from settling.

Temperature Control

For the comfort of the personnel in the factory, it will often be needed to condition the air, at least to some extent. Where food must be processed under chilled conditions, cooling units are used. These units have trays underneath to prevent condensate from dripping onto people and the product. What is not realized but is very important from a food safety point of view is that these trays are perfect places for the selective cultivation of psychotropic bacteria, specifically *Listeria monocytogenes*, a pathogen that may cause listeriosis. It is a fairly selective process because at low temperatures, *L. monocytogenes* grows faster than most other microorganisms. The fans of the cooling unit complete the food safety risk because the circulation of air helps to spread the contaminated condensate over the product. Therefore, collectors of condensate should always slope to one side, from where it is led to a hygienic drain pit. Moreover, they need to be easily accessible for regular inspection, cleaning, and disinfection.

To make the control of the temperature efficient and affordable, as well as to prevent condensation on walls, ceilings, and windows, thermal insulation of walls and roof (and/or service ceiling), and double-glass windows are needed. Care must be taken that the installation of insulation panels does not create a space for the breeding of insects between the wall and the panel. The panels should be smooth for ease of cleaning and their surface should be strong enough to prevent damage under the applicable conditions.

Noise Control

Machinery may produce more noise than is desirable or legally acceptable. Noise absorbing panels may be used and have been used on a large scale. Nevertheless, because they must be cleanable and hence their surface must be smooth, the hygienically acceptable panels are not very effective. Increasingly, food processing machinery is designed to produce significantly less noise than a few decades ago. If however the noise levels are still high (which still is not unusual), the best solution may be encasing the noisy parts using panels with the sound absorbing surface on the inside and having a smooth outer surface. There may also be situations where it is more attractive and efficient to use noise-canceling earphones for personnel working near noisy machinery.

Sewers, Gutters, and Drains

The sewer can be a serious way of contamination of the interior of the building. The design may be such that even large animals, such as rats, gain access, unless appropriate measures are taken to prevent this. The design must also be such that there cannot be pressure differences large enough to cause gases to enter the interior of the factory in any way. It is highly recommendable to keep the sewer system physically separated from other wastewater systems, which are connected to floor drains throughout the factory. Gutters tend to be covered by perforated covers and cleaning the gutters and covers is usually troublesome. Moreover, water, often contaminated with spilled products, tends to be stagnant in most parts of the gutters, allowing microbial growth and nesting of cockroaches and other insects. Gutters

therefore should be avoided and hygienic floor drains should be used instead, while the floors should slope toward these drains. The drains, however, can also become breeding places for insects and bacteria. Therefore, they should be of a design that can be disinfected. In high-care areas they should preferably be of a kind that can hold disinfecting substances.

Internal Zoning, Ventilation, and Air Conditioning

An adequate air supply is needed to ensure a sufficient supply of oxygen and to control the temperature in the production environment. Depending on the temperature required and the amount of heat produced by the machinery or the processing of the product, the amount of air needed may vary greatly between factories and hence also the dimensions and design of air ducts and exhausts. To make the risk of airborne contamination of exposed product or food contact surfaces as low as possible, the air should flow from the exposed final product area, through areas where such contamination is less important, to the area where materials arrive. That requirement influences the differences in pressure needed between the various zones in the building. The air supplied to the cleanest areas should self-evidently be adequately filtered, to the degree needed to meet the product safety requirements. High-efficiency particulate air (HEPA) filters will be required where microbiologically vulnerable products are exposed to the air. Between prefilters, intended to remove course particles and insects, and the fine (HEPA) filters, intended to remove microorganisms, dehumidifiers must be installed to ensure that the fine filters remain dry and to prevent condensation in the production area. The combination of differences in temperature and humidity may result in condensation and hence wet spots in the process area, resulting in the growth of bacteria and molds, which may become airborne and contaminate the product. Hence, care must be taken that either such condensation cannot take place or takes place under control, at easily accessible locations, enabling inspection, cleaning, and disinfection. Air inlets should be positioned at a distance from the air outlets of the factory to prevent contaminated air to unnecessarily burden the air inlet filters and dehumidifiers. Both inlets and outlets must be provided with screens to prevent entry of flying animals and insects.

If at the factory materials that contain allergens are processed, special attention should be paid to airflows to avoid that air from the area where allergenic products are processed can pass to areas where products are processed or packed that must be or are supposed to be free from these allergens.

Zoning of food production premises is important to prevent (re)contamination of exposed food and also proportionate use of protective measures and verifications (e.g., environmental monitoring). Details on zoning can be found in Todd *et al.* (2010). It may be difficult to realize appropriate zoning in very small premises. Nevertheless, if vulnerable products are made in such premises, the zoning rules should be met or such products should not be produced.

Walkways and Stairways

Footwear, including the special footwear that has been put on before entering the production hall of the factory, collects dust and dirt and hence may shed off dirt again when the wearer moves around. It would be safest if the feet of any person who needs to be in the factory during production would stay below the level of any exposed food product and any food contact surface. There are circumstances, however, that necessitates staff to cross over such areas and hence stairways and bridges are needed. It may be required that during production, parts of machinery that are positioned high on a tall machine, must be adjusted or replaced. In such a case, an elevated walkway is needed. Stairs, bridges, and walkways must be designed and constructed such that no dirt from footwear can contaminate the food and food contact surfaces. Consequently, open structures are not acceptable and there must be sides that are high enough to prevent any dust or dirt from falling down.

Process Support and Utility Systems

To operate the food processing and packaging equipment, products, ingredients, cooling or heating media (steam, water), air, electricity, signal cables, packing material, all have to be brought to where they are needed. This requires pipes, cables, conveyor systems, and support structures. Together this can become a nightmare from a hygiene point of view. If put together, which is unavoidable, because they all need to reach the same machine, they form ideal places for insects and other pests to hide and breed. They collect dust and dirt and are a source of dead insects that may fall down to contaminate product underneath. Moreover, this will be very difficult if not impossible to clean.

Cables (electric power supply and signal transfer) and small pipes (compressed air, nitrogen, and lubricants) should best be grouped together in ducts, which can be larger pipes or of special designs. To prevent ingress of insects, these ducts must be effectively sealed at any entry or exit point of a cable or pipe, at least at the processing side. When building a new factory, probably the best way would be to have a service area below the production floor, provided that the space will be high enough for the access of service personnel. From that area, service ducts may go up to the machine and the cables and pipes stay largely below the exposed product level. If a service floor is not possible, a suspended service ceiling can be a good solution. The passage of the ducts through the floor or ceiling must be such that nothing else, even air, can pass through.

Pipes for transport of product or product ingredients may have to pass through walls or ceilings between various processing departments. Care must be taken that such passages are either tight, not allowing anything passing around them, or large enough to allow cleaning and inspection of the passage. If tight, the construction must be such that they remain tight with time and hence the passage can absorb vibrations caused by machinery to which the pipes are connected and the changes in length and diameter of the pipes as a result of thermal expansion.

Where product or packaging material must pass through walls or ceilings using conveyors belts, chains, or slides, care must be taken that the passage itself is cleanable and accessible for cleaning and inspection.

Food Storage Rooms

Food storage must be designed to make certain that insects and other pests cannot reach the food, even if the food is packed. It must be possible to control the humidity to ensure that the area is always dry. Temperature control and monitoring is essential for storage of perishable products. Entrance of insects and small animals can be prevented by building the storage room on an elevated level, but such that the entrance is also higher than the outside pavement. Furthermore, the storage room should meet the general requirements that also hold for the processing area to ensure that the space can be cleaned: smooth walls and ceilings, no ridges, no surface cracks, and other crevices where insects may hide. Walls must be watertight to avoid wet surfaces at the inside. It is important that the lighting is sufficient for inspection to notice any traces of vermin. There must be enough space between the wall and the stored products for inspection.

Storage of Grain

Large qualities of grains (rice, wheat, corn, etc.) usually are stored in silos. Self-evidently, the silos must be sealed to avoid the entrance of vermin. Chances are, however, that there are insects already in the grain. Measures should be effective in ensuring that there will not be any larger animals in the silos. The insects should be prevented from multiplying by keeping the product dry. Insects, like other animals and people, need water to survive and the absence of water may perhaps not kill all insects, but at least the survivors would be dormant. Another, equally important reason to make certain that the grain remains dry is to prevent molds from growing. Molds may produce metabolites that are toxic (mycotoxins) for humans in very low concentrations. For instance, *Aspergillus* species produce a variety of aflatoxins. The European Union regulations require that the concentration in grains for human consumption of all aflatoxins together is below 4 $\mu g\,kg^{-1}$. Ochratoxins are produced by some *Aspergillus* as well as *Penicillium* species. The maximum concentration in grain for human consumption of Ochratoxin A, the most important one, is 3 $\mu g\,kg^{-1}$ (these are parts per billion, $1{:}10^9$!). Mycotoxins are also harmful to animals (e.g., horses) and hence the above also applies to feed.

The problem with large silos is that it is difficult to ensure that the temperature is the same everywhere in the product. Temperature differences, however, will cause transport of moisture to the colder spots, which subsequently may become moldy and thereby toxic. It is therefore recommended to use thermally insulated silos.

Storage of Oils

The solubility of water in oil is strongly temperature dependent. At 20 °C, the solubility in sunflower oil is approximately 75 mg kg^{-1}, and at 40° C it is approximately 50% higher. The consequence is that in oil that contains more water than is soluble at the lowest temperature in the storage tanks, water will come free. In addition, if the tank is not well insulated, water may condense at the inner wall of the tank. Because of its higher density, all water will sink to the bottom of the tank and where there is water and nutrients, microbes will grow and this way the oil becomes contaminated with microorganisms that in turn may produce potentially toxic substances. The message is that it is important to control the temperature of the room for storage of oils and to prevent oil from cooling down. In other words, letting the temperature drop in wintertime to save energy is not a good idea from a microbiological safety point of view.

Storage of Chilled Food

Self-evidently, chilled food storage rooms need adequate temperature control. It is important to take into account that lighting and ventilators produce heat and that as a consequence, despite the temperature control, there are temperature differences in the cold room. Similar to the chilled rooms for food processing, condensate trays underneath cooling units should slope toward a drain, from where it is led to a hygienic drain pit. The trays need regular inspection, cleaning, and disinfection.

Storage of Packing Material

Some packing materials, in particular carton and paper and the increasingly popular biodegradable materials, are substrates for microorganisms and should therefore be kept dry to prevent microbial growth. Other materials, such as glass, metals, and nonbiodegradable polymers, can be stored in areas without temperature and humidity control, unless the humidity at the location is extreme. Switching from nonbiodegradable to biodegradable materials will probably need measures to prevent microbiological problems.

Storage of Chemicals and Lubricants

The design and location of the store, housing chemicals for cleaning and sanitation, must be such that any risk of contamination of the product and packing material with chemicals is avoided. The store must be provided with a lock and there should be no direct connection between product areas and the chemicals store. The same holds in principle for lubricants, glue, and inks needed in the process and packaging areas, unless the lubricants, glues, and ink comply with the requirements for food contact material or they are used only in areas where there the product is packed in well-sealed containers.

Storage of Refuse and Waste Materials

There must be adequate space for storage of accumulating waste materials, such as used cartons and boxes, enabling 'good housekeeping' in the entire factory. Without such spaces, pests (including rodents and cockroaches) will find places to harbor and breed.

See also: Food Safety Assurance Systems: Cleaning and Disinfection; Hygienic Design of Equipment; Infestation Management in Food Production Premises; Management of Allergens in Food Industry. Food Technologies: Aseptic Packaging; Cleaning and Disinfection Technologies (Clean-In-Place, Clean-Out-of-Place); Packaging

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FOOD SAFETY ASSURANCE SYSTEMS

Hygienic Design of Equipment

AN Murray, Hermanus, South Africa

Glossary

Nonproduct contact surface All exposed machinery surfaces other than the product contact surfaces including, where applicable, the splash area.

Product contact surface Machinery surfaces that are exposed to the product and from which the product or other materials can drain, drip, diffuse, or be drawn (self-returned) into the product container.

Splash area Areas composed of surfaces where product may come into contact and not return to the product container.

Introduction

The hygienic design of food processing areas is necessary in order to ensure the safe removal of microorganisms and contaminants during routine sanitation. The hygienic design of equipment is most important because all processed foods make contact with equipment during their manufacture. Poor design can also lead to increased cost, both indirectly and directly as a result of possible food safety failure. The risk of contamination increases where good hygienic design principles are not applied. In addition, poor design results in additional cleaning and sanitation inputs, and thus contributes to environmental risk and unsustainability.

Hygienic design must take account of both the materials of construction and physical design of the product contact surfaces, nonproduct contact surfaces, and splash areas. Those surfaces that do not come into contact with the product have different design requirements compared with those that do make contact.

Strategy for Design

A hazard analysis should be undertaken as a part of the design of food process equipment. This hazard analysis needs to consider the following:

The intended use of the machine,
The product type,
Further processing that will take place,
The application of the product (particularly making a note of the expected consumers of the product),
The cleaning program that will be applied, and
The maintenance program that will be applied.

Once risks have been identified, the design should take these into account and if there are any risks that cannot be eliminated through design these should be addressed by way of cleaning or operating instructions or by limiting the use of the machine. Risks to safety of personnel and environmental risks will need to be assessed simultaneously with hygienic risks and may influence the hygienic design.

Materials of Construction for Product Contact Surfaces

Materials of construction for product contact surfaces may be divided into four types: metals, elastomers, plastics, and other nonmetallic substances.

Metals

Stainless steel is in most cases the preferred construction material for product contact surfaces in the food industry.

Aluminum is not recommended as a product contact material. It also has limitations as a structural material because it is not resistant to some cleaning chemicals.

Copper is generally not suitable for product contact surfaces but is traditionally used in certain industries, particularly distilling and jam making.

Titanium is a possible alternative to stainless steel in cases where the risk of corrosion is severe. This is generally limited to heat transfer surfaces such as heat exchanger plates where welding is not required.

Stainless Steel

Stainless steels are a family of chromium (Cr) containing alloys with a minimum Cr content of 11–12%. The properties of different steels vary considerably as a result of their composition and their treatment during manufacture. Corrosion resistance and cleanability are not the only criteria for the selection of a stainless steel grade. Steel is available in different forms such as plate, bar, or tube and the availability of the required form may also influence the design.

The alloys used in the food industry are generally divided according to their composition onto the following types: martensitic, ferritic, super ferritic, austenitic, super austenitic, and duplex stainless steels.

There are several identification systems for stainless steel. The American Iron and Steel Institute (AISI) grades are also

Table 1 Composition of some stainless steels

AISI	*DIN*	*Typical analysis*				
		%Cr	*%Ni*	*%Mo*	*%N*	*%C*
Martensitic						
410	1.4006	11.5–13.5	0.75			0.08–0.15
Ferritic						
430	1.4016	16.0–18.0				0.08
Super ferritic						
444	1.4521	17.0–20.0		1.8–2.5	0.03	0.025
Austenitic						
304	1.4301	17.0–19.5	8.0–10.5		0.11	0.07
304L	1.4306	18.0–20.0	10.0–12.0		0.11	0.03
316	1.4436	16.5–18.5	10.5–13.0	2.5–3.0	0.11	0.05
316L	1.4435	17.0–19.0	12.5–15	2.5–3.0	0.11	0.03
Duplex						
(SAF) 2205	1.4462	21.0–23.0	4.5–6.5	2.5–3.5	0.1–0.22	0.03

Abbreviations: AISI, American Iron and Steel Institute; DIN, Deutsches Institut für Normung.

used. The composition of the more usual grades used in the food industry are given in Table 1.

Martensitic Stainless Steels

Martensitic stainless steels contain 12–18% Cr and a high carbon content. They have moderate corrosion resistance and poor weldability but are hardenable by heat treatment. For this reason, they find a use in knife blades and springs.

Ferritic Stainless Steels and Super ferritic Stainless Steels

Ferritic stainless steels are high in Cr and low in carbon. They have a moderate to good corrosion resistance and good strength. They cannot be hardened. Their weldability is moderate to poor. The main use for these steels is in domestic hardware, sinks, cutlery, and table tops. Ferritic alloys are used for steel structures, walkways, and stairs.

Super ferritic steels have added molybdenum (Mo) (typically 1–2%). This gives an improvement in the resistance to pitting and stress corrosion cracking (SCC). However, because of their poor weldability, they do not readily replace the austenitic steels for fabricated items.

Austenitic Stainless Steels

Austenitic stainless steels include the 304, 304L, 316, and 316L grades that are commonly used in the food processing industry.

Type 304 has a composition of 18–20% Cr, 8–12% nickel, 0.08% maximum carbon, 2% maximum manganese, and 1% maximum silicon. Grade 316 has a similar composition except that the Cr is reduced to 16–18%, and 2–3% Mo is added. The low carbon grades of each (304L and 316L) have a carbon content reduced to a maximum of 0.03% to avoid sensitization during welding.

Testing for the presence of Mo is a common way of distinguishing between the 304 and 316 grades.

Properties of these steels are as follows:

Excellent corrosion resistance (improved by Mo content),
Excellent weldability particularly the L (low carbon) grades,
Good formability and fabrication properties,
Good strength and hardness, and
Good cryogenic ability.

Their main limitation in the food industry is that these grades are susceptible to halide, particularly chloride ion attack resulting in pitting and SCC.

Duplex Stainless Steels

Typical of this type is the proprietary steel SAF2205. These steels have a high Cr content as well as nickel and in most cases Mo. They do not fall entirely into any of the other categories. They are weldable, have a very good strength and are particularly resistant to pitting and SCC in the presence of chloride ions.

They find application in desalination plants and also in food processing applications where chlorides are present in the presence of acids (e.g., sauces).

Corrosion and Corrosion Resistance of Stainless Steels

Stainless steel is not immune to corrosion but is resistant to many forms of corrosion.

The resistance of stainless steel to corrosion is due to the formation of an extremely thin but uniform continuous film of Cr-rich oxide on the surface of the metal. This film needs to be repaired after any mechanical damage to the surface. Normally, the film will form spontaneously in air but critical areas or components may, after manufacture, be passivated with nitric acid under controlled conditions.

Some of the forms of corrosion on stainless steel are general corrosion, galvanic corrosion, erosion and abrasion, intergranular corrosion, pitting, crevice-shielded corrosion, microbiologically induced corrosion, and SCC. Of these,

intergranular, shielded corrosion, pitting, and SCC are the most important in food industry situations.

Intergranular Corrosion

The formation of complex carbides at welding temperatures (450–850 °C) occurs. The use of the low carbon (L) grades of stainless steel for fabricated items is one of the ways of preventing intergranular corrosion. There are tests available for the susceptibility of steels to this form of corrosion.

Pitting

Pitting refers in particular to chloride attack. Under suitable conditions, the chloride ion possesses the ability to attack any localized weak points in the passive film, resulting in the formation of microanodes in a large surrounding cathodic area. The chlorides are attracted to the pit and hydrochloric acid formed in the pit base accelerates the corrosion.

Crevice (Shielded) Corrosion

This is the corrosion that occurs through concentrations of corrosive material collecting under washers, bolt heads, and other similar places. Crevice corrosion can occur both in splash areas and on product contact surfaces.

Stress Corrosion Cracking

The start of SCC is similar to pitting. If the pit is formed under conditions of tensile stress and particularly at higher temperatures, a crack may be formed leading to a mechanical rupture. The mechanisms are complex. SCC is in practice limited to austenitic crystal structure. SCC will not occur under conditions of compression.

Fabrication and Welding of Stainless Steel

To avoid their contamination by carbon steels, the fabrication of stainless steel components should be carried out in an area separate from any similar work on carbon steels. Wire brushes should therefore be made from stainless, not carbon, steel. Layout or cutting tables that are made from carbon steel should be covered with cardboard or plastic sheets to prevent contact between the two materials. Carbon steel slings and hooks, and the forks of lift-trucks, should have wooden or plastic guards.

During the welding process itself, the molten metal must be shielded from atmospheric oxidation by means of a gas, slag, or vacuum in order to achieve and preserve the optimum corrosion resistance and mechanical properties in the joint.

On either side of a weld-run, the parent metal will have been heated to a temperature approaching its melting point and these areas are known as heat affected zones (HAZ). How the characteristics of the weld-metal and the HAZ will be affected will depend on the composition of the stainless steel and the weld technique, including the use of filler metals, and on subsequent chemical treatments.

All welding of all grades must be followed by an effective postweld cleanup. The heat of welding will leave areas of oxidation (known as heat tint) near to the weld, which will have a significantly reduced resistance to corrosion, and this must be restored by removing the tint, ideally entirely. Pickling is the controlled corrosion of the surface and will remove this undesirable oxidation. The usual medium is 10% nitric/3% hydrofluoric acid in a bath at approximately 50 °C. Local areas may be treated with the use of pickling pastes.

Although not strictly necessary after pickling, a finished component may be passivated. This involves immersion of small components, or the flushing through of pipework, with nitric acid. This does not corrode the stainless steel nor remove the surface layer, but it helps to thicken, and so strengthen, the passive film.

Surface Finish on Stainless Steel

The surface finish on stainless steel is best expressed as the R_a value, which is the arithmetic mean deviation of the readings taken by a surface profile meter measured in microns. Expressed mathematically

$$R_a = \frac{1}{l_m}\int_{x=0}^{x=lm} |y|dx$$

An R_a value of less than 0.8 μm is regarded as adequate for most purposes. In some instances where high turbulence in the fluid product occurs, R_a values higher than 0.8 μm may be acceptable. A sheet that has been cold rolled will usually have an acceptable R_a. The requirement can also be achieved by polishing.

Elastomers

The following are suitable for use in the food industry:

Natural rubber,
Nitrile/butyl rubber.
Hydrogenated nitrile butyl rubber.
Ethylene propylene diene monomer (EPDM) is not resistant to oils and fats,
Silicone rubber, which is suitable for high temperature applications up to 180 °C, and
Viton (fluoroelastomer) which is suitable up to 300 °C.

Plastics

The following plastics are suitable in hygienic design:

Polypropylene,
Polyvinyl chloride (unplasticized),
Acetyl copolymer,
Polycarbonate, and
High-density polyethylene

Note that Teflon (polytetrafluoroethylene, PTFE) is generally porous and is not recommended.

Although these materials are suitable, the requirement of joining or welding them may render them unsuitable due to crevices formed during the joining or welding process.

Glass-reinforced plastics are generally unsuitable due to the presence of filaments of glass.

Other Nonmetallic Substances

Generally, wood is not suitable as a contact material or as a structural material in food processing plants. In certain

industries, such as wine and vinegar making, it is regarded as necessary. It is also suitable for cutting blocks. For anything else, wood should be avoided.

Coatings and adhesives are not generally used for contact areas. Epoxy and urethane-based coatings are used elsewhere. Where coatings are used they should be nonflaking and smooth.

Elements of Hygienic Design

Hygienic equipment may be divided into those items that can be cleaned in place and those items that must be dismantled for cleaning. Where sections of the equipment are cleaned or sanitized at a time when adjacent sections are being used for processing or product storage, there must be positive separation including an air gap between these adjacent sections. This is most often achieved in pipeline construction by the use of swing bends, splitter panels, and mix proof (double seat) valves.

Hygienic design requires that where pasteurized or sterilized product is contained in adjacent sections of the equipment to those containing service fluids or untreated product, the former must be maintained at a higher pressure than the latter. This is to ensure that should a leak occur between the sections, the leak will be from the treated to the untreated product.

Equipment that cannot be cleaned in place must be designed so that it can be inspected after cleaning. All surfaces should be visible.

Design of Product Contact Surfaces

Design of product contact surfaces needs to take the following into account: surface texture, cleaning and inspection, disinfection, pasteurization and sterilization, microbial ingress, draining, dead spaces, joints, coatings, internal angles, corners and grooves, seals, gaskets, *o*-rings and joint rings, fasteners, process flow disruption caused by intrusions, shafts and bearings, sensor and sensor connections, other connections, and openings and covers.

Surface texture: Cracks, pits, and folds are to be avoided and the surfaces should be smooth.
Cleaning and inspection: All equipment must be cleanable either in or out of place and where possible the surfaces should be capable of inspection after cleaning.
Disinfection, pasteurization, and sterilization: It should be possible after cleaning to disinfect, pasteurize, or sterilize product contact surfaces either in place or after disassembly.
Microbial ingress: Where necessary, equipment should be designed to prevent ingress of microorganisms.
Draining: Pipelines and equipment should be completely drainable. This generally requires the use of eccentric reducers in horizontal pipe runs. It also necessitates that certain positive displacement pumps be mounted with their inlets and outlets in the vertical.
Dead spaces: Dead spaces in pipelines and equipment should be avoided. In particular tee pieces for positioning of instruments should be kept as short as possible.
Joints: Permanent metal-to-metal joints should be fully welded. Nonpermanent (dismountable) joints should be flush. Screw threads should not be present in the product contact area as these cannot be easily cleaned.
Coatings: Generally, coatings should be avoided in product contact areas. Where coatings cannot be avoided, they should be nonflaking and smooth.
Internal angles, corners, and grooves: Sharp corners within machinery are difficult to clean. For this reason, all corners should be radiused. In general, grooves, where used, should be wider than their depth.
Seals, gaskets, o-rings, and joint rings: Elastomers have higher coefficients of expansion than steels. This should be taken into account in design. Repeated heating and cooling can otherwise result in sections of the elastomer breaking off in the product. Furthermore, product may be sucked in and trapped behind the seal during cooling.
Fasteners: Fasteners such as screws, bolts, and rivets should be avoided within product areas. During disassembly, some external screw threads may come into contact with product. These should be designed to be cleanable.
Intrusions: In certain instances intrusions such as springs cannot be avoided within the product contact area. If such intrusions cannot be avoided, they should be cleanable.
Shafts and bearings: Shaft entry points require the use of mechanical seals. Where possible, there should be movement of fluid in the seal area. There should be an air gap between the product area and any lubricated bearings.
Sensor and sensor connections: They should be installed in such a way that there are no dead spaces or crevices.
Other connections: All permanent and nonpermanent pipework connections to equipment should be designed to prevent ingress of contamination.
Openings and covers: Hinges that allow crevices for soil to accumulate should be avoided.

Design for Noncontact Surfaces

The design for noncontact surfaces is less stringent than for contact surfaces, particularly with regard to materials of construction. The following should be considered as general requirements:

Horizontal surfaces should be avoided. Supports should be so designed that soil will not remain on the surface.
Insulation on equipment and pipework should be fully sealed to prevent ingress of pests or contamination.
Equipment should be designed to prevent contamination with machinery fluids, such as lubricating fluids and sensor fluids. Where vapor is discharged from machinery, this should be vented outside of the factory area.

Designing for Ease of Maintenance

Wherever possible, equipment should be designed so that there is sufficient clearance between the equipment and the floor to allow adequate cleaning and maintenance. As a

general rule, a clearance of 300 mm should be allowed below major items of equipment.

Major items of equipment should be positioned a minimum of 500 mm from walls to allow maintenance, cleaning, and control of pests.

Pipelines and cable racks should be a minimum of 150 mm from walls to allow cleaning behind the racks.

Verification of Design

The hygienic design of equipment may be verified in one of three ways: the inspection of drawings of the machine, inspection of the machine itself, or by means of a practical test. The cleanability, pasteurization, sterilizability, and bacteria tightness may be practically tested. Methods have been documented by the European Hygienic Equipment Design Group.

As an example, assessment of the in-place cleanability of food processing equipment may be achieved by the following procedure under specific and controlled conditions. The cleanability of the test pieces is compared with a reference piece, which is a straight section of tube of similar diameter.

A thermophilic test strain, *Bacillus stearothermophilus* is prepared. This is added under controlled conditions to a soured milk soil.

The test piece and reference piece are cleaned, degreased and descaled, and sterilized. The equipment is then soiled with the soured milk soil under pressure and dried.

The equipment is then fitted to a test rig and washed under controlled conditions.

The test piece and the reference piece are filled or coated with molten agar and incubated. Yellow coloration is observed where there was residual soil on the test piece and this may be compared with reference piece.

Design of Key Items of Equipment

The requirements listed above are general requirements for all hygienic equipment. For certain equipment, there are specific requirements that supplement the general requirements. Some of these are given below. Requirements of hygienic design require attention to the mechanical design of surfaces, selection of standard items for particular tasks, confirmation of flow rates and physical parameters, and installation of equipment.

Pipelines

Pipelines are used to transfer a fluid or semi-fluid food ingredient or product or a service fluid from one area of the processing plant to another. The energy for the transfer is provided by pumps, gravity, or suction. Cleaning-in-place (CIP) pipelines are considered as a part of the process pipework system. This is consistent with the definition of a product contact surface.

The components of the pipeline system include:

Straight lengths of tube;
Flexible hoses;
Connection pieces in the form of bends, tees, and reduction pieces;
Valves of various types;
Instruments that impinge on the flow to a greater or lesser extent;
Joints either permanent (e.g., welds) or dismountable (e.g., unions); and
Pipeline supports.

For most hygienic applications within the food industry, pipelines should be constructed from stainless steel. The grades that are usually acceptable are AISI-304L and AISI 316L. Glass and copper piping are available as process piping. Plastic piping such as polyvinyl chloride (PVC) is also available and sometimes used. Although these materials may be suitable construction materials for food products, cleanable connections, and valves are often not available.

Stainless steel tubing most often used for hygienic duties in the food industry is seam welded and polished. A number of different dimensional standards are in existence. In particular, these include tube to the following standards:

DIN 11850,
International Organization for Standardization (ISO) 2037/ BS4825 Part 1, and
ISO DIN EN 1127.

In practice, the DIN 11850 standard is the most frequently used. However, dimensions of commercially available tubing vary considerably. It is important that misalignment or variation in dimension of tube components do not exceed 20% of the wall thickness.

As with other product contact surfaces, the pipework and fittings should have an internal surface roughness of 0.8 μm R_a maximum.

Flexible tubing in both plastic materials (notably PVC) and food grade rubbers (notably EPDM) are available. Neither ISO nor European Committee for Standardization (CEN) have standards either for the material or the dimensioning. Guidelines for the use of flexible rubber and plastic materials is given by the 3A Sanitary Standards Inc.

Schedule piping is generally unsuitable for food industry process lines because the surface is rough. In addition, threaded connections are not cleanable by CIP.

Pipelines should be drainable. A slope of 3° to the drainage point is recommended. This is equivalent to 1 in 20.

Standard dimensions for hygienic bends, tees, and reducers for use in conjunction with tubing with DIN 11850 are given in DIN 11851.

Permanent Joints in Pipework

Although expanded connection to dismountable joints was an accepted practice in the past, welded joints are now the norm. Welding is used to connect tube sections to bends, tees, reducers, valves, and also to dismountable joints (unions).

Welding of thin-walled tube may be manual or by means of an orbital welding machine. Tungsten inert gas welding procedures are used. The correct weld type is a butt weld without the use of filler wire.

The preparation of the weld requires proper attention to the cutting, surface treatment, and alignment. The face should

Table 2 Pipeline unions in common use

Type (common name)	*Dimensions of tube (International Organization for Standardization (ISO) – Imperial or DIN)*	*Expand on or weld on available*	*Nut type*	*Seal ring section*	*Standard applicable*
DIN	Either	Metric both Imp. w/o	Slotted	Half round	DIN 11851 Germany
DRT	Imperial	Both	Slotted	Rounded	DS 722 Denmark
SMS	Imperial	Both	Slotted	Square	SMS 1145 Sweden
ISS/IDF	Imperial	Both	Various	T shape	ISO 2853
Clamp Type	Imperial	Weld on	None required	Flat	ISO 2852

be cut exactly at right angles and burrs removed. The area 25 mm on either side of the weld should be cleaned and degreased. Misalignment of the tube ends should be less than 20% of the tube thickness. The gap between the faces should be less than 0.25 mm.

Before welding, the air must be purged with Argon. For short lengths of pipe, the ends of the pipe are sealed with tape or caps and the argon introduced from one end. For longer lengths of pipe, mechanical means of holding the inert gas such as plastic bladders are required.

Typical weld defects are the following: misalignment, cracking, porosity and inclusion, incorrect penetration, lack of sidewall fusion, and lack of gas shield. These will cause unhygienic conditions in the weld area.

Flow Rate in Pipeline Systems

Most thin food liquids (e.g., milk, beer, and cool drinks) should flow in pipelines at approximately 1.5 m s^{-1}. For thick liquids (e.g., tomato paste), this might reduce to as low as 0.2 m s^{-1}. Higher flow rates are important to ensure that dead ends in the pipeline are purged and that air is adequately removed from the system.

For CIP liquids will flow at 1.5 m s^{-1} minimum and flows of 2 m s^{-1} are usually specified.

Dismountable Joints in Pipelines

There are a number of standards for hygienic unions commonly in use. These are listed in Table 2.

It has been found that many of these unions are prone to problems of misalignment, leading to crevices.

For aseptic applications, unions to DIN 11864-1, 11864-2, and 11864.3 should be used. These are flanged, screwed, and clamp-type couplings using *o*-rings.

Reducers in Pipelines

Reducers for pipelines are either concentric or eccentric.

Guidelines for the use of reducers are:

Concentric reducers should be installed in vertical pipes.
Where drainage needs to be facilitated, eccentric reducers should be installed with the flat plane at the bottom.
Where air removal needs to be facilitated, eccentric reducers should be installed with the flat plane at the top. This applies particularly on the inlet to centrifugal pumps.
On the horizontal discharge of centrifugal pumps, eccentric reducers should be installed with the flat pane down.

Tee Pieces and Instrument Connections in Pipelines

Tee pieces often create dead legs in pipelines. If a dead leg is unavoidable, it must be as short as possible. For pipe diameters of 25 mm or more, tee pieces should preferably be less than 28 mm in length. For smaller diameter pipes, the dead leg should be less than the pipe diameter.

When dead ends cannot be avoided, they must be positioned so the flow is into the dead end. Dead ends that point vertically upward can become blocked with air. The result is that they will be almost impossible to clean. If steam sterilization is used, downward sloping dead legs may become filled with condensate and not be sterilized properly.

Swing Bends Splitter Panels

Where there is a risk of contamination or cross-contamination in pipelines, design should allow for a safety break between different sections of the pipeline system. Typically, this allows for a vessel or section of the plant to be cleaned while an adjacent vessel or section contains product. The safety break will be in the form of a swing bend, a splitter panel, or a double seat valve (Figure 1).

A swing bend is a section of pipe or key piece that can be removed to ensure a physical gap between adjacent sections of pipe. A splitter panel is more complex and allows connector pieces to be used to rearrange flows as required.

Design of splitter panel arrangements should ensure that connecting pieces do not allow the operator to make incorrect selections. The distance between the connecting points must be such that only the required connections can be made.

Design must ensure that cleaning fluid flows allow cleaning through each section of splitter panel.

Valves

Valves have a number of purposes in process plant operation. They provide for shut off or modulating control, as well as nonreturn (check), air removal, and safety functions.

Valves should be crevice free and cleanable and be without sharp edges, threads, or dead ends. Valves must, if possible, be drainable without dismantling. Seals must be properly designed and supported. Springs should, if possible, be avoided within the product area but if necessary, must be cleanable.

Sanitary butterfly and seat valves are acceptable for shut off duty. Nonsanitary ball valves must not be used in hygienic

Figure 1 A typical swing bend panel.

areas. During the operation of these valves, product will become trapped behind the ball. This area is not cleanable.

Seat valves include single seat valves and mix proof double seat valves. The area between the seats will be at atmospheric pressure during normal operation. Some valves compensate the pressure on the seat with a thicker shaft. The cleaning of the area between the seats is also important. Flooding the area by movement of either of the seats must be done at very low pressure. At this time, there will only be one seat separating the product from the CIP fluid (Figure 2).

Some valves will allow some leakage into the atmospheric area while the valve is activating.

Where aseptic conditions are required, the seats must be flushed (with a clean fluid such as condensate). Where contamination from outside is unacceptable, the shaft of the valve must be protected by a diaphragm, sterilizing fluid, or some other means.

In general, the operating of double seat mix proof valves should be automatic and without manual override.

Pumps

There are a wide variety of pump types that may be used in hygienic design. These may be divided into positive displacement and centrifugal pumps. Pumps provide a number of challenges both from design and selection points of view.

Hygienic Construction of Pumps

For rigidity the casings of pumps are commonly cast. It is important that the casting is machined to allow surfaces that

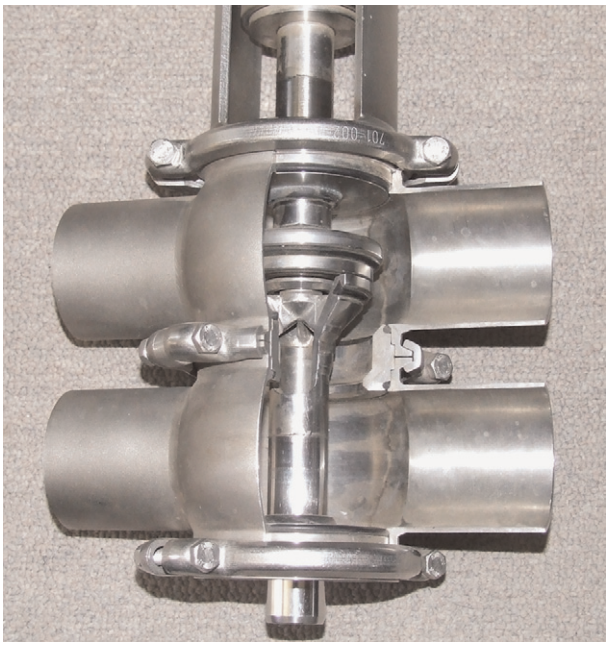

Figure 2 A cutaway of a leak proof valve in the open position.

are sufficiently smooth to be cleanable and to remove any crevices or sharp corners. Owing to the high fluid velocities that exist during the operation of pumps, it may not be necessary that all surfaces have a roughness, R_a less than 0.8 μm.

The seal area is particularly important. The mechanical seal relies on a fluid providing lubrication between a static and a dynamic surface. In the case of a single mechanical seal, this will be product. In the case of a double mechanical seal, this will be a service fluid, which will be at a higher pressure than the product and may then contaminate the product. In the case of the single mechanical seal, slight leakage of product will provide for cleaning of the seal area. In the case of the double seal, the choice of a seal fluid is very important.

Where electric motors are protected by shrouds, such shrouds should be easily removable for cleaning.

Selection of Pumps

Centrifugal pumps are selected whenever possible for hygienic duties. Positive displacement pumps do not usually provide sufficient fluid velocity to be used for CIP duties. Where necessary, a centrifugal CIP pump should be installed in parallel with a positive displacement product pump.

CIP return pumps should be liquid ring-type pumps. These are self-priming, thus ensuring complete emptying of the vessel being cleaned.

Industrial eccentric screw pumps are not suitable for food duties. The usual construction of this type of pump includes a hollow shaft where product can become trapped. Where (hygienic) eccentric screw pumps are used, they should be installed with the seal on the delivery end of the pump.

Where peristaltic (hose) pumps are used, those types that allow retraction of the shoes from the hose are preferable because they allow easy cleaning of the interior of the hose at CIP velocities.

Installation of Pumps

Pumps should be installed so that they are drainable. This requires mounting centrifugal pumps with the outlet in the horizontal plane. Lobe-positive displacement pumps should be installed with the inlet and outlet in a vertical plane.

Where (hygienic) eccentric screw pumps are used, they should be installed with the seal on the delivery end of the pump.

Positive displacement pumps require a pressure relief valve. This should be installed in such a way as to limit the dead ends between the delivery and suction of the pump.

Heat Exchangers

Plate heat exchangers are widely used in the food industry. Hygienic design requires that the flow rate of product within the machines is sufficient to prevent the formation of dead spaces or retention of air within the exchanger. This usually requires that viscous products flow in a single rising pass in the machine.

Flow rates that will be required for cleaning the machine should always be specified.

The gasket arrangement for plate heat exchangers must ensure that there is a double gasket separating the heat exchange fluids. This ensures a gap to atmosphere between the fluids.

Where possible design should ensure that the pressure on the product side of the machine or, in the case of a regenerative section on the treated side of the machine, be higher than the pressure of the fluid on the untreated product or service side.

Pasteurizers

Where heat exchangers are used for pasteurization or sterilization duties, the temperature probe should be so positioned that there is sufficient space between it and the diversion control valve to ensure that the valve will divert before untreated product passes it. The time taken by the temperature probe and valve operation may be several seconds when the plant is clean. This time will increase if there is any fouling on the probe.

Filling Machines

Liquid filling machines provide particular challenges for hygienic design.

Filling machines should, where possible, be designed so that CIP fluids can be returned to the CIP system. For reasons of safety and environmental management, dumping of CIP fluids at the filling machine is not an acceptable practice.

In the case of gravity filling machines, the velocities through the filling nozzles may not be sufficient to allow proper cleaning. In this case, machines should be designed to be disassembled for cleaning.

Equipment for Cleaning in Place

The CIP plant forms and integral part of any pipework system. Thus, the same criteria that apply to other parts of the system will apply equally to the CIP system.

It is preferable to separate cleaning of prepasteurization equipment and postpasteurization equipment.

Cleaning of Equipment for Dry Processing

Equipment may be designed for dry cleaning provided that the product does not present any risk of contaminating subsequent production, that it is nonhygroscopic, and nonsticky. In general, material must have a water activity below 60% for dry cleaning to be an option.

See also: Public Health Measures: Modern Approach to Food Safety Management: An Overview

Further Reading

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Infestation Management in Food Production Premises

CH Bell, The Food and Environment Research Agency, York, UK

Glossary

Diapause A state of arrested development, which can occur at any stage of the insect life cycle (depending on the species) in response to environmental cues, typically those heralding winter such as a lowering of temperature or shortening days.

Fumigant A chemical used in the gaseous stage for pest control, as distinct from insecticidal fogs (finely dispersed liquids) or smokes (finely dispersed solid particles), neither of which have any powers of penetrating commodities.

Instar Term given to the stages of larvae separated by the larval molts and numbered accordingly, the first instar being that emerging from the egg.

Nymph Term given to immature stages of arthropods developing without a complete metamorphosis in the life cycle, i.e., without larval and pupal stages.

Parthenogenesis Reproduction without mating, optional in a few insect species and in one or two cases the only reproductive process possible, there being a complete absence of males.

Introduction

Man is in direct competition with a variety of other species for food. These competitors not only consume the product but also contaminate the product with feces, exuviae or hairs, frass, and microorganisms. They can also alter the physical properties of the product by increasing temperature and possibly moisture content, and pose health threats by acting as vectors of pathogens and parasites. The importance of implementing effective pest management strategies cannot be overemphasized as the discovery of live insect stages or contaminants such as insect fragments and exuviae, or rodent hairs and droppings, has severe health and financial implications. Such incidents usually lead to the recall of the entire distribution of a particular product and may result in expensive litigation procedures, but potentially the greater financial loss is the longer term effect on consumer confidence in the product, which may never be fully restored.

In food production facilities there is a constant threat of pest populations becoming established as food is always present and there are many locations and access points for pests to enter and find refuges. Insect food pests are cosmopolitan whereas food facilities are twice as likely to encounter rodent problems in comparison with domestic premises and are legally bound to practice high food safety standards. Many procedures can be adopted to prevent pest access, to detect their presence on arrival, and to control infestations when they occur, and these are discussed in the following sections.

Pests of Food Processing and Production Facilities and the Risks they Impose

Any site where food is gathered, sorted, processed, or stored, is an attraction to wandering rodents, birds, insects, or mites whose lives depend on the successful location of food sources. Farmers, crop storage and distribution specialists, food processors, and retailers all need to take precautions to render their premises less vulnerable to exploitation by pests.

Although problems from vertebrate pests can largely be addressed by exclusion strategies, the same is not true for insects and mite pests, although exclusion strategies are still an important ingredient of pest management. Incoming supplies often introduce these pests and many species can become established in the fabric of the building, creating microclimates in harborages and wandering out to locate and feed on food residues. In warm climates population increase can be extremely rapid, and urgent action is needed to prevent the spread of infestation.

Vertebrate Pests

Rats, mice, sparrows, and pigeons are ubiquitous and major sources of contamination of food products in food processing facilities. They act as vectors of *Salmonella* and *Leptospira* bacteria, various viruses, rickettsiae causing Q fever and other pathogens. Weils disease caused by *Leptospirosis icterohaemorrhagiae* picked up by contact with rat excreta can be fatal, as can some cases of *Salmonella* food poisoning. Rodents also cause damage by the gnawing of wood, plastics, electric cabling, and even metal water pipes, sometimes with catastrophic consequences. For birds, netting of openings and needlematting of surfaces are well established, effective strategies to prevent ingress, but problems may still occur where continual access for transport is needed or weathering of buildings provides openings in inaccessible areas of roofing where birds such as sparrows, starlings, or pigeons can gain access.

Rodent proofing is a more complex problem as in addition to the obvious exclusion of ground level entry points,

attention needs to be paid to the drainage system as well as roofing eaves as rodents will ascend drain pipes, either internally or externally, and gain access to lofts and then through the whole building via heating ducts or electrical conduit routes. Access of rats from sewerage systems is also not an uncommon occurrence, so screens and other barriers should be in place and regularly maintained. The use of rodenticides for rat control requires the involvement of trained operators and even after careful observance of regulations is still a potential risk to nontarget organisms. Resistance has developed to anticoagulants such as warfarin, and now only second-generation compounds are in widespread use; difenacoum and bromadiolone are used both indoors and outdoors, and the more toxic brodifacoum and flocoumafen are used indoors only under carefully controlled conditions. Formulation and mixture with an appropriate food is of critical importance as baits are readily rejected. All baiting stations should be checked weekly and replaced if necessary.

Anticoagulants have always been less effective against mice because of avoidance following small intakes of bait, and given the loss of calciferol based on Vitamin D_3, no really effective bait is available. Physical traps are used to complement anticoagulant baiting strategies along with single-dose agents based on alphachloralose or zinc phosphide. In addition, sodium cyanide and aluminum phosphide formulations are available for fumigation treatment of rat harborages and burrows away from occupied buildings. However, none of these complementary measures can guarantee adequate control and for each facility an effective exclusion and trapping strategy is therefore a necessity.

Externally, access by rodents to buildings is prevented by clearance of all shrubbery and disused machinery from the vicinity of the exterior walls and the deployment of traps at regular intervals around the property and both inside and outside potential points of entry into buildings. A typical layout of trap deployment for a food facility is presented in Figure 1.

Insect Pests

Beetles

Coleoptera is the largest order of insects and there are more than 20 species of beetle or weevil of worldwide importance in the food industry. Table 1 lists some that are commonly associated with food processing facilities, together with their food preferences and requirements for rapid development. Many species are of tropical origin that have arrived and become established in heated premises since the advent of international trade. Others, such as the biscuit beetle and granary weevil, famous for infesting sailors' biscuits and grain

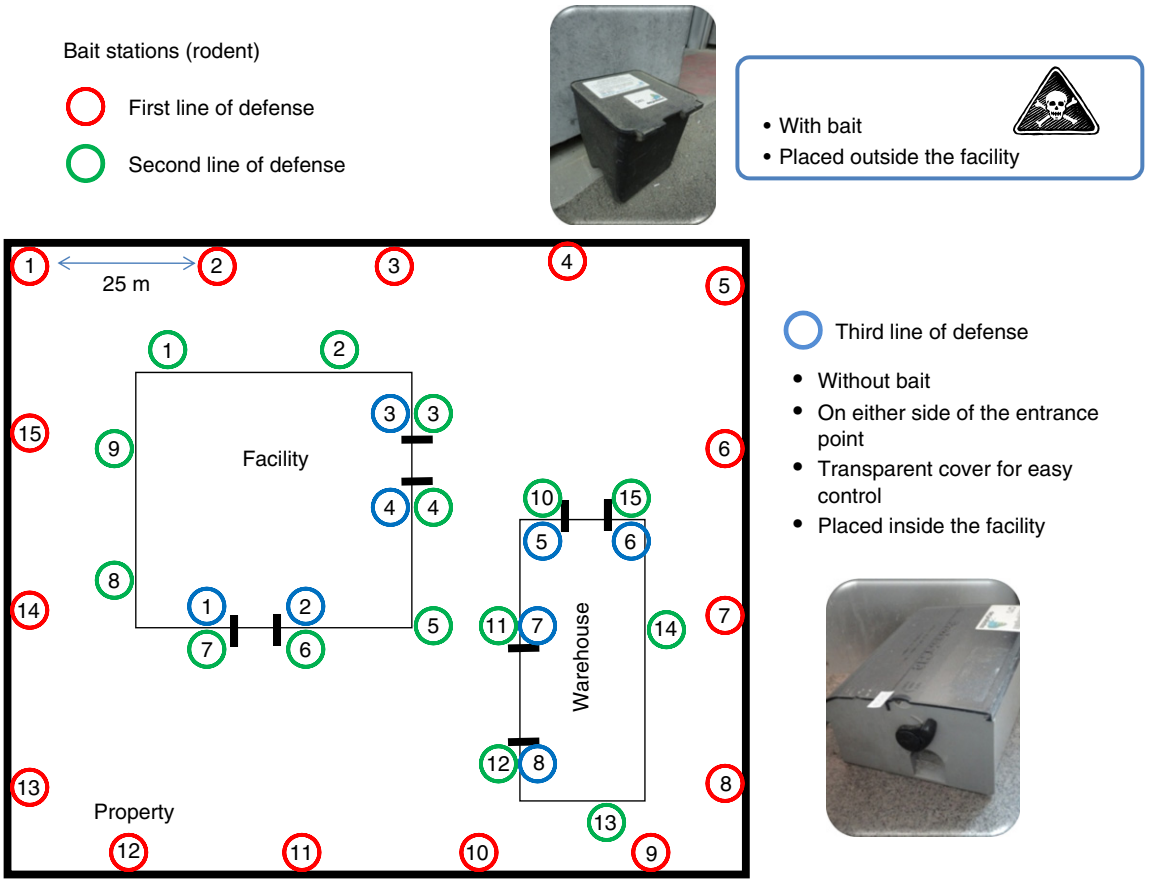

Figure 1 A typical layout of rodent traps for the protection of a food processing enterprise.

Table 1 A compilation of developmental requirements of beetle and moth pests often found in food processing facilities

Species	*Food preferences*	*Developmental range/optimum, and fastest multiplication rate*
Cryptolestes ferrugineus (Stephens) Rust-red grain beetle	Grains, flour, meals, oilseeds, dried fruit, and other dried vegetable materials	20–38 °C, min r.h. *c.* 30%/32–35 °C, 60-fold in 4 weeks
Cryptolestes turcicus (Grouvelle) Turkish grain beetle	Cereal products, notably wheat flour	*c.* 20–36 °C. min r.h. 50%/28–33 °C, *c.* 40-fold in 4 weeks
Gnatocerus cornutus (F.) Broad-horned flour beetle	Cereal products	15–35 °C, min r.h. 30%/*c.* 30 °C, *c.* 20-fold in 4 weeks
Lasioderma serricorne (F.) Cigarette or tobacco beetle	Cocoa, soybeans, tobacco, various cereals, spices, textiles, and many other products	22–38 °C, min. r.h. 30%/32–35 °C, 20-fold in 4 weeks
Oryzaephilus mercator (Fauvel) Merchant grain beetle	Oilseeds, dried fruit, nuts, and cocoa beans	17–38 °C, min 30% r.h./30–35 °C, *c.* 30-fold in 4 weeks
Oryzaephilus surinamensis (L.) Saw-tooth grain beetle	Cereal grains, cereal products, dried fruits, nuts, and some oilseeds	20–38 °C, min r.h. *c.* 40%/31–34 °C, 50-fold in 4 weeks
Rhyzopertha dominica (F.) Lesser grain borer	Cereal grains, flours, meals, and macaroni	19–40 °C, min r.h. 30%/32–35 °C, 40-fold in 4 weeks
Sitophilus granarius (L.) Granary weevil	Cereal grains (exclusively internal grain feeder)	15–30 °C, min r.h. *c.* 50%/25 °C, 15-fold in 4 weeks
Sitophilus oryzae (L.) Rice weevil	Cereal grains (exclusively internal grain feeder)	15–34 °C, min r.h. *c.* 40%/28–30 °C, 30-fold in 4 weeks
Stegobium paniceum (L.) Biscuit or bread beetle	Cereal products and many other dried vegetable and animal products	17–32 °C, min r.h. *c.* 60%/25–28 °C, 7.5-fold in 4 weeks
Tribolium confusum J. du Val Confused flour beetle	Cereal products, copra, groundnuts, sesame, and oilseeds	20–38 °C, min r.h. 20%/30–32 °C, 60-fold in 4 weeks
Tribolium castaneum (Herbst) Rust-red or red flour beetle	Cereal products, groundnuts, cacao, spices, dried figs and dates, copra, dried yam, palm kernels, nuts, and oilseeds	22–40 °C, min r.h. 20%/32–35 °C, 70-fold in 4 weeks
Corcyra cephalonica (Stainton) Rice moth	Cereals, cereal products, dried fruit, seeds, cocoa, and groundnuts	18–35 °C, min r.h. 50%/30 °C, 50-fold in 4 weeks
Ephestia cautella (Walker) Tropical warehouse moth, almond moth	Dried fruit, nuts, cereals and cereal products, cocoa beans, spices, copra, carobs, pulses, and dried vegetables	17–36 °C, min r.h. 25%/30–32 °C, 60-fold in 4 weeks
Ephestia elutella (Hubner) Warehouse or tobacco moth	Grain, cocoa, dried vegetable products	10–30 °C, min r.h. 20%/25 °C, 20-fold in 4 weeks
Ephestia kuehniella Zeller The Mediterranean flour moth or mill moth	Cereals, cereal products	10–30 °C, min r.h. 20%/25–28 °C, 50-fold in 4 weeks
Plodia interpunctella (Hubner) Indian meal moth	Dried fruit and nuts, cereals and cereal products, cocoa, oilseeds, confectionery, citrus pulp, dried vegetables, pulses, seeds, and carobs	18–36 °C, min r.h. 20%/30–32 °C, 50-fold in 4 weeks

supplies in the days of sailing ships, are native to temperate regions. Excavations of archeological sites have found dead specimens of the biscuit beetle in leather artifacts dating back to Roman times and in the remains of food left in tombs in ancient Egypt.

Stored-product beetles may be divided into those developing externally on semiprocessed foods and in finely divided products, and those internal feeders developing within whole seeds such as cereal grains and legumes. The latter group includes bruchids, weevils (Curculionidae), and grain borers (Bostrichidae), which cause problems because of infested raw materials and rarely become endemic in the structure of the food production facility. However, they are notoriously difficult to eradicate because they avoid detection and are protected from direct contact with control measures. The lesser grain borer and the granary and rice weevils occur as pests of rice and flour mills in this manner (Figure 2).

Those beetles feeding on semiprocessed materials or foods may again be divided into two groups, those with relatively short-lived adults (anobiids such as cigarette beetle, and dermestids such as Khapra beetle) and those whose adult stage may last a year or longer. In this latter group, including the *Tribolium* and *Cryptolestes* species, which are serious flour mill pests, and the *Oryzaephilus* (and also *Tribolium*) species (Figure 3) occurring widely in breakfast cereal, pet food, and confectionery manufacturers, both larval and adult stages actively feed on food products. It is this group that often establishes residual infestations in premises, entering cracks, crevices, and voids where food material escaping from processing machinery may accumulate. The long-lived adults seek out harborages from which they wander, often in a daily cycle, to scavenge for food and locate additional oviposition sites from which fresh infestations may start.

Despite their tropical origin and need of warm conditions for breeding, adults of many species of stored-product beetle,

(a) (b)

Figure 2 Two internally feeding grain beetles: (a) lesser grain borer *Rhyzopertha dominica* and (b) granary weevil *Sitophilus granarius*.

(a) (b)

Figure 3 Two externally feeding grain beetles: (a) saw-toothed grain beetle *Oryzaephilus surinamensis* and (b) rust-red flour beetle *Tribolium castaneum*.

both 'internal' and 'external' feeders, are highly cold tolerant and can readily overwinter in parts of the facility. Long-term infestation problems are revealed if the mealworm *Tenebrio molitor* L., at 12–17 mm in length the largest of all stored-product beetles, *Gnatocerus* spp. flour beetles, or spider beetles (Ptinidae) are present in the facility.

(a) (b)

Figure 4 Two moth pests of stored products: (a) brown house moth *H. pseudospretella* and (b) almond moth *Ephestia cautella*.

Moths

Most moth pests of food processing facilities belong to the family Pyralidae, although two oecophorid moths, the brown house moth *Hofmannophila pseudospretella* (Stainton) (Figure 4(a)), and white-shouldered house moth *Endrosis sarcitrella* (L.), are commonly encountered in damper, cooler situations such as mill basements and storage areas. Adult moths do not feed and damage is caused by the larval stage, which features a heavily sclerotized head capsule with biting and chewing mouthparts whereas the rest of the elongated body is flexible, unpigmented, and unsclerotized. In addition to the consumption and contamination of food, moth larvae produce silk from glands in the mouth, which builds into webbing that can obstruct machinery and clog production lines.

Oviposition in pyralid moths occurs mainly at dusk. The egg stage lasts a maximum of 7 days at 25 °C and there are five larval instars. The duration of the larval stage is influenced by temperature, food source, humidity, and whether or not a larval overwintering diapause occurs at the fifth instar. On completing their development, in preparation for pupation or diapause larvae spin a tough cocoon, which may be double layered. The pupal stage lasts about twice the duration of the egg stage at a particular temperature and adults are short lived, females laying most of their 2–300 eggs within 3 days.

The rice moth is a serious pest of mills in hot damp climates but can become established in heated premises anywhere in the world. The tropical warehouse or almond moth (Figure 4(b)) is the most frequently intercepted moth pest on food imports into the developed world and a common pest of food processing facilities. The Mediterranean flour moth is the principal moth pest of flour mills and bakeries in temperate regions of the world whereas the warehouse moth, as its name suggests, is largely confined to warehouse storage areas where it overwinters as a diapausing larva, able to tolerate temperatures down to − 10 °C. The arrest is triggered by late summer daylengths of less than 14 h. The Indian meal moth, perhaps the most versatile of all moth species in occupying niches in the food industry, can also overwinter as a diapausing larva. Details of the developmental limits and optima for each species are provided in Table 1, together with their food preferences.

Other Insects

Cockroaches, flies, ants, and psocids can also cause problems in food processing facilities. The principal cockroach pests belong to the genera *Periplaneta*, *Blatta*, *Blatella*, or *Supella*. Eggs are produced in capsules or oothecae, which remain attached to the female until deposited near a suitable substrate. Females produce a series of such egg cases, each containing up to 40 eggs. Nymphs can mature to adults within 12 weeks, though typically it takes several months for the life cycle to be completed. Because of their relatively large size and scurrying mobility cockroaches are the most noticed and hence most disliked of all insect pests. The American cockroach *Periplaneta americana* L. at approximately 3 cm in length is one of the larger species. All the pest species are of warm climate origin and though many are named after countries or regions, this does not necessarily reflect their true origin or range. Cockroaches are notable disease vectors and, particularly the German cockroach *Blatella germanica* (L.), can cause serious allergenic problems, besides contributing to the contamination and spoilage of food. With the notable exception of the Asian cockroach *Blatella asahinai* Mizukubo, few species fly and all need access to water. The waxy cuticle is more permeable to water loss than in more advanced insects and therefore wax-absorbing dusts can be effective means of enhancing control measures. Most species are cryptic, hiding from light in refuges with proximity to food sources. Though

some cockroaches respond at close range to aggregation pheromones, movements are more influenced by the physical environment than by odors, joints between walls and floors or shelving being favored for runs, particularly where open space is avoided. Complex or cluttered environments greatly contribute to their becoming established in premises.

Many different flies are hygiene threats in industry, including house flies, blow flies, fruit flies, and drain flies, each originating from different sources of hygiene failure. They can transmit many fecal and oral-borne pathogens. UV-light traps are widely employed to monitor and control flies in bakeries, restaurants, and food processing plants. The traps usually attract flies to an electrocuting grid but some incorporate a motorized glue paper roll, which allows the recording of catch over specific time periods. Siting of traps is important to minimize the chances of attracting insects from outside the building or contaminating food with biological material falling out from the grid or tray. Properly used, light traps can keep problems under control as long as adequate attention is also paid to remove potential sites for larval development.

Ants have caused problems at nearly every type of food production or processing premises at some time or other. Worker ants forage for food and carry it back to a central nest often at a considerable distance from the food source, leaving a chemical trail on the return journey. The result is that increasing numbers of workers appear in the facility, all following the same path. Two species regularly causing problems in houses, hotels, restaurants, hospitals, warehouses, and food production and processing facilities are the common black ant *Lasius niger* (L.) and the pharaoh's ant *Monomorium pharaonis* (L.). The latter can be controlled by insecticide baits based on juvenile hormone activity because, unlike the *Lasius* spp., there is usually only a single queen producing eggs in the nest.

Psocids are tiny, primitive insects feeding mainly on molds and decaying vegetable material in damp situations. They sometimes appear in huge numbers on food materials in commercial or domestic premises. The smallest opening in a food package can provide a point of entry for the minute nymphal stages. The commonest species is *Liposcelis bostrychophila* Badonnel, a rapid-moving, wingless, pale-colored insect approximately 1 mm long for which only females are known. Parthenogenetic multiplication can be rapid, but temperatures higher than 20 °C and high humidity are needed for egg production.

Mites

Mites, more closely related to spiders than insects and extremely small, utilize microenvironments of moderate temperature and raised humidity. The most important family associated with food storage problems is *Acaridae*, though the dried fruit mite *Carpoglyphus lactis* (L.) (Carpoglyphidae) and cosmopolitan food mite *Lepidoglyphus destructor* (Schrank) (Glycyphagidae) are also common pests. The life cycle includes a brief larval stage typically followed by three nymphal stages before the reproductive adult stage. Their rate of increase is unparalleled by any insect, with only 14 days being needed to complete development under optimal conditions and with a single female being able to produce 5–600 eggs. Eggs are cold tolerant and in some species development can proceed down to 5 °C, but in all species low humidity prevents development.

The flour mite *Acarus siro* L. is able to infest any food used by man if the local environmental conditions are suitable. The mold mite *Tyrophagus putrescentiae* (Schrank) is perhaps the most cosmopolitan mite pest of stored products, occurring in any product with a high fat or protein content. The tiniest opening permits entry of mites into packaged products and, once inside, an unpleasant taint is produced in the substrate. Many mites are also strongly allergenic.

Minimizing Pest Occurrence in Food Premises

It can be seen from the optimum requirements of insect pest species that the ideal environment of food processing premises should be low temperatures, low humidity, and an absence of accessible food sources. Unfortunately, none of these parameters can be maintained throughout a site and so there are always tensions in striving for the right balance between production needs and pest avoidance. Most food ingredients are vulnerable to pest attack, especially those with an equilibrium relative humidity greater than 65%, and the continual movement of commodities to and from trade premises poses a constant threat of importing pests. In nearly every country legislation demands the highest standards for any food product destined for human consumption, so the elimination of pest contamination of food is of paramount importance for the industry.

Effective control measures carried out at the source of raw ingredients provide a vital start to the chain that leads to the final processed product. Buildings need to be designed to avoid access points from outside, and doors and windows need to be precision-fitted and kept closed whenever possible. Recessing of external drain pipes prevents a ready access route to the eaves for rodents and wall surfaces should have a smooth finish both inside and outside. Internally, minimization of voids, ledges, crevices, or dead spaces is an important aspect at the planning stage as these provide locations where insect pests can establish refuges. Similarly, all machinery should be designed to avoid pest harborage. Rigorous, systematic cleaning of machinery and food production areas on a regular basis helps to prevent infestations becoming established. Timely and appropriate removal of accumulating waste and debris by vacuum cleaning, sweeping, and washing is another vital aspect to be built into management practice. Streamlining product distribution to reduce residence time in store and avoidance of storage alongside other less secure products are other useful measures to avoid problems. Care should also be taken to avoid stacking products in corners or near to walls, which reduces access for cleaning and provides harborages for pests.

Packaging can be an effective measure for reducing access of pests to food materials after processing, but standard carton designs generally provide little protection against stored-product insects. The spot weld glue patterns commonly used tend to leave channels through which smaller insect or mite stages can enter and does not provide a complete seal. Card, paper and cellophane wrappings are the least resistant to insect

penetration, whereas polycarbonate, some polyesters, polyurethane, and aluminum foil are much more resistant. All packaging is vulnerable to damage by rodents, and insects such as the lesser grain borer, biscuit beetle, cigarette beetle, and larger larvae of pyralid moth species possess powerful biting mouthparts and are also able to penetrate most films. Any measures to improve packaging design by reducing the chance of an incomplete seal, and removing joins, folds, and corners that are susceptible to mechanical damage or provide leverage for insect mouthparts should be implemented. Overwraps also improve resistance, particularly if applied as shrink-wraps fitting tightly around the package. A higher level of protection is provided by the 'form-fill-seal' machines employed in modified atmosphere (MA) or vacuum packaging. A heat-molded base tray is filled with product and a flat lid is heat-sealed across the top in the relevant atmosphere for the product.

All the above measures have economic implications and require there to be an adequate profit margin for the final product. Although the presence of pests can be minimized, total elimination of pest incidence can never be guaranteed. Therefore, there is a need for measures to detect pests at an early stage before they locate and damage the product. Meanwhile, research continues to refine methods of excluding and controlling pests, but problems can only be avoided if vigilance is maintained and management procedures are optimized and rigorously applied.

Pest Detection Strategies

A vital part of pest management programs is the early detection of pests. Many systems of trapping have been employed over the years, such as sticky papers and tapes, baited traps of various kind, and thin lines of grease or food-grade mineral oil around processing machinery or other vulnerable areas. The present focus is on the use of pheromones, the volatile chemicals released by insects themselves that function as a means of communication between individuals, and on food volatiles. Pheromones are particularly important for insect reproduction, both in long-range attraction of the opposite sex and in short-range mate location.

The chemical structure of pheromones has been analyzed for a large number of species of concern in stored-product protection. A list of some of the materials that have been isolated and identified is given in Table 2. There are two basic types of pheromone involved in pest detection systems: sex pheromones and aggregation pheromones.

Sex Pheromones

Sex pheromones are usually emitted by females to attract males for mating. They have been reported from many moths and certain families of beetles including Anobiidae, Bruchidae, and Dermestidae in which adults are relatively short lived and feed very little or not at all.

Sex pheromone activity may be exclusive to a single species but commonly may be shared between several related species. Thus, the sex pheromone, (*Z*,*E*)-9,12-tetradecadienyl acetate (TDA, also known as ZETA), is active not only against *Plodia interpunctella* but also against at least four other of its pyralid relatives. Similarly, the anobiids *Stegobium paniceum* and *Anobium punctatum* share stegobinone, and several *Trogoderma* spp. share (Z)-14-methyl-8-hexadecenal.

Aggregation Pheromones

Aggregation pheromones are usually produced by males and attract both sexes to suitable habitats and food sources where mating can then proceed. Beetles of the families Bostrichidae, Cucujidae, Curculionidae, and Tenebrionidae, which have adults that are relatively long lived, release pheromones of this type. Aggregation pheromones are also produced by some mite species. As with the sex pheromones, the aggregation pheromones may involve mixtures of materials and be shared by related species (Table 2).

Food Volatiles

A wide range of volatiles and aromas emitted from food materials are attractive to stored-product insects, and even plain water is effective in attracting moth species in dry conditions. Food bait traps have been employed widely in food processing facilities to monitor for the presence of beetle pests with varying degrees of success. The combined use of pheromones and food attractants offers the prospect of a monitoring system for a wide range of pests.

Pheromones as Pest Management Tools for Detection and Monitoring of Pest Populations

Pheromones are powerful attractants because of the extreme sensitivity of insects to these cues, and enable infestations to be detected at very low levels when visual or other forms of inspection are unlikely to be successful. This information is of critical importance for pest management programs in the food industry.

Pheromones are often complex mixtures of related compounds and their stereoisomers can evoke very different responses, so accurate identification, synthesis, and blending of the components is essential. Efficient delivery mechanisms for pheromones are also crucial. They must be adjusted to produce the appropriate concentration level for the species concerned, release the pheromone at a uniform rate, and have a capacity and operational life time consistent with the particular application. Trap design is important for both walking and flying insects. The distribution of traps in the treatment area is also a key factor. A vital issue after detecting the presence of insects in a facility is the accurate location of the infestation origin and to this end, spatial analysis of trapping data has proved useful, enabling precision targeting of infestation sources at an early stage.

Pest Control Strategies

Chemical Control Methods

Recently chemicals were the mainstay for pest control in the food and agricultural industry but various adverse side effects

Table 2 Attractants produced by some stored-product beetles and moths

Species	*Attractant*	*Details*
Lasioderma serricorne Cigarette beetle	Serricornin: (4,6-dimethyl-7-hydroxynonan-3-one)	Sex pheromone produced by females. Commercially available
Stegobium paniceum Biscuit beetle	Stegobinone: (2,3-dihydro-2,3,5-trimethyl-6(1-methyl-2-oxobutyl)-4*H*-pyran-4-one)	Sex pheromone produced by females. Commercially available
Rhyzopertha dominica Lesser grain borer	Dominicalure: (1-methylbutyl-(*E*)-2-methyl-2-pentenoate)	Aggregation pheromone produced by males. Commercially available
Cryptolestes ferrugineus Rust-red grain beetle	Ferrulactones I and II: [(*E,E*)-4,8-dimethyl-4,8-decadien-10-olide, and (3*Z*, 11 S)-3-dodecen-11-olide, respectively]	Two-component aggregation pheromone produced by males. Commercially available
C. turcicus Turkish grain beetle	(*Z,Z*)-5,8-tetradecadien-13-olide	Aggregation pheromone produced by males
Sitophilus granarius Granary weevil	Sitophilate: (1-ethylpropyl-2-methyl-3-hydroxy-pentanoate)	Aggregation pheromone produced by males. Commercially available
Sitophilus oryzae and *S. zeamais* Rice weevil and Maize weevil	Both species; Sitophinone: (5-hydroxy-4-methyl-3-heptanone, the 4*S*, 5*R* enantiomer)	Aggregation pheromone produced by males Commercially available
Trogoderma granarium Khapra beetle	92:8 mixture of (*Z*)- and (*E*)-14-methyl-8-hexadecenal	Sex pheromone produced by females
Carpophilus hemipterus Dried fruit beetle	(2,4,6,8)*E*-3,5,7-trimethyl-2,4,6,8-decatetraene and related compounds	Aggregation pheromone produced by males. Commercial lure available
C. dimidiatus Corn sap beetle	(3,5,7,9)*E*-6,8-diethyl-4-methyl-3,5,7,9-dodecatetraene	Aggregation pheromone produced by males. Commercial lure available
Oryzaephilus mercator Merchant grain beetle	*R* enantiomers of *Z*-3-dodecen-11-olide and *Z,Z*-3,6-dodecadien-11-olide	Aggregation pheromone produced by males. Commercial lure available
O. surinamensis Saw-toothed grain beetle	*R* enantiomers of *Z,Z*-3,6-dodecadien-11-olide, *Z,Z*-3,6-dodecadienolide and *Z,Z*-5,8-tetradecadien-13-olide	Aggregation pheromone produced by males. Commercial lure available
Tenebrio molitor Yellow mealworm	4-methyl-1-nonanol	Sex pheromone produced by females
Gnathocerus cornutus Broad-horned flour beetle	(*R*)-acoradiene	Aggregation pheromone produced by males
Tribolium confusum, *T. castaneum* Confused flour beetle, Rust-red flour beetle	Both species: 4*R*,8*R*-dimethyldecanal	Aggregation pheromone produced by males. Commercial lure available
Trogoderma spp. Warehouse beetles	(*Z*)-14-methyl-8-hexadecenal	Sex pheromone produced by females. Commercial lure available
Corcyra cephalonica Rice moth	Farnesal: (*E,E*-3,7,11-trimethyl-2,6,10-dodecatrienal)	Sex pheromone produced by males. Commercial lure available
Ephestia cautella Tropical warehouse or almond moth	ZETA: (*Z,E*-9,12-tetradecadienyl-acetate) and *Z*-9-tetradecenyl-acetate	Sex pheromone produced by females. Commercial lure available
E. elutella Warehouse moth	ZETA: (*Z,E*-9,12-tetradecadienyl acetate) and ZETOH: (*Z,E*-9,12-tetradecadienol)	Sex pheromone produced by females. Commercial lure available
E. kuehniella Mediterranean flour moth	ZETA: (*Z,E*-9,12-tetradecadienyl acetate)	Sex pheromone produced by females. Commercial lure available
Plodia interpunctella Indian meal moth	ZETOH: (*Z,E*-9,12-tetradecadienol), ZETA: (*Z,E*-9,12-tetradecadienyl acetate), and *Z,E*-9,12-tetradecadienal	Sex pheromone produced by females. Commercial lure available

of many compounds are causing concern. Hence, the more toxic substances have largely been replaced and the use of the remaining materials is being confined to application to surfaces or areas where subsequent contact with food or packaging is unlikely, thus avoiding the problem of chemical transfer to the food.

Insect Growth Regulators

In recent years the focus has been on developing compounds of highly specific action, based on the physiology of the pest. In this area, chemicals that act by disrupting insect life cycles have been developed. Insect growth regulators have come into use for the protection of many stored products such as grain. Methoprene, fenoxycarb, and hydroprene are commercially available juvenile hormone agonists. These cause the terminal disruption of insect development but have little or no mammalian toxicity. Their use in admixture on grain or on surfaces such as fabrics can confer protection against pests for over a year.

A second group of insect growth regulators, effective against Lepidoptera, act by interfering with the molting

hormone ecdysone with consequent prevention of normal metamorphosis. A third group, effective against cockroaches, act by inhibiting the synthesis of chitin, which also prevents normal molting of immature stages. Besides the very long life of the compounds, which can be an issue in international trade if residues are detected, another constraint for the use of insect growth regulators has been in integrated pest management programs where economically important biocontrol agents may be adversely affected.

Insecticides and Repellents

The use of insecticidal sprays and dusts has been a routine measure for spot treatment of localized infestations and surface application to areas of high risk. Organophosphorus and pyrethroid compounds remain in use for this purpose though registrations on some compounds are lapsing in many countries, restricting the choice available. Much effort is being placed on the search for new insecticidal compounds of botanical origin and some such as azadirachtin from the neem tree have joined with pyrethrins as registered botanical insecticides. The bacterial metabolite-based product, spinosad, is also now commercially available as a dust formulation. Dichlorvos space sprays have now been replaced by ultra low-volume (ULV) or aerosol treatments of synergized pyrethrins or pyrethroids in food production facilities, sometimes in mixture with an insect growth regulator such as methoprene, but are only effective against flying insects. The field of insect repellency is one still under investigation, a nontoxic, nonspecific insect and mite repellent being the goal.

Fumigants

For many years fumigants have been relied on for the whole site treatment option when infestation problems get out of control. Flour mills and chocolate factories would typically rely on an annual fumigation by a licensed company with registered pest control operators. To be effective the fumigant had to be suitable for rapid and even distribution throughout the treatment area and in order to minimize production downtime it had to be effective against pests within 24 h. The first fumigant in widespread use for treatment of structures, hydrogen cyanide, was replaced in the 1960s by methyl bromide, which, though less of an acute toxic risk to operators was still a highly toxic compound. It was nevertheless extremely effective when used in a well-sealed structure, being an excellent penetrant of voids containing food residues and highly toxic to all pests, achieving control within 24 h.

Methyl bromide, listed as an ozone-depleting compound under the Montreal Protocol in 1992, was phased out from all but a few specialist uses in non-Article 5 (developed) countries in January 2005. Developing countries can continue using methyl bromide until 2015, beyond which their use also will be confined to a few quarantine-related circumstances. The only other fumigant widely registered at the start of this century was phosphine, which is an excellent fumigant for commodities in store where the longer residence times permit the long exposure periods (up to 3 weeks at 15 °C) required for effective control of pests. The gas is released from aluminum or magnesium phosphide formulations on contact with atmospheric moisture. Phosphine is, however, difficult to use in food processing premises because of its corrosive properties against electronic equipment and the long exposure times required, especially at temperatures below 25 °C.

Sulfuryl fluoride (trade name Profume), has been registered for use instead of methyl bromide in empty flour mills, starting in Switzerland in 2003, and now in many European countries, Australia, the US, and Canada, but concerns over its global warming potential and the significance of fluoride residues has limited registrations for use on food materials or in structures where raw or processed food is present. Its use also requires additional heating as insect eggs are tolerant and would otherwise require long exposure times for control. With increasing pressures for the safe and effective use of chemicals, any move away from heavy reliance on them is obviously desirable.

Physical Control Methods

There are opportunities and limitations for the use of physical control methods in structures. For example, the use of MA techniques for space treatments is restricted to specialist chambers because while buildings can be sealed sufficiently for fumigation, they cannot be sealed to the much higher standard required for MA applications (very high CO_2 or very low O_2 levels). Scope for use of sonic, microwave, or radiation technologies is also very limited. Nevertheless, several physical methods are of value in the controlling of pest outbreaks.

Heat

For the food processing industry, the downtime and production loss arising from whole site treatments to combat pest problems has restricted control options to those which act most rapidly and effectively. Heating to 47 °C or above results in rapid immobilization and death of insect and mite stages within a few hours. Heat is thus one of the few options offering a similar rate of action to fumigation with methyl bromide. The principal problem for heat disinfestation, though, is the planning of heating requirements and heat source deployment to obtain a uniform heat profile throughout the structure without causing high localized temperatures, which would cause damage to structural or electronic components. The temperature of air from heaters needs to be limited to 65–70 °C to avoid activating sprinklers or causing expansion and cracking; and air speeds should not exceed 5 m s^{-1} to avoid dust explosions. Structural heat treatment involves raising the building temperature to 50–55 °C at a rate of 5 °C h^{-1}. Sufficient heaters to ensure that 50 °C is reached within 6–8 h are required. Spot heat treatments may also be carried out where a zone of a processing facility or an item of machinery is heated to above 50 °C with a forced hot air stream.

Much progress has been made using a combination of heating strategies, often in conjunction with the use of inert dusts to treat areas difficult to heat such as voids and cracks, a procedure first tested in Canada and further developed in Europe. Residual infestations in deep-seated harborages in the basement or elsewhere remain a particular problem. It must be remembered that the target temperatures for control must be reached at the point where the insects reside in the

structure, a process that may take 24 h, and that the presence of protective material such as food residues can lower the temperature experienced by the insect.

Cold

The intense periods of winter cold have long been used by millers and warehouse keepers in Canada and the Northern USA for a freeze-out of pests, and there is seldom any need for additional control methods in the first few months after treatment. Cold can also be used as a spot treatment by the injection of liquid nitrogen into confined spaces such as wall voids. However, insulation in walls can affect cold distribution, leaving protected warm spots. Also, surfaces can be stained and warping of wooden structural components may occur.

Most insects succumb to exposure at temperatures below − 10 °C within a few days whereas below 10 °C insect reproduction ceases and population levels of most pests decline. The cold resistance of the pest depends on the stage of development: eggs are more sensitive, and adults or larvae, especially those in diapause, are the most cold tolerant. Nevertheless, adults of most species can survive temperatures around 4 °C for many months and so can readily overwinter in buildings in temperate climates. In consequence, cold exposure requires very long holding times to be effective and this is rarely achievable in the production areas of food processing facilities. Nevertheless, the use of designated cold storage areas for incoming ingredients is a widely practiced measure in many industries in spite of the high capital investment required.

Impaction

Many situations in which agricultural products are mechanically conveyed during food processing offer the opportunity for control of insects by shock, abrasion, and impaction. The principle was developed more than 70 years ago for use in the flour milling industry and impaction machines such as the 'Entoleter' became a routine fixture in facilities such as flour mills. In the Entoleter, flour falls between two rapidly spinning disks. Centrifugal force pushes the flour to the edges of the disks where it impacts a row of steel pegs mounted on the rims, and is thrown against the outer steel casing before falling into the basal receiving hopper. The material passing through the Entoleter thus encounters two major impactions and this effectively controls all free-living insect stages. Impaction machines can also kill a high percentage of insects such as weevils developing inside cereal kernels.

Inert Dusts

Inert dusts cover a wide range of materials including clays, sands, ashes, diatomaceous earths (DEs; fossilized remains of diatoms consisting mainly of silica with small amounts of other minerals), silica aerogels and nonsilica dusts, such as phosphate and lime. Inert dusts have a long history of use for grain protection. Their lethal action against pests is caused by dehydration, the cuticular waxes being adsorbed by the desiccant on prolonged contact. Abrasion of the cuticular joints in mobile stages may also be a contributory factor but recent formulations are being designed to minimize their abrasive properties to protect conveying machinery.

Inert dusts are registered in many countries for treatment of grain and pulses against insect pests and for use as sprays applied to the fabric of food premises to minimize residual infestation and migration of pests. They form a useful part of integrated pest management (IPM) strategies providing an alternative to chemical protectants for pest control. Some formulations are accepted as suitable for use on foods certified as 'organic' in some countries. DEs are widely used as food and processing additives.

Irradiation

Irradiation from a cobalt-60 source has been used primarily as a bactericide for many years for treatment of some commodities, mainly spices, and also for dried and fresh fruit, potatoes, onions, and poultry. It requires proximity to a commercial treatment source to be practical and consumer acceptance has limited its widespread use.

Exposure of a commodity to irradiation may be by continuous flow through an irradiator or by batch treatment of cartons by pallet load. A 10-MeV electron beam unit has also been in use for certain applications but the reduced safety concerns are outweighed by the very low penetrability of commodities, restricting the form in which they can be presented for treatment.

Biological Control Methods

Many organisms are known to attack, infect, or parasitize stored-product insects some of which are listed in Table 3. The use of such organisms in food processing facilities is limited by the need to ensure that their presence does not itself lead to problems as discovery of any insect fragments in a finished product is unacceptable. The need for a constant low population level of the host also rules out many applications. Nevertheless, opportunities exist for deployment of parasitoids and predators in receival facilities to deal with background pest levels in empty stores as an alternative to cold storage or fabric treatments with insecticides. Also pathogens can be used in conjunction with attractants to provide a control system for flying pests, or as pest-specific additives to bulk commodities such as cereals.

Use of Pheromones for Population Control

Pheromones can be used to provide the attraction agent for mass trapping to physically remove insects, by disrupting mating to prevent breeding, or by acting as an attracticide to a point where pesticides, pathogens, or sterilizing agents are used as the control agent. The technique is used to reduce pest populations to manageable levels rather than eliminate them and is most suited to confined areas. It is most effective at relatively low starting population densities. Aggregation pheromones are more effective than sex pheromones because both sexes are attracted to the traps. Nevertheless, mass trapping has been successfully trialed with sex pheromones against moths in flour mills to reduce pest populations to a constant low level. The pheromone trap is baited with an insecticide such as cypermethrin or another quick knock-down agent or arrestant to retain the attracted moth. Alternatively, a

Table 3 Potential biocontrol agents and their possible target food pest species

Parasite/predator/pathogen	*Description*	*Host species/prey*
Anisopteromalus calandrae (Howard)	Pteromalid wasp, endoparasite attacking larvae	*L. serricorne*, *R. dominica*, *Sitophilus* spp.
Choetospila elegans Westwood	Pteromalid wasp, endoparasite attacking larvae	*L. serricorne*, *R. dominica*, *Sitophilus* spp., *T. granarium*
Dimachus discolor (Walker)	Pteromalid wasp, endoparasite attacking larvae	*S. paniceum*
Lariophagus distinguendus (Foerster)	Pteromalid wasp, endoparasite attacking larvae	*L. serricorne*, *S. paniceum*, *R. dominica*, *S. granarius*
Pteromalus cerealellae Ashmead	Pteromalid wasp, endoparasite of larvae and pupae	*L. serricorne*, *Sitophilus* spp.
Zatropus incertus (Ashmead)	Pteromalid wasp, endoparasite attacking larvae	*S. oryzae*
Cephalonomia gallicola Ashmead	Bethylid wasp, ectoparasite attacking larvae	*L. serricorne*, *S. paniceum*
Cephalonomia tarsalis Ashmead	Bethylid wasp, ectoparasite attacking larvae	*Oryzaephilus* spp.
Cephalonomia waterstoni Gahan	Bethylid wasp, ectoparasite attacking larvae	*C. ferrugineus*, *C. turcicus*
Habrobracon brevicornis (Wesmael) and *Habrobracon hebetor* Say	Ichneumonoid (Braconid) wasps attacking larvae	Pyralid moths
Venturia canescens (Gravenhorst)	Ichneumonid wasp attacking larvae	Pyralid moths
Trichogramma cacoeciae Marschal, *Trichogramma evanescens* Westwood, and *Trichogramma pretiosum* (Riley)	Trichogrammatid wasps attacking eggs	Pyralid moths
Acarophenax tribolii Newstead and Duvall	Predatory mite attacking eggs and small larvae	Tenebrionid beetles
Cheyletus eruditus (Schrank) and *Pyemotes tritici* L. Fossat & Montagne	Predatory mites attacking eggs and small larvae	Stored-product beetles and moths other than internal grain feeders
Pyemotes ventricosus (Newport)	Predatory mite attacking eggs and small larvae	Most stored-product beetles and moths
Xylocoris flavipes (Reuter)	Predatory bug	All free-living stages of stored-product beetles and moths
Adelina spp., *Farinocystis tribolii*, *Mattesia dispora*, *Mattesia oryzaephili*, and *Nosema* spp.	Pathenogenic schizogregarines	*C. ferrugineus*, *Oryzaephilus* spp., *Tribolium* spp., *P. interpunctella*
Bacillus thuringiensis and Bacillus cereus	Entomopathogenic bacteria	*L. serricorne*, Pyralid moth larvae
Polyhedrosis viruses	Larval pathogens	Pyralid moths
Beauveria bassiana (Balsamo) Vuillemin	Entomopathogenic fungus	*Sitophilus* spp., *Tribolium* spp., *Oryzaephilus* spp.

pathogen source may be incorporated to disseminate disease through the pest population.

Another approach is to use sex pheromones to disrupt mating. It is achieved by flooding the environment with the sex pheromone of the target species so that mating behavior is disrupted by false trail following and sensory fatigue so that mate location and reproduction is minimized. The dispensers need to release adequate amounts of pheromone over a prolonged period and treatments need to be applied before emergence of the target species over a wide area for successful results.

IPM

IPM is a pest risk-management approach combining a selection of the methods described above in a way that addresses socioeconomic, health, and environmental risks in a sustainable manner while maintaining an acceptable level of productivity. It is highly information based, integrating knowledge about the pests with knowledge about the facility to avoid pest problems and maintain high product quality. For successful implementation, adequate training of industry staff on the tools employed is necessary. In many cases pest management is contracted out by companies to a registered pest control company with specialist trained staff, but for any management strategy to work the minimum requirement is that a weekly inspection of facilities, and particularly trapping and baiting locations, is carried out and coupled with a clearly defined course of action if there is evidence of pest presence.

Emerging Threats for the Successful Maintenance of Pest Management

The big issue regarding the continued successful use of chemicals for control of stored-product pests is the development of resistance. Pests have become resistant to insecticides, insects growth regulators, fumigants such as phosphine and even to some bacteria-based sprays. The problem is often compounded by cross resistance to other groups of compound.

Adoption of alternative strategies that avoid chemical control tends to be costly and labor intensive. This places a burden on the manufacturer that cannot always be passed on

to the consumer and can result in lower standards of pest management than when chemicals were in wider use. A related effect that is often overlooked is that the reduced market for chemicals results in products being withdrawn from the market, particularly when an existing compound comes up for regulatory review on a prefixed timetable. Product registration is required in most countries for each chemical intended for use in pest control. Significant efforts have to be undertaken by commercial companies to conduct research, assemble, and submit a registration package to obtain a label for legal use of a new compound or to extend the use of one that is under review. The registration process is very costly with lengthy delays and requires that the company developing the product has the relevant technically qualified personnel. Applications are often returned with requests for more data, increasing the expenditure. Where the company can only see a small market in a particular country or application, they are unlikely to proceed with registration. This can result in the disappearance of existing compounds from the market, reducing the options for pest control.

Although some problems remain, pest management standards in the food industry have never been higher and research is actively in progress to keep abreast of developments as new products and procedures, and new pests, come into being.

See also: Food Safety Assurance Systems: Building Design; Food Safety and Quality Management Systems; Hygienic Design of Equipment; Investigation of Incidents in Industry; Management of Allergens in Food Industry; Management of Supplier and Raw Material. Food Technologies: Food Irradiation; Packaging. Hazards of Food Contact Material: Food Packaging Contaminants. Pesticide Residues: Dithiocarbamates; Organochlorines; Organophosphates and Carbamates; Pyrethroids. Public Health Measures: Challenges of Developing Countries in Management of Food Safety; Challenges of Industrialized Countries in Food Safety Management; Food Inspections and Enforcement Systems; Modern Approach to Food Safety Management: An Overview; Monitoring of Contaminants. Safety of Food and Beverages: Cereals and Derived Products; Coffee, Tea and Herbals, Cocoa and Derived Products; Nuts; Packaging Material and Auxiliary Items; Spices and Seasonings

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Personal Hygiene and Employee Health

ECD Todd, Ewen Todd Consulting LLC, Okemos, MI, USA

Glossary

Antiseptic A substance that inhibits the growth and development of microorganisms, typically used as a topical agent for application to the skin.

Asymptomatic shedders Food employees who do not exhibit the symptoms of foodborne illness, but excrete a pathogen; these are typically only identified during a foodborne illness outbreak investigation through microbiological testing.

Food workers Individuals who harvest, process, prepare, and/or serve food, i.e., across the whole food chain to retail/food service; this category is broader than that of 'food handlers', who typically work in food-service establishments, typically foodservice; however, the two terms are used interchangeably in the literature.

Nasopharyngeal secretions Secretions from the area of the upper throat that lies behind the nose.

Oropharyngeal secretions Secretions from the area of the throat that lies behind the mouth.

Food Workers as Cause of Enteric Illness

Outbreaks Contributed by Food Workers

Outbreaks of foodborne illness involving infected food workers in many food-service settings have been widely reported, with some resulting in many cases and deaths. A recent review of such outbreaks by Greig et al. (2007) and Todd et al. (2007a, b, 2008a, b, 2009) shows that food workers have been responsible for foodborne disease outbreaks for decades, and there is no indication that these are diminishing. These authors collected 816 reports comprising 80 682 cases from events spanning 1927 to the first quarter of 2006, with most of these occurring in the last three decades. Most of the outbreaks reviewed were from the US, Canada, Europe, and Australia, with relatively few from other parts of the world, indicating a skewed set of data because of the availability of relevant information in the published literature or through personal contact. These outbreaks were caused by 14 agents: norovirus, Norwalk-like viruses or probable norovirus (338), *Salmonella enterica* (151), hepatitis A virus (84), *Staphylococcus aureus* (53), *Shigella* spp. (33), *Streptococcus* Lancefield groups A and G (17), and the parasites *Cyclospora*, *Giardia*, and *Cryptosporidium* (23). It appears from these data that the frequency of streptococcal, staphylococcal, and typhoid outbreaks has diminished over time and those involving hepatitis A virus (HAV) have little change, but those with norovirus and maybe nontyphoidal *Salmonella* is increasing. Norovirus terminology changed over the time of the reporting of the 816 outbreaks and it is only in the more recent years that the agent could be definitively be called norovirus; thus, many older reports used terms such as Norwalk or Norwalk-like viruses (called here probably norovirus). Table 1 shows the main agents implicated in food worker outbreaks and their frequency as the cause of these outbreaks. The outbreaks involving bacterial

Table 1 Pathogens identified in outbreaks where food workers have been implicated

Agents	*Frequency of worker infection leading to outbreaks*
Bacterial	
Campylobacter jejuni	Rare
Escherichia coli O157:H7 and other VTEC/STEC	Rare
Salmonella spp (nontyphoidal)	Frequent
Salmonella Typhi/Paratyphi	Occasional
Shigella spp.	Occasional
S. aureus	Frequent
Streptococcus pyogenes Group A	Rare
V. cholerae	Rare (in most countries)
Yersinia enterocolitica	Rare
Viral	
Hepatitis A virus	Frequent
Norovirus	Frequent
Rotavirus	Rare
Parasitic	
Cryptosporidium parvum	Rare
Cyclospora cayetanensis	Rare
Giardia lamblia	Rare

Abbreviations: VTEC, verotoxin-producing *Escherichia coli*; STEC, Shiga toxin-producing *Escherichia coli*.

pathogens were typically associated with potentially hazardous foods that allowed growth. Specifically, multiple foods and multi-ingredient foods were identified most frequently with outbreaks, perhaps because of more frequent hand contact during preparation and serving.

In some cases, the worker may have been a victim rather than the cause of the infection, becoming ill at the same time as the customers or later. In other situations, the worker blatantly disregarded normal hygienic practices, which may have been a result of inappropriate individual actions or the accepted way of doing business in the establishment. Practices leading to these actions have previously been documented, such as workers being asymptomatic and excreting the pathogen unknowingly while working, or they continued to prepare food when it is obvious to them, and sometimes others, that they were ill with a high probability of contaminating food. This seems to be in contrast to the 1988 World Health Organization study conducted by an international working group, which concluded that asymptomatic carriers of nontyphoidal *Salmonella*, *Shigella*, *Vibrio cholerae*, and enteric viruses pose only minimal risks as long as good hygiene is practiced (*55*), although it is recognized that proper handwashing does reduce the microbial load on fingers and palms.

Some of the outbreaks were very large; 11 involved more than 1000 persons, 4 with more than 3000 ill. The larger outbreaks tended to be extended over several days with a continuing source of infections, such as at festivals, resorts, and community events, or the contaminated product had been accessed by a large number of customers. There were five outbreaks with more than 100 persons hospitalized, with attack rates ranging from 9.9% to 100%. However, overall, the hospitalization rate was low (1.4%), and deaths were rare (0.11% of the 80 682 cases). Many of the deaths were associated with high-risk persons (i.e., those who had underlying diseases, malnutrition, or both, as in a refugee camp, or young children), but a few occurred with apparently healthy adults.

Food Operations and Foods Implicated

An analysis of the settings for the food worker-related events showed that most of the outbreaks came from food-service facilities (376 outbreaks (46.1%)), followed by catered events (126 outbreaks (15.4%)), the home (83 outbreaks (10.2%)), schools and child care centers (49 (6.0%)), and health care institutions (43 outbreaks (5.3%)). However, many cases resulted from relatively few outbreaks (<30 each) associated with community events (9726), processing plants (8580), mobile/temporary service (5367), and camps/armed forces (5117). The single most frequently reported setting was restaurants, with 324 outbreaks and 16 938 cases. Improper hygienic practices in homes, on picnics, or at community events accounted for 89 of the 816 outbreaks. Case numbers in outbreaks in homes or at community events are probably underestimated because they are less likely to be reported than those involving commercial establishments. Sixteen outbreaks occurred where food, primarily produce, was harvested and shipped from one country to another.

Sometimes the presence of an infected worker preparing food was only one of several factors contributing to the outbreak. Large outbreaks frequently occurred because of the continual exposure of large groups to a pathogen, either because the source had not been identified soon enough or because control measures had been insufficient to eliminate the agent, such as at refugee camps or large outdoor events. However, in several other large outbreaks, the amount of contaminated food was so great that thousands of persons were exposed by the same batch of food; this occurred with frosting on cakes, imported raspberries used in a variety of dessert dishes, and items served at large receptions or commissaries. The agents in large outbreaks also tended to be highly infectious, such as *Shigella* or norovirus. Because ready-to-eat (RTE) foods are not further processed or cooked, subsequent contamination by infected food workers frequently led to outbreaks. These included produce and baked goods, as well as beverages that would not normally allow the growth of pathogens. However, many of these were of viral origin with sufficient particles to cause an infection without further multiplication. Food-service outlets, such as restaurants, and catering companies, and schools that served large numbers of patrons, were the most frequent settings implicated. However, episodes linked to bakeries, hospitals, camps, homes, and church meals highlighted the necessity for those who prepare and serve meals in these operations to excuse themselves from food preparation if they are ill or exposed to infected individuals. There were 18 outbreaks associated with commercial travel in air flights, trains, and cruise ships over several decades, although only the last seems to be a major concern today. Because the patrons have limited choices in these carriers and are confined within their means of transportation, the impact of illness is often quite dramatic and newsworthy. However, the role of the infected food worker in outbreaks on cruise ships is difficult to determine, because the passengers may be as much a source of infectious agents, such as norovirus, as the crew members.

Factors Contributing to Outbreaks

The most frequently reported factor associated with the involvement of the infected worker was bare hand contact with the food, followed by failure to properly wash hands, inadequate cleaning of processing or preparation equipment or utensils, crosscontamination of RTE foods by contaminated raw ingredients, and (for bacterial pathogens) temperature abuse. Many of the workers were asymptomatic shedders or had infected family members and/or used improper hygienic practices. Outbreaks were sorted into categories based on how many workers were implicated, the origin of the infective agent (outbreak setting or off site), the degree of certainty that the workers were the cause or were victims, whether or not the workers denied illness, the ability of the agent to grow in the food, whether only the workers and not the patrons were ill, and whether patrons were more responsible for their illnesses than were the workers. The most frequent scenarios were (1) a single worker causing an outbreak by directly infecting patrons; (2) an infected worker fecally contaminating foods that were then temperature abused, leading to an outbreak; and (3) multiple workers linked to an outbreak, but with no clear initiating source.

Sources of Pathogens

These include vomitus, diarrhea, and nasopharyngeal or oropharyngeal secretions, often being transmitted to food or food contact surfaces. The likelihood that workers cause illness in patrons and fellow workers depends on several factors: the numbers of organisms required to initiate an infection, the site of colonization, and the length of their carriage in infected persons. Pathogens of nose, throat, skin, or fecal origin are most likely to be transmitted by the hands, as hands are the parts of the body that frequently touch the mouth, skin, and anal areas. The pathogens most likely to be transmitted by food workers are norovirus, HAV, *Salmonella*, *Shigella*, and *S. aureus*. Unfortunately, such pathogens can be in high numbers in or on the body during an infection. This is particularly true for intestinal infections, where levels can reach 10^{11} infectious cells or particles per gram of feces, although 10^5–10^9 g^{-1} is more frequent. Some pathogens appear to be able to infect at doses as low as 1–100 units, including viruses, parasites, and some bacteria. Although parasitic foodborne episodes of illness are rare, the dose for *Cyclospora*, *Cryptosprodium*, and *Entamoeba* may be as low as one cyst/oocyst. Based on outbreak data and other infectious disease studies, other pathogens with low minimum infections doses are *Campylobacter*, *Escherichia coli* O157:H7, *Salmonella* Typhi (and a few other *Salmonella* serotypes), *Shigella dysenteriae*, HAV, norovirus, and rotavirus (<100 cfu or particles). Interestingly, only rarely have *Campylobacter*, *E. coli* O157:H7, and other *E. coli* serotypes been implicated in food worker associated outbreaks. Infectious dose data for pathogens transmitted by food workers and implicated in outbreaks are shown in Table 2.

Table 2 Infectious dose data for pathogens implicated in outbreaks transmitted by food workers

Pathogen	*Infectious dose from volunteer studies (cfu/viral particles)*	*Infectious dose from outbreak data and estimates (cfu/viral particles)*	*Levels in outbreak food samples (cfu/g or ml)*	*Pathogen implicated in food worker outbreaks*
Campylobacter jejuni	10^3			Rare
Enteropathogenic *E. coli* (EPEC), Enterotoxigenic *E. coli* (ETEC) Enteroaggregative *E. coli* (EAEC)	10^6–10^{10}	$>10^2$ (estimated)		Unknown or rare in developed countries, maybe more frequent in developing countries
Enteroinvasive *E. coli* (EIEC)		10^0–10^3 (estimated)		Unknown, maybe in developing countries
Enterohemorrhagic *E. coli* (EHEC)		10^0–10^2 (estimated)		Occasional
E. coli O157:H7		10^0–10^4	0.04–93	Occasional
E. coli O111			0.1	Not known
Salmonella Typhi	10^5–10^9	10^1–10^3 (estimated)		Occasional but declining
Salmonella spp. (nontyphoidal)	10^5–10^9	$<10^1$–10^{11}	0.03–$10^{4.5}$ mostly low	Frequent
S. dysenteriae	$<10^1$–10^4	$\geq$1(estimated)		Probable in developing countries
Shigella flexneri and *S. sonnei*	10^2–10^3			Occasional
S. aureus		$<$1–5 μg enterotoxin produced with $\geq10^5$ cells	10^5–10^8	Frequent
S. pyogenes (Group A)		$\leq10^3$ (estimated)		Occasional
Streptococcus Group D		$>10^7$ (estimated)		Rare
Vibrio cholerae O1 and O139	10^3–10^{11}	10^2–10^3		Likely in endemic areas
V. cholerae non-O1	10^6–10^9	10^6–10^{11} (estimated)		Not known
Y. enterocolitica	10^9	10^2–10^9 (estimated)		Occasional
Hepatitis A virus		10^1–10^2 (estimated)		Frequent
Norovirus	$\leq10^4$–10^8	10^1–10^2 (estimated)		Frequent and increasing
Rotavirus	10^1–10^4	10^1–10^2 (estimated)		Occasional
Cryptosporidium parvum	10^2–10^3 oocysts	10^0–10^1 oocysts	$<10^2$ oocysts	Rare
Cyclospora cayetanensis	Unknown (up to 10^4 oocysts did not cause symptoms)	Assumed to be low		Not known but probable
Entamoeba coli	1 cyst	1 cyst (estimated)		Not known, but probable
Giardia lamblia	10^1–10^2 cysts	1 cyst (estimated)		Rare

Source: Based on Todd et al. (2008a, b), with permission from International Association for Food Protection.

Table 3 Infection characteristics of foodborne pathogens transmitted by infected food workers

Pathogen	*Incubation period (days)*	*Duration of illness (days)*	*Carriage rates reported (%)*	*Postsymptomatic shedding (days)*	*Percentage of individuals who excrete the pathogen but are asymptomatic*
C. jejuni	2–5 (1–10)	2–10	0–38% of GI[a] cases, 0.5–0.9% asymptomatic controls	2–9 weeks; if untreated, relapses occur in 20% of patients	0.1–77
Enterohemorrhagic *E. coli* (STEC[b], VTEC[c]); *E. coli* O157:H7	1–10	5–21	0.0–0.6% (verotoxin in 2.1% of GI cases), 0.0% of asymptomatic controls	<10–62	Up to 53
E. coli non-O157 VTEC/ STEC			0.2–8.6% of GI cases, 0.5–1.0% of controls		0.2–1.2
Enteroaggregative *E. coli* (EAEC)			2.9–15% of GI cases, 0.5–1.8% of asymptomatic controls		2–9
Enteroinvasive *E. coli* (EIEC)	0.5–3	3–>7	0.4–1% of child diarrheal cases		
Enteropathogenic *E. coli* (EPEC)	0.5–3	3–14	0.1–20.7% of GI cases		1.2 (children)
Enterotoxigenic *E. coli* (ETEC)	0.5–3	3–14	0.6–32% of GI cases, 0% of asymptomatic controls		1.6–2.5 (children)
Salmonella Typhi/ Paratyphi	Typhi: 4–60 Paratyphi: 1–10	Many days to weeks	37% with typhoid fever symptoms; 4% chronic carriage, higher in older age groups	>7 years; more women than men as chronic symptomatic carriers occur, more women than men	
Nontyphoidal *Salmonella*	0.5–3	3–5	• 0.0–16% of GI cases • 0.2–4.6% of asymptomatic controls	4 weeks–12 months (up to 22 weeks in infants), 0.5% of asymptomatic controls excrete at 12 months	0.23–18.7

Shigella spp.	0.5–6.1	2–90	0–52% of GI cases, 0.0% of asymptomatic controls	Up to 17 months	
S. aureus	0.5–8 h,	1–2	0.1–0.4% of GI cases, 0.1–0.2% of controls (fecal counts $>10^6$/g)		
S. pyogenes				21–60	20–65
V. cholerae 01	0.25–5	3–9	33% of GI cases	2 weeks–7 months	4
V. cholerae non-01	5.5–96 h	3.5–48 h			4
Y. enterocolitica	1–11	1–3 weeks	0.1–3.4% of GI cases, 2.4–3.1% of controls (*Yersinia* spp.)	Intermittent carriage by asymptomatic children up to 14 weeks with pathogenic strains	0.2–6% with pathogenic strains
Hepatitis A virus	10–50	2–3 weeks (months)	9.7/100,000	21–6 months	8.2
Norovirus (Caliciviruses Norwalk-like virus)	0.6–77	0.5–3.5	0.1–6.8% of GI cases, 0.2% of asymptomatic controls	2–>14	<2 weeks
Rotavirus	0.5–6	4–8	0.2–0.3% of GI cases, 0.0% of asymptomatic controls (up to 83% of child diarrheal cases)	≤2 weeks	0.7–48
Cryptosporidium spp.	2–22	Weeks–months	0.4–13.% of GI cases, 0.0% of asymptomatic controls (higher for immunodeficient persons)	≤2 months	0.1–71%, 15–99% with past infections
Cyclospora cayetanensis	1–14	Weeks–months	0.0–6.1%		0.0–94
Entamoeba histolytica	2–3 days to 4 weeks	Weeks– months	0.0–7.4%	Years	0.0–43.7
Giardia lamblia/ intestinalis	1–3 weeks	Days–months/years	0.4–3.8% of GI cases 0.3–0.5% of asymptomatic controls (up to 33.3% of children)	5–41	1.6–% 37%

[a]GI = gastroenteritis.
[b]STEC = Shiga toxin-producing *Escherichia coli*.
[c]VTEC = verotoxin-producing *Escherichia coli*.

Incubation Periods

For ill persons, these can range from a few hours, e.g., *S. aureus* enterotoxin, to many weeks, e.g., HAV and *S.* Typhi. The longer the incubation period, the more opportunities there are that an infected person will excrete the pathogen. This is equally important for worker's contact persons, mostly likely the family or fellow workers, who may be the persons initially infected and excreting. The duration of illnesses is important too. Gastroenteritis symptoms may last many days or even weeks or months, e.g., chronic diarrhea, as in cases of infection with *Salmonella* Typhi, *Shigella* spp., HAV, and the protozoan parasites. Because employees want to return to work quickly after illness, and they do not usually receive paid sick leave, they may work while continuing to be ill or only having mild symptoms like occasional diarrhea, without reporting their conditions to management. Postsymptomatic long-term shedding can also occur with *Campylobacter, Salmonella, Shigella, V. cholerae, Yersinia*, enteric viruses, and parasites. Characteristics of foodborne pathogens transmitted by infected food workers with incubation period, duration of illness, carriage rate, and asymptomatic shedding are shown in Table 3.

Pathogen Survival

The soil matrix, relative humidity, and temperature all influence pathogen survival. Declines can be rapid on hands, but most pathogens that cause foodborne illness survive long enough on hands and contact surfaces to allow some transfer to food or fellow workers during a shift. *Salmonella* can survive for several hours on fingertips if they are not washed. Nonenveloped viruses such as norovirus, rhinovirus, and enterovirus are more stable on skin than are viruses with envelopes, such as the influenza virus.

All this information indicates that the risk of workers hands becoming contaminated and transferring them to food or food contact surfaces may not be unusual, and it is incumbent on these workers and their managers to develop good hygienic practices and continually monitor them during food production and preparation. Survival of pathogens and other enteric microorganisms on finger tip skin and different food contact surfaces is shown in Table 4.

Personal Hygiene and Employee Health

Barriers to Contamination

Barriers to contamination of food by food workers include glove use, and hand hygiene and its compliance, which have been reviewed by Todd et al. (2010a–e). Hands can carry billions of enteric bacteria or viruses (up to 10^9), especially if the individual is ill, caring for a sick person, changing diaper, or handling raw foods of animal origin. Thus, multiple barriers to limit transfer of pathogens to foods are essential. There are both physical and chemical barriers. Physical barriers include the use of properly engineered building walls and doors to minimize the flow of outside dust particles, microorganisms, and pests to food storage and food preparation areas; food shields (sneeze guards) to prevent aerosol contamination of displayed food by customers and workers; work clothing designated strictly for work, because clothing worn outdoors can carry undesirable microorganisms, including pathogens from infected family members, into the work environment; and utensils, such as spoons, tongs, and deli papers, (thin papers for grasping and weighing deli meats and serving bakery items) to prevent direct contact of hands and the food being prepared or served. Handling money and RTE foods should be two separate operations, preferably carried out by two workers. Chemical barriers include sanitizing solutions used to remove microorganisms, including pathogens, from objects or material used during food production and preparation; and laundering of uniforms, work clothes, and soiled linens. However, laundering, as normally practiced, may not effectively eliminate viral pathogens.

Glove Use is Another Well-Established Barrier

To prevent direct bare hand contact with food and food surfaces, many jurisdictions have made glove use compulsory for food production and preparation; if properly used, gloves can substantially reduce opportunities for food contamination. However, gloves have limitations and may become a source of contamination if they are punctured or improperly used. Experiments conducted in clinical and dental settings have demonstrated occasional pinhole leaks in gloves, and observations after many hours of the same glove use have shown that sufficient damage can occur to pose a risk for contamination of patients by the user. Wearing jewelry, rings, and artificial nails are discouraged, because these can puncture gloves and allow accumulation of microbial populations under them. Although such loss of glove integrity can lead to contamination of foods and surfaces in the food industry, improper use of gloves is more likely to lead to food contamination and outbreaks than leakage. Examples of these are not using gloves for all the required food handling operations, not washing gloved hands as frequently as desired, or allowing accumulated perspiration from the hands (through occlusion trapping of moisture with low oxygen levels, causing skin maceration) to contact food on removal of the gloves. Occlusion, trapping of moisture by the glove on the skin, during long-term glove use in food operations creates the warm moist conditions necessary for microbial proliferation and can increase pathogen transfer onto foods either through leaks and exposed skin or during glove removal. Most importantly, glove use can create a false sense of security resulting in more high-risk behavior that can lead to crosscontamination, especially if employees are not adequately trained.

Exclusions

Some jurisdictions have guidelines for employees working with food. These include being clean with trimmed fingernails, no artificial nails or nail polish, no jewelry, having short hair, and wearing clean uniforms or overalls. Employees are not allowed to work with boils or infected wounds or cuts, but may do so if they have an impermeable cover and a single-use glove over the impermeable cover. The most important set of conditions, but most difficult to supervise, is for an employee

Table 4 Survival of pathogens and other enteric microorganisms on skin and food contact surfaces

Infective agent	*Surfaces*	*Suspending media*	*Log or percentage loss*
Campylobacter	Glass slides and moist cloths	Room temperature	Decimal reduction times: glass, 0.5–24 h
	Poultry		Decimal reduction times: raw and cooked meats at 4 °C, ≥3 days
C. jejuni	Hands	Peptone water with chicken broth and 50% blood	3–7 log loss in 2–45 min
	Dry inanimate surfaces		Up to 6 days
E. coli	Fingertips	Broth, saline, milk	>90% after 5 min >99.9% after 1 h
	Coins, Teflon, glass	Broth	Survived 7, 9, and 11 days on the surfaces of pennies, nickels, and dimes/quarters, respectively; Teflon/glass for 4–7 days
Klebsiella aerogenes	Skin/fingertips	Broth, Ringer's solution	99% loss after 5–10 min
	Contaminated donor fabrics to hands	Broth	0.29% transferred and 66% loss on skin after 5 min
Listeria spp	Dry inanimate surfaces		1 day to months
Listeria monocytogenes	Fingertips	Broth, saline, milk	Loss: 0.45–99% after 15–45 min
Salmonella Typhi	Dry inanimate surfaces		6 h–4 weeks
Salmonella (nontyphoidal)	Dry inanimate surfaces		1 day–years
	Currency notes in Myanmar		*Salmonella*, enterotoxigenic *E. coli*, and *Vibrio* isolated from notes from butchers and fishmongers
	Coins		Survived 1, 2, 4, and 9 days on the surfaces of pennies, nickels, quarters, and dimes, respectively
	Fingertips	Broth	Still present 3 h later
	Formica™ surface, utensils, Teflon, glass	Egg white and yoke	Survived up to 17 days
	Dishcloths	Egg or blood	Survived >1 year
Shigella spp.	Dry inanimate surfaces		2 days–5 months
S. aureus	Glass	Broth diluted with distilled water at room temperature	29–89% loss after 12 h
	Skin of volunteers	Broth diluted with distilled water at room temperature	7–94% loss after 5–12 h
	Fingertips	Broth	99–99.99% loss after 5–90 min
	Dust exposed to different light intensities	Naturally contaminated sweepings from hospital wards	Up to 0.6 log in low daylight, 2 log in sunshine, 0.13–0.64 log in artificial light after 10 days
	Glass cover slip	Serum, water	0.0–95% loss after 100 min, up to 99% loss after 2–3 days
	Dry inanimate surfaces		7 days–7 months
S. pyogenes	Dust exposed to different daylight intensities	Naturally contaminated sweepings from hospital wards	Up to 0.5 loss in low daylight, 1.0 log loss in sunshine after 10 days
	Glass cover slip	Serum, water	0.0–91.6% loss in 100 min, 0.0–99% loss in 2–3 days
	Contaminated donor fabrics to hands	Broth	0.01–0.02% transferred and 38–77% loss on skin after 5 min
	Dry inanimate surfaces		3 days–6.5 months

(*Continued*)

Table 4 Continued

Infective agent	*Surfaces*	*Suspending media*	*Log or percentage loss*
V. cholerae	Dry inanimate surfaces		1–7 days
Hepatitis A virus	Fingertips	Fecal suspension	70–84% loss in 4 h
	Hands	Fecal suspension	50% loss in 5.5–7.7 h
	Stainless steel, copper Polythene, PVC, ceramic tile	Phosphate buffered saline	0.6–3 log reduction after 8 h at 20 °C (survival lowest on copper),
	Stainless steel	Fecal suspension	50% loss in 51–187 min at 20 °C
	Cloth	Phosphate buffered saline, 20% feces, drying for 3–5 h	0.8–1.6 log reduction
	Dry inanimate surfaces		2 h–60 days
Rotavirus	Fingertips	10% fecal suspension	43%, 57%, and 93% loss after 20, 60, and 260 min, respectively
	Aluminum, ceramic tile cloth	Phosphate buffered saline, 20% feces, drying for 3–5 h	0.3–1.2 log reductions
	Dry inanimate surfaces		6–60 days
Norovirus and feline calicivirus	Office surfaces	Feline calicivirus in culture medium with fetal bovine serum	90% loss after 0–4 h on computer keys, mouse, brass, and telephone wire; after 4–8 h on telephone receiver; after 12–24 h on telephone buttons, nondetectable at 72 h
	Metal disk	Feline calicivirus 10% fecal suspension	89% loss after 30 min, still detectable at 7 days
	Ham, lettuce, strawberries	Feline calicivirus 10% fecal suspension	57–99% loss after 30 min, still detectable in ham and lettuce after 7 days, and in strawberries after 5 days
	Dry inanimate surfaces	Norovirus (20% fecal solution) and feline calicivirus (cell lysates)	8 h–7 days
E. histolytica	Nail region of hands	Fecal suspension	Survival up to 45 min
	Fingers and thumbs	Fecal suspension	$\geq$95% in 10 min

Source: Based on Todd et al. (2008a, b), with permission from International Association for Food Protection.

not to have any communicable disease (as determined by gastrointestinal symptoms, and if necessary stool testing). If an employee has such a condition, the person must be excused from work until completely well, which may depend on the working conditions (e.g., 24 or 72 h after symptoms are absent or 7 days after being jaundiced, or with a medical form proclaiming them free from infection).Those working with food for highly vulnerable populations will require stricter control. Unfortunately, routine stool examinations have limited value for exclusion purposes. Asymptomatic-food workers may excrete pathogens without any indication of their health status, and tests rarely detect positive workers; for instance 1.6% of 307 954 workers in military food-service operations in Turkey were positive for enteric pathogens in stool specimens, based on tests mostly carried out on routine periodic examinations. Therefore, an in-depth knowledge of the behavior of workers by supervisors and careful monitoring of employees,for example, frequent visits to the toilet are important to minimize the risk of pathogen transmission.

Hand Hygiene

Personal hygiene, including showering and wearing hair nets, is a necessary component of performing safe food preparation and handling operations. However, many foodborne outbreak investigations have identified the hands of food workers as the source of pathogens in the implicated food. During various daily activities at home and work, hands can quickly become contaminated. Some of these activities increase the risk of finger contamination by pathogens more than others, such as the use of toilet paper to clean up following a diarrheal episode, changing the diaper of a sick infant, blowing a nose, or touching raw food materials. The most convenient and efficient way of removing pathogens from hands is through handwashing. Important components of handwashing include potable water for rinsing, and soaps to loosen microbes from the skin, followed by drying. Handwashing should occur after any activity that soils hands and certainly before preparing, serving, or eating food. Antimicrobial soaps are only marginally more effective than plain soaps at any one wash, but constant use with these soaps results in a build-up of the antimicrobial compound on the skin. The time taken to wash hands and the degree of friction generated during lathering are more important elements for removing soil and microorganisms than water temperature. However, excessive washing and scrubbing can cause skin damage and infections. Drying hands with a towel removes pathogens first by friction during rubbing with the drying material, and then by wicking away the moisture into that material. Paper rather than cloth towels should be encouraged, although single-use cloth towels are present in the washrooms of higher class hotels and restaurants. Warm-air dryers remove moisture from hands by evaporation and any surface microorganisms loosened by washing and vigorously rubbing the hands together; however, these take too long for efficient use. The newer high-speed air blades can achieve dryness in 10–15 s without hand rubbing. An effective handwash should remove most transient and some resident microorganisms and is typically facilitated by the use of soaps, detergents, and antimicrobial compounds. However, hands are never sterile and can become recontaminated if they come into contact with a contaminated surface or infected person as soon as the washing process is over. The overall efficacy of hand hygiene depends on many factors, including soil types, antimicrobial soap strength, or alcohol gel composition. In the food industry, alcohol-based antiseptics should be combined with regular washing of hands and should not replace handwashing and drying, or use of fingernail brushes. Wipes containing alcohol and antimicrobial compounds, as well as moisturizers, wetting compounds, etc., are widely used by the public. These have been shown to be more effective than plain soap and water, and should be considered as a feasible, practical hand hygiene intervention for remote food-service situations or where water availability is limited. Unfortunately compliance for handwashing in health care settings, food operations, and in the general society is not good, and a targeted management plan for hand sanitation in specific food operations needs to be developed and enforced as a part of the corporate culture of the company.

See also: Disciplines Associated with Food Safety: Epidemiology. Food Safety Assurance Systems: Cleaning and Disinfection; Hazard Analysis and Critical Control Point System (HACCP): Principles and Practice; Hygienic Design of Equipment. Food Technologies: Cleaning and Disinfection Technologies (Clean-In-Place, Clean-Out-of-Place). Public Health Measures: Food Safety in Hospitals and Other Healthcare Settings; Foodborne Disease Outbreak Investigation; Management of Food Safety in Food Service Sector; Modern Approach to Food Safety Management: An Overview; Surveillance of Foodborne Diseases

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FOOD SAFETY ASSURANCE SYSTEMS

Cleaning and Disinfection

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Glossary

Cleaning The complete removal of food soil using appropriate detergent, physical methods, and chemicals under recommended conditions.

Disinfection Reduction, by means of chemical agents and/or physical methods, of the number of microorganisms in the environment to a level that does not compromise food safety or suitability.

Food hygiene These are measures and conditions necessary to control hazards and to ensure fitness for human consumption of a foodstuff, taking into account its intended use.

Sanitation It is cleaning and disinfection.

Sanitation program The planned way of practicing sanitation.

Soil Any material in an incorrect location.

Introduction

Cleaning and disinfection, or more precisely sanitation, is multidisciplinary applied science. Multidisciplinary because it incorporates knowledge from many sciences like: microbiology, chemistry, physics, mechanics, etc. Applied because it is the first and the most important line of defense of human health and it is related to environmental factors that are affecting health. Its principal goal is to combine design principles, development, implementation, modification, maintenance, restoration, and improvement of hygienic conditions and practices. As applied science, sanitation is directly connected with biological, chemical, and physical hazards control in food production chain and represents one of the most important tools for assuring safe and quality food products.

Sanitation usually refers to hygienic practices and procedures which are designed to create and maintain clean environment for food production, processing, preparation, storage, and distribution. However, sanitation is much more than cleanliness. Properly done sanitation is improving food products' quality, economics, shelf life, and esthetics. Also, sanitation is improving waste disposal, which is beneficial for the environment.

Speaking of cleaning, there are two types of processes:

1. Wet cleaning and
2. Dry cleaning

Wet Cleaning

Wet cleaning is a cleaning in which water is used. When speaking about the food industry, all food areas and food support areas has to be wet cleaned, except where dry cleaning is otherwise permitted. Increasingly, it is recognized that inappropriate use of water for cleaning in dry product food process environments can increase food safety risk by creating microbiological growth conditions. However, in many countries, government inspectors do not recognize a 'flush' or dry cleaning as providing a sufficient clean break in a production cycle. So wet cleaning usually needs to occur at intervals.

Generally speaking, cleaning and disinfection can be performed in five different stages:

1. Wetting and penetration of cleaning solution in the present soil and cleaned surface.
2. Reaction between cleaning solution with present soil and cleaned surface and facilitation of fats saponification, protein peptidization, and minerals dissolution, and displacement of solid and liquid soils from the cleaned surfaces.
3. Preventing redeposition of dispersed soils onto cleaned surfaces by provision of good rinsing properties.
4. Wetting cleaned surfaces with disinfection solution and its reaction or penetration with/into present microbial cells and completion of microbiostatic or microbicidal action.
5. In some cases (where disinfectant should be rinsed), dispersion of microorganisms from disinfected surface.

Fundamentals

Wet cleaning and disinfection are primarily affected by three factors: soil, surfaces type, and water.

Soil

As stated in the definition, soil is any material in an incorrect location. Examples are: organic deposits inside processing equipment, lubricants on conveyer belts, dust on equipment, etc.

Soil Nature

Soil nature will determine cleaning method, cleaning compound composition, and its concentration. That is why it is very important that personnel involved in cleaning process have a deep knowledge of the nature of the soil to be removed. Food production soils result from food product ingredients and composition and food production process. Regardless of used cleaning and disinfection method, increased mortality among microorganisms is achieved when soil is completely removed from the working surface.

More details about the organic (sugars, fats, and proteins) and inorganic soil (mineral substances) in terms of their solubility, degree of removal, and problems connected with heating are given in Table 1.

However, besides soil with pure composition (only sugars, proteins, etc.), it is very common to find complex soils or films which are combinations of food components, dust, oil, cleaner components, and/or hard water salts. These films solubility properties will depend on combination of different factors such as age, heat, dryness, time, etc.

Table 1 Characteristics of organic and inorganic pollutants

Substance	*Solubility*	*Removal*	*Problems iduced by heating*
Sugars	Soluble	Easy	Caramelization Hard for cleaning
Fats	Insoluble in water Soluble in bases	Partly hard	Polimerization Hard for cleaning
Proteins	Insoluble in water Soluble in bases and partialy in acids	Very hard	Denaturation Very hard for cleaning
Minerals	Solubility in water varies Soluble in bases	Easy and hard	Generaly not significant

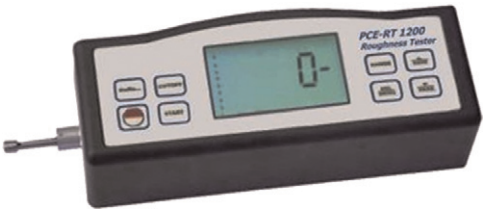

Figure 1 Surface roughness tester.

Soil Quantity

The other important factor affecting cleanability is soil quantity. The most important factors affecting soil buildup are improper hygienic design and cleaning practices. That is why in order to remove soluble soil, it is very important to rinse food contact surfaces before cleaning. Also, heavy deposits require more mechanical energy and detergent to be removed.

Surfaces Type

Surface materials have very big influence on cleaning compound selection and cleaning effectiveness evaluation. Surface characteristics are:

- *Surface composition*: Food industry equipment contact surfaces are constructed from many different materials as: plastics, rubber, metal, marble, wood, etc. Most preferred surfaces are constructed metal or more specific – stainless steel. For highly corrosive products, high corrosion metal such as titanium is often used.
- *Surface finish*: Equipment product contact surfaces should have a finish of an acceptable roughness. According to European Hygienic Engineering and Design Group Doc. 8, large areas of product contact surface should have a surface finish of 0.8-μm Ra, or better. Surface finish can be determined by surface roughness tester (Figure 1) or surface roughness comparator (Figure 2).
- *Surface condition*: Product contact surfaces must be free of imperfections such as crevices, pits, racks, corrosion, etc. These kinds of surfaces are very difficult for cleaning and disinfection. To avoid this kind of defects, personnel responsible for cleaning should be educated and trained how to use and handle surface materials.

Water

Water is the most common solvent for cleaning and disinfection agents and is used both for start and final rinsing. It is a basic ingredient of all wet cleaning systems. Water is considered as an active ingredient that adds to the detergency of cleaners. It performs several very important functions such as being solvent of ionic compounds (salts and sugars) and

Figure 2 Surface roughness comparator.

fats (if used temperature is above its melting point) and can even be abrasive agent (if high pressure cleaning is used). Also, water aids in the suspension and antiredeposition of soils. Once the soil has been dissolved and emulsified away from the surface, water keeps the soil suspended away from the clean surface so that it can be carried away easily during the rinsing process. It is clear that without water, cleaning formulas would be much less effective.

Therefore, microbiological and chemical quality of water is very important. In principle, water used for cleaning should have drinking water quality. Ideally, food processing establishments should use pure water, but there is no ideal water supply. Therefore, cleaning compounds used have to be customized for the individual water supply and type of operation.

Water impurities have adverse effects on cleaning. For example, soluble manganese and iron salts concentrations more than 0.3 ppm will result in appearance of colored deposits on working surfaces.

Besides water's purity and potable quality, it is very important to know the hardness of water. Hard water contains large amounts of calcium and magnesium salts. If hard water is heated, the result will be sedimentation of calcium and magnesium salts. This property is also common for some cleaning agents, especially for basic ones.

Moreover, hard water is reduces detergents effects and creates scale. Besides unpleasant appearance, scale is unwanted because it:

- Harbors microorganisms (contains and protects them);
- Reduces transfer of heat energy, this can result in insufficient cooking, pasteurization, or sterilization; and
- Stimulates corrosion.

Scale formation can be reduced with addition of chelating or sequestering agents, which bind calcium and magnesium in insoluble complex. However, water softening is highly recommended because soft water is ideal for general food plant cleaning in terms of savings and effectiveness. This can be done with ion exchangers, which are replacing calcium and magnesium with sodium ions and thus forming soluble salts. Latest, and also the most expensive, water softening method is reverse osmosis. However, in some food processing such as canning of certain vegetables, a degree of hardness is often preferable.

One of the important water properties for cleaning and disinfection is its pH. Usually, pH of water ranges from pH 5 to 8.5 and there are no serious consequences to most detergents and sanitizers. However, if water is highly alkaline or highly acidic, addition of buffering agents is required.

Cleaning

The primary function of cleaning is to remove the food deposits, microorganisms, foreign bodies, etc. Under normal conditions, however, the removal of microorganisms and spores is not complete and that is why the cleaning has to be followed by disinfection.

In the preparatory phase, the production facility is cleaned from all residues, spillings, etc. Machines, conveyers, etc. are dismantled in a way which allows exposure of everything that is likely to collect microorganisms. Electric installation and all other sensitive systems should be covered and protected from contact with water and/or chemicals. At the start of the cleaning, pouring of water on the floor and machines should be avoided (nevertheless, whether it is pressurized water from a hose or water from water containers) before all food products are removed.

Before using cleaning chemical, all food and other types of residues should be removed by brushing, rubbing, etc., and then all the surfaces should be washed with water. Use of cold water is recommended because hot one can caramelize sugars and coagulate proteins and can create invisible film which does not allow full cleaning and represents potential niche of microorganisms and produces unpleasant odors.

After this, process of cleaning should be inspected and cleaning should be recorded.

Cleaning Agents

Efficient cleaning can be performed only if appropriate detergents or cleaning agents are used. The ideal detergent should:

- Possess enough chemical energy to dissolve the material to be removed.
- Possess small surface tension which allows penetration in all crevices and indentations. It should break down waste materials and maintain them in a state of suspension.
- Be capable to perform water softening, which prevents scale formation, if used with hard water.
- Be rinsed freely from the facility.
- Leave no chemical residue on the equipment.
- Cause no corrosion and other harmful effects in the facility.
- Be not hazardous for people.
- Be appropriate for the specified cleaning procedures.
- Be easily dissolved in water, and their concentration should be easily checked, if they are in solid state.
- Be in accordance with existing legal requirements regarding safety and environment.

All cleaning methods including foam and submersion require adequate contact time.

It has to be said that there is no detergent with all listed features. Therefore, for different types of equipment and production, detergent that contains most of the above mentioned properties or their combinations, which will serve best for their intended purpose, should be used. A cleaning solution or detergent is blend composed from a number of components or more precisely, from water as a solvent with:

- Surfactants,
- Alkaline agents,
- Acid agents, and
- Chelating (sequestering) agents.

Surfactants are usually organic compounds with amphipolar nature, i.e., they are composed of both hydrophobic or lyophilic (nonpolar) tail and hydrophilic or lyophobic head. According to the composition of their head, they are classified as: nonionic (no charge groups in its head), anionic (negative

charge), cationic (positive charge), and zwitterionic (a head with two oppositely charged groups). Most common surfactants are anionic and nonionics. Surfactant's amphipolar nature facilitates cleaning by reducing surface tension and fat's emulsification. When surfactant is added on the water surface, its polar heads disrupt water's hydrogen bonds, which lead to reducing water surface tension and allow water droplets to collapse and wet the cleaning surface. This so-called 'wettability' enhances penetration in present soil and surface, regardless its irregularities. When surfactant is added in water/fat emulsion, its hydrophilic heads are dissolved into water and hydrophobic ends are dissolved in the fat, causing fats/oils emulsification. In the case when fat is bound to a surface, the forces which act on water/fat interface force fat particle to take form of a sphere, which leads to fat detachment from the cleaning surface.

Alkaline agents are cheap cleaning agents. Generally, they can be divided into two groups: highly alkaline and moderate detergents. Highly alkaline detergents are caustic soda (NaOH) or caustic potash (KOH). Very important property of these detergents is that they saponify fats (forming soap) and break down proteins. They are used in many clean in-place (CIP) systems or bottle-washing applications. However, they are highly corrosive and hazardous for operators. Moderately alkaline detergents are less effective than highly alkaline detergents. They include sodium, potassium, or ammonium salts of carbonates, silicates, or phosphates. One of the most effective is trisodium phosphate. Silicates are most often used as a corrosion inhibitor. The main disadvantages of alkalis are their tendency to precipitate hard water ions, formation of scum's with soaps, and poor rinsability.

Acid's major functions are control of mineral deposits and water softening. When speaking about deposits, acids are very useful in dissolving carbonate and mineral scales, hard water salts, and proteinaceous deposits. They are more effective when they are more concentrated, but this means that they will be more corrosive. Acids are not used very frequently; they are used more for periodic cleaning.

Chelating (sequestering) agents' primary function is control of water hardness. They are usually added to surfactant ions as aid to their capacity for dispersion and rinsability. In fact, they are used to prevent precipitation of mineral ions by forming soluble complexes with them. Some common chelating agents used in industrial cleaning compounds include ethylene diamine tetra acetate (EDTA), sodium citrate, and zeolite compounds. Most widely chelating base is EDTA. The only drawback is that this substance is expensive. There are cheaper alternatives but most of them are phosphate based, which are banned because of their adverse effect on environment.

Cleaning Efficiency

Cleaning procedure efficiency mainly depends on:

- Type and amount of waste to be removed;
- Chemical and physicochemical properties of the cleaning agents (strength, surface activity, etc.) and their concentration and temperature used during application;
- Applied mechanical energy (less or more brushing, higher or lower water pressure, etc.);
- Condition of the cleaned surfaces (new/old, damaged/not damaged);
- Type and condition of surfaces. Some surfaces are very difficult to (e.g., corroded steel or galvanized aluminum) to clean, , which means that disinfection will be ineffective. The same applies for other surfaces such as wood, rubber, etc.;
- Residues which are usually removed from working surfaces. Usually they are organic substances such as sugars, fats, and proteins. These matters are most efficiently cleaned with strong base detergents (especially caustic soda, NaOH);
- Types of inorganic substances such as calcium salts, etc. Best agent for removal of these substances are acids; and
- Presence of biofilms, created by bacteria, fungi, yeasts, and algae (removal agents same as for organic substances).

Disinfection

Traditionally, the terms 'disinfection' and 'disinfectants' are used for description of procedures and means in the food industry to achieve acceptable standard of hygiene from microbiological point of view. It has to be said that these procedures and materials cannot ensure 'sterility' – complete absence or elimination of microorganisms in a given environment.

Disinfection Agents

Ideal disinfectant should have the capacity to:

- Possess antimicrobial effect to kill all microorganisms present in a given environment;
- Have sufficient surface tension that allows better penetration in material pits and pores;
- Be rinsed freely, should flow out of the facility, and should not cause any kind of pollution (products or environment);
- Promote creation of resistant strains of microorganisms or microorganisms which can survive its action;
- Not cause corrosion or other type of damages in the facility. It is recommended to consult equipment manufacturers and disinfectant suppliers.;
- Not discolorate food or other materials;
- Not be hazardous to employees;
- Be compatible with disinfection procedures that are used in building;
- Be easily dissolved in water and their concentration should easily be determined, if in solid state;
- Be stored for extended period of time;
- Be in compliance with legal requirements regarding safety, environment, and biodegradability; and
- Be reasonably economical. When it comes to economics, disinfectant concentration should always be considered. Food producers often purchase cheaper packaging of disinfectant, not taking into account the required concentration (because ready to use solution is normally cheaper than concentrate), how long is the period of use of a single package, and how many packages are needed monthly or yearly.

There are number of disinfection techniques such as steam (water at elevated temperature, widely used in aseptic food production), hot water (immersion of small equipment, parts, utensils, and containers into water heated to 80 °C or higher – sterilization), irradiation (radiation at a wavelength of 2500 Å in the form of ultraviolet light or high-energy cathode or γ-rays), ozone (use of O_3 as oxidizing agent), dry cleaning (mechanical removal of soils by sweeping, brushing, wiping, and vacuuming), and chemical disinfection (different chemicals with disinfection effects). Chemical disinfection and steam/hot water disinfection are most popular. Usual chemicals used for disinfection are listed in **Table 2**.

Table 2 Types of disinfectants

Disinfectants types	*Forms/description*	*Advantages*	*Disadvantages*
Chlorine	Hypochlorite's gas form organic chlorine (e.g., chloramines)	1. Kills many types of microorganisms 2. Do not create film on surfaces 3. Relatively cheap 4. Easy to determine the concentrations	1. May cause metal corrosion and rubber weakening 2. Irritates skin, eyes, and throat 3. Unstable and readily decomposable 4. Liquid chlorine loses its efficiency during storage 5. Reduced activity in hard water 6. Sensitive to pH 7. Leads to food discoloration 8. Oxidize fats
Iodophores	Iodine dissolved in surfactant and acid	1. Kills many types of microorganisms 2. Organic matter has low influence on their efficiency 3. Less sensitive to pH than chlorine types disinfectants 4. Solution color indicates disinfectant activity 5. Easy to determine the concentrations	1. May color plastic and porous materials 2. Inactivated more than 50 °C 3. Reduced activity at alkaline pH 4. More explosive than hypochlorites 5. May be inadequate for clean in-place (CIP) (foam creation)
Quaternary ammonia compounds	Benzalkonium chloride and related compounds, sometimes named as quats or qaqs (QAQs)	1. Efficient against *Listeria monocytogenes* 2. Have residual antimicrobial activity if not rinsed 3. Organic matter have low influence on their efficiency 4. Can be used in form of foam 5. They are not corrosive 6. Efficient in odor control 7. Easy to determine the concentrations	1. Inactivated by most of the detergents 2. May not be efficient against some microorganisms 3. May be inactivated by hard water 4. Efficiency depends on the formulation 5. Not efficient like others on low temperatures 6. May be inadequate for CIP (foam creation)
Acid – anionic compounds	Combination of some surfactants and acids	1. Performs sanitation and acid rinsing in one step 2. Hard water slightly reduces their efficiency 3. Very stable 4. Organic matter have low influence on their efficiency 5. Can be applied at high temperatures	1. May cause corrosion of some metals 2. Their efficiency depends on type of present microorganisms 3. Sensitive to pH (applications below pH 3.0) 4. More explosive than others 5. May be inadequate for CIP (foam creation)
Peroxy compounds	Acetic acid and hydrogen peroxide combined to create peroxyacetic acid	1. Kills many types of microorganisms 2. Best against bacteria's in biofilms 3. Efficient at low temperatures 4. Relatively stable 5. Complies with regulations for direct removal in water supply networks 6. Low foaming (appropriate for CIP)	1. May cause corrosion of some metals 2. Inactivated by some microorganisms and organic matter 3. Low efficiency against yeasts and molds 4. More explosive than others
Carboxilic acids	Fatty acids combined with other acids	1. Kills many types of microorganisms 2. Hard water slightly reduces their efficiency 3. Performs sanitation and acid rinsing in one step 4. Stable in the presence of organic matter 5. Low foaming (appropriate for CIP)	1. May damage common steel 2. Inactivated by some detergents 3. Sensitive to pH (applications below pH 3.5) 4. At low temperatures less efficient than chlorine disinfectants 5. Low efficiency against yeasts and molds

(*Continued*)

Table 2 Continued

Disinfectants types	*Forms/description*	*Advantages*	*Disadvantages*
Chlorine dioxide	Gas diluted in solvent or with acidification of chlorites and chlorate salts	1. Kills many types of microorganisms 2. Less sensitive at pH 3. Organic matter have low influence on their efficiency 4. Stronger oxidizing agent than chlorine 5. Less corosive than chlorine	1. Not stable and can not be stored 2. Potentially explosive and toxic 3. Relatively expensive
Ozone	Gas diltued in solvent	1. Kills many types of microorganisms 2. Stronger oxidizing agent than chlorine and chlorine dioxide	1. May cause metal corrosion and rubber weakening 2. Potentialy toxic 3. Inactivated by organic matter (similar as chlorine) 4. Not stable and can not be stored
Hot water and heated solutions	Water at 78–88 °C	1. Kills many types of microorganisms 2. Penetrates in irregular surfaces 3. Relatively cheap 4. Appropriate for CIP	1. May form film or scale 2. May cause burning (of employees) 3. Efficiency depends from contact time

Source: Reproduced from Anonymous (2000) *Sanitation Control Procedures for Processing Fish and Fishery Products Manual*. Gainesville, FL: Florida Sea Grant College Program.

Following are the most used disinfectants:

Chlorine

This is the most frequently used and the most common disinfectant. Chorine is available in several forms, for example, sodium hypochlorite solutions, chloramines, etc. and can also be used in the gaseous forms (e.g., chlorine dioxide).

Chlorine disinfectants at a concentration of 200 ppm free chlorine are very active. These solvents at room temperature (23–28 °C) do not corrode high quality stainless steel but can be corrosive for less resistant materials. If organic materials are present, then its disinfection effect declines rapidly. If the ingredients are dissolved in water, hypochlorite acid is formed and has a strong disinfection action expressed through oxidation. However, the solution is very unstable, especially if they are acidic solutions, because poisonous chlorine gas is created.

Unfortunately, chlorine's antimicrobial effect is stronger in acid than it is in alkaline solutions, which means that working pH should be chosen as a compromise between stability and efficiency.

Iodophores

These disinfectants contain iodine, which is bound to a carrier. Common iodophor solutions are formulated with phosphoric acid because that way the pH of these disinfectants is lowered from 2 to 4. This is because of the greatest effect of iodine in this pH range. Most effective concentration is 25 ppm free iodine.

Iodophores are disinfectants with broad antimicrobial spectrum. However, they are less effective against sporogenous bacteria and viruses. They are inactivated by organic matter.

Commercial solutions are often acidic. They can be corrosive, which depends mostly on their formulation. It should not be used at temperatures more than 45 °C because that leads to release of free iodine. If iodophores come in contact with caustic agent residue (mostly because of inappropriate rinsing and being in the dead zones), unpleasant smell is created (so-called phenol smell).

Hydrogen Peroxide and Peracetic Acid

These disinfectants have broad antimicrobial spectrum. Hydrogen peroxide readily kills sporogenous bacteria at high temperatures and peracetic acid act on bacteria and viruses within timeframe of 30 min, whereas they act on vegetative bacteria within 5 min.

Solutions can be used independently or in combination with other disinfectants. Usually they are used on previously cleaned surfaces because presence of organic matters lowers their efficiency rapidly. They should be used in concentrations of 200–300 ppm.

Quaternary Ammonia Compounds

These disinfectants are effective fungicides (killing fungi) and bactericides (killing bacteria). However, they are less effective toward Gram-negative bacteria. To avoid creation of resistant microbes, occasionally, quaternary ammonia compounds should be replaced with other disinfectants.

Their low surface tension allows them excellent penetration in different materials, but the drawback is that they are hard for rinsing. If they come in contact with the active anionic detergents, then it is possible that they will be inactivated and therefore subsequent use of these disinfectants should be avoided. For working surfaces, concentrations of 200 ppm should be used.

Disinfection Efficiency

There are number of factors that are affecting microbicidal effectiveness of chemical disinfectants. Most important are:

1. Concentration (minimal, which is required for effective disinfection).
2. pH (water pH and actual pH of resulting solution for optimal effect).
3. Temperature (warm = 36–45 °C, hot = 50–75 °C).
4. Exposure time (minimal time needed for complete disinfection).

Table 3 Commonly used concentrations of certain disinfectants

Disinfectant	*Working surfaces into contact with food (ppm)*	*Working surface without contact with food (ppm)*	*Water (ppm)*
Chlorine	100–200[a]	400	3–10
Iodine	25[a]	25	
Quat's	200[a]	400–800	
Chlorine dioxide	100–200[a,b]	100–200	1–3[b]
Peroxyacetic acid	200–315[a]	200–315	

[a]Higher concentrations of the specified range is the maximum concentration without rinsing (surfaces have to be dried).
[b]Includes a mixture of oxichlorine ingredients.
Source: Reproduced from Anonymous (2000) *Sanitation Control Procedures for Processing Fish and Fishery Products Manual*. Gainesville, FL: Florida Sea Grant College Program.

5. Equipment cleanliness (soil can affect disinfectant effect).
6. Water hardness (different disinfectants for different water hardness for optimal effect).
7. Incompatibility (disinfectants mostly are incompatible with each other and often with soaps or other additives).

In Table 3, generic concentrations for some disinfectants are shown.

Application of Cleaning and Disinfection Agents

Cleaning and disinfection can be performed with various methods. Usually, simple manual cleaning performed with simple tools is sufficient, but having in mind that there are more than few surfaces to be cleaned and disinfected, special equipment for providing mechanical energy and chemicals' dispersion is necessary. There are many methods and means of subjecting the surfaces to cleaning compounds and solutions. Depending on the economy and effectiveness, in general, following methods are used:

1. *Manual method:* This method is used for cleaning small items; chemical is applied and spread with brushes, sponges or cloths, scrubbers, etc. Positive aspects of this method are that a high degree of mechanical energy can be applied directly where it is needed, and if used with soak tanks (see below, Cleaning out of place (COP) system), contact time can be prolonged and chemical and temperature input can be increased. If larger areas are to be cleaned, only low levels of temperature and chemical energy can be applied in order to keep the operator safe. Main advantages of this method are: low-cost equipment is required; it is adaptable to all types and sizes of facilities, equipment, and tools; and it is affordable in small food-producing operations. However, this method is labor intensive and time consuming; its effectiveness depends on human factor; most of the time it is inconsistent; and there is greater opportunity for cross-contamination.
2. *Soaking:* Small equipment/fittings/valves are immersed in cleaning solutions in a small vessels or sinks, whereas larger vessels (tanks and vats) are partially filled with a cleaning solution. This solution should have high temperature (50–52 °C) and the equipment should be soaked for 20–30 min before being manually or mechanically scrubbed. One relatively recent approach is the ultrasonic cleaning tanks. In these tanks, equipment immersed in a cleaning solution is cleaned by the scrubbing action of microscopic bubbles produced by high frequency vibrations (20 000–40 000 cycles per s).
3. *Spray method:* This is the most used method in food industry. Using fixed or portable spraying units, cleaning solution is applied on equipment surfaces.
4. *CIP systems.* This automated cleaning system represents cleaning method where contact surfaces of vessels, equipment, and pipe work are cleaned in place, i.e., without dismantling. Primary energy source required for soil removal in this system is fluid turbulence in pipelines.
5. *COP system*. Most effective cleaning for small parts (e.g., pump rotors, hoses, tubing, piping, filter housings, needles, diaphragm valves, fittings, gasket, mixers, blenders, filler components, tools, bowls, belts, etc.) is in recirculating parts washer (called COP). These sanitary tanks are utilized in combination with a recirculating pump and distribution headers, which provide agitation of cleaning solution. Sometimes parts of this washer are serving as recirculating unit for CIP cleaning.
6. *Foaming:* This method uses concentrated blend of surfactants, which are added to highly concentrated alkaline or acid cleaner solutions. When applied with foam generator, this cleaner will have form of stable foam. This foam is clinging at surfaces. That way, contact time between liquid and soil is increased and rapid drying and liquid cleaner runoff is prevented.
7. *Gelling:* In this method, concentrated gelling agent is dissolved in hot water, which results in viscous gel formation. Afterward, appropriate cleaning agent is dissolved in the hot gel. Resulting gelled acid/alkaline detergent is sprayed on surface which is to be cleaned. Gelled cleaner forms thin film on the cleaned surface for 30 min or more and attacks present soil. At the end, soil and gel are rinsed with pressurized hot water.
8. *Fogging systems:* This method is used to create and disperse a disinfectant aerosol which reduces the number of airborne microorganisms (2–3 log in 30–60 min) and to apply disinfectant to difficult-to-reach surfaces, especially overhead surfaces. For surface disinfection, fogging will be effective only if sufficient amount of chemical is deposited onto the surface. Application of this method represents potential health risk (inhalation). To avoid this risk, at least 45–60 min are required for settling of disinfectant fog and reentering of operatives in production area.
9. *High- and low-pressure water jets:* This method can be used for application of different cleaners, especially foam cleaners and rinsing of cleaning chemicals. Main advantages of this method is fast application of cleaners on walls, floors, and stationary equipment and allowing easy reach to hard-to-clean areas. However, during application, there is a loss of water temperature. Also, low-pressure systems generally require higher water volumes and high pressure may cause cross-contamination with resulting aerosol's overspray (Figure 3).

Figure 3 Aerosols creation resulting from very high pressure.

10. *Abrasive cleaning:* Abrasive-type powders and pastes are still available and used for removing difficult soil. Complete rinsing is necessary and care should be taken to avoid scratching stainless steel surfaces. Scouring pads should not be used on food contact surfaces because small metal pieces from the pads may serve as focal points for corrosion or may be picked up in the food.

Sanitation equipment should be dedicated to tasks for which they are designed. They should optimize cleaning effectiveness and minimize risks from cross-contamination between different food-producing areas. For example, brushes should have proper stiffness and they should not be used simultaneously for floor scrubbing, application of cleaners' solution, and equipment cleaning.

Very important feature for sanitation equipment is their hygienic design. This equipment should be constructed from nonporous, nonoffensive, smooth, and easily cleanable materials. Crevices, pits, and gaps are not allowed because they are niches where microorganisms are collected, multiplied and spreading around. Most desirable construction material is not only the stainless steel but also mild steel or other corrosion subject materials can also be used, provided that they are suitably protected (painted, coated, etc.). Wood as a construction material should be avoided.

After their use, cleaning tools and equipment should be thoroughly cleaned and, if appropriate, disinfected and dried.

Pathogen Resistance to Disinfectants

As mentioned before in Section Cleaning, cleaning always antecede disinfection, because without proper cleaning, disinfection cannot fulfill its primary goal – inactivation and/or killing of microorganisms present. In practice, 90 ± 95% of the present microorganisms are removed by an efficient cleaning protocol. Following process of disinfection reduces the amount of remaining microorganisms to acceptable level.

When disinfectants are applied in optimal conditions such as concentration, pH, temperature, and time, susceptible microbial cells are killed. Generally speaking, disinfectants have broad spectrum of targets and mechanisms when attacking microbial cell. The most known are:

- Breaking down cell membrane and its outer layers, resulting in rapid cell death/inactivation of the microorganism.
- Damaging enzymes and important metabolic processes. Some heavy metals (e.g., silver, mercury, and copper) are poisonous to enzymes. If they are added as salts or organic mixtures, they bind to enzymes' SH groups, which results in changes in their tertiary and quaternary structure.
- Affecting proteins' synthesis, resulting in prohibition of growth.
- Inhibiting deoxyribonucleic acid (DNA) synthesis or DNA strands' breakage, resulting in the blockage of cell growth.

Just like every other living organisms, microorganisms are protecting themselves against all kinds of influence from the environment. When speaking about their response to disinfectant, they can generally respond with:

1. Alteration of target,
2. Reduction of target access,
3. Inactivation of disinfectant, and
4. Low or nonsensitivity to applied disinfectant.

As disinfectants have broad spectrum of action, it is very unlikely that their response will be alteration of the target. Possibilities are that they will protect themselves by one or the other mentioned responses or with combination of resistance mechanisms. This kind of microorganisms' resistance responses to disinfectants can be intrinsic (innate), apparent (pseudo-), or extrinsic (acquired).

Intrinsic (Innate) Resistance

Intrinsic resistance is resistance that is naturally present in the microorganism. It is a property controlled by chromosomes and is related to the general physiology of the microorganism.

Differences in resistance to antimicrobials occurring among different types, genera, species, and strains of microorganisms under identical environmental conditions and antimicrobial concentrations are most likely controlled innately.

Different types of microorganisms show different innate resistances to chemical biocides. This is indicator for relative activity of different levels of disinfection. Highest innate resistance are showing sporogenous bacteria (e.g., *Bacillus cereus* and *Clostridium perfringens*), followed by coccidia (e.g., *Cryptosporidium* sp.), mycobacteria (e.g., *Mycobacterium tuberculosis*), nonlipid or small viruses (e.g., poliovirus and coxsackievirus), fungi (e.g., *Aspergillus* sp. and *Candida* sp.), lipid or medium-sized viruses (e.g., herpes and human immunodeficiency virus), and vegetative bacteria (e.g., *Staphylococcus* sp. and *Pseudomonas* sp).

Mechanisms of intrinsic resistance in microorganisms to disinfectants vary. For example, some microorganisms have cellular barriers, which prevent entry or uptake of disinfectants (e.g., spore coat and cortex in sporogenous bacteria, waxy cell wall in mycobacteria, outer membrane of Gram-negative bacteria, and teichoic acids in Gram-positive bacteria), some of them possess cellular efflux (i.e., mechanisms that pump

compounds out of the cell), which reduces efficacy of a number of microbicides, including benzalkonium chloride and related compounds, phenolics parabens, and intercalating agents (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*), or there is lack of a biochemical target for antimicrobial attachment or microbial inactivation, and some microbes have enzymes which inactivate microbicide applied.

Proven examples for intrinsic resistance to disinfectants are: bacterial spores and *Cryptosporidium* sp. having innate chlorine resistance, *Bacillus* sp. being intrinsically resistant to benzoates due to their ability to metabolize this compound, *P. aeruginosa* being resistant to many disinfectants, and *Coxiella burneti* surviving standard disinfectants.

Apparent (Pseudo) Resistance

Apparent resistance is term for resistance which occurs when microorganism appears as resistant to a certain kind of microbicide, but when the same microorganism is placed in a microbicide-free environment, the resistance disappears. This means that this kind of resistance is not stable and is considered as pseudo resistance.

There are two main reasons which are causing apparent resistance:

1. Presence of interacting stress conditions (e.g., low pH and polarity). The lower the pH of a food product, the greater the proportion of acid in an undissociated form, and the greater the antimicrobial activity. Another important factor that affects apparent activity is polarity. Polarity relates to the molecule ionization and the contribution of any hydrocarbon side groups or hydrophobic parent molecules. To attach and pass through the cell membrane, antimicrobials have to be soluble in the aqueous phase and lipophilic phase. Microorganisms exposed to food antimicrobials in lipid-containing food systems, due to the solubilization or binding of the antimicrobials by the lipids, demonstrates apparent increased resistance.
2. Conditions of the disinfection application. Incorrect application of disinfectant are as follows:
   - Application of disinfectant on surfaces with biofilm,
   - Use of an inappropriate disinfectant (i.e., disinfectant with limited spectrum of activity),
   - Incorrect disinfectant use (not according to the recommended conditions like concentration, contact time, and temperature), and
   - Insufficient availability of the reactive agent.

Obviously, these reasons easily can result with survival of microorganisms and although it is not considered as genuine microbial resistance, probably it is the most widespread form of perceived resistance.

Extrinsic (Acquired) Resistance

Extrinsic resistance is the result of the acquisition of new genetic information in the existing genome. This information can be plasmid or nonplasmid encoded.

Nonplasmid-encoded resistance usually occurrs when microorganism is exposed to gradually increasing concentrations of a certain microbicide. In most cases, plasmid-encoded resistance is a form of resistance against metal-based microbicides (silver, copper, or mercury). Nevertheless, it is shown that microorganisms may develop acquired resistance to chlorine. Conditions which are contributing to the development of resistance include application of sublethal concentrations of chlorine used in error or neutralization of the compound during use. Studies report that *Salmonella* show resistance against hypochlorous acid concentrations of up to 72 $\mu g\,ml^{-1}$. Development of resistance to other compounds commonly used as disinfectants in food processing environments is also possible.

Pickett and Murano exposed *Listeria monocytogenes* to sublethal concentrations of different disinfectants. Results showed that *L. monocytogenes* developed resistance to the minimal inhibitory concentration (MIC) of the acidic anionic disinfectant (500 $\mu g\,ml^{-1}$) when challenged after an initial shock of 350 $\mu g\,ml^{-1}$ with the same disinfectant. They suggested that preexposure of the cells to the dissociated form of the acid, but not to the undissociated form, caused *L. monocytogenes* to become resistant.

There are few authors who suggested that use of a single type of disinfectant may allow selection and persistence of resistant strains. For example, Lemaître *et al.*, showed that strains of *L. monocytogenes* isolated from poultry were shown to be resistant (MIC = 16 $\mu g\,ml^{-1}$) to the quaternary ammonium disinfectant – benzalkonium chloride. Resistance was plasmidborne and could be transferred to other microorganisms such as *Listeria* and *S. aureus*. The plasmid genes may code for an energy-dependent efflux system.

A number of authors suggest that there is a relationship between microbial resistance to disinfectants and microbial resistance to antibiotics that are used therapeutically. It is already reported that cross-resistance exists between microbicides and antibiotics, and antibiotic-resistant *S. aureus* and *Staphylococcus epidermidis* developed plasmid-mediated resistance to chlorhexidine and quaternary ammonium compounds. Also, methicillin-resistant *S. aureus* were significantly more resistant than methicillin-sensitive strains to both povidone-iodine and hypochlorite.

Microbial resistance to cleaning and disinfection agents and measurements are major consideration when developing sanitation plan and strategies. In that regard, users are always looking for cleaning and disinfection agents' concentration, contact time, and temperature, which eliminate most resistant types of microorganisms.

Sanitation

Sanitation is a sum of cleaning and disinfection. Sanitation is the appropriate treatment of the working surface that efficiently destroys all vegetative and some sporogenous microorganisms that can have adverse effect on human health or is reduces the number of other undesirable microorganisms, without any harm to consumers and safety of the product.

Different procedures are an integral part of the sanitation. Brief description of the subsequent processes or sanitation operations that are generally performed is following:

- Removing food product(s);
- Cleaning the surrounding, including waste beans;

- Dismantling the equipment and exposing the working surface to be cleaned;
- Removing and transferring the smaller equipment, parts, and connections in a separate facility intended for their cleaning;
- Covering sensitive installations and their protection from water;
- Cleaning the workspace, machines, and equipment from the food residues with jet of water and use of brushes, brooms, etc. (it can be cold and/or hot water. Recommendation is to first clean with cold water and brush, and then to apply hot water);
- Applying the cleaning agent and use of mechanical energy (e.g., pressure or rubbing);
- Rinsing the cleaning agent completely with water after recommended contact time, because the residues from this agent can inhibit the action of disinfectant;
- Cleaning control;
- Disinfecting with chemical disinfectant or hot water;
- Rinsing the chemical disinfectant completely with water after recommended contact time. (This is not necessary for some disinfectants which have very short time of decomposition, for example, hydrogen peroxide);
- Reassembling the equipment and leaving enough time for drying;
- Cleaning and disinfection control; and
- In some cases redisinfecting (e.g., with hot water or low concentration of chlorine) before starting the production.

Sanitation operations have to be carried out in a manner that does not contaminate food and/or packaging material during or after cleaning and disinfection (e.g., aerosols, chemical residues, etc.). Also, these operations have to be performed at room level, i.e., all environmental and equipment surfaces in one area should be cleaned at the same time.

It is essential for each food producer to have their own written sanitation program. Scope of this program should be premises, production, and storage areas. Within this program, special sanitation and housekeeping procedures required during production should be specified. This written sanitation program should at least include:

- Area/line which has to be cleaned; frequency; and responsible person;
- Specified special procedures for cleaning and disinfection that are performed during production (e.g., cleaning between shifts, with procedures, and responsible person);
- Special instructions for specific equipment cleaning and responsible person;
- Used cleaning equipment, together with instructions (e.g., pressure, volume, etc.);
- Disassembly and assembly instructions;
- Detergents/sanitation materials that are used. In the description, their generic names can be used and their solution factors, water temperature, etc. should be clearly stated;
- Mode in which chemicals are used (according to manufacturer's instructions);
- Methods of application, contact times, foam consistency, high/low pressure, etc.;
- Instructions for rinsing, water temperature, etc.;
- Instructions for sanitation, commercial and generic names, dissolving factors, time of action, etc.;
- Instructions for final rinsing (if appropriate); and
- Instructions for safe handling of hazardous products.

For each production section and piece of equipment in this written program, name of responsible person, used chemicals, procedures, and frequency of cleaning and disinfection should be specified.

This program should be documented and recorded. Records for monitoring corrective actions and verification should be available on demand.

Very important issue in sanitation programs is timing. Generally, when in production period, facilities should be cleaned and/or disinfected at least once a day, and equipment which is cleaned manually or out of place, at least once a day should be dissembled, cleaned, and monitored. CIP equipment should be dissembled and controlled according to CIP program. Also, there are periodical sanitations (weekly and monthly), which means that cleaning and disinfection should be performed thoroughly and beyond the level of sanitation on daily basis.

Methods of cleaning and sanitation program have to be monitored and verified (e.g., microbiological analysis, etc.) by authorized institutions and/or responsible person/laboratory.

Records for monitoring corrective actions and verification should be available on demand.

Assigned person is responsible for the sanitation program. This person has to possess deep technical hygiene knowledge. Scope of his/her job description includes: selection of sanitation chemicals, equipment and methodology, selection of a appropriate supplier of chemicals, ensuring that adequate supplies and cleaning materials are always available, internal training of sanitation operatives, development and implementation of cleaning schedules and monitoring system, and presentation of hygiene issues to senior management.

Monitoring Sanitation Programs

A prerequisite for effective disinfection is effective cleaning. This just shows how important the process of monitoring is. Most important control is sensory (visual, touch, and smell) inspection that show that all working surfaces:

- Are visibly clean;
- On touch and do not contain food residues, scale, or other material; and
- Do not have unpleasant odor.

However, although this method of assessing hygiene of cleaned surfaces enable detecting significant residues, small amounts (not visible) of soil and microorganisms' contamination cannot be revealed.

Besides visual check, there are few other cleaning parameters which can be monitored. If cleaning is performed with hot water, then concentrations, pH values, temperature, and contact time of the cleaning agent has to be monitored and recorded. Rinsed water pH value measuring (or similar tests) is used as a proof that the cleaning agent is completely removed (to avoid mixing with disinfection agent). These controls are rapid and are enabling decision making in the sense whether cleaning should be completely or partly repeated whole or in part or should be continued with

disinfection. All the above mentioned monitoring and control measures should be integral part of the factory sanitation program and quality system control. At this stage, there is no need for microbiological analysis because to date there are no really fast and precise methods.

Provided that cleaning control is appropriate, disinfection control should at least include the following parameters:

- Time and temperature control (if heat disinfection is used);
- Used chemical active concentrations control;
- Control whether all surfaces to be disinfected are covered with disinfectant; and
- Contact time control.

Common means of verification of sanitation program effectiveness and degree of cleanliness of equipment and foodstuffs are microbiological tests and controls. Selection of the most appropriate microbiological technique is based on desired accuracy and precision, desired results, and the amount of effort and expenses available. More techniques are available, but none of them is ideal and cannot derive rapid result.

Precise microbiological analysis takes between 24 and 72 h and sometimes 168 h (7 days). However, these analyses should be performed in predetermined periods of time and frequency and should include critical control points. Most used methods are:

Swabbing

This method employs use of sterile stick with sterile cotton (sometimes sponge) on one end. The part is swabbed on the disinfected working surface with the cotton and submerged in solvent which can be used for further dilutions. From the final dilution, usually 1 ml is poured on/into appropriate nutritive media and numbers of microbes are determined. Sterile swabs are convenient for taking samples from difficult-to-reach places such as corners, dead legs, valves, etc.).

Contact Plate Method

This method employs ready-to-use petri dishes or contact slides with different nutrient Media. They are applied on disinfected surface, cultivated, and then the number of microbes is determined. This technique is used for flat surfaces.

Membrane Filtration

Final rinsing water is passed through system of special material membranes in the form of a circle. After that, these circles are placed on appropriate nutrient Media and the number of microorganisms present is determined. This technique is most useful for CIP system or when there are places from which it is possible to collect final rinsing water.

Adenosine Triphosphate (ATP) or Bioluminescence Tests

This is the fastest method possible which gains result within minutes (Figure 4). It is very sensitive and can be used in combination with swabbing method. However, this method is not specific, i.e., it does not make differences between food residues and microorganisms. If used under defined conditions, it is possible to be a useful method.

Regardless of which technique is used, microbiological analysis will clearly indicate where and why the mistakes are made, which will allow food producers to take corrective actions before losing control over the production process.

Figure 4 ATP test.

Dry Cleaning

Facilities and premises (including rooms holding or storing packaged product and packaging material) which are dedicated to processing, handling, and storing dry foods may be solely cleaned by dry methods.

Dry cleaning can be applied in the cases where:

- The equipment and environment remains dry.
- Remaining dry, material does not present any risk of microbial growth to occur due to the present temperature, moisture, and humidity.
- Dry material which remains on the equipment contact surfaces as dust coverings or layers does not present any risk for quality of dry material subsequently produced.

- Dry material is nonhygroscopic and nonsticky.
- Good housekeeping procedures are preventing dust layers' accumulation in the environment or on the equipment where they may serve as breeding sites for vermins, especially insects.

Dry Cleaning Frequency

Cleaning frequency has to be determined from the:

- Microbiological evaluation of the types of raw materials used,
- Characteristics of the production process, and
- Nature of the food soils and the environmental residues.

Production process scraps has to be removed from dry processing areas at least once per day. Waste containers have to be clean and dry before they are returned to the processing area.

Dry Cleaning Equipment

Usual equipment for dry cleaning is:

- Brushes, scrapers, etc.,
- Vacuum cleaners, and
- Pressurized air.

Generally speaking, equipment which is used to clean food contact surfaces shall not be used for other purposes. Dry cleaning equipment has to be clearly marked and stored in a clean and dry location. In the cases where this equipment is removed from a dry processing area in the other area for the purposes of wet cleaning, then it has to be dried before it is returned in dry processing area.

If there are fatty food residues on the equipment surfaces, then brushes and scrapers can be used together with edible oil or appropriate food grade solvent (e.g., 70% ethanol). Brushes, scrapers, etc. shall be cleaned and sanitized in regular intervals and appropriate to the type of equipment.

When vacuum cleaning systems are used, for the purposes of efficient and frequent cleaning, there should be sufficient number of vacuum cleaners. Filters and dust bags have to be changed regularly, provided that removal of dust bags should be done away from food areas. Portable vacuum cleaners have to be protected from moisture during storage and transportation and in no way they should be dismantled for cleaning in food production and storage areas.

In the cases where pressurized air is used, special attention should be given to the fact that the air has to be contained within the internal equipment surfaces. Compressed air which comes in direct contact with food contact surfaces has to be filtered.

Dry Cleaning Methods

Dry cleaning methods have to be designed to minimize the creation of dust and airborne contamination. Also, there has to be a regular cleaning program for the filters and air-handling systems where dust extraction systems are installed. Generally, there are two dry cleaning methods: Manual cleaning and semiautomatic cleaning.

Manual Dry Cleaning

This method is focused on the main deposits and product layers removal by a vacuum cleaner, which is followed by brushing and/or scraping the surfaces.

As much as possible, dust formation should be avoided. Also, use of pressurized air should be avoided because this will result in creation of dust clouds that can be vector for transfer of contaminants to other areas.

Brushes, scrapers, and vacuum cleaners have to be easy accessible, regularly cleaned, and maintained. In the cases where equipment has to be dismantled in order to gain access, dismantling should be possible without the use of special tools. Use of damaged cleaning tools is prohibited because there is a risk of contamination with foreign material (broken parts from the cleaning equipment) that can remain in the equipment, pass to the dry food discharge area during subsequent operation, and contaminate product.

Nevertheless, the use of brushes and scrapers usually results in the recovery of a secondary quality grade material.

Semiautomatic Dry Cleaning

Semiautomatic cleaning procedures that are acceptable include the use of:

- Vacuum cleaners;
- dense particles (e.g., rice, plastic pellets, etc.) which are conveyed as an abrasive medium through ductwork handling dry material; and
- retractable nozzles and in-place air jets that sweep or blow dry material off a contact surface into the product collecting area. Use of air jets usually results with the recovery of dry material within the quality specification (provided that the surfaces being cleaned are not fouled with other material and the air used is filtered and have processing air quality).

This method requires availability of inspection ports, access points, and manholes for visual inspection of surfaces after cleaning.

Wet Cleaning in Dry Food Areas

Sometimes, in the dry food areas, wet cleaning is necessary as indicated earlier. The main reasons for this are when:

- Owing to present environmental temperature, humidity, and moisture content, dry material remaining in the equipment are present a real risk of microbial growth.
- Dry food material is hygroscopic and/or has a low softening point that gives rise to deposit formation on contact surfaces.
- Dry material remaining in the equipment represents a real risk of degrading the quality of the subsequently produced dry material.
- Any cross-contamination of dry material during a production change to another material cannot be permitted.
- Type of fixed and/or mounted processing equipment requires only wet cleaning.

In this case, the amount of used water and/or steam shall be kept to a minimum and contained within the immediate

area that is being wet cleaned. If the entire dry food area, including walls, floors, ceiling, equipment, etc., requires wet cleaning, then the amount of used water should be adequate to ensure that all residues are removed.

Before dry food production restart, everything that was wet cleaned (e.g., equipment, food surfaces, etc.) has to be free from food residues and moisture. Drying time should be shortened as much as possible. That is why forced hot air ventilation should be used where practical.

During wet cleaning in dry food areas, the use of sanitizers is recommended because they minimize the risk of microbial growth during drying. Also, after wet or dry cleaning, a 70% aqueous solution of ethanol or isopropanol may be used on equipment. This kind of solution is bactericidal, and the water vaporizes quickly, with the alcohol leaving the treated surface dry.

Air Disinfection

To clean the whole volume of the premises, air should also be disinfected. There are a few air disinfection systems such as:

Ultraviolet (UV) light irradiation,
Fogging, and
Ozone.

It should be noted that all the above mentioned air disinfection systems are not replacement for traditional methods for cleaning and/or disinfection.

UV Light Irradiation

UV light is one of the systems used for air disinfection as UV germicidal irradiation (UVGI) is considered UV light with wavelength of 2537 Å (254 nm). UV lamps for air disinfection can be part of low-power systems (lamp ratings from 15 to 100 W) or part of more powerful systems (medium pressure arc tubes with ratings from 0.5 to 5 kW). Most commonly used in UVGI applications are low-pressure mercury (Hg) discharge lamps.

According to Brown, dose needed for one decimal reduction varies widely between species (e.g., for *Legionella pneumofilia* – 2 mW s cm^{-2} and for *Aspergillus niger* – 132 mW s cm^{-2}).

UV light irradiation has two mayor drawbacks. One is that microorganisms which are in the shade will be not destroyed. That means that the UVGI lamps should be located in a manner that does not allow occurrence of shadows. The second drawback is that UV light with high intensity can cause eye cataracts and skin cancer. That is why, as a part of the designing systems, it is essential to establish proper screening and interlock devices.

Fogging

Fogging in the food production area has two goals:

1. To reduce the number of airborne microorganisms and
2. To apply disinfectant to surfaces that are difficult to clean (e.g., overhead surfaces).

This air disinfection system can be applied in chillers, freezers, process lines, processing areas, etc. This system is common for factories producing ready meals, dairy products, and sandwiches.

In general, there are two types of fogging solutions: wet (cold) and dry (thermal). The wet solution is a mist with particles that is applied in a fine spray. The dry type delivers the disinfectant as a gas with minimal moisture and can even be applied as a powder. Both types of fogger are effective as disinfecting agents for large areas where hand disinfection is not possible or practical. Both types of fogger materials are measured in microns to describe the miniscule size of the droplets or particles. Most effective fogging is when the medium diameter of fog droplets are between 10 and 20 μm, because they are dispersing well and settling within the timeframe of 45 min.

This air disinfection system also has its own drawbacks. First one is that during this procedure, personnel are excluded from the premises and the production process should stop. The second drawback is that this system is effective in reducing microbes only on upward facing surfaces, but in general, this is not effective on microbes on downward-facing surfaces.

Ozone

In the past decade, there was great interest in the food industry for using ozone as air disinfection agent. As antimicrobial agent, ozone was approved for use by the US Food and Drug Administration in 2001.

Various authors investigated effect of ozone on different microorganisms. Moore *et al.* found that gaseous ozone is effective against Gram-positive and Gram-negative vegetative bacteria at levels between 0.005 and 2 ppm. However, it is proven that the effect is time dependent. Bacterial endospores are much more resistant to ozone. According to Broadwater *et al.*, *Bacillus* spp. spores are 15 times more resistant than their vegetative cells. Different studies show that increased relative humidity increases the ozone effectiveness against bacterial spores. Most effective ozone disinfection takes place when the relative humidity is between 80% and 100%; no significant spores' inactivation occurs when the relative humidity is less than 50%.

Positive aspects of ozone air disinfection are that ozone:

1. Reduces the number of viable microbes attached to stainless steel surfaces and
2. Leaves no residue on the surface and can be used as terminal sanitizer for food contact surfaces.

However, ozone also has drawbacks. One of these drawbacks is that ozone is toxic to humans and even at 0.5 ppm can cause headaches and nausea. Exposure at 50 ppm for 30 min is fatal. The Health and Safety Executive (HSE) Guidance – Note EH 38 recommended exposure limit of 0.1 ppm as an 8 h weighted average and 0.3 ppm as a 15 min average for short exposure. This is the main reason to take special measures and care which will ensure that operators will not be exposed to ozone at the levels recommended previously. Second drawback is that ozone is highly reactive with many materials such as metals, textiles, organic dues, paints, different plastics, and natural rubber. The ozone can even react explosively with oils and grease. Therefore, thorough audit has

to be conducted before using ozone in order to replace the materials with alternatives that will not be degraded by ozone.

Allergen Cleaning and Validation

Food allergies are a food safety concern in the food industry. They represent one of the most important world health problems. Prevalence of allergic reactions worldwide is indicating an increasing trend over the past years. Studies elaborated in Europe and the USA is showing that food allergies affect up to 2% of adult population and up to 8% of children. Specifics of food allergens are that very low concentrations of allergen ingredients or components can trigger allergy reaction and the severity of the possible consequences for allergic people can be extreme. According to a European legislation, all food producers have to claim the presence of allergen ingredients on the declarations/labels of their packaged products, regardless of their concentration.

That is why each food producer has to establish an effective allergen management program that contains many elements including hygienic engineering and design, controls, formulations, operational methods, storage practices, scheduling, labeling, personnel practices, etc. The main pillar of this program is the allergen cleaning program. In this program allergen cleaning procedure has to be written in details.

Allergen Cleaning

Effective allergen cleaning is depending on following variables:

1. Soil type,
2. Surface texture, and
3. Cleaning method

Soil

The soil type depends not only on the allergen type but also on its form (e.g., different methods for removal of liquid eggs residue versus the removal of powdered eggs). Tree nut and peanut protein in most forms have high oil content, which require use of a detergent. Allergens which are in powdered form are typically creating a greater risk in product zones. The dusty nature of the ingredient/s is allowing airborne dispersal. That way, affected surfaces for powdered allergens will expand beyond the direct food contact surfaces. Particulate allergens are a problem in that they are not evenly dispersed throughout the product or the manufacturing equipment; therefore, if a particulate gets 'hung up' in the system, the contamination will be unevenly distributed in products which follow, i.e., contamination could be high in a small number of products.

Surface Type

Most of the food processing equipment is constructed from metals (stainless steel), elastomers, and plastics. Beside the fact that machinery and equipment should be free from any flaw (pits, imperfection, cracks, gaps, etc.), key issues regarding its cleanliness (including allergens) are correct selection of materials for equipment construction, matching the choice of material to the working environment, and their hygienic design.

Allergen Cleaning Methods

Allergens can be removed using following types of cleaning:

1. Wet cleaning,
2. Dry cleaning, and
3. Product purge (wet or dry).

As explained above in Section Wet Cleaning, wet cleaning involves water, often in combination with different types of chemicals. Nevertheless, it has to be clear that sanitizers do not remove residue, including allergen protein.

Dry cleaning does not involve water or chemicals. When speaking of allergen removal, used methods may include brushing, wiping, or vacuuming. The use of air hoses is strongly discouraged for allergen removal because the risk of dispersing the allergen is too great. Dry cleaning is especially appropriate for dry allergens with little to no oil content.

As cleaning method, product purge usually becomes an option when the surfaces that need to be cleaned are enclosed and not easily accessed, and the material that will be used for the purge can be recovered and reused in a formula that contains all of the material purged or is inexpensive and can be discarded. If product purge is used as the allergen removal method, then the amount of purged product has to be established in the appropriate procedure and validated through testing. It has to be stressed that product purge may address an allergen concern without addressing a microbial issue.

Allergen Cleaning Validation

Evidence of an effective allergen cleaning program cannot be the lack and/or absence of regulatory action or customer complaints. That is why the allergen cleaning program has to be validated and tested for each combination of soil, surface type, and cleaning method used. Validation process has to be precise and complete. Having in mind that any undeclared allergen/s is in fact a life-threatening contaminant, incomplete validation data or inaccurate data will result in risking human lives.

Generally, there are two approaches to testing:

1. Testing of cleaned surface and
2. Testing the product which is produced after cleaning.

Both testing methods have their own limitations, but if both types of testing are performed, then the most variables will be addressed.

Testing can be performed on site (with purchased test kits) or samples taken can be sent to outside laboratory/ies. Allergen-specific testing is necessary when validating allergen changeover cleaning because other types of postcleaning validation tests (ATP tests, general protein residual) will not provide the information which will demonstrate that allergen has been removed.

Usual procedures involved in testing are:

1. Running a product with an allergen.
2. Cleaning the line or equipment with the previously established method.
3. Swabbing the surfaces after cleaning and testing for the allergen in question and/or

   a. Sampling the first quantities of product from the subsequent run (product which is not containing the same allergen) and testing it for the allergen in question.
4. Holding all products produced in the subsequent run, until results have been obtained.
5. The results can show:
   a. Absence of detectable allergen, in which case the line or equipment may be used further and any product which was held may be released.
   b. Allergen carryover, in which case the held product has to be destroyed (or sometimes can be used in a formula which is declaring all carryover ingredients).
6. The line or equipment has to be recleaned (with a different method or can be variation of the same – modification of time, temperature, and/or chemical, etc.).

Allergen Cleaning Validation Frequency

The best approach is to perform validation of allergen cleaning at least once a year and whenever there are any significant changes in variables mentioned above (soil type, surface type, or cleaning method).

Also, if in the meantime more sensitive allergen test method has been developed, it is recommended to include that method in allergen cleaning validation.

Allergen Cleaning Verification

It is typically done using a documented observation by responsible staff, often the Quality Assurance personnel.

See also: Analytical Methods: Overview of Methods of Analysis for Chemical Hazards. Bacteria: *Bacillus cereus* and Other Pathogenic *Bacillus* Species; *Clostridium perfringens*; *Listeria monocytogenes*; *Mycobacterium avium* ssp. *paratuberculosis*; *Mycobacterium bovis*; *Pseudomonas*; *Staphylococcus aureus*. Food Safety Assurance Systems: Management of Allergens in Food Industry; Management of Biofilm Risk; Microbiological Testing, Sampling Plans, and Microbiological Criteria. Food Technologies: Aseptic Packaging; Cleaning and Disinfection Technologies (Clean-In-Place, Clean-Out-of-Place). Other Significant Hazards: Food Allergies and Intolerances; Physical Hazards in Foods. Protozoa: *Cryptosporidium* spp.. Risk Analysis: Risk Assessment: Chemical Hazards; Risk Assessment: Microbiological Hazards. Viruses: Hepatitis A Virus; Hepatitis E Virus

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Hazard Analysis and Critical Control Point System (HACCP): Principles and Practice

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Glossary

Control measure An action or activity that can be used to prevent, eliminate, or reduce a hazard to an acceptable level.
Corrective action Any action to be taken when the results of monitoring at the CCP indicate a loss of control.
Critical control point (CCP) A step at which control can be applied and is essential to prevent or eliminate a food safety hazard or reduce it to an acceptable level.
Critical limit A criterion that separates acceptability from unacceptability.
HACCP plan A document prepared in accordance with the principles of HACCP to ensure control of hazards that are significant for food safety in the segment of the food chain under consideration.
Hazard A biological, chemical, or physical agent in, or condition of, food with the potential to cause an adverse health effect.
Hazard analysis The process of collecting and evaluating information on hazards and conditions leading to their presence to decide which are significant for food safety and therefore should be addressed in the HACCP plan.
Monitoring The act of conducting a planned sequence of observations or measurements of control parameters to assess whether a CCP is under control.
Prerequisite program Practices and conditions needed before and during the implementation of HACCP and that are essential to food safety. Alternative definitions: universal steps or procedures that control the operating conditions within a food establishment, allowing for environmental conditions that are favorable for the production of safe food; or, procedures, including good manufacturing practice, that address operational conditions, providing the foundation for the HACCP system.
Significant hazard Hazards that are of such a nature that their elimination or reduction to an acceptable level is essential to the production of safe foods.
Validation Obtaining evidence that the elements of the HACCP plan are effective.
Verification The application of methods, procedures, tests, and other evaluations, in addition to monitoring, to determine compliance with the HACCP plan.

An Introduction to the Internationally Agreed Approach to Food Safety Control

The hazard analysis and critical control point (HACCP) system is the internationally agreed approach to food safety control. The reference standard for implementation of HACCP is published by the Codex Alimentarius Commission of the joint United Nations Food and Agriculture Organization/World Health Organization Food Standards Programme. The HACCP approach is enshrined into legislation in many countries, for example, the European Commission Regulation on the hygiene of foodstuffs (EC N0. 852.2004), and it is widely recognized that the principles of HACCP are flexible and can be applied at any stage of the food chain anywhere in the world. HACCP can be applied by all food businesses, large and small, and also to improving food safety control in developing countries and even to food safety control in the home.

HACCP was developed as part of the US manned space program, where the National Aeronautics and Space Administration (NASA) collaborated with the US Army Natick Laboratories and the Pillsbury Company to develop an approach that would protect astronauts from foodborne illness. Following its success in the space program, HACCP was further developed by the Pillsbury Company who applied it to its own operations and launched the system to the food industry in the USA at the first National Conference on Food Protection in 1971. Although the principles of HACCP were further developed to become the internationally agreed approach to food safety management, the methods for HACCP principle application using multidisciplinary HACCP teams are still based on the original approach taken by Pillsbury in the USA in the 1970s.

The HACCP System and Food Safety Management

HACCP is a preventative approach to food safety management. It is designed to control significant food safety hazards, i.e., those hazards that are likely to cause an adverse health effect when products are consumed. This is achieved through the development, implementation, and maintenance of

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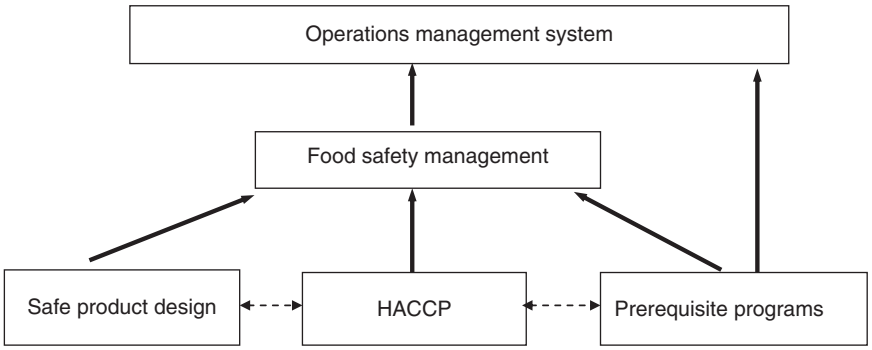

Figure 1 Manufacturing food safety management model. Adapted with permission from Mortimore SE and Wallace CA (2001) *HACCP – Food Industry Briefing*. Oxford: Blackwell Science.

the HACCP plan, a document that states how food safety hazards will be controlled in the food production operation. The HACCP System comprises the HACCP plan plus associated monitoring and verification records, which demonstrate that the HACCP plan is working in practice at all times. HACCP systems are developed through the application of the internationally agreed HACCP principles.

For food safety management to be effective, it is essential that HACCP be supported by good manufacturing practice or prerequisite programs (PRPs) that control the general hygiene and environmental conditions in a food processing operation. In a manufacturing operation, food safety management is achieved through the application of system 'building blocks' – safe product design, PRPs, and HACCP – operating under the framework of the overall operations management system (Figure 1).

The systems of the HACCP 'building block' are developed through application of the internationally agreed HACCP principles. For effective food safety management, all three 'building blocks' need to be adequately designed and their implementation be verified. Safe product design and PRPs are covered in more detail in other articles of this encyclopedia; however, it is useful to consider some key points here.

Safe product design relates to the intrinsic characteristics of the product, as determined by its recipe/composition, and to the processing methods and technologies used in its manufacture and the packaging, storage, and distribution mechanisms that protect the product once made. Although there are no defined principles of safe product design in the Codex documents, there is much published information about the elements of safe product design in other source documents. For example, the relationship between product intrinsic characteristics and the growth and survival of pathogenic microorganisms has been widely studied and is discussed in detail in texts such as those published by the International Commission on Microbiological Specifications for Foods. Similarly the effects of heat processes on microbial survival are widely cited in the literature and principles of hygienic design in food processing can be found in a wide range of publications. A key point is that product developers need to be considering food safety in the design of their recipe formulations and processes and that appropriate skills, knowledge, and experience are necessary to perform this adequately.

PRPs are the practices and conditions needed before and during the implementation of HACCP and which are essential to food safety. PRPs provide a hygienic foundation for the HACCP system by enabling environmental conditions that are favorable for the production of safe food. Like the HACCP system, there is an international agreement on the general principles required published by Codex and these essential characteristics of PRPs are laid out under the following headings for application to food chain establishments:

- Design and facilities.
- Control of operation.
- Maintenance and sanitation.
- Personal hygiene.
- Transportation.
- Product information and consumer awareness.
- Training.

Although Codex does not itself use the term PRPs, these requirements are generally accepted around the world as the essential areas where PRP elements must be developed, implemented, and maintained to provide environmental conditions that are favorable to the production of safe food and thus the foundations needed for effective HACCP systems.

PRPs are essential elements of any food safety management system and need to consist of formalized procedures and actions that will really provide a hygienic foundation for the HACCP system. To develop effective PRPs, personnel must have knowledge and experience of the current best practice in food hygiene management, as well as an appreciation of the key issues to be managed in their operation, for example, likely pest issues or constraints from building fabric.

PRPs are generally managed separately from the HACCP plan and are not normally involved in the management of CCPs because they are rarely designed to control specific significant hazards.

The HACCP Application Process

The process of HACCP plan development and implementation, through the application of the Codex HACCP principles, involves a number of interlinked stages (Figure 2).

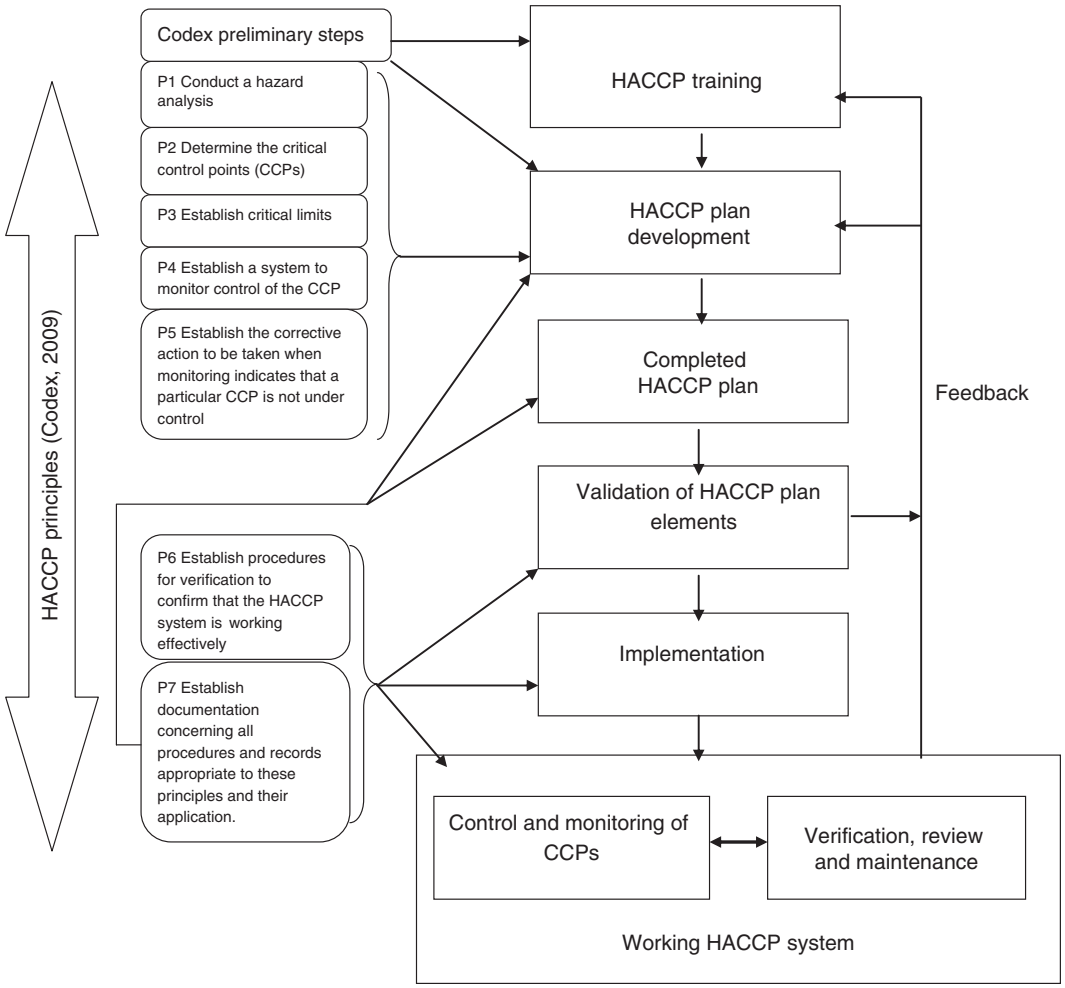

Figure 2 HACCP application process. Adapted with permission from Wallace CA, Powell SC, and Holyoak L (2005) Post-training assessment of HACCP knowledge: Its use as a predictor of effective HACCP development, implementation and maintenance in food manufacturing. *British Food Journal* 107(10): 743–759.

The HACCP Principles Explained

As indicated in Figure 2, the HACCP plan is established by applying the seven HACCP principles. Before examining in detail how to develop an HACCP plan, it is useful to briefly consider the requirements of each Principle (Table 1).

HACCP Documentation

The HACCP plan is defined as:

A document prepared in accordance with the principles of HACCP to ensure control of hazards that are significant for food safety in the segment of the food chain under consideration.

Put simply, the HACCP plan is the documentation produced that shows how significant hazards will be controlled. The HACCP plan is a formal document holding all details of areas critical to food safety management for a product or process. It consists of the:

Core Plan

- Valid process flow diagram.
- Documented CCP management details – this is usually captured in a table known as an HACCP Control Chart or CCP management table.

Support Documentation

This comprises the preparatory documentation that has been used in developing the HACCP plan as well as details of the verification requirements, including:

- HACCP team details.
- Product/process description (including terms of reference, consumer target group, and intended use of product).
- Hazard analysis details.
- CCP identification – details of approach and justification.
- HACCP verification plan.
- HACCP audit and review data.

Documenting the HACCP Study and HACCP Plan Development

Codex guidance on document organization (the HACCP Worksheet) is widely used as a basis for HACCP study records

Table 1 The HACCP principles explained

HACCP principle		*Clarification*
Principle 1	Conduct a hazard analysis	This requires the team to look at each process step one at a time, consider which hazards might occur, evaluate their significance, and establish how best to control them
Principle 2	Determine the CCPs	At this stage, the points that are critical to product safety are identified. This can be done through judgment and experience or using a structured tool – the Codex Decision Tree
Principle 3	Establish critical limit(s)	Critical limits are the safety limits that form the boundary between safe and potentially unsafe food. These need to be established to manage all CCPs
Principle 4	Establish a system to monitor control of the CCP	The monitoring system needs to demonstrate that the CCP is under control on a day-to-day basis and must be capable of detecting loss of control
Principle 5	Establish the corrective action to be taken when monitoring indicates that a particular CCP is not under control	If the CCP is not working, action needs to be taken to protect the consumer and to put right the cause of the deviation
Principle 6	Establish procedures for verification to confirm that the HACCP system is working effectively	This requires checking that the system is capable of controlling relevant hazards, is working in practice, and is up-to-date on an ongoing basis
Principle 7	Establish documentation concerning all procedures and records appropriate to these principles and their application	Documentation will include the process flow diagrams and tables created during the HACCP study (HACCP plans and development records) as well as monitoring records

Source: Reproduced with permission from Table 1 in Wallace CA, Sperber WH, and Mortimore SE (2011) *Food Safety for the 21st Century.* Oxford, UK: Wiley-Blackwell.

but there is no prescribed format and most companies use their own adaptations of recommended tables. A variety of templates are available in textbooks and in HACCP plan examples published on the Internet. The key requirement is to understand what needs to be documented not only to help the HACCP team in their deliberations during the HACCP study but also to ensure that all food safety hazards are identified, evaluated, and effectively controlled, and to provide evidence of an effective food safety system, for example, when being assessed by external auditors.

Developing a HACCP Plan

Application of the HACCP principles is done using a logical, step-by-step approach such that each step builds on the work done in applying the previous step. As shown in **Figure 2**, the HACCP principles are involved not only in the development of the HACCP plan but also in HACCP implementation and maintenance.

HACCP Study Terms of Reference and Scope

It is a standard practice to establish the scope or terms of reference at the start of any HACCP study. This should include the types of hazards to be studied (normally microbiological, chemical, and physical hazards); however, a particular study could focus on a specific hazard group, for example, when a specialist needs to be brought in to support the team.

Another important part of the terms of reference or scope is to identify exactly which part of the operation is to be covered by the HACCP study. This involves considering where the start and end points need to be and whether the HACCP study covers one product, a process involving several products, or a process module. Modular (or process-led) systems are practical to develop and are used in most manufacturing businesses, particularly those with complex processing operations, as well as in many foodservice operations. This is where the operation is split into a number of process sections and HACCP is applied to each section rather than to each individual product (the individual product approach is referred to as linear or product-led HACCP). With modular HACCP, a key point is to ensure that the modules add up to the entire operation and that no processes are missed out, so it is important to identify the start and end points accurately for each HACCP study and this is defined as part of setting the terms of reference and scope.

These details may be listed as an introduction to the HACCP plan or may be included in the product/process description.

Applying HACCP Principles – The Codex Logic Sequence

Before applying the HACCP principles, there are a number of preparatory steps that must be completed. These are described in the Codex logic sequence for the application of HACCP, as shown in **Table 2**.

Steps 1–5 are also known as the Codex preliminary steps to HACCP application and these were applied as part of the preparatory process before the use of HACCP principles.

Codex Logic Sequence Step 1: Assemble HACCP Team

Although this step tells us to 'Assemble HACCP Team,' it follows that before an HACCP team can be assembled, the correct people need to be identified and trained and it is therefore important to consider the key aspects of HACCP teams. The multidisciplinary HACCP team is believed to be one of the most powerful strengths of HACCP. Use of a team

Table 2 Logic sequence for application of the Codex HACCP principles

Logic sequence for application of HACCP	
Step 1	Assemble HACCP team
Step 2	Describe product
Step 3	Identify intended use
Step 4	Construct flow diagram
Step 5	On-site confirmation of flow diagram
Step 6	List all potential hazards, conduct a hazard analysis and consider control measures
Step 7	Determine CCPs
Step 8	Establish critical limits for each CCP
Step 9	Establish a monitoring system for each CCP
Step 10	Establish corrective actions
Step 11	Establish verification procedures
Step 12	Establish documentation and record keeping

Source: Reproduced with permission from Codex (Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission) (2009a) *Food Hygiene Basic Texts*, 4th edn. Rome: Joint FAO/WHO Food Standards Programme, Food and Agriculture Organization of the United Nations.

ensures that HACCP plans are developed by a group of people who, collectively, have the knowledge and experience to take decisions about food safety.

The essential expertise within the HACCP team, therefore, includes:

- Personnel who understand the process operations, ingredients, and products on-site.
- Personnel who have knowledge and experience of the equipment, how it works to achieve process conditions, and the likely failure modes.
- Personnel who understand the likely hazards and appropriate control mechanisms, including how to validate process controls, including the necessary validation requirements.
- Personnel who have knowledge and experience of HACCP principle application.

This expertise is most likely to be gained by including personnel from a range of different disciplines within the food company, including Manufacturing/Operations, Quality/Technical, and Engineering disciplines. Additional specialists may also be required to provide knowledge and experience of specific aspects. Within the HACCP team, a leader needs to be appointed and a scribe or secretary identified. These are two crucial roles to the success of HACCP, ensuring that the HACCP development program is coordinated and kept on track, and that accurate records of all team discussions are maintained.

The total size of a HACCP team is normally kept to a maximum of four to six personnel for ease of management, although this 'core team' may not include the additional specialists who may be called in for specific tasks. In small operations, and even in some larger ones, it may be difficult to achieve a multidisciplinary HACCP team of this nature due to the limited number of appropriate personnel on-site. It is also likely that personnel in small businesses will have less knowledge of food safety hazards, and this will need to be compensated for by bringing in external support.

The multidisciplinary approach to HACCP works well and ensures that the system does not rely on the knowledge and experience of one individual. However, it is important that a balance of individuals is found and a 'sharing' environment promoted where job roles are left outside the door. This helps to overcome any difficulties from existing group norms such as inability to challenge more senior/dominant staff when necessary.

For an HACCP team to work effectively, all team members need to understand the application of HACCP principles. For best results, the whole team should be trained using a practical training intervention that covers both theory and practical application of HACCP. It is important to understand the balance of HACCP knowledge within the team such that the HACCP study process is guided by the team members with the best knowledge of HACCP principles. This might mean that one to two people with good HACCP knowledge are given the task of ensuring that HACCP plan development proceeds effectively whereas the remaining team members focus on their functional input to the team deliberations.

Having identified and trained the HACCP team members, the team will assemble to discuss and apply HACCP principles to the operation in order to develop HACCP plans.

Codex Logic Sequence Step 2: Product/Process Descriptions

This Codex Preliminary Step tells us to 'Describe Product'. In practice, this step considers information both about the product(s) and the process. The product/process description helps all HACCP team members to understand the background to the operations that they are about to study. It is most useful if the information is recorded formally as a 'product description' or 'process description.' This document then becomes a historical point of reference to the situation when the HACCP plan was developed and is useful both as a training tool for new personnel and briefing aid for internal or third-party auditors or regulatory inspectors.

The product/process description should include:

- Main ingredient groups to be used or 'work in progress' (WIP) inputs to process modules.
- Main processes and how materials are prepared/handled.
- Production environment and equipment layout.
- Hazard types to be considered, if known.
- Key control measures available through processes and prerequisites.
- Packaging/wrapping if appropriate to the scope of study.

When applying HACCP in foodservice operations, in addition to the general product description information listed above, it is a normal practice to group all the different menu/food items into like process groups at this stage, as this will help in developing process flow diagrams (Codex Logic Sequence Step 4).

Codex Logic Sequence Step 3: Identify Intended Use

It is important to identify the intended use of the product, including the intended consumer target group. Different consumer groups may have varying susceptibilities to the potential hazards, for example, the elderly, young children, or

immunocompromised individuals. However it must be emphasized that all products should be safe for all consumers.

Intended use considerations need to be examined throughout the product supply chain, including further manufacturers/processors, foodservice, retailers, through to handling and use by the final food preparer and consumer. Different uses of the food items may also need to involve different hazard considerations, for example, food items that may be cooked or used without any further heat process. The HACCP team needs to think about any ways that the product could be abused or used other than that intended.

Intended use and consumer group information is usually included as part of the process description record (from Step 2). In many cases it will be important to provide information to the consumer about how to handle, store, and prepare (including cooking as appropriate) the food item safely; and this can be derived once the intended use and potential misuse of the product is established.

Codex Logic Sequence Step 4: Construct Process Flow Diagram(s)

A process flow diagram, outlining all the process activities in the operation being studied, needs to be constructed. This should list all the individual activities in a stepwise manner and should show the interactions of the different activities. The purpose of the process flow diagram is to document the process and provide a foundation for the hazard analysis (Step 5).

To produce a flow diagram, it is necessary to separate the process into a series of steps. In the context of HACCP, the word 'step' refers not only to obvious processing operations but also to all stages that the product goes through, for example, incoming raw materials, storage, etc. The diagram should progress logically and relate to how the product is actually produced, and should contain enough detail to allow an understanding of the process. The steps should be listed as 'activities,' i.e., what is happening at this step, and the time and temperature information should be included where relevant. A common error in HACCP is to list the names of the process equipment rather than the process activity and to miss out transfer steps. This often results in an incomplete process flow diagram, which makes the process difficult to follow.

The most commonly used type of flow diagram for use in HACCP studies shows ingredients or groups of ingredients along the top of the page through to the end point with the finished product(s) at the bottom. This gives a realistic interpretation of what actually happens from the starting point of listing ingredients to the end of production.

The style of process flow diagram will also depend on how the HACCP system is structured for the operation and the terms of reference/scope of the HACCP study. In most manufacturing operations, unless the process is very simple, the modular approach to HACCP will be used and this means that there will be a series of process flow diagrams comprising the different processes. Only the initial modules will show the handling of ingredients but later modules should show the incoming inputs from the previous module, for example, work-in-progress (WIP) or part-produced items. In foodservice operations, process flow diagrams will be generalized to cover the processes for the key recipe groupings but will not show individual ingredients or specific menu items.

Full detail of all process activities, storage, and transfer steps are needed in the HACCP study to allow a thorough hazard analysis to take place. This requires a very detailed layout to be prepared by the HACCP team, ensuring both that the process is easy to understand and that the diagram is representative of what actually happens.

Codex Logic Sequence Step 5: On-Site Confirmation of Flow Diagram

As the process flow diagram will usually be developed in the office away from the processing activities and it will be used as a tool to structure the hazard analysis, it is important to check and confirm that it is correct. This is done simply by going into the process area and comparing the documented diagram with the actual process activities, noting any changes necessary, and making sure that all variations, for example, on different shifts, are covered. This exercise is normally done by members of the HACCP team or production personnel, but it is good to have someone independent to confirm the process flow as the on-site HACCP/production team may be too close to the processes and either miss out points or make assumptions. The completed process flow diagram should then be signed off and dated as valid and it is important to make sure that this is done before the hazard analysis commences.

Codex Logic Sequence Step 6: List all Potential Hazards, Conduct a Hazard Analysis and Consider Control Measures (Apply HACCP Principle 1)

Using the process flow diagram(s), the HACCP team considers each process activity in turn and lists any potential hazards that might occur, then performs an analysis to identify the significant hazards and suitable control measures. A number of key HACCP terms are introduced at this stage and these are defined by Codex as follows:

Hazard: A biological, chemical, or physical agent in, or condition of, food with the potential to cause an adverse health effect.

Hazard analysis: The process of collecting and evaluating information on hazards and conditions leading to their presence to decide which are significant for food safety and therefore should be addressed in the HACCP plan.

Control measure: An action or activity that can be used to prevent, eliminate, or reduce a hazard to an acceptable level.

Hazard analysis is a key element of HACCP and will determine the strength of the resulting HACCP plan. The hazard analysis needs to be accurate and specific – including detail about the type of hazard and its source or cause, as well how the significance of specific hazards was determined and justified. If the hazard analysis is too brief or too general, then the following steps in the HACCP study will be more difficult and the HACCP plan is likely to be weak.

For microbiological hazards, it is possible to generalize to a certain extent, but consideration should be given to specific pathogens. Microbial hazards are normally listed as specific organisms, for example, *Listeria monocytogenes* or *Salmonella* spp., or using the collective terms, vegetative and spore-forming pathogens. The cause or source of the hazard needs to

be established along with how the hazard is manifested in the process, that is:

- Presence of the hazard in a raw material.
- Contamination with the hazard during processing and handling.
- Growth of microorganisms during production.
- Survival of microorganisms through a failure in a process designed to destroy them.

This detail is important to understand the most appropriate control measures as, for example, growth of a particular hazard will need a different control measure for contamination with a hazard.

For physical hazards, it is important to consider whether the item would genuinely cause physical harm to the consumer. Physical hazards are:

- Items that are sharp and may cause injury.
- Items that are hard and may cause dental damage.
- Items that could block airways and cause choking.

For chemical hazards, the hazard analysis will consider the likelihood or presence of toxic chemicals in the raw materials and contamination by chemicals during processing, which may raise the toxicity to an unacceptable level. The issues involved with food allergens that may cause hypersensitivity reactions in susceptible consumers are normally considered under the chemical hazards group, and will be managed by HACCP and PRPs.

Chemical contaminant hazards are, perhaps, the least well-understood group of hazards within food company HACCP teams as few companies employ toxicology specialists. Therefore, this is an area of HACCP where most companies need to seek external advice to understand the likely chemical hazards in their operations. Nevertheless, HACCP can be applied successfully to the control of chemical hazards both as raw material hazards and processing contaminants. Motarjemi *et al.* (2009), provided a case study on the application of HACCP in managing process contaminants that HACCP teams may find useful as a basis for discussion.

The process of hazard analysis includes:

- Hazard identification – Identifying which hazards may occur and where.
- Assessment of significance – Establishing which hazards are likely to occur and cause an adverse health effect.
- Identification of control measures – Establishing an effective mechanism for ongoing control of each significant hazard.

A common approach to documentation of the hazard analysis is the use of Hazard Analysis Charts. Hazard analysis charts (**Table 3**) are used to help structure the hazard analysis, allowing HACCP teams to document the important aspects with respect to potential hazard identification, reasoning, and decision making regarding significance and determination of appropriate control actions. This level of detail is important to the production of the HACCP plan and provides useful information for any future challenges of the HACCP plan, for example, through external audit.

Determination of hazard significance

Codex requires 'control of hazards that are of such a nature that their elimination or reduction to acceptable levels is essential for the production of a safe food' and states that the process of hazard analysis is intended to 'identify those hazards that are significant for food safety and therefore should be addressed in the HACCP Plan.' Although the term significant hazard is not defined by Codex, the International Life Sciences Institute has put these two phrases together to form a useful definition:

Significant hazard: Hazards that are of such a nature that their elimination or reduction to an acceptable level is essential for the production of safe foods.

To identify the significant hazards, it is necessary to consider the likelihood of occurrence of the hazard in the type of operation being studied as well as the severity of the potential adverse effect. A significant hazard, therefore, is one that is both likely to occur and cause harm to the consumer (**Figure 3**).

Most companies will assess significance of hazards using judgment and experience but structured 'risk evaluation' methods, where different degrees of likelihood and severity

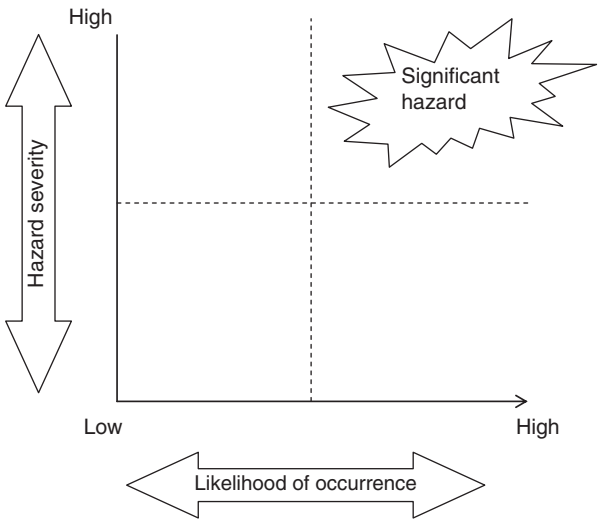

Figure 3 Hazard significance assessment. Reproduced with permission from Mortimore SE and Wallace CA (1998) *HACCP – A Practical Approach*, 2nd edn. Gaithersburg, USA: Aspen Publishers Inc.

Table 3 Possible Hazard Analysis Chart headings

Process step	*Hazard: Source, cause, and manifestation*	*Likelihood of occurrence (high/low)*	*Severity of outcome (high/low)*	*Significant? (yes/no)*	*Justification of significance decision*	*Control Measure(s)*	*Justification of control measures*

are weighted, are sometimes used to help with the significant decision. Risk evaluation decisions should be taken from a sensible viewpoint based on knowledge and experience. Structured risk evaluation methods often involve significance assessment tables, which aim to consider the degree of likelihood and the severity of effect by rating these as 'high,' 'medium,' or 'low' (Table 4). This is similar to the concept shown in Figure 3 but aims to put individual hazards in boxes to assist with the significance decision.

Although these tools are generally believed to make significant assessment more straightforward by the companies using them, individual tools need to be proven to be effective and require training in their application and use of judgment to position the identified hazards in the correct subcategories, i.e., a tool such as Table 4 is only useful if there is also guidance on which boxes are equivalent to significant hazards. Currently, there are no definitive rules on this within HACCP. There has been a resurgence in the use of these risk evaluation tools in recent years, which is believed may be linked to uptake of the International Organization for Standardization 22 000 audit standard, Food safety management systems – Requirements for any organization in the food chain. This requires formal records of hazard assessment to be maintained, although there is no specific requirement in the standard for any particular tool to be used.

The most important point is that the decision on hazard significance is based on appropriate knowledge and experience, and is not clouded by the inappropriate use of unproven decision-making tools. Further assistance to consider when carrying out the hazard analysis is provided by Codex, which lists some brief points (Table 5) to help the HACCP team discuss different hazard issues.

Table 4 Example significance assessment table

		Likelihood of occurrence		
		High	Medium	Low
Severity of effect	High			
	Medium			
	Low			

Control measures

Once the significant hazards have been established, effective control measures need to be identified for each significant hazard. As defined above, control measures are the actions that can be used to prevent, eliminate, or reduce a hazard to an acceptable level. Control for each significant hazard is essential but there may be more than one control measure for any hazard. There will also be control measures operating for PRPs.

Control measure options include:

- Process steps, for example, cooking, sieving, and metal detection.
- Product intrinsic factors.
- Use of approved suppliers.
- Temperature controlled storage or holding.
- Handling procedures.
- Controlled segregation.

An important point about control measures is to make sure that they are capable of ongoing control of the hazard at all times. Often HACCP teams mistakenly identify monitoring checks as 'control measures' rather than genuine controls – the measure must be control not monitoring and effective control measures must relate to the hazard and source, be comprehensive and appropriate, and be effective at controlling the significant hazard.

When deciding on control measures, it is important to consider the different options that may be available to control the particular hazard in order to establish the best method for control. This can include an evaluation of the measures currently in place, but it is important to decide whether these are strong enough or if additional control is necessary.

Codex Logic Sequence Step 7: Determine CCPs (HACCP Principle 2)

CCPs are the points in the process where the significant hazards must be controlled, and are defined by Codex as follows:

CCP: A step at which control can be applied and is essential to prevent or eliminate a food safety hazard or reduce it to an acceptable level.

Table 5 Codex guidance on application of HACCP Principle 1

List all potential hazards associated with each step, conduct a hazard analysis, and consider any measures to control identified hazards

The HACCP team should list all of the hazards that may be reasonably expected to occur at each step according to the scope from primary production, processing, manufacture, and distribution until the point of consumption. The HACCP team should next conduct a hazard analysis to identify for the HACCP plan whose hazards are of such a nature that their elimination or reduction to acceptable levels is essential to the production of a safe food

In conducting the hazard analysis, wherever possible the following should be included:

- The likely occurrence of hazards and severity of their adverse health effects
- The qualitative and/or quantitative evaluation of the presence of hazards
- Survival or multiplication of microorganisms of concern
- Production or persistence in foods of toxins, chemicals, or physical agents
- Conditions leading to the above

Consideration should be given to what control measures, if any exist, can be applied for each hazard.

More than one control measure may be required to control a specific hazard(s) and more than one hazard may be controlled by a specified control measure

Source: Reproduced with permission from Codex (Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission) (2009a) *Food Hygiene Basic Texts*, 4th edn. Rome: Joint FAO/WHO Food Standards Programme, Food and Agriculture Organization of the United Nations.

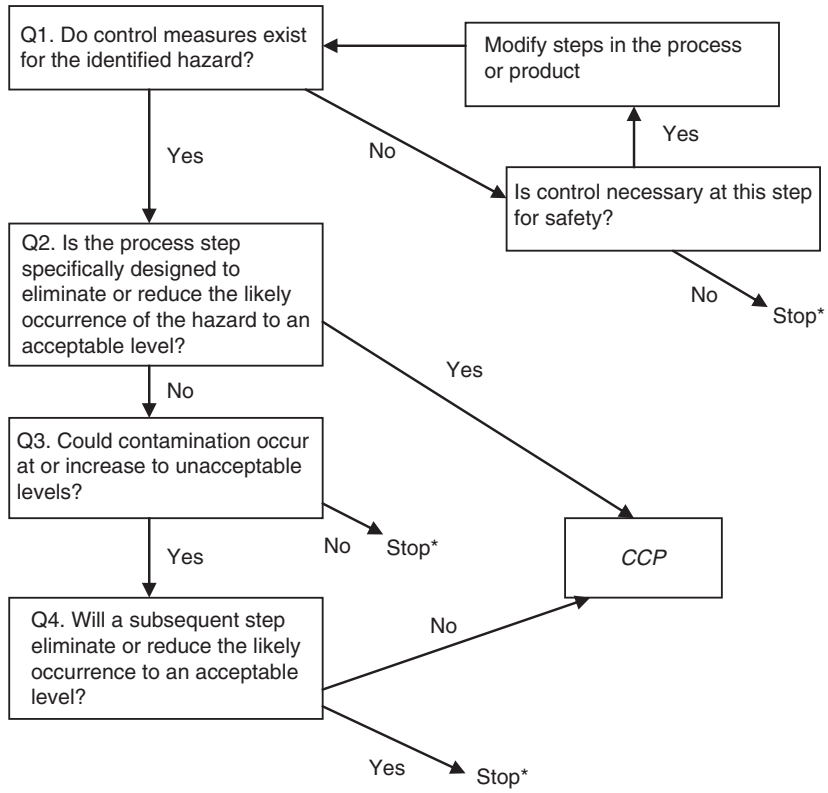

Figure 4 CCP decision tree. Adapted from Codex (Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission) (2009a) *Food Hygiene Basic Texts*, 4th edn. Rome: Joint FAO/WHO Food Standards Programme, Food and Agriculture Organization of the United Nations.

CCPs can be identified using HACCP team knowledge or experience and by using tools such as the Codex CCP Decision Tree (Figure 4).

The Codex CCP decision tree is a useful tool that is widely used by HACCP teams. To use the decision tree, the questions are asked in sequence for each significant hazard that has been identified for each process activity (Table 6). This discussion of Codex decision tree application is reproduced from Wallace *et al*. (2011).

When working with the decision tree, it is useful to keep a record of the team's discussions and justification of the decisions for future reference. This is normally done using a CCP Decision Record Sheet such as the following example (Table 7). Even if the team is not using the decision tree, it will be important to keep a record of the decisions so that full evidence of the HACCP process is available to show regulators and auditors.

Once the HACCP team has worked through the processes for all the hazards, a list of CCPs will be available. These are the points in the processes that must be carefully managed to make sure that the food produced is safe. For each of the CCPs, it is now important to define how they will be controlled and managed on a day-to-day basis. HACCP Principles 3–5 are applied to set these standards, and normally this information is recorded in a HACCP Control Chart or Table such as the following example (Table 8).

Codex Logic Sequence Step 8: Establish Critical Limits for Each CCP (HACCP Principle 3)

Critical limits are the safety limits that must be achieved for each CCP to ensure that the food is safe. If the process operates beyond the critical limits, then products made will be potentially unsafe. Critical limits are defined by Codex as follows:

Critical limit: A criterion that separates acceptability from unacceptability.

Critical limits are expressed as absolute values (never a range) that define the barrier between 'safe' and 'potentially unsafe.' They often involve criteria such as temperature and time, pH and acidity, moisture, etc. Critical limits must be measurable and must be established for all CCPs. The choice of critical limit can be based on scientific and experimental data, industry or legislative standards, and historical evidence. Another measure that is often used for practical purposes in food operations is the 'target level' or 'operational limit.' The difference between critical limits and operational limits is that operational limits are set at 'tighter' parameters than required for safety, thus providing a buffer zone for process management by indicating if a CCP is moving out of control.

It is important to know that everyday process parameters would achieve the critical limit, along with what the margin of error is, and this is done by validating the process, which will be discussed in more detail later in Codex Logic Sequence Step 11.

Table 6 Applying the Codex decision tree questions

Q1: Do control measures exist for the identified hazard?	In most cases, when conducting the hazard analysis (Step 6), control measures will have been identified for the significant hazards. Therefore, it is most common to answer 'yes' to Q1. However, if the HACCP team could not identify a control measure, then it is necessary to answer 'no' and move on to the sub-question Q1a
Q1a: Is control at this step necessary for safety?	Perhaps control is not necessary at this process step for safety, for example, there might be a control measure later in the process. In this, the answer 'no' results in the decision that this step is not a CCP for that particular hazard and the instruction to stop/proceed (move on to the next hazard). However, if the HACCP team considers that control is necessary at this step for safety (perhaps there is no control later), then the answer 'yes' results in the decision tree instruction to 'Modify step, process or product,' that is carry out some modification to allow a control measure to be built in For example, if you were concerned about metal hazards entering a process but had no control measure later on that could remove them, it would be possible to carry out a modification to build in control through a suitable metal hazard removal system such as magnets or metal detection later on in the process Once a modification has been determined, the team needs to go back and ask Q1 again. Now they will be able to answer 'yes' and move on to Q2. A key point to remember is that if you cannot establish a CCP for a significant hazard, then you cannot make the product as it would always be potentially unsafe for consumption
Q2: Is the process step specifically designed to eliminate or reduce the likely occurrence of the hazard to an acceptable level?	This is a key question in the decision tree and one that people often have difficulty with. The question provides, in effect, a shortcut to a CCP decision for those process steps that are designed to control hazards, for example, most cooking processes. The important thing to remember is that the question is asking about the process step and not the control measure. This is because control measures are always designed to control hazards so would always result in the answer 'yes.' Because you are asking the question about control measures, and a CCP is identified every time you answer 'yes,' the result could be many more CCPs than are actually required. To make matters slightly more confusing some process steps are also control measures – these are the ones that this question is designed to find Where cooking processes are not specifically designed to control hazards, then the team should answer 'no' rather than 'yes.' Not all cooking steps are CCPs – some are at such high heat processes, designed to change the physical structure of the product rather than for safety (e.g., some baking processes), that a significant hazard such as vegetative pathogens simply could not survive. In other words, the likelihood of occurrence should have been established as 'low' during the hazard analysis and it will not be a significant hazard. Although this should have been identified at the hazard analysis stage, and therefore not be an issue during CCP decisions, some companies find in practice that customers and/or regulators insist on such steps being CCPs, even when there is clear justification that this is not required If the team believes the answer to this question is 'no,' they should progress onto Q3
Q3: Could contamination with identified hazard(s) occur in excess of acceptable level(s) or could these increase to unacceptable levels? Note: Acceptable levels are safe for consumption and unacceptable levels may cause harm to the consumer	If a significant hazard has been identified, then this is really already saying that something unacceptable could occur. Therefore, in most cases the answer to this question will be 'yes.' However, the question does give the chance to just think again and confirm whether it is unacceptable or acceptable. Sometimes use of the loop at Q1a resulting in a process modification, might mean that the hazard(s) are no longer considered unacceptable (perhaps they have been designed out of the process completely) and so the answer would be 'no' in this case. Where you have answered 'yes' to this question move onto Q4, otherwise stop and proceed with the decision tree for the next hazard
Q4: Will a subsequent step eliminate the identified hazard(s) or reduce their occurrence to acceptable levels?	This last question allows the presence of a hazard at one process step if it is going to be effectively controlled at a later process step. It is helpful in keeping the CCPs to a manageable number, whilst making sure that the essential ones are identified. If there is a subsequent step in the process where the hazard will be controlled ('yes' answer) then the current process step is not the CCP but the later step will be. It is important to check that the later process step is properly identified as a CCP when the team gets to the end of the study. If there is no subsequent process that will control the hazard then the current step needs to be made a CCP and managed accordingly

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Table 7 CCP decision record

Process Step (hazard)	*Control measure*	*Q1*	*Q1a*	*Q2*	*Q3*	*Q4*	*CCP (Yes/No?)*	*HACCP team notes (justification)*

Table 8 Example HACCP Control Chart

CCP number	*Process step*	*Hazard*	*Control measure*	*Critical limit*	*Monitoring*			*Corrective action*	
					Procedure	*Frequency*	*Responsibility*	*Procedure*	*Responsibility*

Codex Logic Sequence Step 9: Establish a Monitoring System for Each CCP (HACCP Principle 4)

Once the critical limits, and operational limits, have been established, the next step in HACCP involves developing a monitoring system for ongoing measurement, which will demonstrate that the CCPs are working effectively. Codex defines monitoring as:

Monitoring: The act of conducting a planned sequence of observations or measurements of control parameters to assess whether a CCP is under control.

Monitoring is necessary to demonstrate that the CCPs are being controlled within the appropriate critical limits and monitoring requirements need to be specified by the HACCP team during the HACCP study. Each monitoring activity should have a person who is allocated to carry it out (CCP monitor) and record the results and take any necessary actions. In manufacturing, monitoring is usually done by production line personnel who are involved in operating the processes where the CCPs are located. The frequency of monitoring should also be defined and this will require consideration of the process speed/throughput. The ideal situation is to have continuous monitoring systems linked to alarm and action systems.

Codex Logic Sequence Step 10: Establish Corrective Actions (HACCP Principle 5)

Corrective action needs to be taken where monitoring shows that there is a deviation from a defined critical limit. Corrective actions must deal with both the product produced while the process is out of control (it may need to be destroyed or reprocessed) and with the process fault that has caused the CCP deviation in order to bring the process back under control. Codex defines corrective action as follows:

Corrective action: Any action to be taken when the results of monitoring at the CCP indicate a loss of control.

Corrective action procedures and responsibility need to be identified by the HACCP team during the HACCP study such that they can be implemented by the appropriate operations personnel if deviation occurs. Corrective action is not 'contact the Quality Manager' for every event but specific actions that will handle potentially unsafe product and bring the process back under control. The effectiveness of the proposed corrective action plan needs to be verified and challenged as this is the last defense mechanism protecting the consumer from receiving potentially unsafe product should a CCP fail.

Codex Logic Sequence Step 11: Establish Verification Procedures (HACCP Principle 6)

Verification requires that procedures are developed to confirm that the HACCP system can and is working effectively. There are actually two different types of confirmation required – validation and verification. Many people find the terms validation and verification confusing, partly because the words sound similar and partly because they are both part of the verification principle (Principle 6). They are separate and different activities and it is helpful to consider the definitions in more detail to help understand the difference.

Validation — Codex definition: Obtaining evidence that the elements of the HACCP plan are effective

Clarification

- Is the HACCP plan capable of controlling all relevant hazards if correctly implemented? or
- Will it work?

Verification — Codex definition: The application of methods, procedures, tests, and other evaluations, in addition to monitoring, to determine compliance with the HACCP plan.

Clarification

- Is there compliance with food safety requirements defined in the HACCP plan or
- Is it working in practice?

Validation will include:

- Cross-checking through the HACCP plan to make sure that all the principles have been correctly applied.
- Checking that the hazards will be controlled, i.e.,
  - The control measures are suitable.
  - Correct CCPs have been identified.
  - Critical limits are set correctly for the hazard, for example, using literature values, challenge testing, etc.
  - Process will achieve the critical limit(s), for example, the process is capable of always achieving this limit within normal process variation.
  - Monitoring will detect loss of control if it happens.
  - Corrective action will prevent the potentially unsafe food being consumed.

Validation can be done by HACCP team members working with other managers within the business. As with preparing the HACCP plan, it will be better to involve more than one

person if possible and, like hazard analysis, this will be an area where many companies will need to use expert resource from outside the company to assist in validation.

Commonly used verification procedures include:

- HACCP audits.
- Review of CCP monitoring records.
- Product testing – microbiological and chemical tests.
- Review of deviations, including product disposition and customer complaints.

Verification can also be done by HACCP team members or other personnel within the business, for example, supervisory staff. It is important to have independence from the system to audit effectively so consideration can be given to using external resource or other personnel who were not involved in developing or in the day-to-day running of HACCP. Auditors should be competent in both HACCP application and audit skills.

Codex Logic Sequence Step 12: Establish Documentation and Record Keeping (HACCP Principle 7)

It is important to document the HACCP system and to keep adequate records. The HACCP plan will form a key part of the documentation, outlining the CCPs and their management procedures (critical limits, monitoring, and corrective action). It is also good practice to keep documentation showing how the HACCP plan was developed, i.e., the hazard analysis, CCP determination, and critical limit identification processes.

When the HACCP plan is implemented in the operation records, will be kept on an ongoing basis. Essential records include:

- CCP monitoring records.
- Records of corrective actions associated with critical limit deviation.
- Records of verification activities.
- Records of modifications to processes and the HACCP plans.

The key consideration for all businesses should be to have sufficient documentation to demonstrate the effective working of the HACCP system. Maintenance and archiving of HACCP records is therefore an important element of effective HACCP. Records may be kept as paper archives; however, increasingly companies are turning toward computerized record-keeping systems.

Activities for Implementation of an HACCP Plan

The implementation stage is where the HACCP plan documents are translated from paper documents owned by the HACCP team to a working system managed by operations personnel. Training is therefore a key requirement, including training for the personnel who will monitor CCPs and take corrective action, along with HACCP awareness training for the wider operations workforce. Implementation needs to be carefully planned, with responsibility for various actions given to the appropriate people. It is not simply a case of handing

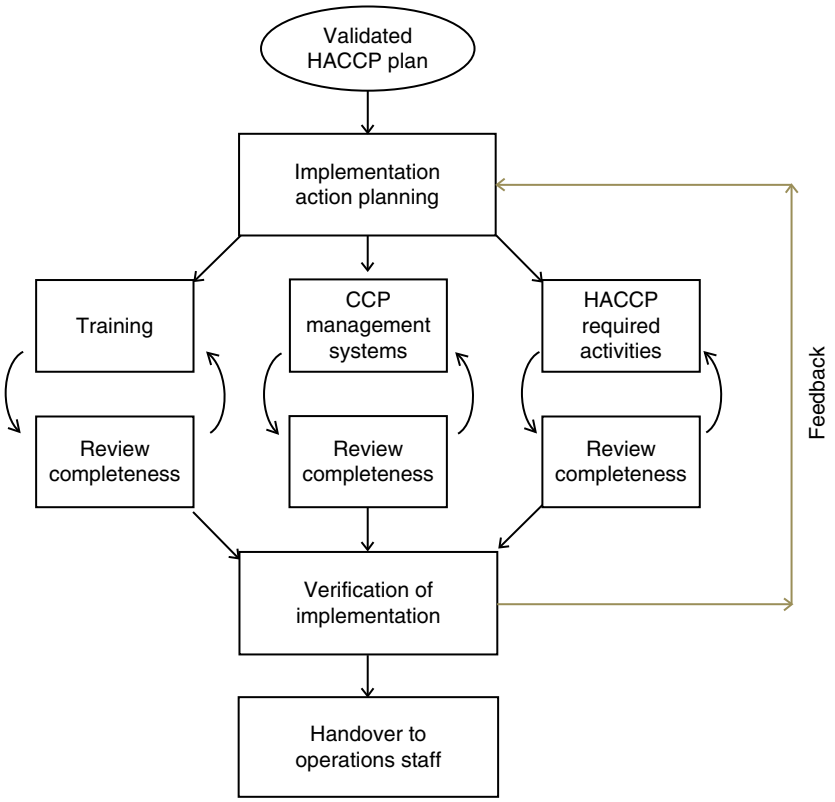

Figure 5 Steps to HACCP implementation. Reproduced with permission from Wallace CA, Sperber WH, and Mortimore SE (2011) *Food Safety for the 21st Century*. Oxford, UK: Wiley-Blackwell.

the HACCP plan documentation over to the operations personnel; rather there is a need for detailed and careful planning such that all the required activities for successful implementation can be identified and progressed. This is best achieved by breaking the necessary activities down into individual steps (Figure 5).

Maintaining HACCP and Food Safety Management Systems

Maintenance of a food safety program requires several key fundamentals:

- Challenging the effectiveness of the program elements.
- Ensuring that the program remains up-to-date, both with the ingredients, processes, and operations on-site; and with changing knowledge on food hygiene and food safety hazards.
- Making certain that the program remains suitable, both for the provision of adequate hygiene foundations (PRPs) and for the effective control of all relevant food safety hazards (HACCP plans).

Effective food safety program maintenance requires the application of a range of different techniques and approaches and the involvement of personnel from different roles and areas of the operation. Tools will include audit and management review alongside a variety of specific test procedures, and it will be important for personnel to have appropriate skills, for example, in auditing and information searching/update. It is important that all maintenance procedures are formally managed as part of the company's ongoing operations management procedures.

The key elements of food safety program maintenance are illustrated in Figure 6.

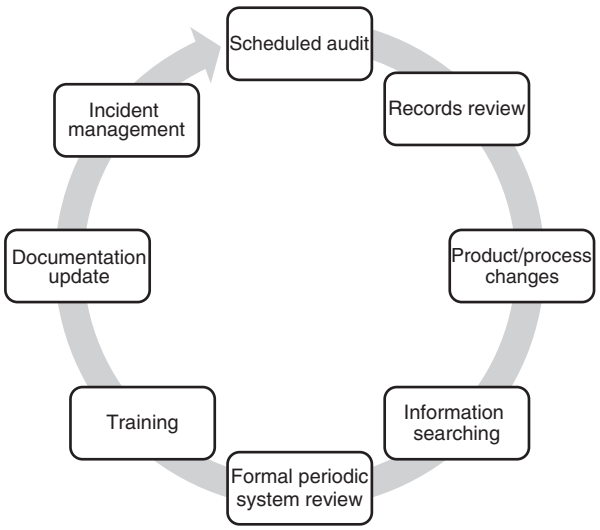

Figure 6 Elements of food safety program maintenance. Reproduced with permission from Wallace CA, Sperber WH, and Mortimore SE (2011) *Food Safety for the 21st Century*. Oxford, UK: Wiley-Blackwell.

Effective HACCP-Based Food Safety Management

As discussed in this article, application of HACCP principles is achieved by following a straightforward step-wise procedure outlined by the Codex Logic Sequence. This will only result in an effective HACCP system if performed by HACCP teams made up of personnel with the correct blend of training, skills, and experience. The outcome of this HACCP study process should be a HACCP plan that clearly defines how all significant hazards relevant to the operation will be controlled.

The preventative nature of the HACCP approach to the management of food safety comes through its identification, evaluation, and control of hazards that could cause harm to the consumer. HACCP should be effective in that it proactively identifies potential food safety hazards and implements control systems before the hazards are realized. However, HACCP cannot guarantee zero tolerance for all food hazards 100% of the time due to variability in materials and processes in conjunction with the potential for control procedure failure and human error, so it should be considered a risk management system that can minimize the likelihood of food safety hazards occurring. A rigorously designed, fully implemented, and securely managed and controlled HACCP system – i.e., 'effective HACCP' – should come as close to zero tolerance as technically and operationally feasible in a food operation. This, combined with safe product design and proven PRPs, will provide effective HACCP-based food safety management programmers as a cornerstone of public health protection.

Disclaimer

As the author of this article is an established writer in the field of HACCP, out of necessity this article draws on previously written source material, in particular the following: Mortimore and Wallace (1998), Mortimore and Wallace (2001), Wallace (2009), and Wallace *et al*. (2011).

See also: Food Safety Assurance Systems: Food Safety and Quality Management Systems. Public Health Measures: Food Safety in Hospitals and Other Healthcare Settings; Management of Food Safety in Food Service Sector; Modern Approach to Food Safety Management: An Overview

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FOOD SAFETY ASSURANCE SYSTEMS

Management of Biofilm Risk

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Glossary

Biofilm Surface-attached structured matrix-enclosed microbial communities.
Biofouling Biofilm formation including organic matter on equipment surface.
Cleanroom A room in which the concentration of airborne particles is controlled, and which is constructed and used in a manner to minimize the introduction, generation, and retention of particles inside the room, and in which other relevant parameters, for example, temperature, humidity, and pressure, are controlled as necessary.
Contact agar method Detection method for microbes on surfaces based on contact sampling with suitable nutrition agar of microbes on surfaces and their growth on the agar substrate.
Hygienic design Simple and cleanable structure for equipment, which is utilized according to rules stated in guidelines.

Biofilm Formation

Microorganisms exist predominantly as sessile multispecies communities in natural habitats. These surface-attached highly structured matrix-enclosed microbial communities are known as biofilms.

Biofilms occur in a wide range of environments, on every surface when supplied with moisture and nutrients. Biofilms can exist in natural ecosystems, for example, river stones and surfaces of a plant, or in ecosystems connected with disease, for example, teeth and the mucosal membranes of animals, as well as in manmade ecosystem, for example, steel surfaces of industrial equipment, heat exchangers, and water pipes.

The biofilms usually consist of microcolonies formed by multiple microbial species embedded in an extracellular matrix. The composition of the extracellular matrix, i.e., extracellular polymeric substance, is as diverse as the biofilm-forming microbial species. It is usually composed from polysaccharides, proteins, nucleic acids, and cell components with concentrated minerals and nutrients from the surrounding environment. As the population in a biofilm grows, the individual microbial cells may differentiate and take on specific tasks enabling the formation of a defined architecture of shapes resembling mushrooms or towers connected by a network of water channels responsible for the transport of nutrients, oxygen, and wastes.

The complex structures of biofilms with a high level of differentiation among biofilm cells require cell-to-cell signaling, i.e., quorum sensing. When a bacterium starts to form a biofilm, it switches from single cell to multicellular lifestyle by up and down regulating specific genes. Even in single-species biofilms, individual cells express their genes in a pattern that differs from one cell to another and from the planktonic cells of the same species. It is suggested that biofilms hold microniches with varying gradients of nutrients due to metabolic activity of sessile cells. These small ecosystems create conditions for spontaneous mutations to occur. Different cell types in biofilm provide genetic diversity for adaptation to sudden environmental changes.

For a human point of view, biofilms can be beneficial or detrimental. Biofilms are utilized in water purification and bioremediation of hazardous substances in the environment. However, they cause inconvenience when formed on industrial surfaces or when causing persistent infections in humans, animals, or plants.

Biofouling is a term describing unwanted biofilm formation on equipment surfaces. This can occur in wide range of manmade environments and can significantly decrease equipment performance and lifetime and cause contamination and impaired quality of the products. Because biofilms are more resistant against external forces than free swimming cells, they are more difficult to eradicate from industrial processes by biocides or cleaning agents. Therefore, they create diverse problems. In the food industry, biofilms may provide a habitat for many pathogenic bacteria and are common sources of contamination. Because biofilms are attached and grow on the surfaces of food-processing equipment, other food contact surfaces, and pipelines, they are not always removed by routine cleaning procedures. This may lead to serious hygienic problems and food spoilage.

Pathogens in Biofilms

Microbes inhabiting contact and environmental sites in food processing are mainly harmful, as attached microbial

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communities lead to contamination of surfaces and the product produced in the process. However, it is even more alarming to know that pathogens such as *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* Typhimurium, *Campylobacter jejuni*, and *Yersinia enterocolitica* can easily produce biofilms or be a part of biofilm communities. According to the literature survey, all the common pathogens are reported to be detected on food process surfaces. Specific properties of pathogens in biofilms have been studied in laboratory studies and, for instance, it has been found that biofilm cells of *Listeria* are more resistant than planktonic cells to disinfectants containing, for example, chlorine, iodine, quaternary ammonium, and anionic acid compounds.

Pathogen contamination can be defined as prolonged or persistent contamination when a strain has caused food plant contamination for long periods of up to several years. Persistent *Listeria* strains have been reported in several food industry areas, for example, meat, poultry, fish, dairy, and fresh sauces. *Escherichia coli* and *Salmonella* isolates are also known to be persistent in food and fish-feed factories. Persistent plant contamination can be caused by properties that influence survival, including enhanced adherence to food contact surfaces and adaptation to disinfectants, in addition to such predisposing factors in the processing line as complex processing machines and poor zoning.

Biofilm Detection

In assessing hygiene, it is essential to detect the microbes remaining on surfaces after cleaning. Hygiene assessment includes checking the amount of surface-attached soil including protein, polysaccharide, other organic and inorganic residues, biofilm, dead and/or living microbes in general, or specific pathogens and other harmful microbes. Hygiene assessment also helps with tracing contamination sources and in optimizing cleaning systems. The sources of microbial contaminants in the final product can be several: raw materials, process equipment, environmental surfaces, air, animals, and personnel. Quantification of the actual number of microbes from surfaces is challenging. Detection methods for measuring microbes directly from the surface are rare and unpractical for field use (i.e., microscopy) and detachment of microbes completely without damaging the surface or the microbes is difficult due to strong microbial adherence of biofilms. The traditional detachment method is swabbing not only with cotton-tipped swab but also other sterile swabs, sponges, and nonwoven cloths can be used as well as ultrasonication. The disadvantage of culturing-based methods is long incubation periods since results are needed immediately. Microscopy is very often used as a reference method for swabbing and culturing (Figure 1). It has been reported that the cells counted by direct microscopy consistently give results one log unit higher than the culturing methods. Epifluorescence microscopy has clearly revealed that with swabbing only a small part of the biofilm is detached. To detach surface-adherent cells, using excessive agitation and strong chemicals may harm the cells, thus, making them unable to grow in the culturing procedure. Surfaces can be tested using contact agar methods by pressing relevant nutrient agar against the surface for few seconds. According to a collaborative study for validation of total aerobic bacterial and enterobacterial count using Hygicult® dipslides (commercial contact agar application), contact agar dishes and swabbing revealed that 15–20% of the theoretical yield of contaminants on artificially soiled stainless-steel surfaces were detected with all used methods. Adenosine triphosphate bioluminescence and protein detection kits can provide a real-time estimation of overall cleaning efficacy of organic soil or protein residues respectively. However, these methods are unable to detect the presence of low numbers of bacteria on surfaces where other organic soil is not present. Visual observation of cleanliness is also recommended in many cases. Visual observation can be improved by using test foam, which contains dyeing agents that attach selectively to the protective matrix of most biofilms commonly found in food industry or by using ultraviolet-lamp, which makes some organic soil fluorescent, or by wiping the surface with white tissue.

Microbial biofilms can be detected and quantified using microscopy techniques like fluorescence or scanning electron microscopy (Figure 2) in combination with appropriate software for image analysis. Also, by microscopy it is possible to distinguish different components of biofilms (microorganisms, protein, and carbohydrates) by specific dyes/probes. However, microscopy requires samples that can be easily transported to laboratory and is not always suitable to field conditions.

Real-time polymerase chain reaction (PCR), also called quantitative real-time PCR (qPCR), is a technique which is used to amplify and simultaneously quantify a targeted deoxyribonucleic acid (DNA) molecule. The procedure for qPCR follows the general principle of PCR excepting that the amplified DNA is detected as the reaction progresses. The methods for detection of amplification products are using a double-stranded DNA-specific fluorescent dyes or sequence-specific DNA probes consisting of oligonucleotides that are labeled with a fluorescent reporter, which permits detection only after hybridization of the probe with its complementary DNA target. The qPCR method can be used for detection and quantification of bacterial species forming a biofilm. This method being very sensitive might be useful in food industry, where excluding the presence of certain pathogenic species is important.

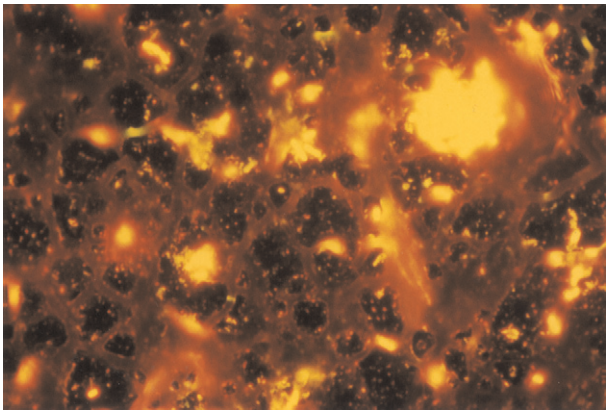

Figure 1 Biofilm on stainless-steel surface, which is stained with acridine orange and observed using epifluorescence microscopy. Bright orange rods and clumps are microbe cells and the clouded orange net is extracellular matrix (slime produced by microbes).

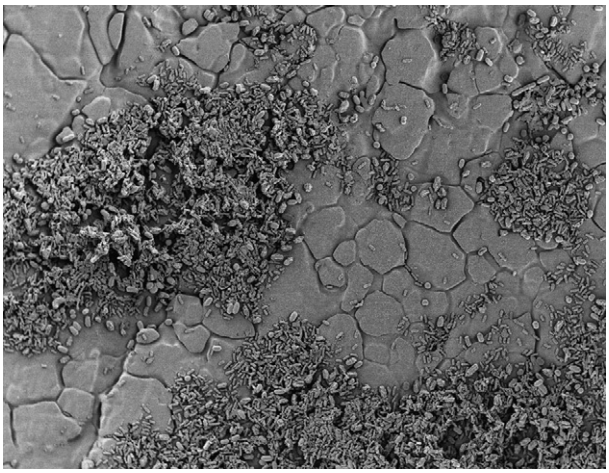

Figure 2 Scanning electron micrograph of bacterial biofilm on stainless steel.

Biofilm Prevention

Hygienic Design

The hygienic design of process equipment has a significant impact on reducing the risks of contamination of food during production. Poorly designed equipment which is difficult to clean, can harbor spoilage microbes and pathogens. This can be avoided through hygienic equipment design, which should be taken into consideration when purchasing new or redesigning old process lines. The basic rule in hygienic design is to use simple structure which is stated in most common guidelines. The process equipment is easy to clean if the surface materials are smooth and in good condition. Dead ends, corners, cracks, crevices, gaskets, valves, and joints are vulnerable points for biofilm accumulation. Equipment that causes problems in food processing and packaging include slicing and cutting equipment, filling and packing machines, conveyors, plate heat exchangers, and tanks with piping. These types of equipment can cause contamination through spoilage microbes and pathogens as they are difficult to clean, for example, the pathogen *L. monocytogenes* is often associated with harborage in poorly designed equipment.

The European Commission Regulations 852/2004, 853/2004, and 854/2004 cover the principal objective of the new general and specific hygiene rules to ensure a high level of consumer protection with regard to food safety. Legislative demands set the basic requirements for the manufacturing of safe food products, whereas food safety management systems and food safety guidelines and standards based on given legislation help the food industry to keep up with the current food safety requirements. Hygienic requirements should be adopted at the initial stage of developing process equipment and components because upgrading existing designs to meet hygienic requirements is often both expensive and unsuccessful. Legislation states that strict requirements regarding technical specifications for hygienic designs, constructions, and installations of process equipment are needed. It is important for process equipment manufacturers to have their design or prototype tested to the given requirements. European Hygienic Engineering and Design Group (EHEDG) has developed an evaluation and certification program in which certified equipment and components have been evaluated and tested according to relevant design criteria and legislation available, for example, the EHEDG document no. 8 (1993) on design criteria for hygienic equipment. This guarantees that the equipment is of a good hygienic design and is easy to clean. There is also a need for criteria and procedures for testing, assessment, and certification of such process equipment such that the formal mark guarantees that the equipment functions in conformity with predefined requirements.

The cleanability results can be used for optimizing the cleaning parameters and plant design. Simulations obtained using computational fluid dynamics (CFD) can be used to relate information on cleaning efficiency to the physical design of processes. CFD is used in many applications to model the bulk parameters of fluid flows. Recently model developments have made it possible to resolve local flow phenomena in specific positions and near walls, which is of interest in the study of cleaning processes. Results of the CFD simulations yield information about wall shear stresses and the flow rates in different parts of the system. A combination of wall shear stress, fluid exchange, and turbulence conditions can be used to predict areas that are not properly cleaned in both simple and complex flow systems.

Cleaning Procedures

It is important to address the cleaning procedures in food industries due to economics, efficacy, and safety issues. The cleaning procedure of food-processing factories may occasionally need to be improved, for example, if pathogens have been detected on process surfaces. Improvement of regular cleaning programs may also be needed in order to improve the quality and safety of the product. Once developed, biofilms are difficult to remove completely. Moreover, increased resistance to chemicals will occur due to changes in physiology when microbes are attached on the surfaces. It is not sufficient to kill the microbes; the remaining matrix must also be removed, because it provides an excellent opportunity for rapid reestablishment of new biofouling. Parameters effecting the cleaning of surfaces are chemical agents, mechanical forces, temperature, and duration of the cleaning procedure. The selection of detergents and disinfectants in the food industry depends on the efficacy, safety, and rinsability of the agent as well as where it is corrosive or affects the sensory values of the products manufactured. Disinfectants allowed for use in food factories also needs to be accepted by authorities. The European Union (EU) biocide directive states procedure for acceptable disinfectants and keeps record on accepted disinfectants. The key to effective cleaning and disinfection of food plants lies in the understanding of the type and nature of the soil (sugar, fat, protein, mineral salts, etc.) and the microbial contamination on the surface. In a wet open process, the gross soil should be removed by dry methods, for example, brushing, scraping, or vacuuming and visible soil rinsed off with low-pressure water. Disinfectants approved for use in the food industry are alcohols, chlorine-based compounds, quaternary ammonium compounds, oxidants

(peracetic acid, hydrogen peroxide, and ozone), persulphates, surfactants, and iodophors (the new registration of disinfectants according to EU biocide directive is in active state at the time this list is written).

Biofilm Management

Wet industrial processes provide environments for formation of microbial biofilms because open processes are impossible to keep sterile. In processes like food manufacturing, control of biofouling with chemical biocides is not possible. Engineering surfaces and coating materials to repel microbial adhesion or using physical methods (e.g., photocatalysis, electrochemical, and polarization) rather than chemicals, is therefore of great interest for antifouling. However, designing materials or coatings effectively repelling toward all kinds of microbial colonization on long-term basis has proven to be impossible. The countermeasures against biofouling should be separately tailored for each individual process situation.

Systematically operated process line ensures optimal runability. One way to ensure high performance is to implement hazard analysis and critical control points (CCPs) and good manufacturing practices (GMPs), which primarily deal with hygiene, cleaning, and CCP monitoring. The commitment from the management and motivated personnel is essential in order to benefit from these quality systems.

Processes or products can be protected from contaminants in the air by operating in the cleanroom. Cleanroom is defined as a room in which the concentration of airborne particles is controlled, and which is constructed and used in a manner to minimize the introduction, generation, and retention of particles inside the room, and in which other relevant parameters, for example, temperature, humidity, and pressure, are controlled as necessary. The cleanroom class needed should be chosen according to the process step and the processed product. Packaging step in food processing is a typical process step in which cleanroom technology can be applied. The shelf life of product increases when the product is packed correctly in a controlled room. It is important to follow the GMP rules about clothing and behavior, especially when working in cleanroom. Basic standards for designing cleanrooms and associated controlled environments are standard series ISO 14698 on biocontamination control and ISO 14644 dealing with topics such as general principles and methods; evaluation and interpretation of biocontamination data; classification of air cleanliness; specifications for testing and monitoring; test methods; design, construction, and start-up; operations; vocabulary; separative devices (clean air hoods, glove boxes, isolators, and minienvironments); classification of airborne molecular contamination; and classification of surface particle cleanliness.

See also: Food Safety Assurance Systems: Cleaning and Disinfection; Hygienic Design of Equipment

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Relevant Websites

www.Bacfoodnet.org
BacFoodNet: A European network for mitigating bacterial colonization and persistence on foods and food processing environments.

http://puffin.vtt.fi
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http://safoodnet.vtt.fi
SAFOODNET: Food safety and hygiene networking within new member states and associated candidate countries.

FOOD SAFETY ASSURANCE SYSTEMS

Microbiological Testing, Sampling Plans, and Microbiological Criteria

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Glossary

Analytical unit The actual volume/mass/area etc., of a sample unit that is tested in the specified method: An analytical unit may comprise the entire sample unit, or may be an aliquot of the sample unit.

Food safety objective (FSO) A metric advocated by Codex Alimentarius and established by a competent authority that represents the maximum level of a hazard in a food at the point of consumption that may be tolerated in the light of an appropriate level of (health) protection (ALOP) or relevant policy decisions on public health protection.

Guideline An advisory criterion that may be established by regulators, industry, or trade associations that articulates expected quality and/or safety when best practices are applied to the manufacture of the food.

Homogeneous Uniformly distributed, for example, when referring to the characteristic of the distribution of microorganisms in the lot.

Lot A specified quantity of some commodity manufactured or produced under conditions which are presumed to be, or have been, uniform for the purpose of application of sampling plans.

Microbiological criterion A statement or specification that defines the acceptability of a product or a food lot, based on the absence or presence, or number of specified microorganisms including parasites, and/or quantity of their toxins/metabolites, per unit(s) of mass, volume, area, or lot. Complete specification of a microbiological criterion includes definition of n (the number of sample units taken), m (the microbiological limit for any individual sample units), c (the maximum number of analytical units (*q.v.*) in the sample that are permitted to not comply with the attribute m) and, for a three class plan, M (a limit that if exceeded in any analytical unit in the sample renders the entire lot unacceptable), as well as: Statement of the microorganism(s) of concern and/or their toxins/metabolites and the reason for that concern; the food to which the criterion applies; the specific point(s) in the food chain to which the criterion applies; the analytical methods to be used and any actions to be taken when the criterion is not met.

Operating characteristic (OC) curve A graph that relates the probability of acceptance of a lot as a function of its actual quality (rate of defects or concentration level). The shape of the curve depends on the stringency of the sampling plan (number of sample units n, number of positive sample units acceptable c).

Sample One or several units, or portions, of the material comprising the lot.

Sampling plan A specification of the number and size of samples to be taken from a lot, and the method of testing in order to obtain information needed to decide whether that lot complies with some particular criteria for microbiological quality or safety; a sampling plan is part of a microbiological criterion.

Specification A microbiological criterion which is applied as a condition of acceptance of a food or ingredient by a food manufacturer or public, or private agency;
it can be mandatory in a supplier–buyer relationship.

Standard A microbiological criterion which is part of a law or regulation (a mandatory criterion enforceable by the regulatory agency having jurisdiction).

Microbiological Testing

Microbiological testing is frequently used to determine the microbiological quality and safety of foods. The results of such testing are sometimes (incorrectly) interpreted as absolute, but due to simple statistical effects and generally substantial heterogeneity in the distribution of microorganisms in food, the results should definitely not be considered as such. In many cases, the number of samples required to prove a certain absolute level of control is impractically large.

Nevertheless, microbiological testing generally is an important part of a food safety management system. For instance, in the import and export of shipments for which no information is available on the food safety management system used in the production of a food, no other means of food safety assessment is possible. For food safety management systems in which the control of food safety is assured by other

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means, like the application of best practices and Hazard analysis and critical control points (HACCP) principles, microbiological testing is key for verification purposes. Microbiological testing can also be usefully applied for other purposes (in many different contexts, see Table 1) and at various locations in the food chain (Table 2).

Depending on the specific purpose for testing (e.g., specific batch testing, verification, and investigational sampling) of the food and food production situation under consideration, the expected variability in results, frequency of sampling, the numbers of samples to be taken, and the specific limits to test against will vary.

Table 1 Microbiological testing in various contexts

National and international governmental context:
Epidemiological investigations
Investigational testing regarding outbreaks, recalls
Baseline studies for food safety policy setting
Import, export food safety assessments
Industry context:
Trade association studies
Retail surveys
Company specific context:
Across different production facilities and lines
Customer–supplier/purchase specifications'
Validation of safe product design, control measures
Process validation
Facility/product specific context:
HACCP
Prerequisite programs (GMP, GHP)
Compliance with microbiological criteria
Environmental testing
Verification of food safety management systems
Investigational testing

Table 2 Microbiological testing at various locations in the food chain

Raw material:
Verify effectiveness of supplier HACCP/GHP
Assess suitability of incoming raw material for use in product
Contribution to finished product criteria
Impact on effectiveness of control measures
Communicate to supplier of raw material expectations for quality and safety
Environment/process hygiene criteria:
Verify effectiveness of HACCP, GHP programmes
Verify functioning of production process
Finished product:
Verify effectiveness of HACCP, GHP programmes
Verify compliance of lot against regulatory standards
Verify compliance of lot to agreed specifications
Port-of-entry assessments in import/export

Microbiological Criteria

A microbiological criterion includes a number of elements that together establish the microbiological acceptability of a product for a specific purpose, whether as an ingredient in a particular food product or for a final food product intended for consumption with or without particular preparation. In establishing whether a final food product is microbiologically acceptable, therefore, the expected further handling and treatment of the food is an important consideration. Microbiological testing is carried out against a sampling plan specified in a microbiological criterion by the limit or limits to the level of a particular microorganism for each analytical unit and the proportion of the analytical units representing the batch or lot that must be below the microbiological limit(s).

According to Codex Alimentarius, microbiological criteria define the acceptability of a product or a food lot, based on the absence or presence, or number of microorganisms, per unit(s) of mass, volume, area, or lot. They should specify all the following elements:

- The food and the point(s) in the food chain where the criterion applies;
- Any actions to be taken when the criterion is not met;
- A statement of the microorganism(s) of concern;
- A plan defining the number of field samples to be taken;
- The size of the analytical unit to be tested for each field sample;
- The analytical method(s) for detection/quantification;
- Microbiological limit(s) considered appropriate to the number of microorganisms found in the analytical units; and
- The proportion of analytical units that should conform to the(se) limit(s).

The last five elements together form the sampling plan underlying a microbiological criterion. The sampling plan is a key part of the microbiological criterion as it determines the stringency of microbiological acceptability. So far, the performance of sampling plans has not been a required element of a microbiological criterion as defined by Codex, but in the ongoing revision of the Codex guidelines on establishing microbiological criteria, this additional element may be considered.

Microbiological criteria may have different standings in different contexts. A standard is a microbiological criterion which is part of a law or regulation; it is a mandatory criterion that is legally enforceable by the regulatory agency having jurisdiction. A guideline is an advisory criterion that may be established by regulators, industry, or trade associations to articulate microbiological levels that are achievable when best practices are applied to the production or manufacture of foods, whereas a specification is a microbiological criterion that is applied as a condition of acceptance of a food or ingredient by a food manufacturer or public, or private agency. A specification can be mandatory in a supplier–buyer relationship.

Two types of sampling plans can be distinguished, attribute plans and variable plans. In attribute plans experimental results are attributed to certain classes (desirable, marginal, and/or unacceptable). In variable plans all quantitative results of

the test are quantitatively used in the criterion. The focus here is on attribute plans.

Two- and Three-Class Sampling Plans

The International Commission on Microbiological Specifications for Foods (ICMSF) defined 15 risk categories ('cases'; see Table 3) that differentiate acceptable levels of microorganisms in foods based on the degree of health risk possibly posed by food containing specific microorganisms as dictated by: (1) the inherent severity of infection by the pathogen or ingestion of a microbial toxin, (2) the conditions of handling and use of the foods, and (3) the susceptibility to foodborne illness of the population intended to consume the food. These cases use different sampling plans to reflect the stringency required for microbiological acceptance. For lower degrees of risks, 'three class' sampling plans are advocated, although 'two class' sampling plans are more usual for higher degrees of risk. Three class plans are used where some tolerance of the presence of the specific microorganism is acceptable and does not present a risk to consumers. In three class plans, 'm' represents the desirable level that is consistent with a product of good microbiological acceptability. Recognizing that deviations may occur in food production, some margin of tolerance is allowed. However, there is a second limit in three class plans that should never be exceeded as this would mean that the product is unacceptable. This intolerable level is defined by 'M.' In two class plans, there is only one limit, 'm,' which renders the unit nonconforming when exceeded. Usually, three class plans are used where higher levels of the target organism are acceptable and quantitative methods are used, whereas two class plans are more commonly used when only very low levels of microorganisms are tolerated and qualitative ('presence/absence') methods are used.

Thus, whereas both kinds of sampling plans specify the methodology, the number of analytical units, microbiological limits, and the proportion of analytical units that must satisfy the microbiological limit(s), three class plans additionally specify desirable levels, completely unacceptable levels and, by inference, marginally acceptable levels.

In Figure 1, both types of sampling plans are illustrated. The x-axis shows increasing concentrations of microorganisms that could occur in the food product. The y-axis shows the likelihood of a particular concentration occurring. The curve in the graphs shows a lognormal distribution of the concentration of microorganisms. This kind of distribution is often assumed to reflect natural variation or heterogeneity. The curve shows that concentrations of 2.8 log cfu g^{-1} are most likely to occur, whereas concentrations at the lower end of the scale, for example, 1 log cfu g^{-1}, or at the higher end of the scale, i.e., 5 log cfu g^{-1}, are much less likely to occur. In both graphs, an indication is given of the proportion of concentrations that, when found, would indicate that the unit is nonconforming (i.e., often meaning the lot being qualified as defective); for three class plans, also the proportion is shown that would be in the marginally acceptable range.

Characteristic Numbers Defining a Sampling Plan

The characteristic numbers for a two class sampling plan are the number of analytical units to sample (n), the microbiological limit (m) that determines the acceptability of each of the analytical units, and the number of analytical units (c) that are allowed not to meet the limit. A three class plan specifies the number of analytical units to be sampled (n), a lower microbiological limit defining acceptable analytical units (m), an upper microbiological limit defining completely unacceptable analytical units (M), thereby defining the defective lots, and the number of analytical units (c) that are allowed to be in the 'marginal' range, thus between 'm' and 'M.' In a three class plan, a lot or batch can be rejected if too many ($>c$) of the sampled analytical units are found to be in the marginal range, or if any of the analytical units exceeds 'M.' It is desirable that the values selected for m and M are not too close together for the analytical method to be able to discriminate between them.

Microbiological criteria should be defined only where there is a need to articulate criteria for microbiological acceptability, whether for reasons of trade or public health. Next to the sampling plan details discussed above, they should clearly state or describe all characteristic elements defining the

Table 3 Cases for sampling plans as described by ICMSF

Hazard/microorganism	*Effect on risk due to conditions in which the food is expected to be handled after sampling*		
	Reduction of risk	*No change in risk*	*Increase of risk*
Utility	Case 1 Three class, $n=5$, $c=3$	Case 2 Three class, $n=5$, $c=2$	Case 3 Three class, $n=5$, $c=1$
Indicator	Case 4 Three class, $n=5$, $c=3$	Case 5 Three class, $n=5$, $c=2$	Case 6 Three class, $n=5$, $c=1$
Moderate hazard	Case 7 Three class, $n=5$, $c=2$	Case 8 Three class, $n=5$, $c=1$	Case 9 Three class, $n=10$, $c=1$
Serious hazard	Case 10 Two class, $n=5$, $c=0$	Case 11 Two class, $n=10$, $c=0$	Case 12 Two class, $n=20$, $c=0$
Severe hazard	Case 13 Two class, $n=15$, $c=0$	Case 14 Two class, $n=30$, $c=0$	Case 15 Two class, $n=60$, $c=0$

Source: Reproduced from ICMSF (International Commission on Microbiological Specifications for Foods) (2002) *Microorganisms in Foods 7. Microbiological Testing in Food Safety Management*, pp. 363. New York, USA: Kluwer Academic/Plenum Publishers.

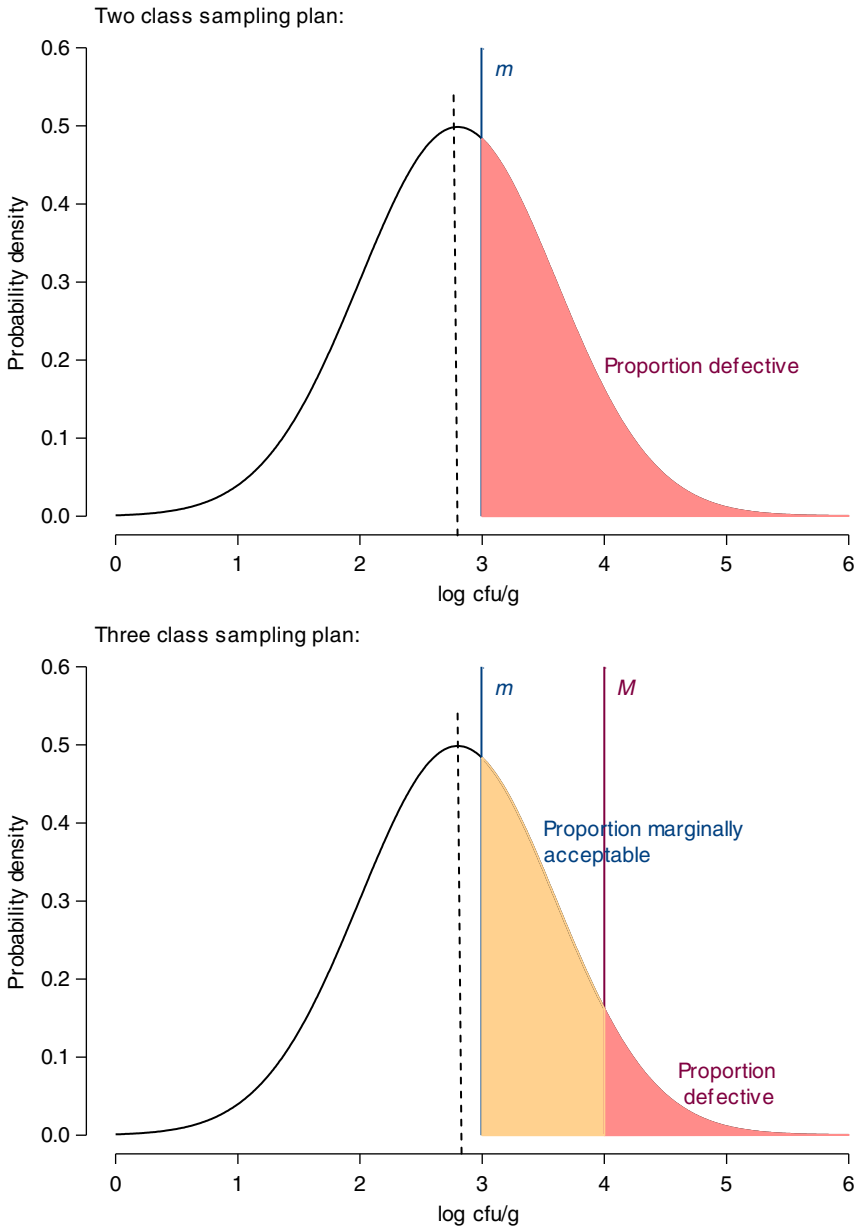

Figure 1 Graphical representation of two and three class sampling plans. Dashed line is the mean log concentration. Furthermore the microbiological limits *m* and *M* are indicated.

criterion. These include the microorganism targeted, the point in the food supply chain it applies, the analytical method advised and actions taken regarding defective lots/batches. Microbiological criteria should also include the field sample size, particularly for presence/absence of testing.

Sampling Plan Cases

To select values for *n* and *c*, the risk categories ('cases') as defined by the ICMSF (Table 3) can be used as guidelines, as these clearly are taking into account the degree of concern of the hazard and the conditions of storage and use.

The cases in Table 3 differentiate between different types of hazards and microorganisms. Utility organisms are measures of general contamination not directly related to safety, like total plate count, cold tolerant organisms, and lactic acid bacteria. Indicator microorganisms, like Enterobacteriaceae or coliforms, usually are not directly harmful, but may indicate the likely presence of pathogens. Moderate hazards cause illness symptoms of short duration, which are rarely life threatening, and do not result in serious sequelae (e.g., *Bacillus cereus*, *Staphylococcus aureus*). Serious hazards result in disease of moderate duration and do not normally result in sequelae (e.g., *Salmonella enteritidis*, hepatitis A). Severe hazards can result in substantial chronic sequelae or effects that can be of long duration (e.g., *botulinum* toxin, enterohemorrhagic

Escherichia coli). Notably, hazards that are termed moderate for the general population may be serious hazards for particularly sensitive subgroups (e.g., *Salmonella* in infant food).

Going from case 1 to 15, the level of public health concern increases, as it also increases going from the lower case to the higher case per row or from the first to the last row in the table. For each case, a sampling plan type and typical characteristic numbers for the sampling plans are proposed to give guidance on the level of stringency that could be applied for lot acceptance. However, suitable microbiological limits, and the appropriate size of the analytical units need to be established for each case additionally according to the required performance of the sampling plan for the specific purpose that the microbiological criterion is established.

Performance of Sampling Plans

Although the ICMSF cases have proven useful as general guidance, the specific number of samples, the proportion of compliant analytical units, the microbiological limits and the size of analytical units proposed cannot be directly related to public health risk. Therefore, the approach has been taken further by determining the performance of sampling plans in greater detail. Depending on the level of heterogeneity expected (i.e., as characterized by the standard deviation of the assumed log normal distribution of counts of bacteria within the lot) the average level of contamination that can be detected with a specified level of certainty (or 'confidence') can be determined. The decisions to be taken on the basis of assumptions or additional data generation for the specific situation are much the responsibility of risk managers, aided with data brought forward by risk assessors. In particular, the actual standard deviation of contamination levels within the batch would be essential as well as the target level of contamination tolerated for microbiological acceptability linked to the feasibility to detect such a level. Guidance here can be obtained from recent studies on the actual distribution of microorganisms in foods and how best to model these.

Example of Different Aspects of a Sampling Plan

The application of microbiological criteria with full specification of all elements, such as the underlying sampling plan, as advisory standards for the control of relevant microorganisms, is clearly exemplified in the recently published Codex criteria for powdered formulae for infants and young children, as shown in the following textboxes.

In Textbox 1 the sampling plans are defined for *Cronobacter* and *Salmonella* as these two microorganisms are considered significant pathogens that need to be controlled. In each sampling plan, the number of sample units (n) to be tested and the analytical unit (10 or 25 g) are specified. Because both organisms are considered capable of causing severe disease for the highest risk-group within the intended population (i.e., new borne infants), a two class plan is used and no sample units are permitted to be positive ($c=0$). In effect, this sampling plan requires absence in 10 g in each of the 30 sample units taken for *Cronobacter* and absence in 25 g for *Salmonella* in each of 60 sample units taken.

Although not currently a requirement for microbiological criteria, the performance of the sampling plans is also specified for both microorganisms, which depends on the standard deviation for the distribution of the microbial cells in the food lot and the probability of detection of defective lots selected (Textbox 2). The sampling plan performance is expressed as the sensitivity of detection giving the selected standard deviation and probability of detection.

The analytical methods deemed most appropriate for detection of the two different microorganisms are duly described and criteria for the selection of alternative methods are provided (Textbox 3), as is the stage in the food chain where the microbiological criteria apply (Textbox 4), and the action to be taken when products are found not to conform to the sampling plan (Textbox 5).

Textbox 1 Definition of a sampling plan for powdered formulae for infants and young children

Microorganisms	*n*	*c*	*m*	*Class plan*
Enterobacter sakazakii (*Cronobacter* species)*	30	0	0/10 g	2
*Salmonella***	60	0	0/25 g	2

Where n=number of samples that must conform to the criteria: c=the maximum allowable number of defective sample units in a two class plan. m=a microbiological limit which, in a two class plan, separates good quality from defective quality.

Textbox 2 Performance of the sampling plan for *Cronobacter* and *Salmonella*

*The mean concentration detected is 1 cfu in 340 g (if the assumed standard deviation is 0.8 and probability of detection is 95%) or 1 cfu in 100 g (if the assumed standard deviation is 0.5 and probability of detection is 99%).

**The mean concentration detected is 1 cfu in 526 g (if the assumed standard deviation is 0.8 and probability of detection is 95%).

Textbox 3 Description of the methods to be used

The methods to be employed for *E. sakazakii* (*Cronobacter* species) and *Salmonella* should be the most recent editions of ISO/TS 22964: 2006 and ISO 6579, respectively, or other validated methods that provide equivalent sensitivity, reproducibility, reliability, etc.

Textbox 4 Locations where the criteria apply

These are to be applied to the finished product (powder form) after primary packaging or anytime thereafter, up to the moment when the primary package is opened.

These Codex guidelines furthermore define process hygiene criteria to determine the quality of the manufacturing conditions under which the product is produced (Textbox 6). These criteria target two groups of microorganisms (i.e., Mesophilic aerobic bacteria and Enterobacteriaceae) that themselves do not necessarily pose a risk to consumers, but that are utility microorganisms, or possibly indicate the presence of harmful microorganisms due to insufficient conditions of general hygiene in the operation. When product lots do not comply with these hygiene criteria, it signals a high probability that the manufacturing operation is not under sufficient control and appropriate action needs to be taken by the manufacturer to improve the situation before new food lots are produced. Also when food lots are found to be of marginally acceptable quality, the manufacturer should consider appropriate action, such as investigating the hygienic conditions in place and more tightly monitoring food lot quality parameters as to avoid production of defective lots.

A three class plan is used for the 'utility' microorganisms (mesophilic aerobic bacteria) with $n=5$ samples, of which none should be above $M=5000\ g^{-1}$, with no more than two sample units between $m=500\ g^{-1}$ and $M=5000\ g^{-1}$. An exception for this criterion is made, of course, for microorganisms that are intentionally added such as probiotics (explained in the footnote '∗' in the criteria). For the indicator microorganisms (Enterobacteriaceae), a two class plan with $n=10$ samples is defined, of which maximally two sample units can be found positive in a 10 g enrichment. Although for other foods it is more common to use a three class plan approach for indicator microorganisms, in the case of powdered formulae for infants and young children a two class plan is defined due because only very low levels of Enterobacteriaceae are typically occurring when stringent hygiene conditions are maintained.

The performance of this plan for detecting Enterobacteriaceae is described in Textbox 7.

The appropriate methods for analysis are given in Textbox 8.

The locations and scope of use of these microbiological criteria are articulated in Textbox 9 and the action to be taken when samples are found not to conform to the process hygiene criteria (Textbox 10).

Statistical Aspects

Microbiological criteria are used as a tool that is powerful enough to detect defective food products but limits the level of sampling and testing required for this. The level of testing relates much to the statistical aspects of sampling and testing. The first important aspect to realize is that there is a clear distinction between the power of one test ($n=1$), and the result of a sampling plan with a certain n and c value. A single test of a relatively small sample of a food lot or batch obviously would not give much assurance of product safety, considering that lot sizes in a manufacturing operation are very large (e.g., from hundreds of kilograms upward to hundreds of

Textbox 5 Description of the actions to be taken if the product is not conform

The typical action to be taken when there is a failure to meet the above criteria would be to: (1) prevent the affected lot from being released for human consumption, (2) recall the product if it has been released for human consumption, and (3) determine and correct the root cause of the failure.

Textbox 6 Process hygiene criteria

Microorganisms	*n*	*c*	*m*	*M*	*Class plan*
Mesophilic aerobic Bacteria*	5	2	$500\ g^{-1}$	$5000\ g^{-1}$	3
Enterobacteriaceae**	10	2	0/10 g	Not applicable	2

Where n=number of samples that must conform to the criteria: c=the maximum allowable number of defective sample units in a two class plan or marginally acceptable sample units in a three class plan: m=a microbiological limit which, in a two class plan, separates good quality from defective quality or, in a three class plan, separates good quality from marginally acceptable quality: M=a microbiological limit which, in a three class plan, separates marginally acceptable quality from defective quality.

Textbox 7 Performance of Enterobacteriaceae hygiene criteria

**The mean concentration detected is 1 cfu in 16 g (if the assumed standard deviation is 0.8 and probability of detection is 95%) or 1 cfu in 10 g (if the assumed standard deviation is 0.5 and probability of detection is 99%).

Textbox 8 Description of the methods for process hygiene criteria

The methods to be employed for mesophilic aerobic bacteria and Enterobacteriaceae should be the most recent editions of ISO 4833: 2003 and ISO 21528–1/21528–2, respectively, or other validated methods that provide equivalent sensitivity, reproducibility, reliability, etc.

Textbox 9 Description of point of application and scope of process hygiene criteria

These are to be applied to the finished product (powder form) or at any other previous point that provides the information necessary for the purpose of the verification.

The safe production of these products is dependent on maintaining a high level of hygienic control. The following additional microbiological criteria are intended to be used by the manufacturer as a means of ongoing assessment of their hygiene programs, and not by the competent authority. As such these tests are not intended to be used for assessing the safety of a specific lot of product, but instead are intended to be used for verification of the hygiene programs.

tons). To ensure safety of the product only by testing, every part of a lot would have to be tested and no product would be left to sell. Importantly, when a safe product and process design are implemented in a food production operation that is managed with proper hygienic practices and an adequate HACCP principles approach, sampling and testing serves much more the purpose of verifying whether the operation is in control and thus the product is safe. Even for batches for which the management is not known, such as port-of-entry situations, a somewhat limited amount of sampling and testing can give sufficient confidence that the product was produced under appropriate conditions. However, good knowledge of the statistical aspects of sampling and testing is essential to determine what limited level is sufficient for food safety assurance through the use of microbiological criteria.

Acceptance of the Batch Following a Certain Sampling Plan

The curve representing the probability of accepting a batch based on the n and c value as function of the proportion defective sample units is named the operating characteristic curve (OC-curve).

This probability can be described as a Binomial distribution:

$$P_{\text{accept}}(n,c,P_{\text{defective}}) = \text{Bionomial}\ (k \leq c, n = n,\ P = P_{\text{defective}})$$

Determining the cumulative probability of all possible numeric outcomes k, smaller than or equal to the c-value given the probability to be defective ($P_{\text{defective}}$) per sample unit and the number of sample units (n).

Textbox 10 Description of the actions to be taken if the samples are not conform with the process hygiene criteria

The typical action to be taken when there is a failure to meet the above criteria would be to determine and correct the root cause of the failure and, as appropriate, review monitoring procedures, environmental surveillance, and review prerequisite programs in particular the hygienic conditions from the drying step up to the packaging step (Enterobacteriaceae) and the process conditions during wet processing (mesophilic aerobes). Continued failures should be accompanied by increased sampling of the product for *E. sakazakii* (*Cronobacter* species) and *Salmonella* and potential revalidation of the control measures.

For sampling plans in which $c=0$, as is often used for pathogens, this equation equals:

$$P_{\text{accept}}(n,c,P_{\text{defective}}) = \text{Binomial}\ (k = 0, n = n,\ P = P_{\text{defective}})$$

$$= (1 - P_{\text{defective}})^n$$

From this equation it can be clearly shown that the performance of sampling is often rather poor even when high numbers of samples are tested, if the sampling is required to be able to detect a low rate of defective (i.e., contaminated) products (Table 4), which generally should be the case for pathogens which would be occurring infrequently and at low concentration.

From Table 4 it can be seen that even a sampling plan with 60 sample units has quite a low probability of detecting contamination rates of 1% or 2% as the probabilities of acceptance are still 55% and 30%, respectively. To put this in to a practical example: In a batch of 100 000 chocolate bars of which 1% are contaminated with *Salmonella*, i.e., 1000 bars, the probability that this rate of contamination would be detected with 60 sample units is only 45%, meaning that such a batch will go undetected in 55% of the cases. Obviously, in practice, *Salmonella* contamination rates of 1% or 2% in chocolate would already be rather high. Also, the statistics presented in Table 4 assume that the contamination is homogenously distributed throughout the batch, and that $P_{\text{defective}}$ is equal for every sample unit taken. If the contamination however is heterogeneously distributed, $P_{\text{defective}}$ is not equal for every sample taken and there will be an impact on the underlying statistics. This is discussed in the Section 'Probability that a Sample Unit is Defective or Conform'.

When sampling plans are compared and their stringency in making decisions is considered, such as in the examples given in the Section Example of Different Aspects of a Sampling Plan, different aspects of their performance can be addressed. In the ideal situation, the OC curve would switch from a 100% probability of acceptance to a 100% probability of rejection just at the limit of the proportion defective that distinguishes between conforming and nonconforming lots. In practice, as in the previous examples, it is unlikely that a sampling plan can achieve this ideal, but the steeper the curve, the closer the sampling plan comes to approaching the ideal. In general, steeper curves can only be achieved by increasing the number of sample units n to be drawn from a lot. Also a lower value for the acceptance number c will result in a general reduction of the failure to reject a noncompliant lot (i.e., the consumer's risk). From a consumer's or a regulator's point of view, the

Table 4 Probability of accepting a lot with a specified rate of nonconforming units (i.e., $P_{\text{defective}}$) depending on the numbers of sample units analyzed (n), and when no sample unit is permitted to be positive ($c=0$)

% Defective samples ($P_{defective}$)	n = 1	n = 2	n = 5	n = 10	n = 15	n = 20	n = 30	n = 60
0 (0.00)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1 (0.01)	0.99	0.98	0.95	0.90	0.86	0.82	0.74	0.55
2 (0.02)	0.98	0.96	0.90	0.82	0.74	0.67	0.55	0.30
5 (0.05)	0.95	0.90	0.77	0.60	0.46	0.36	0.21	0.05
10 (0.10)	0.90	0.81	0.59	0.35	0.21	0.12	0.04	<0.01
20 (0.20)	0.80	0.64	0.33	0.11	0.04	0.01	<0.01	<0.01
30 (0.30)	0.70	0.49	0.17	0.03	<0.01	<0.01	<0.01	<0.01

confidence in ensuring food safety by applying a sampling plan will tend to focus on a rejection of noncompliant lots with high probability, for example, 95% (or accepted with low probability). Food producers, however, will be more interested in examining which lot quality would be accepted with high probability, say 95%, to adjust their production processes accordingly. This is explained in more detail elsewhere.

Probability that a Sample Unit is Defective or Conform

A second aspect to consider is the probability that any sample unit is either defective or compliant with the microbiological limit. For determining this, the (statistical) distribution of the contaminant in the batch should be known. If the contamination is homogeneously distributed due to complete mixing, the probability that a sample unit is positive is equal for every sample. But if the contamination is heterogeneous, $P_{\text{defective}}$ can be different for every sample and is in itself statistically 'distributed'. Often it is assumed that the contaminants are lognormally distributed. Also other distributions can be assumed or found to be preferable, but due to the fact that many microbial processes are of exponential nature, the assumption that a log transformation makes the distribution normal is reasonable.

Two situations have to be distinguished, first the use of an attribute plan with a given quantitative value of the *m*-value. In this case, the probability that a sample unit is defective equals:

$$P_{\text{defective}} = P_{\text{normal}}\,(\log C > m,\ \text{mean}_{\log C},\ \sigma_{\log C})$$
$$= 1 - P_{\text{normal}}\,(\log C \le m,\ \text{mean}_{\log C},\ \sigma_{\log C})$$

In the case an absence/presence test is carried out (e.g., for two class plans), the probability can be determined by:

$$P_{\text{defective}} = \int_{-\infty}^{\infty} P_{\text{normal}}\,(\log C,\ \text{mean}_{\log C},\ \sigma_{\log C}) \times (1 - \exp\,(-C \times \text{samplesize}))\ \text{d}\log C$$

(In this case the *m* value can be seen as *m* = absence in sample size grams).

Based on the mean log concentration (geometric mean, so the mean on log scale) and the standard deviation (and for counts the *m* value and for enrichments the sample size) $P_{\text{defective}}$ can be determined and can be used to determine the overall performance of the sample plan (based on *n* and *c*) as described in Section Acceptance of the batch following a certain sampling plan. This whole process is exemplified in Figure 2. In this manner the OC-curve is presented as a function of the quality of the batch defined as mean log concentration, having a better relation with risk than the proportion defective samples, because this proportion depends also on factors like the size of the analytical unit.

Difference between a Microbiological Criterion and an FSO/PO

A food safety objective (FSO) specifies the maximum permissible level of a microbiological hazard in a food commodity at the moment of consumption and is based on a management decision regarding the acceptable risk of the hazard to the population or on a public health goal. Maximum hazard levels at other points along the food chain are called performance objectives (POs) and can be derived from the FSO. The current definitions for FSO and PO are that an FSO is: 'the maximum frequency and/or concentration of a hazard in a food at the time of consumption that provides or contributes to the appropriate level of (health) protection (ALOP),' whereas a PO is: 'the maximum frequency and/or concentration of a hazard in a food at a specified step in the food chain before consumption that provides or contributes to an FSO or ALOP, as applicable.' FSOs and POs are general risk-based objectives for a food commodity. An FSO can for example stipulate that the probability of survival of *Clostridium botulinum* in sterilized foods at maximum is 1 spore in 10^{10} cans. Such an objective cannot be tested by any practical microbiological method.

Although FSOs and POs are expressed in quantitative terms, they are not microbiological criteria, which are defined as the acceptability of a product or a food lot, based on the absence/presence or number of microorganisms including parasites, and/or quantity of their toxins/metabolites, per unit(s) of mass, volume, area, or lot. A microbiological criterion is designed such that the underlying microbiological test can be used in practice to verify that a batch meets a predefined level of safety. There are many specific elements defined in a microbiological criterion other than a particular microbiological method.

The microbiological criterion includes for instance the specific parameters of the sampling plan (*n*, *m*, *c*, and *M*), and also the analytical methods to be used and any actions to be taken when the criterion is not met.

The *m* value of a microbiological criterion is also a microbiological limit, but this is the limit related to the results of one sample that is taken. So *m* is the limit in one sample, the MC is the criterion for a lot and the FSO for all batches of a certain commodity in a country or region.

As an example we could define an FSO/PO and a microbiological criterion for *Salmonella* in apple juice:

The general quality/safety level can be expressed in an FSO or PO, that is required, for example, absence of *Salmonella* in 99.99% of 100 mL packages of apple juice. So the FSO is a very generic global target for all food products in a specific category and related to an acceptable level of risk or a public health goal.

For a specific product lot the microbiological criterion can be for example from the European legislation, as described in Textbox 11.

The microbiological criterion is thus clearly related to one specific batch of apple juice, including the *m* value which is in this case the absence in 25 g. Depending on the management of the processing related to this criterion, resulting from both the rejection of positive lots (direct effect) and actions to prevent too many lots being positive (indirect effect), it can contribute to the FSO/PO as given above.

Both microbiological criteria, FSOs and POs need to state the microorganism(s) of concern and/or their toxins/metabolites and the reason for that concern; the food to which the criterion applies; and the specific point(s) in the food chain to which the objective/criterion applies. The microbiological criterion however is clearly related to a specific batch of food,

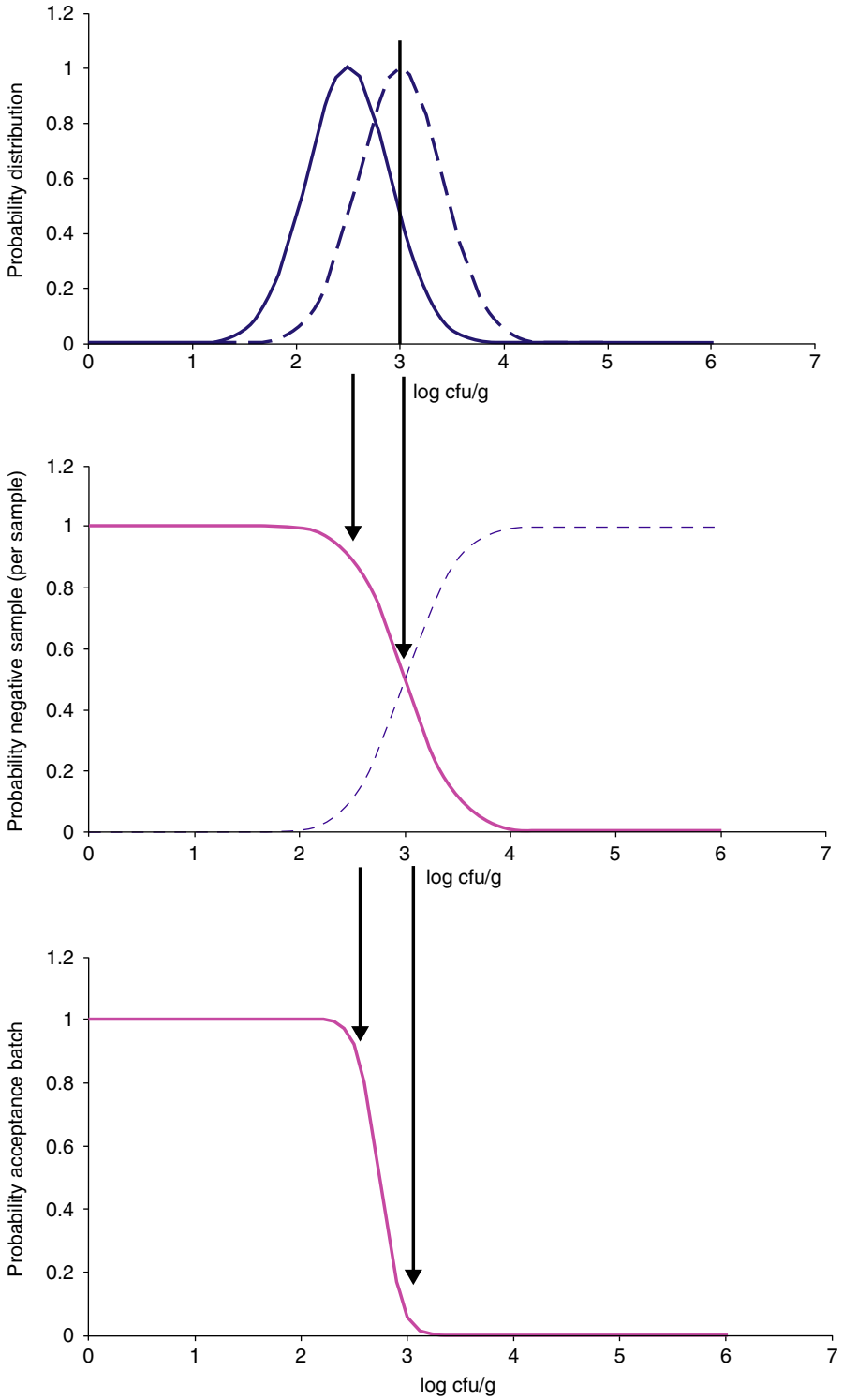

Figure 2 Two distributions describing the heterogeneity in a batch (with mean log$C$ 2.5 and 3 log cfu g^{-1} respectively and with a standard deviation of 0.4 log cfu g^{-1}), with below the probability when taking one sample from these distributions, to be either below (—) and above (—— equal to $P_{defective}$) an m value of 3 log cfu g^{-1}). With this $P_{defective}$ then in the bottom graph the probability to accept a batch giving a sampling plan with $n=10$ and $c=2$ is represented.

Textbox 11 Definition of an EU sampling plan for unpasteurized fruit and vegetable juices (ready-to-eat) for products placed on the market during their shelf life using as analytical method EN/ISO 6579

Microorganisms	*n*	*c*	*m*	*Class plan*
Salmonella	5	0	0/25 g	2

Where n = number of samples that must conform to the criteria: c = the maximum allowable number of defective sample units in a two class plan. m = a microbiological limit which, in a two class plan, separates good quality from defective quality.

whereas the FSO and the PO are general objectives for all batches of a commodity in a specific region or country.

Conclusions

Sampling plans are useful components within food safety management and quality control, however results should be interpreted with care, because results do not give an absolute indication of the quality of the batch under study. The cases as defined by the ICMSF give useful guidelines for selecting the characteristics of sampling plans, taking into account the degree of concern of the hazard and the conditions of storage and use of the product. By determining the performance of sampling plans or the OC-curves, a quantitative indication of the performance of sampling plans can be obtained.

See also: Food Safety Assurance Systems: Management of Supplier and Raw Material. Public Health Measures: Modern Approach to Food Safety Management: An Overview

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FOOD SAFETY ASSURANCE SYSTEMS

Management of Allergens in Food Industry

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Glossary

Hazard A biological, chemical, or physical agent in, or condition of, food with the potential to cause an adverse health effect.

Hazard analysis critical control point (HACCP) A system which identifies, evaluates, and controls hazards which are significant for food safety.

Minimum eliciting dose (MED) The lowest dose of an allergenic food or protein demonstrated to cause a reaction in a food challenge (normally a double-blind, placebo-controlled food challenge)

Probabilistic (risk assessment) Risk assessment methods that produce quantitative risk estimates based on the distributions associated with the input variables (e.g., MEDs and food consumption) rather than point estimates such as the No Observed Adverse Effect Level or the mean consumption of a food.

Risk A function of the probability of an adverse health effect and the severity of that effect, consequential to a hazard(s) in food.

Risk assessment A scientifically based process consisting of the following steps: (1) hazard identification, (2) hazard characterization, (3) exposure assessment, and (4) risk characterization.

Introduction

The recognition of food allergy as a food safety issue by the food industry dates back to the 1990s, and its public health importance was affirmed in a Food and Agriculture Organization of the United Nations–World Health Organization (FAO–WHO) Consultation in 1995. The growing prevalence of allergies and the severity of reported reactions to foods combined to highlight its public health significance and spurred regulatory efforts to address it. Recent evidence indicates that the implications of food allergies to the affected individuals extend beyond the immediate potential health impacts to significant influence on quality of life.

For practical purposes, no cure exists for food allergy, and allergic consumers can therefore only manage their condition by avoiding foods, which contain the relevant allergenic ingredient(s). These may be present deliberately, as part of a product formulation. However, food production and manufacturing practices mean that allergenic constituents may also be present inadvertently, perhaps because they occur in the raw materials used or are carried over in small amounts from a previous product during manufacture. This inadvertent presence is not formally regulated under labeling legislation, but is arguably covered under more general food safety law, such as the EU's Regulation 178/2002 (EC).

A key issue has been how to assess the risk from this unintended presence of allergenic material and protect allergic consumers. Lack of data and knowledge, together with the potentially severe consequences, resulted in the widespread use of precautionary labeling (e.g., 'may contain peanuts'), a somewhat unique feature of allergen labeling and management. However, recent years have seen the generation of data and development of models that offer the possibility of quantitative risk assessment. Although this is a critical aspect of managing the risks from the unintended presence of allergenic residues, equally critical is knowing how allergenic residues may find themselves in products in the first place. An important insight was that effective allergen management needed to be integrated into overall food safety management not only so that allergens are taken into account at all points, but also so that the impact of allergen control measures on other food safety endpoints can be considered and, where necessary, mitigated.

Although allergens can provoke reactions rapidly and in small doses, a particular challenge for allergen management remains that, for practical purposes, allergenic constituents cannot be excluded from manufactured food products, and therefore most manufacturing sites, as many are important and valuable sources of nutrients.

Food Allergy

Food allergy describes a condition where an immune response to a food occurs in an individual and a subsequent encounter with the food provokes an adverse reaction. Foods can induce many different types of immune and allergic responses, but the current public health concern lies principally with those in which IgE antibodies are produced to proteins in the food. These antibodies are then implicated in immediate-type

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reactions on subsequent exposure. Such reactions can vary from very slight to severe and occasionally fatal, depending on the dose, the individual, and other factors. Food allergy affects a higher proportion of children than adults, and reactivity to some allergenic foods, such as milk and egg, tends to be largely outgrown, whereas allergy to others such as peanuts generally persists. Little is known about why allergy to certain foods develops, although exposure and its pattern, the characteristics of the implicated proteins, and also the characteristics of the allergic individual, such as atopy, play a role. The minimum doses required to elicit an observable reaction range from microgram to gram amounts. Until recently, the distribution of sensitivity in the allergic population, as expressed by minimum eliciting doses (MEDs) remained uncharacterized, making risk assessments arduous and fraught with uncertainty. However, recent work analyzing results from double-blind placebo-controlled food challenges (DBPCFC) conducted under well-defined conditions demonstrates that sufficient data are available to characterize the response to many allergenic foods of public health importance, such as peanut.

Where prevalence has been estimated, data indicate that food allergy affects approximately 2–4% of the overall population, with a higher proportion among young children. Milk and egg are common allergenic foods among infants worldwide, but considerable diversity exists among older population groups, both with regard to the prevalence of allergy to different foods within populations and between populations. Management of the public health risk from allergenic foods also requires an understanding of which foods provoke allergies in particular regions or countries.

Avoidance of the offending food remains the only way of preventing reactions. Accurate labeling of food products, where the allergenic constituent is not obvious, is therefore critical to the safety of people with a food allergy. Legislation now recognizes this in countries covering over a third of the world population and mandates the labeling of allergens of public health importance among their populations. In contrast, the unintended presence of allergenic constituents is not specifically regulated, but instead relies on the voluntary use of precautionary labeling to warn allergic people to avoid the food. However, the absence of commonly agreed standards for its application and the uncertainty over the risk posed has led to its pervasive use. Evidence indicates that this extensive use impairs the quality of life of people with food allergies, particularly through severely limiting their food choices, and leads to risk taking. This situation feeds the fear of reaction, which is a documented feature of the environment of a proportion of food-allergic people.

Food Allergens as Contaminants

Unintended allergenic constituents in a food can unquestionably be considered as contaminants. However, they differ from the more usual chemical or microbiological contaminants. First, any one allergenic substance poses a risk to a comparatively small proportion of the population, the prevalence of allergy to any particular food rarely exceeding 2%. Second, and significantly, many allergenic substances constitute important sources of nutrients, which could not be readily replaced and are used in often large quantities in food manufacturing. They are therefore ubiquitous and often extremely difficult to remove completely while maintaining the economic viability and sustainability of the operation. Because allergens are natural components of foods, the term cross-contact is usually used to refer to unintended presence of allergens, particularly as a result of transfer during manufacturing processes. Most stakeholders now accept the impossibility of reducing risk to zero, particularly in view of risk-taking behaviors resulting from the widespread use of precautionary labeling. Furthermore, measures to reduce allergen contamination can also impact on other aspects of food safety (e.g., microbiological safety) as well as the environment and use of resources (e.g., water). Thus, risk minimization is probably a more appropriate objective of allergen management.

Food Safety Systems and Their Requirements and Limitations

Several international as well as national documents provide direction and guidance on the requirements for food safety systems, including the Global Food Safety Initiative (GFSI) Guidance document and ISO 22000:2005. Associated with those documents are prerequisite programs (PRPs), an example of which is the Publically Available Specification 220:2008, developed by the British Standards Institute in collaboration with the food industry. All these documents recognize that the effective management of allergens can only be achieved if it is integrated with food safety management systems to manage other food safety hazards. Integration not only ensures that food allergy is properly considered as a hazard, but also that measures taken to mitigate it do not aggravate other food safety hazards. Prerequisite programs defined in the context of good manufacturing practices also provide a sound basis for allergen management but are not sufficient on their own. The principles of hazard analysis critical control point (HACCP) analysis can be readily applied to allergens, even though the current lack of agreed management action levels remains a significant drawback. Examples of possible critical control points include sanitation and labeling verification.

Practical Application: What Needs to be Done?

Allergen management implies actively dealing with allergens when making food products so that allergic consumers can make safe choices. It requires knowing where and what allergens are present throughout the food manufacturing process, deliberately or, perhaps even more importantly, unintentionally. It is also about assessing the residual risk if an unintended allergen cannot be completely removed from a product and communicating it clearly and accurately to consumers. Allergen management thus concerns the whole supply chain from the farm to the final consumer and requires accurate and comprehensive information about allergens from all those stages. Assessment starts from an analysis of all the

stages leading from raw materials to finished products to identify the probability and extent to which this happens. It follows that a manufacturer must consider and understand the allergen status of all raw materials and processes involved in manufacturing the product in question. Implementation of allergen management demands significant resources and therefore requires engagement of senior management within companies, as recognized by the Food Safety Management Standard ISO 22000:2005.

Underlying allergen management, as well as food safety generally, are PRPs, which describe the basic conditions considered necessary to assure safe food production. These include considerations of premises design, hygiene, etc. and will not be described here as they are general food safety requirements.

Allergen management requires firstly identification of all sources of the allergen risks, then assessment of those risks and subsequently their management. This will be an iterative process, since, having identified a risk and determined that it is significant, the first step will be to look at ways of reducing it. Allergen risks can occur at all stages of the food manufacturing process, which can be summarized as design, sourcing, manufacture, and delivery.

At the design stage, key considerations include product composition and ingredient specification. An allergen's contribution to product functionality should be critically assessed and the feasibility of substitution or omission considered. Ingredient specifications are also critical, particularly in respect of unintentional presence of allergens. For instance, a 'gluten-free' claim would require assurance from suppliers of absence of gluten, supported by evidence that their ingredients meet appropriate specifications. The measures required to manufacture the product so that no additional allergen risks created need to be considered, particularly if a new allergen is introduced to a site.

At the sourcing stage, comprehensive, accurate, and reliable information about the ingredients is critical. Supplier questionnaires should provide information about their allergen management, such as their understanding and application of processes such as HACCP. Scrutiny of 'may contain' disclaimers and statements may be needed better to understand the resulting risk, whereas quantitative information may need to be sought to permit a quantitative risk assessment. Periodic audits, either by the company's own auditors or by auditors, accredited under the major standards (e.g., British Retail Consortium, International Featured Standards (IFS), GFSI) should support this process. Suppliers must also understand that they cannot change a formulation or specification without agreement. Making provision of appropriate (i.e., suitable for use in risk assessment) allergen information a contractual requirement is strongly recommended.

At the manufacturing stage, detailed knowledge of the design and operation of the plant are imperative to the successful management of allergens. Critical elements include identifying where the risk of allergen cross-contact arises and devising systems to minimize it. Parts of the manufacturing system through which the ingredients do not obviously flow can seriously challenge attempts to manage allergens as well as validation studies. The filters used to protect machinery in vacuum/pneumatic material transport systems are a good example. If they are not cleaned or replaced at appropriate intervals, product residues build up on them, which can be released at random intervals into product. As a result, the quantitative risk assessment based on validation studies can be totally negated. Measures to minimize cross-contact include allergen segregation in both space and time, including careful design of storage areas to minimize potential contamination in the event of spills, dedicated equipment, and, occasionally, whole lines or facilities, where feasible. Production scheduling effects separation in time and is a powerful measure in allergen management. However, the complexity involved should not be underestimated because allergens are not the only variables that need to be taken into account, with flavor and color among two other important considerations. Of course, each allergen needs to be considered individually too.

Cleaning also separates allergens from other components and each other, both in time and space. Protocols need to be validated to ensure they achieve their purpose and then need to be periodically verified. Indeed cleaning can also be considered as a critical control point in a HACCP plan. Validation, discussed in more detail later, usually involves analytical measurements, but a visually clean standard may be sufficient for accessible parts.

Finally, measures that can only be implemented as part of a longer term plan include equipment selection and factory design.

The delivery stage is the one at which the product is brought to the consumer. Failures at this point account for a significant proportion of allergen alerts (e.g., UK Food Standards Agency reports). Critical attention to artwork is needed to ensure that the correct packaging has been used and that all allergens are listed and clear to the consumer or purchaser. Controls include, for instance, ensuring that label rolls for previous products are removed from packing line after a product run. Use of bar code recognition systems can reduce the possibility of errors at this point. At this stage the packaging should be checked for incompatible elements, such as a 'dairy-free' logo but with milk in the ingredients. The packaging is also the vehicle for any precautionary labeling that a risk assessment has shown to be required for the product. This will include verifying whether suppliers' precautionary statements on ingredients and raw materials warrant carrying through to the final product. However, precautionary labeling can never be a substitute for good allergen management measures and does not, of itself, exonerate the manufacturer from any legal liability. If an allergen box is used, then this also needs to accord with the ingredients list: product recalls have occurred as a result of discrepancies in this area.

Allergen Management Plans

Allergen control (management) plans summarize all the necessary elements that must be checked in order to determine the allergen status of a specific facility and define the control measures that may be needed. It can thus be developed as part of the more general HACCP plan, considering the flow of materials through the factory. The Allergen Control Plan can therefore follow the schema outlined in Table 1.

Table 1 Components of an allergen management plan

Raw material sourcing
● Is the specification of raw materials and semifinished ingredients accurate and comprehensive with regard to allergens?
● Can the allergen risks be accurately assessed from the specification?
● Have all allergenic materials that are used at the facility been categorized?
Raw material receipt and storage
● Could cross-contact occur between raw materials during transport?
● Could raw materials be stored in the wrong location?
● Is segregation of allergens from other raw materials and each other ensured during storage, even in case of failure to contain them?
Manufacturing operations
● Are material flows comprehensively described and understood so that all possibilities for cross-contact have been identified?
● Have all operations where cross-contact can take place been identified?
● Have the opportunities for scheduling (e.g., nonallergen before allergen) been explored and implemented?
● Are formulations positively checked?
● Is work in progress properly labeled?
● Is rework of products containing allergens controlled?
● Do procedures exist to avoid mispackaging?
● Do protocols exist for all cleaning operations and have they been validated?
● Are cleaning operations verified and how?
● Has a study to validate allergen management at the facility been conducted and documented?
● Are there procedures to avoid inadvertent introduction of allergens into manufacturing areas (e.g., on clothing, tools, etc.)?
Personnel and training
● Have all personnel, including part-time and temporary staff, trained in allergen management to a level appropriate to their role?
● Is basic allergen training included in staff induction procedures?

Evaluating the Risk from Allergens

Thresholds and Hazard Characterization

Knowing what amount of an allergenic food can be tolerated by what proportion of the allergic population is a critical consideration for assessment and management of the resulting public health risk. For many years, this remained the most striking knowledge gap. Although regulatory authorities and others considered that thresholds below which reactions do not occur exist, they cannot be defined. Considerable data have now been acquired from studies using DBPCFC for many of the most important allergenic foods. Statistical modeling of dose distributions using data on MEDs from food challenges has proved very successful in filling this gap for several allergens while avoiding the difficulties of defining an absolute (population) threshold or no observed effect level experimentally and is now widely accepted. The principle of this approach consists of plotting the individual MEDs from controlled food challenges against the frequency of reaction to derive a cumulative population dose distribution, which can then be fitted to different statistical models. This approach helped to define eliciting doses corresponding to amounts of allergen predicted to cause reactions in small proportions of the allergic population (5% or less). These studies reveal that the range of reactivity spans amounts from micrograms to grams, but the proportion reacting to very low amounts is actually quite small. For peanut, the most extensively studied food, data on upward of 450 patients have now been identified and modeled (Figure 1). Indications are that 10% of peanut-allergic patients from clinics react to a dose of between 10 and 15 mg of whole peanut and 5% to a dose of approximately 5 mg.

Risk Assessment for Food Allergens

Risk assessment implies probability and, therefore, involves a number of factors in addition to dose distribution. At the population level, the probability of a reaction to an allergen depends on the prevalence of allergy to the food, the amount present, the likelihood that an allergic person will consume the product (in sufficient amount) and that person is sensitive enough to react to the amount of allergen consumed. Both the probability that an allergic person will consume the product and the amount present can be influenced by other factors. Ideally, a precautionary label would result in avoidance of the product by the relevant allergic individuals. In practice, observance of the warning can be quite low ($<50\%$). The reasons for this are complex, but they include lack of credibility as well as consumer confusion over the message, no doubt exacerbated by the large number of different precautionary statements. The statistical modeling approach can help to define quantitative action levels because information about the extent of consumer compliance with precautionary labeling as a function of its prevalence can be factored in as an additional quantitative factor (Figure 2). The distribution of unintended allergen in the product population is another variable, which can be included. The same estimate of cross-contact for products made according to different processes, using worst-case assumptions, may also mask completely different allergen distributions and consequently risk profiles. Probabilistic approaches open the possibility not only for more refined quantitative assessment of the global public health risk from a category of products, but they can also be applied to specific processes and actively help to improve management of allergens by enabling the investigation of particular scenarios to see which are most effective in minimizing risk.

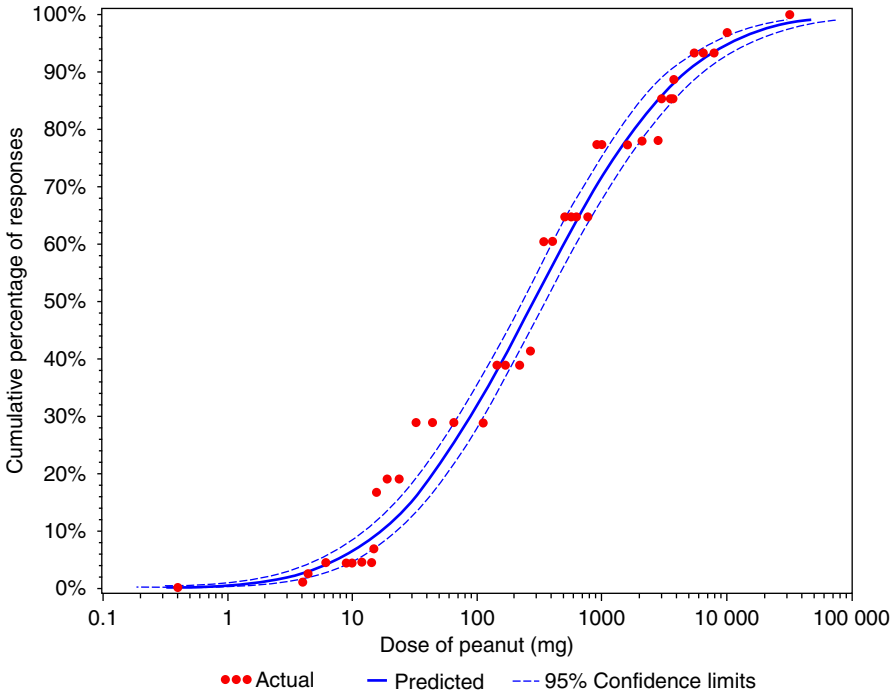

Figure 1 Dose distribution for peanut. Reproduced with permission from Taylor SL, Moneret-Vautrin DA, Crevel RW, *et al.* (2010) Threshold dose for peanut: Risk characterization based upon diagnostic oral challenge of a series of 286 peanut-allergic individuals. *Food and Chemical Toxicology* 48: 814–819.

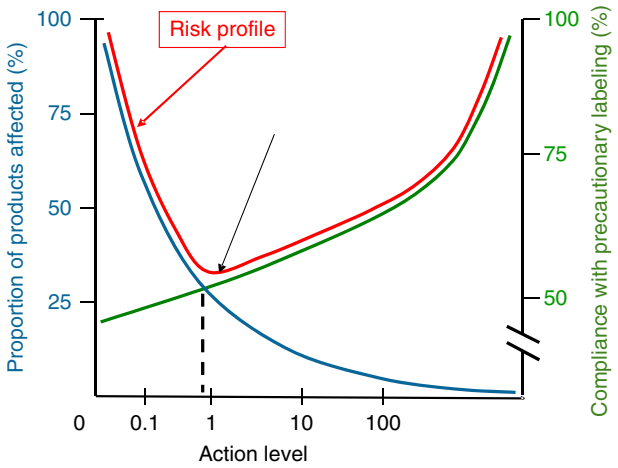

Figure 2 Postulated relationship between extent of use of precautionary labeling and observance.

Currently, there are neither generally agreed quantitative standards defining when unintended food allergens should be declared nor how validation results should be interpreted. However, there have been some attempts in different parts of world to provide labeling guidance. In Switzerland, an action limit for labeling of 1 g kg^{-1} (one part per thousand) was defined in 2001, and in Australia and New Zealand, the Allergen Bureau (an initiative of the Australian Food and Grocery Council) developed and recently revised the voluntary incidental trace allergen labeling (VITAL) system. The system includes a set of action levels that define when a precautionary label is deemed necessary to protect allergic consumers, based on clear levels of risk derived from available food challenge data.

Severity is also an important component of risk, but the relationship between dose and severity remains ill defined. In food allergy, consumption of the allergen may result in barely perceptible signs at one extreme and death at the other. For many years, not only was the proportion likely to react to a defined dose unknown, but also reactions to any particular dose were also thought to be unpredictable. Although a number of extrinsic factors (e.g., infection, stress, etc.) affect

the outcome of exposure to allergen, the dose itself is now recognized to be critical. Other recent studies provide information about the likely proportion of severe reactions in particular groups, which can form the basis of some reference points for setting limits in a public health context, such as the reference dose recently proposed as part of the Australia–New Zealand VITAL program. Once defined, the probability of a severe reaction could easily be incorporated into the probabilistic models described as an additional criterion.

Operational Aspects

Risk assessment as described above requires access to significant resource and expertise, which may not be readily available to smaller or even medium-sized food operators. The starting point in these cases will be identifying where allergen risks arise throughout the production process and managing them according to their likelihood and the consequences of possible failure to address them. These can be ranked on relatively simple qualitative scales, such as 'unlikely' to 'likely' and 'mild' to 'severe'. Likelihood that failure would be undetected can also be incorporated into this assessment. Taken overall, an assessment based on those elements will help the operator focus on managing those situations, which are most likely to result in harm to the allergic consumer. Table 1, summarizing the elements of an allergen management plan, provides an outline of relevant sources of allergen risks.

Allergen Cleaning Validation Studies

Validation and Verification

As described earlier, unintended allergenic constituents in a product can pose a risk to allergic individuals. Total avoidance of cross-contact and therefore absence of specific allergens from products where they are not part of the formulation is not always practicable. Therefore, an analysis of the risk arising from the residual allergen is required to ensure that precautionary ('may contain') labeling is only applied when absolutely necessary in order to maintain the value of such warnings.

Validation is the process of checking whether or not current allergen management procedures, particularly cleaning procedures, control allergen cross-contact to an acceptable level. Verification is checking and recording that validated procedures, which are being implemented.

Allergen Detection Methods

Allergen detection down to appropriately low concentrations is critical to effective validation and verification. A variety of methods are used for allergen cleaning validation studies, and the most common are listed in Table 2 along with their major advantages and drawbacks.

Enzyme-linked immunosorbent assays (ELISAs) are currently the most commonly used allergen detection tests, but although quick, sensitive, and relatively simple, they suffer from a number of disadvantages that require careful consideration.

- ELISAs rely on an antibody reaction with a protein(s). Proteins exist in different forms and relative abundances in different foodstuffs. Thus, antibodies in an ELISA may have been raised against a different mixture of proteins to those present in the potential contaminating material. Target protein(s) can also differ between ELISA kits that have the same purpose, for example, ELISAs for milk may detect beta-lactoglobulin or casein or a mixture of milk proteins. Therefore, knowledge of the protein composition of the allergen source is required in order to ensure the correct choice of kit and for the interpretation of the data.

Table 2 Common methods used in allergen cleaning validation studies

Method	*Target*	*Main advantages*	*Main disadvantages*
Non-specific methods ● Visual check ● Adenosine triphosphate (ATP) ● Total protein	N/A ATP Protein	Rapid and cheap	Only apply to accessible areas and equipment dependent Lack of specificity Possible false negative results Positive results difficult to interpret
DNA Detection methods ● Polymerase chain reaction (PCR)	Species-specific DNA sequences	DNA is relatively stable Usable when in absence of suitable ELISA Confirmatory technique alongside an ELISA	Indirect indication of potential allergen presence Negative result does not confirm lack of allergen Requires highly trained laboratory staff and equipment: expensive, and time consuming
Antibody-based detection methods/immunochemistry ● Enzyme-linked immunosorbent assay (ELISA) ● Lateral flow/dipstick devices	Allergenic/antigenic protein	Measures allergenic component, i.e., protein Sensitive (ppm) Relatively fast Quantitative Relatively cheap, rapid, and simple to use	Not available for all common allergens Different protein targets for the same allergen and different performance Extraction of target proteins from samples and/or ability to detect them variable Lateral flow devices qualitative (semiquantitative at best)

Positive control standards of similar composition to the contaminating material should be used to calibrate the assay.

- Food processing can alter proteins and their ability to be detected or extracted and therefore measured. ELISAs may thus produce a false negative result or quantify inaccurately for heated, fermented, and hydrolyzed products.
- ELISAs require the extraction of the protein into an aqueous environment before analysis. The efficiency of this extraction can vary considerably and should be checked using a 'spike and recovery test' where the allergen is mixed into and recovered from the matrix of interest, known to be free of the allergen. The food matrix can also affect ELISAs directly, for example, some ingredients could cross-react with the antibodies in the ELISA to give a false positive reading and others may produce color backgrounds that need to be controlled for.
- Both ELISA and PCR require a degree of technical expertise and comprehensive understanding of the materials and matrices involved. Thus, whether an in-house laboratory or an external one is used, it should be accredited with a recognized scheme and participate in proficiency testing.

Design of Validation Studies

Starting with a qualitative risk assessment and then moving onto a semiquantitative is recommended in order to determine whether or not an analytically based validation study is required or applicable. For example, it is sometimes possible to estimate levels of allergen carryover from one production run to another by 'worst-case scenario calculations,' i.e., measuring how much material is left behind in a process (e.g., based on film thickness on equipment or weighing brushed out residual), what the levels of such material would be after dilution with the next product (or in the next process step), and what amount of the material is allergen and therefore allergen levels in the final product that could be consumed.

If an analytically based study is required, accurate and robust analytical results are only useful if the samples analyzed have been taken as part of a correctly designed study. This will include the sampling locations and procedures and subsequent analyses.

For a food product, development of a scientifically sound sampling plan includes a statistical analysis of the probability that all allergens are detected and ensures that any allergens present are accurately measured. Important sampling questions that need to be considered include whether the allergen is likely to be evenly distributed within the batch; the number of samples per batch that should be tested; which batches should be tested; which portion of a run should be tested; and how to obtain a specific degree of confidence about allergen presence (e.g., the potential carryover from skimmed milk powder, a nonparticulate material can be assessed analytically, which would be inappropriate if the contamination were particulate (e.g., nut pieces). For the latter, a visual inspection should take place after cleaning to ensure that no particulates are left, supplemented by enumeration to determine probability of occurrence. The build-up of allergenic material on process line/equipment (e.g., heat exchange plate) also needs to be assessed, as it can be a source of random contamination).

Table 3 Considerations for an allergen validation study

1. Define and document procedure to be validated
2. Define and understand the potential 'contaminating' material
3. Define what and how to sample
   a. Surface sampling (swabbing)
   b. Rinse/push materials
   c. Final product
4. Include appropriate control samples
   a. Negative control – matrix free from the allergenic ingredient
   b. Control containing known amount of allergen (positive control)
   c. Standards of the same composition as contaminating material (in addition to kit standards)
5. Define in the study protocol how to take, label, and store samples
6. Define how results will be interpreted in terms of risk assessment

Table 3 summarizes the six main points to consider in the design of an analytical validation study to ensure that it is fit for purpose.

Verification

Cleaning processes should be periodically verified to confirm that they remain effective. If changes are made that might impact allergen management, for example, design alterations to a process line/equipment a revalidation should be performed.

Examples of Validation Studies

Example 1. A worst-case scenario calculation

Does soy lecithin carried over into non-soy-containing products made on a shared line, present a risk to consumers?

- Highest soy lecithin content in any recipe: 25 mg kg^{-1}
- Protein content of soy lecithin: 1000 mg kg^{-1}
- Worst case carryover: 3%
  - Maximum carryover: $25 \times 0.001 \times 0.03 = 0.00075$ mg kg^{-1} soy protein
  - Amount of soy protein in a 200 g serving of product: 0.000 15 mg
  - Soy reference dose based on VITAL 2.0: 1.0 mg
- Conclusion: negligible risk

Example 2. An analytically based cleaning validation study

Are the cleaning protocols adequate to control unintended milk presence in nonmilk products?

- HACCP assessment suggested a minimal extent of cross-contact
- Validation study discussed and designed with experienced accredited laboratory, who then conducted the analyses
- Rinse water sampled in all loops of cleaning-in-place (CIP) system, as well as final product
- Rinse water contained 2 mg kg^{-1} beta-lactoglobulin (equivalent to 3–4 mg kg^{-1} whey protein)
- Rinse water volume is 20% of batch size component of product and that constitutes 25% of final product.

- Concentration of whey protein in product: $3–4 \times 0.2 \times 0.25 = 0.15–0.2$ mg kg^{-1}
- Product serving size: approximately 20 g
- Whey protein per portion: 0.003–0.004 mg
- ED1 for milk (VITAL 2.0): 0.1 mg, i.e., approximately 0.02 mg whey protein
- Conclusion: risk negligible, no precautionary labeling required.

See also: Food Safety Assurance Systems: Cleaning and Disinfection; Food Safety and Quality Management Systems; Hazard Analysis and Critical Control Point System (HACCP): Principles and Practice; Hygienic Design of Equipment; Labeling and Information for Consumers; Recall Systems and Disposal of Food. Institutions Involved in Food Safety: FAO/WHO Codex Alimentarius Commission (CAC). Other Significant Hazards: Food Allergies and Intolerances. Public Health Measures: Fundamentals of Food Legislation; Health Education, Information, and Risk Communication

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Relevant Website

www.allergenbureau.net/vital/vital

VITAL: Voluntary Incidental Trace Allergen Labelling system.

FOOD SAFETY ASSURANCE SYSTEMS

Management of Supplier and Raw Material

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Glossary

Culture Attributes exhibited by people through their behavior as a result of what was inherited, formally studied, and experienced.

Global Food Safety Initiative (GFSI) It was launched as a nonprofit making foundation in 2000, to achieve harmonization of food safety standards that would drive and reduce audit duplication throughout the supply chain.

Good hygienic practice (GHP) These are standard practices often used in order to achieve/maintain good hygiene.

Good manufacturing practice (GMP) These are standard practices often used in order to achieve/maintain good manufacturing.

Hazard analysis critical control point (HACCP) A food safety management tool focusing on the hazards and the steps in food manufacturing which are critical to food safety.

Supply chain The successive stages in food production, often beginning at the farm/field through to customer delivery.

Introduction

Every business establishment has a supplier and a customer; together they form the supply chain. Depending on the scope under discussion, the supply chain may have two or more components. A simplified generic linear supply chain may have six components consisting of the farm/field, raw material processor (e.g., miller), secondary processor (e.g., bakery), warehousing, retail, and consumer. This is presented graphically in Figure 1. In reality, this chain is not entirely linear, but cyclic, with waste material forming one of the foundational inputs of the growing step at farm/field. Also each component would often have more than one supplier and one customer. For simplicity and more focused approach to explore supplier management, reference would be made to one supplier providing raw material to one customer.

As each component of the supply chain has raw material supplier(s) upstream, and customer(s) downstream, it has to be able to manage its supplier(s) and the ingredient(s). Naturally, the selected component of the supply chain is itself a supplier of raw material to its customer downstream; as such similar expectation should be in place in its own facility as those it would impose on its supplier.

Several management system controls used by each supplier in the chain are similar, if not the same, with respect to food safety. The same would apply with regards to the tools used for management of food safety. Several studies have delved in and described these management tools in great detail, such as good practices and hazard analysis and critical control point (HACCP). These tools shall be addressed here, but not in great depth, because they will be covered in other articles of this encyclopedia.

Management of suppliers and ingredients are assisted by the tools described above (GMP, HACCP, etc.), and others such as ingredient and product specifications, regular reviews of performance and audits. But often, a major part of management requirement is overlooked, specifically the organization and the personnel within it and their expertise. In this article some of the requirements for supplier and raw material management for all three perspectives will be reviewed, i.e., organization, personnel, and supplier management tools used by the latter two entities.

The Organization

A manufacturer may be large, medium, or small in size, but their ethos toward management of their suppliers and raw

Figure 1 Supply chain example.

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material could be similar. It is often this similarity that attracts them to each other. This is so because they all believe that 'rubbish in rubbish out' is not a cliché, but a fact that cannot be ignored if one is aiming for the provision of a good product. Therefore, to provide top quality product to the customer, one has no choice but to choose top quality raw ingredients provided by top quality suppliers.

However, identification of a quality supplier who can provide the desired ingredients is not an easy task. How would one identify a good supplier? What is it that one would look for in an organization? Would one recognize a top quality organization if one saw it? These are fundamental questions that need to be answered before the search can begin. The answers are often initially found when one begins to look inwards and identify its own standards. The application of 'lead by example' strategy is often a good start. This works because an organization, like people, would at first work with those of similar standing. In time, the organization will work with those who are better than itself, to initiate self-improvement. And when it has achieved a certain secure standing, it will begin to work with those with potential to rise to similar standards.

It is, therefore, the ability of an organization to be able to see itself for what it is and what it can achieve; that begins the process of setting standards for supplier and ingredient management, based on its own internal standards. All this begins with the company ethics that dictates their attitude toward their vendors and customers.

Naturally an organization is made up of people and it is the people who carry out the investigations necessary for identification, selection, approval, engagement, and management of the supplier and the ingredients.

The Personnel

One of the key components of a successful management system are the managers who are responsible for supplier and raw material management. Just as there are specific criteria associated with the managers of each department, the same applies for those assigned to food safety. As in any other profession, there are desirable attributes that are necessary to be able to manage food safety systems of a supplier and the raw material successfully. Listed below are some of the qualities that are sought when identifying a supplier and raw material manager with regards to food safety:

- Relevant education.
- Appropriate hands-on experience.
- Ability to translate science into layman's terminology.
- Resourcefulness.
- Reporting capabilities.
- Sense of responsibility.
- Sense of accountability.
- Honesty.
- Sharing similar ethics with that of the company.
- Cool headed.
- Respectful.

Some of the above-listed requirements appear as personality traits, which are arguably more important than formal education. It is these 'soft' competencies that are derived from a person's culture, and it is a person's culture that may be very influential when managing suppliers. The subject of culture is an important and intriguing one, which will be covered in other sections of this encyclopedia. With the necessary personnel selected and appropriately integrated into the company's culture, the manager can begin the process of supplier and raw material management.

The Supplier Management Tools

Managing suppliers and raw material within the supply chain is carried out by the organization's personnel under the 'personality' of the organization. These traits define the approach a manager takes, which are important in the overall management process. The tools and their use by the organization and its personnel form the practical application in management.

Supplier and raw material management has many facets and these can be divided and assessed separately. One of the directions that may be taken is defined below:

1. Supplier identification – The organizations identified for possible selection should be based on:
   a. Their ability to supply desired raw material(s).
   b. Their reputability.
2. Supplier selection – Of the identified suppliers, those who meet the necessary attributes listed below would be suited for selection:
   a. Share selecting organization's values.
   b. Assured supply.
   c. Assured quality (including food safety) of product.
   d. Appropriately qualified personnel.
   e. Works under a good quality (including food safety) management systems.

With the supplier being selected, engagement and working with the supplier begins. Approaching the supplier as a long-term partner in business is a winning approach. This is logical when one realizes that the success of one will naturally lead to the success of the other. Therefore, working together as partners would have a greater chance of success than working alone. Beginning the partnership requires building trust and it is at this point that verification of the food safety system is necessary to move the supplier into the approved phase.

3. Supplier approval – The supplier approval process is of vital importance for the vendor, as it provides information on the customer's expectations. By knowing the expectations the vendor can more effectively and efficiently meet the customer's needs. The requirements within the expectations will come in the form of internal process assessment through their quality (including food safety) management system. This system must have the criteria listed below:
   a. Prerequisite programme (PRP) – The PRPs needed depend on the segment of the food chain in which the organization operates and the type of organization. PRPs have historically been referred to as good practices and identified by the sector they apply to. Some examples of equivalent terms are good agricultural practice (GAP), good manufacturing practice (GMP), good hygienic practice (GHP) and good distribution

practice (GDP). PRPs consist of numerous requirements which are bundled together under one heading. Each requirement needs to be appropriately defined, developed and managed before establishing an HACCP system. PRPs for manufacturers may include topics such as, but not limited to:

i. Facilities and grounds.
   - Building exterior – The design, construction and maintenance must be in such a way as to protect the interior from contamination.
   - Building interior – The layout must be designed, constructed and maintained to facilitate GMP. Included in this area are walls, ceilings, air systems, processing area, lighting, and employee facilities.
   - Personnel and equipment movement – The movement patterns of materials, products and people shall be designed to protect against potential product contamination.
   - Waste handling – There must be systems to ensure that waste materials are identified, collected, removed and disposed in a manner that prevents contamination of products.

ii. Facility security – The facility must take appropriate security measures to prevent intentional harm to staff, product, processes, and building.

iii. Work environment – The working environment must be suitable to facilitate the production of safe and quality food. This would involve providing the appropriate layout, lighting, staff changing rooms and toilets, fit-for-purpose walls, ceilings, floors and drains.

iv. Equipment – All equipments used for the manufacturing of food must be of good hygienic design, calibrated, and well maintained.

v. Training – Appropriate training for new and existing employees.

vi. Material handling – Procedures must be in place to protect the food and food ingredients from contamination by all hazards. This involves storage, processing and transport.

vii. Pest management – An integrated program to prevent and eliminate pests.

viii. GHP – Personnel must abide by the set standards to prevent product contamination. The standards under GHP cover personnel cleanliness, hand hygiene, glove use, apparels, behavior, and health.

ix. Sanitation – A program must be in place to keep the facility, all food handling equipments and utensils under sanitary conditions appropriate to the processes undertaken.

x. Foreign material – All necessary steps must be taken to prohibit the contamination of product. This would require control of such items as glass, hard plastic, jewelry and loose items (production and personal).

xi. Chemicals – All necessary steps must be taken to prevent the contamination of product. This would include the control of cleaning and disinfecting chemicals.

xii. Good laboratory practices (GLP) – Accessing accredited laboratories that use standard analytical methods is recommended. The laboratory must have processes in place to ensure accurate and precise results.

xiii. Nonconforming products – Procedures must be established to prevent nonconforming products being shipped to customer.

xiv. Traceability – Systems must be in place to allow full traceability of the product and the ingredients forward and backward.

xv. Contractors – All such persons are to be given appropriate training to avoid increasing potential risk to product.

b. HACCP – This management tool would focus attention on the critical steps in food manufacturing and establish methods for their control. It would cover biological, chemical (including allergens), and physical hazards. In the initial stages of establishing an HACCP system, hazard analysis would be conducted to identify the potential hazard(s) associated with food production at all stages, from growth, processing, manufacture, and distribution, until the point of consumption. Also, an assessment of the likelihood of occurrence of the hazard(s) and identify the preventative measures for their control. At this stage some identified hazard will be deemed to be controlled through PRPs such as sanitation and segregation. Hazards not controlled through PRPs are managed through the establishment of CCPs which are the determined points/procedures/operational steps that can be controlled to eliminate the hazard(s) or minimize its likelihood of occurrence.

c. Full regulation adherence – All relevant applicable laws and regulation should be known and complied with.

d. Crisis management – An effective plan must be in place to manage contingency in light of a crisis. The plan should include such items as current contact details of customer and relevant employees and action plan protocol.

e. Internal and vendor verification – Similar processes that have been required from this organization should be transferred downstream.

f. Continuous improvement – The organization must establish processes to improve the effectiveness of its food safety (and quality) management system continually.

g. Management commitment and responsibility – The organization must have its management, at all levels, show commitment to the development and implementation of food safety (as well as quality) systems. This is demonstrated through appropriate policies, availability of resources, management reviews and timely and appropriate communication.

The provision for approval should be the successful verification of the above criteria. The verification is best performed by the appropriate customer personnel. It is important to note that for the approval of suppliers, it is best to verify the food safety management systems internally (by the customer) and not through a third party, where possible. The advantages are numerous, and to highlight but a few when approving the supplier with customer's staff, one would recognize clearly:

- Supplier attitude toward food safety.

- Does the supplier operate under the same culture as the customer?
- Do both parties share the same values?

On having approved the supplier, the next step would be to assess the quality of product, the customer's raw material that is being supplied. The authors will resume the discussion on raw material management later in this article.

Approved suppliers are often forgotten and not routinely inspected, especially to verify whether the materials they supply meet the requirements. It is of great importance to closely monitor the supplier standards and regularly verify, internally or externally, through audits and visits. It is always desirable to trust that the vendor shall provide material exactly to the specification. However, knowingly or unknowingly raw materials will at one time or other deviate from the set specification. It is therefore necessary to trust but verify.

One of the important measures in understanding supplier food safety management system is to take a video recording during the process verification activity. Subsequently, assessing the video would enable one to understand the strengths and weaknesses within the system. Also, identifying threats to the system and where opportunities for contentious improvement exist is important. In other words, conduct a strengths, weaknesses, opportunities, threats (SWOT) analysis. A good verifier would be able to conduct a SWOT analysis, naturally if permitted by the rules of the verification process. The competencies needed for such a person are defined in the Global Food Safety Initiative (GFSI) Guidance Document version 6.

Based on the findings of an audit, it will be possible to judge the frequency of future audits. The frequency can be based on qualitative or quantitative risk assessment, if needed. Auditing is by far not the only method of supplier adherence verification in supplier management. Maintaining and improving supplier relation can be managed through other means such as:

- Formal meetings:
  - reviewing and updating goals and objectives;
  - updating each other on the progress within each company;
  - exploring new projects;
  - reviewing audit results; and
  - reviewing customer complaints.
- Informal visits:
  - building relationships for more open interaction;
  - updating on the process and sharing best practices; and
  - improving communication process.

With good results from materials supplied and audits, the supplier can be retained on the approved list. Staying on the approved list would inevitably create opportunities for developing the partnership. Apart from the named process above in building on the existing relationship, further strength may be achieved through sharing values and aligning goals and objectives and even adopting each others' cultures.

On the contrary, if supplier performance deteriorates, steps must be taken to disapprove them. A judgment call must be made based on the risk the supplier and the raw material pose to the customer's business. The criteria for this action must be clearly defined at the supplier approval stage and agreed to. Listed below is a nonexhaustive list of criteria that may lead to supplier being delisted from a food safety perspective:

- Failing a food safety audit and failing again on the re-audit.
- Providing materials that do not meet the agreed specification.
- Deliberate provision of false information.
- Holding vital information that may lead to food safety concerns.
- Lack of commitment to food safety.
- Disalignment of cultures.

It should be possible to bring a delisted supplier to an approved list. The criteria for the same must also be clearly defined and agreed to during the initial approval stage. These may include:

- Correction of all nonconformities.
- Replacement of staff responsible for creating the issue leading to delisting.
- Improved commitment and its demonstration.

With the criteria for reapproval met, the customer may wish to keep the supplier on a temporary approval to rebuild trust. As such, more frequent visits and verifications would be necessary to regain trust in the supplier and the raw material is reinstated. Following this, the procedures for the supplier maintenance management would be in place as described earlier.

The Raw Material Management Tools

Suppliers receive raw material which they process into a product which in turn becomes the raw material of the customer for their product. The food safety management of the supplier's product, here referred to as the raw material, has to be reviewed and verified by the customer. After all the quality of the raw material defines the process needed to establish the necessary food safety standard of the final product for the customer.

To establish a baseline of the customer's requirement with respect to the raw material, a product specification has to be designed, shared, agreed and signed by both parties. This will be a legally binding document which will ensure what the customer wants and hence expects from the vendor. The specification must clearly define the product attributes. Listed below is a nonexhaustive list of the contents of the raw material specification:

- Name.
- Appearance.
  - Color.
  - Shape.
  - Dimensions.
- Weight.
- Packaging.
- Ingredients.
  - Allergens.
  - Preservatives.
- Chemical profile.

  - pH.
  - Water activity.
- Microbiological criteria.
  - Targets and limits.
- Sensory attributes.
  - Taste.
  - Mouth feel.
  - Odor.
- Transport and storage.
- Shelf life.
- Further processing (if applicable).

The product (raw material) specification is the agreement between the two parties, agreeing on one hand, what will be delivered and on the other, what is needed to be delivered. Therefore, it is important that both parties regularly analyze the raw material to verify that it meets the specification. With internal verification the frequency and sample size would vary depending on the material and batch size. With respect to external verification the frequency and sample numbers vary depending on the trust placed on the vendor. The level of trust comes with time and track record of meeting the specification. It is must be noted that pathogen testing by customer is rare. This is owing to the difficulties that the customer faces in taking correct and meaningful action when faced with a positive result for pathogens. In such circumstances, the customer and supplier must rapidly recall the product and address the issue with those who have already consumed the product. It is for such reasons that where pathogen testing is required, positive release procedures would need to be established at the supplier.

Depending on the criteria being assessed, appropriate laboratories need to be selected. Both the supplier and customer need to use a laboratory that is accredited against all relevant standards, where possible, and of reputable status.

The supplier should keep records of the laboratory tests conducted on the raw material and where necessary the corrective actions taken.

The customer should also maintain records of the laboratory tests conducted on the raw material on receipt of the material. If there are discrepancies, the supplier should be informed, so that they may implement corrective actions. This database would be defined as part of the customer complaint file and can be used to further develop the supplier approval status described earlier.

Conclusion

Managing suppliers and raw materials with respect to food safety and quality is a logical and common sense process. It consists of verifying adherence to standards such as GP, HACCP, and crisis management. These standards are common to all components of the supply chain with slight variation in focus depending on its position in the supply chain. Because of this commonality, it is always useful to select a supplier who has similar level of adherence to the standards as oneself. Although the food safety and quality management tools described is useful and necessary, there are two other factors in management that are also of great importance. The quality of the managers as well as the ethos of the organization is of key importance. It is this manager who has to define, review, and verify such systems.

The managerial attributes which would allow them to successfully manage the supplier and raw material, comes from their culture, education, and experience. Establishing the correct criteria for supplier and raw material approval, maintenance, and removal is fully based on the skills of the food safety manager.

The organizational culture plays a very important role on how the personnel behave and grow. The systems within are the defining criteria for employees to carry out the tasks for supplier selection.

Specifically defined and agreed to standards and measurements have to be verified regularly to ensure that the supplier is providing safe raw material. Based on internal and external verification of the supplier food safety and quality management system as well as raw material safety, the customer could establish the risk the supplier and the raw material pose. This risk is then managed through regular visits and verification processes.

In conclusion, an organization's ethics, selection of the appropriate food safety personnel, and providing them with the necessary tools are key factors in achieving successful supplier and raw material management. Having established this, the rest is relatively simple!

See also: Characteristics of Foodborne Hazard and Diseases: Cost of Foodborne Diseases. Disciplines Associated with Food Safety: Food Microbiology. Food Safety Assurance Systems: Audits of Food Safety Management Systems; Building Design; Cleaning and Disinfection; Documentation and Record Keeping; Food Safety and Ethics; Food Safety and Quality Management Systems; Hazard Analysis and Critical Control Point System (HACCP): Principles and Practice; Hazard Analysis and Critical Control Point System (HACCP): Principles and Practice; Hygienic Design of Equipment; Infestation Management in Food Production Premises; Investigation of Incidents in Industry; Microbiological Testing, Sampling Plans, and Microbiological Criteria; Personal Hygiene and Employee Health; Root Cause Analysis of Incidents. Food Technologies: Chilling; Drying; Food Irradiation; Freezing; High Pressure Processing; Pasteurization; Pulsed Electric Field Technology; Pulsed Ultraviolet Radiation Processing; Sterilization. Other Significant Hazards: Food Allergies and Intolerances; Food-Related Choking. Public Health Measures: Management of Food Safety in Food Service Sector; Modern Approach to Food Safety Management: An Overview. Risk Analysis: Estimating the Burden of Foodborne Disease; Food Safety Training and Health Education: Principles and Methods; Risk Analysis of Hazards in Food: An Overview; Risk Assessment: Microbiological Hazards; Risk Assessment: Chemical Hazards; Risk Assessment: Principles, Methods, and Applications; Risk Communication: Biological Hazards; Risk Communication: Chemical Hazards; Risk Communication; Risk Management: Application to Biological Hazards; Risk Management: Application to Chemical Hazards

Further Reading

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Relevant Website

http://www.mygfsi.com
Global Food Safety Initiative.

FOOD SAFETY ASSURANCE SYSTEMS

Documentation and Record Keeping

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Glossary

Critical Control Point A step at which control can be applied and is essential to prevent or eliminate a food safety hazard or reduce it to an acceptable level.

Document Any written procedure or protocol that describes how something is done, demonstrates that a measurement or observation was made or supports a program.

HACCP plan A document prepared in accordance with the principles of hazard analysis critical control points (HACCPs) to ensure control of hazards that are significant for food safety in the segment of the food chain under consideration.

Hazard analysis The process of collecting and evaluating information on hazards and conditions leading to their presence to decide which are significant for food safety and therefore should be addressed in the HACCP plan.

Monitoring The act of conducting a planned sequence of observations or measurements of control parameters to assess whether a critical control point (CCP) is under control.

Record Written or electronic information that provides documentation that status on a procedure, whether an action has been conducted properly or has been completed or supports a program. Most records are maintained on forms designed for that purpose.

Step A point, procedure, operation, or stage in the food chain including raw materials, from primary production to final consumption.

Validation Obtaining evidence that the elements of the HACCP plan are effective.

Verification The application of methods, procedures, tests and other evaluations, in addition to monitoring to determine compliance with the HACCP plan.

Introduction

It is a law the world over that food, ingredient, and beverage processors must produce safe foods. Processors develop, document, implement, and maintain a wide range of programs to ensure that the foods they manufacture are safe and wholesome. These programs include prerequisite programs, such as cleaning and sanitation and pest management, a food safety management system (hazard analysis critical control point (HACCP)), quality management, production control, and food defense programs. The latter are aimed at minimizing the potential of an act of bioterrorism. Monitoring and the records that are the end result of the monitoring program demonstrate how effective the system truly is. Government officials and others have said:

> If it is not written down, it never happened.

This directly applies to records, but a proper documentation system is essential for all phases of a food processing operation. Procedures describe how programs are to be established or managed; work instructions provide line workers, the sanitation crew and others with detailed instructions as to how a task should be conducted, what records to keep, and what to do in the event if there is a problem. Forms, both hard copy and electronic, provide a means to monitor and record the activities related to quality, safety, and sanitation. These forms are filed as records, which provide a history that allows the processor, third-party auditors, customers and potential customers, and regulators to verify that the system is working as designed. Of course, these same records may also demonstrate lack of compliance and provide evidence as to why problems, such as foodborne illness, occurred.

Food processors are expected to develop many different programs. Table 1 shows a list of the kind of programs a processor should have. This is based on what must be done to ensure quality and safety, and meet the expectations of most of the third-party audit schemes. Procedures and work instructions, if needed, must be developed in each of these areas. The area, such as cleaning and sanitation, might have a general requirement for managing the program, plus a long list of specific work instructions as to how each piece of equipment can be kept properly cleaned along with floors, walls, drains, overheads, utensils, and whatever else needs cleaning. All procedures and work instructions must include the means of evaluating the activity to ensure that it is done properly and a form or some other means of recording that the work was done. The processor must also commit to training the work force on each procedure, which must be recorded. Training not only ensures that the worker understands how to do his or

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Table 1 Records required for a food safety management system

Document/program title
1. Organizational Chart
2. Quality Manual: Mission & Quality Policies
3. Stakeholders List
4. Communication Guidelines – Internal/External
5. Food Safety Committee/HACCP Team
6. Master Cleaning Schedules
7. Cleaning Procedures
8. Ingredient Shipping & Receiving Procedures & Records
9. Specification Manual: Ingredients, Packaging & Finished Goods
10. Letters of Continuing Guarantee (COA)
11. HACCP Program
12. GMPs
13. Allergen Program
14. Consumer Complaint Program
15. Recall & Traceability Program
16. Non-Conforming Products Procedures
17. Regulatory Inspection Program
18. Processing SOPs & Record Keeping
19. Change Control Procedure
20. Process Deviation Procedures & Log
21. Internal and External Audit/Inspection
22. Product Testing
23. Vendor Approval Program
24. Glass & Brittle Plastic Program
25. Preventive Maintenance Program: Including Emergency Repairs
26. Pest Control Program
27. Sampling Procedures
28. Environmental Monitoring
29. Metal Detection Program
30. Foreign Material Detection
31. Shipping and Distribution
32. Receiving Programs
33. Calibration Procedures
34. Retain Samples Program
35. Water Quality Program
36. Warehousing and Storage
37. Food Defense: Security Assessment & Plan
38. Corrective and Preventive Actions (CAPA)
39. Legal Requirements: File of Laws & Regulations
40. Education and Training

her task safely and effectively, but it also protects the company and the worker. In an increasingly litigious world, failure to properly train workers could be deemed negligence if there is an injury or foodborne illness.

So, a well-managed document program will provide a large number of benefits to the company.

1. It describes how work should be done.
2. It provides a record that the work was done.
3. It provides materials to verify that the system is working.
4. It provides evidence when the system is not working.
5. It provides evidence that corrective actions were taken.
6. It provides support that the programs are properly designed (validation).
7. It provides evidence that workers are trained and competent.
8. It protects the business, employees, and customers.

Developing a Document-Control Program

Each and every processor should make a commitment to developing a document-control program. This program will help ensure the following.

1. Procedures and work instructions are developed using a standard format.
2. All documents and forms are subject to a review process before being put into use in the facility.
3. Only current protocols and forms are used.
4. Old or outdated documents are removed from circulation.
5. A record of how protocols have evolved is maintained.
6. There is an updated master list of all procedures, work instructions, and forms used in the facility.
7. Documents and records are protected from damage and are secure.

The processor should also appoint someone to manage the document-control program. This could be someone from the quality group or it could be someone whose title is simply Document Control Manager. This person's job is to manage the program. He or she need not be a technical person, unless part of their job description will be to review procedures, work instructions, and forms for content. If this is the case, someone with a technical background should be assigned to manage the program. If a company does not have a document-control program, the Document Control Manager will be responsible for developing the program incorporating the elements noted earlier. If the program is already established, their task will be to manage the program. He or she will also be responsible for developing a team to review and approve documents. Some companies assign responsibility for creating documents to specific persons in each operating group, whereas others allow anyone to do so provided they follow the procedures that have been established.

Each company has their own format for creating documents. There are, however, elements that are common to most. These include

1. Title – What is the name of the procedure, work instruction, or form?
2. Objective – What is the program supposed to do and why?
3. Procedure – How is the protocol to be done? This step provides the details and establishes expectations. It would also include information such as tools required, personal protective equipment (PPE) required, safety issues and required records, and how they should be monitored. If a specific form is required, that form should be referenced and even attached to the procedure itself.
4. Responsibility – Who is responsible for managing this area or doing the work described in the protocol.
5. Corrective actions – What should be done in the event that there is a problem, a critical factor is not achieved or the evaluation is unsatisfactory.
6. Footers – Many forms will also include information such as the date when the form was generated, a tracking number, revision number, who created the document, and who signed off on it.

Other sections that may be included are a scope, key words, and references. Some companies like to refere other procedures, supporting documents, or regulations in their procedures. The format is really up to each individual operation.

All of the procedures, work instructions, and forms should be entered into a Master List, which is updated as required. Headers in the Master List might include the document title, the number, creation date, last revision date, document number, department, and who created the document. All materials can be kept in one Master List, but for many companies, it is often easier to maintain separate lists for each department.

Another element that needs to be established is how to ensure security if the procedures and forms are maintained in electronic files. The Document Control Officer will need to work with the computer group to establish passwords, establish levels of access, and ensure that the system is backed up and protected.

Procedures and Work Instructions

Procedures and work instructions provide the road map for plant operations. They should be written so they clearly inform management and staff how things should be done, when things should be done, and how to document the work. These documents also form the basic for training and for internal audits. Auditors, both internal and external, must watch different operations to determine whether procedures are being followed as written. When an external auditor sees that one procedure is not being followed, he or she will often dig much deeper. They will assume that there are others that are in doubt.

As noted earlier, procedures often describe the expectations of a program, whereas the work instructions define how the tasks within the program shall be performed.

Table 2 shows a procedure for a glass and brittle plastic program. This procedure includes all the elements expected

Table 2 Sample format for procedures

Subject: Glass and brittle plastic program

Objective: Minimize the potential for crosscontamination of foods and ingredients with glass or brittle plastic to ensure that foods and ingredients are safe and will not result in injury to end users.

Procedure:

1. It is a company policy that there shall be no glass or ceramics carried into or used in the food processing area or warehouse at any time. Glass and ceramic shall be confined to the offices and break area. Watches must be removed before entering the plant. Eye glass lenses shall be manufactured from shatterproof materials.
2. Windows in production or warehouse areas must be shatterproof or coated/covered with plastic to contain breakage.
3. Inventory all glass and brittle plastics in areas where foods and ingredients are stored or handled. This includes the warehouse, production, and packaging areas. When conducting the inventory, note location of glass or plastic, the type of glass or plastic (if possible) and whether it is shielded or strengthened in any way. This inventory must also include the area or areas where glass is stored.
4. Transfer all information gathered in step #1 into a Master List.
5. Plant Management shall then examine the Master List for glass and brittle plastic and determine whether any of the situations existing within the plant pose a realistic threat of contamination and potential injury.
6. The potential risk (high, medium, low) shall be noted in the Master List with the date(s) of review.
7. Situations that pose a realistic threat (high) shall be addressed as soon as possible and corrective actions noted in the Master List. Corrective actions may include but need not be limited to the use of shields of covers, replacement with stronger or shatterproof plastics, or removal of the situation from the production or holding area.
8. The company shall establish a regular audit program to examine all glass and brittle plastic noted on the Master List to determine its condition. If any breakage is noted, it shall be noted on the Master List and repairs made immediately.
9. Employees shall be trained to report any broken glass or brittle plastic such as cracked gage covers or windows.
10. Whenever glass must be transported through the plant, it must be protected and contained in some way. For example, lights must be carried in their original box when being moved for replacement.

Responsibility: Production supervisor
Corrective actions:

a. Glass and brittle plastic breakage and cleanup
   If glass or brittle plastic breaks in the production area or warehouse, operations shall cease immediately.
   b. All exposed product shall be segregated and placed on hold.
   c. Workers in the area of the breakage shall not move throughout the plant so as not to spread the glass and brittle plastic through the plant. Shoes shall be inspected before leaving the area and cleaned if needed.
   d. Broken glass and brittle plastic shall be cleaned using designated tools and disposed of into designated containers.
   e. Glass and plastic breakage shall be recorded in a Glass and Brittle Plastic Breakage Log. This shall note the type of breakage, the location, whether any product was affected and the disposition of said product.
   f. Plant Management shall meet to discuss all incidents, determine the cause and implement a corrective/preventive action plan to minimize the potential for future incidents.
g. Discovery of damaged or broken glass or brittle plastic
   If cracked, damaged, or broken glass or brittle plastic is observed during audits, internal inspections or routine examinations of operations, it must be noted and evaluated immediately. Repairs shall be prioritized based on potential risk to the business.
   h. These incidents shall be recorded in the Glass Breakage Log.

from a glass and brittle plastic program. There are elements in this procedure that would require additional information. Work instructions for how to develop the glass and brittle plastic register, how to establish the degree of risk for each item on the register, and how often internal inspections should be done are not defined. These points need to be established and documented in a separate work instruction. There should also be procedures detailing how the glass breakage log should be establish and utilized.

From a food safety perspective, it is especially important that the work instructions that are developed for monitoring critical control points (CCPs) are clearly written and that they reference the record-keeping forms. Those responsible for monitoring should:

- be trained in CCP monitoring techniques, (company must document training and competence),
- fully understand the importance of CCP monitoring,
- have ready access to the monitoring activity,
- accurately report each monitoring activity,
- immediately report critical limit infractions so that immediate corrective actions can be taken, and
- understand the corrective and/or preventive actions that must be taken.

The procedures for doing the work need to be clearly written. Forms for monitoring should be simple to use, provide a space for entering the information being monitored, and an area where the operator can sign the form. It is a good idea to include the critical limits on the monitoring form so that information is easily accessible.

Not all records are done manually. Even if the records are continuously recorded on a chart or data are entered into an electronic system, there must be procedures for how the work is to be done.

One of the best tools that food processors can use when developing good, easy to use procedures is the digital camera. Incorporating pictures of instruments, equipment, or gages can help clarify procedures and minimize the potential for them being misunderstood. Pictures can also be used to emphasize what are good practices and what is unacceptable. As an example, most food processors have basic requirements for hair restraints, garments, eye protection, ear plugs, and other gear. Many facilities are seen that post pictures in their locker rooms or at the entrance to the plant to show workers what is and what is not acceptable. But the best use of photographs is with equipment operation. Many operations utilize startup checklists that provide the operators with step-by-step actions to follow and information that must be recorded. Using photographs reinforces the protocol and is an invaluable tool when it comes to training new operators.

As noted, forms should be considered controlled documents and should be included in the document-control program. There are a wide variety of forms that are used throughout the industry. There are several elements that should be part of each. These are

- form title,
- firm name and location,
- area to enter the time and date,
- product information (including product type, package size, processing line, and product code where applicable),
- area for actual observations or measurement,
- critical limits,
- area for operator's signature or initials, and
- space for reviewer's signature or initials, and date of review.

If a company has forms that they are using do not have a space for reviewer's signature and date, a stamp may be used with spaces for the entries.

One thing that should never be done is to print forms with preprinted information, such as time. The operator must enter the time, and when the measurement, or reading was made. It should not be on the form. In the reality of the production environment, record keeping is not done every hour on hour.

Monitoring and Record Keeping

Monitoring records are the heart of the HACCP program. Monitoring records show the following:

1. that the system is in control,
2. that the system may be going out of control, and
3. that the system has lost control.

As noted earlier, persons monitoring CCPs must be trained on the procedures and fully understand the importance of monitoring. This applies not only to persons doing monitoring CCPs, but also to all responsible for monitoring prerequisite programs, production operations, and any other element of the quality, safety, and sanitation program. They are all part of the food safety management system (FSMS).

One set of documents that each processor must maintain are calibration records for all instruments or equipment used to monitor CCPs, plus protocols for how these instruments or pieces of equipment are to be calibrated. The procedures for calibration must include how to do the work, who is responsible for doing the work, the acceptable tolerances and corrective actions to be taken in the event if an instrument or equipment is found to be out of calibration. The corrective actions must examine whether any food has been affected by an instrument that is out-of-calibration and describe what should be done with any affected foods.

There are basic requirements for how records should be kept. Many companies mandate that monitors use only blue or black pens to record information and go so far as to define what kind of pen is safe to use in the processing area. They will also define how corrections should be made.

"If operators make an error on a form, the error shall be noted by lining out the incorrect entry, inserting the proper result and initialing and dating the change" (Figure 1).

The procedures should state that corrections cannot be made by erasing an entry, the use of liquid paper, or tape. In the eyes of the regulators, these would be falsified records (Figures 2 and 3).

Monitors must also be taught that there are some things that they should never do. Many persons charged with monitoring will use entries such as ditto marks or lines to

indicate that the numbers are the same (Figures 4 and 5). This is unacceptable. Records have to be recorded at the time they were measured or observed. Ditto marks or lines drawn down the page do not provide that information. This is why forms with preprinted data are unacceptable.

Corrective Action Records

If monitoring shows a deviation at a CCP, the product in question is deemed to be unsafe or potentially hazardous. The processor must now take corrective actions to bring the system back into control and make a disposition of the product that was affected. Records of all actions to evaluate the deviation and dispose of the product must be maintained.

Corrective actions records must be maintained for all quality and prerequisite monitoring activities. In many cases, a corrective action may be noted on the record-keeping form. As an example, if a processor was using adenosine triphosphate (ATP) swabs to verify the efficacy of cleaning and a swab indicated that an area was not clean, the sanitation supervisor could record the swab results and the results from retesting after the suspect area was recleaned. In this case, the corrective action would be captured on the original monitoring form.

Corrective actions and records of these actions are one of the most common failings in food processing plants. All too often, the processor makes a correction but fails to document that it was done. They fail to close the loop. Failures to conduct corrective actions are very common when evaluating records of prerequisite programs. As an example, the pest control operator observes that a door is broken and does not fit tightly. He notes it on his report and the plant fixes it, but the plant fails to record that this issue was closed on the report or elsewhere.

It is expected that all deficiencies on internal audits, routine audits, and third-party audits will be addressed and there will be corrective action records of all actions taken to fix the problem. The quality group should verify that the work was completed properly by signing off on the corrective action report or in the corrective and preventive action (CAPA) logbook.

For routine corrective action issues, such as properly cleaning a piece of equipment or making a repair, how these issues are addressed is quite simple. They are simply cleaned or fixed. However, if there is a significant problem, such as a

4/3/11	6:05	41	RFS
4/4/11	6:01	~~51~~ 41 RFS 4/4/11	RFS
4/5	6:00	39	RFS
4/6	5:58	37	RFS

Figure 1 Properly corrected record: cross out, date and initial.

4/11	6:01	39	RFS
4/12	6:03	39	RFS
4/19	6:04	41	RFS
4/20	5:59	39	RFS

Figure 3 Improperly corrected record: Liquid Paper and tape.

Figure 2 What not to use when correcting records.

Improper Record

AREA OF EVALUATION	OK	N	N/A	COMMENTS	IN
1. Garments/Uniforms a. Clean	✓				RFS
b. Properly stored	"				
2. Handwashing Stations a. Conveniently located	"				
b. Adequately supplied; clean	"				
c. Sufficient water pressure	"				
d. Adequate water temperature	"				
e. Handwash signs posted	"				
f. Employees complying	"				
g. Enough stations for needs	"				
3. Hand Sanitizing Stations (Yes/No) a. Conveniently located	"				
b. Adequate sanitizer	"				
c. Concentration maintained	"				
4. Loose jewelry, watches	"				
5. Gloves - Used (Yes/No) a. Appropriate material	"				
b. Clean	"				
c. Well maintained	"				
d. Properly handled/stored	"				
6. Hair & beard restraints	"				
7. Belongings properly stored	"				

OK – Acceptable N – Not Acceptable N/A – Not Applicable

Figure 4 A sample of improper records.

Improper Record

AREA OF EVALUATION	OK	N	N/A	COMMENTS	IN
1. Garments/Uniforms a. Clean	✓				RFS
b. Properly stored					
2. Handwashing Stations a. Conveniently located					
b. Adequately supplied; clean					
c. Sufficient water pressure					
d. Adequate water temperature					
e. Handwash signs posted					
f. Employees complying					
g. Enough stations for needs					
3. Hand Sanitizing Stations (Yes/No) a. Conveniently located					
b. Adequate sanitizer					
c. Concentration maintained					
4. Loose jewelry, watches					
5. Gloves - Used (Yes/No) a. Appropriate material					
b. Clean					
c. Well maintained					
d. Properly handled/stored					
6. Hair & beard restraints					
7. Belongings properly stored					

Figure 5 A sample of improper records.

major deviation at CCP or a serious quality issue, the corrective action program and how it is documented becomes more complex. Again, how these issues are addressed must be defined in the procedures. With complex issues, the processor should define the problem, develop a written corrective action plan, create a time line, and move forward on the activity. When the work is done, they will notify the quality team who will then review the records and the results and approve or disapprove the work. Depending on the type of problem, they may recheck the issue at a later date to verify the actions were successful. Again, this must all be documented.

One tool used by many processors is a CAPA logbook. This may be retained as a hard copy or electronically. This log serves as a reservoir for all issues that required corrective actions and is used to track progress. Electronic logs seem to be more popular as they allow the quality group to easily track the status of corrective action activities. They can see what has been done and what is still pending. In addition, a well-designed electronic log allows problems to be sorted and high priority or regularly occurring issues can be addressed at greater length. The CAPA log is something that the HACCP team should review each time they meet.

Record Review

One of the seven HACCP principles is verification and part of verification is ensuring that all CCPS have been properly monitored. Food processors must be sure that a member of the management team has been trained to review all records of CCP monitoring. Ideally, this review should take place before the product is shipped. The reviewer must examine the monitoring records, be sure that the values are correct, and that they have not only been filled out properly, but were also signed by the monitor.

All calibration records, especially those done on instruments used to monitor CCPs, should also be reviewed on a regular schedule. Most companies will conduct such a review at intervals ranging form a week to a month.

The reviewer must sign and date the records following his/her review. If a space has not been provided for a signature and date, the stamp mentioned earlier may be used. It is also acceptable simply to sign and date the records.

Procedures should be drafted that describe how records should be reviewed and what should be done if deviations are observed. This is especially important when reviewing more complicated records such as those required for the production of low-acid foods. There may be many CCPs for such records. In addition, there may also be several different pieces that will require review. As an example, there may be the operator retort record, the recorder chart printout, and seam integrity records. All need to be carefully examined and issues highlighted.

Many companies bundle their daily production records and require that their quality personnel review all the records in the bundle, that is, food safety, quality records, and sanitation records. Processors who utilize this kind of procedure usually attach a cover sheet to the bundle listing all the required records that must be in the packet and are subject to review. The absence of a record would then be considered a deviation. The completed packet should include evidence that each record in the packet was not only present, but was also reviewed.

There are other situations where documents and records are subject to review: third-party audits and regulatory inspections. Each and every third-party audit, whether it is a company-owned system or one of the six schemes approved by the Global Food Safety Initiative (GFSI), entail review of procedures and records, plus evaluation of how the protocols are actually implemented. With these audits, all procedures, work instructions, and all kinds of records are subject to examination. The six schemes currently approved by are the British Retail Consortium (BRC), International Food Standard (IFS), Dutch HACCP, Safe Quality Foods (SQF), FSSC 22000, and Synergy 22000. The most stringent of all the food safety audits is that for International Organization for Standardization (ISO) 22000. Companies who make the commitment to meet the ISO 22000, FSMS are usually looking at 12–18 months preparation at a minimum, plus an intensive audit by the certifying body of up to 5 days.

Regulatory investigations also include review of records. The records that are available for review depend on the type of investigation and what the company is manufacturing. For example, in the US, there will be many more records available for review for a processor of low-acid foods than there would be for a manufacturer of soft drinks. Food processors need to understand which records the regulators are allowed to access and which are not. With the passage of the Food Safety Modernization Act of 2010, HACCP will become mandatory for food processors in the US. These records and others will, therefore, be accessible to regulators.

Therefore, since documents and records are an integral part of both third-party audits and regulatory investigations, it behooves a processor to properly develop, implement, and maintain these materials.

Record Storage and Retention

An integral part of the document-control and record-keeping program is storage and record retention. All records must be stored in a secure (locked) area where they are protected from water damage, fire, pests, and theft. Some companies have actually constructed what amounts to block houses for record storage. These facilities are built in a warehouse of cinder blocks and brick with a sturdy roof. They are air conditioned and have fire abatement systems; systems that rely on chemicals not water. The records themselves are filed in locked cabinets in the area and the companies adopt checkout systems if anything is taken out of the area. The document-storage areas are usually built with a reading room, so records can be viewed within the secure area. The storage facilities at most plants are nowhere near this elaborate.

There are also many companies that are turning to firms that will store documents for them off site. There are, however, regulations that mandate how long certain regulated products must retain records on site. These operations have all the features noted earlier. They also manage their files so that they can access things quickly and easily. If the processor contacts them and asks for specific records, they can usually be located and delivered within 2 h. The record storage companies will also notify the

processor when the records are scheduled for destruction. When they get approval to move forward, they will destroy those records scheduled for destruction and provide the processor with documentation that verifies which records were destroyed.

This leads to another requirement for records. How long should they be kept? The juice HACCP regulation in 21 CFR Part 120. 12 (d) states the following:

> (d) Record retention. (1) All records required by this part shall be retained at the processing facility or at the importer's place of business in the United States for, in the case of perishable or refrigerated juices, at least 1 year after the date that such products were prepared, and for, in the case of frozen, preserved, or shelf stable products, 2 years or the shelf life of the product, whichever is greater, after the date that the products were prepared.

Records for low-acid canned foods must be retained for 3 years; one of which must be on-site.

For products that are not regulated, one rule of thumb is that records should be maintained for the shelf-life of the product, plus 1 year. When determining how long to keep records, companies need to talk to their clients and the legal department. Processors who do contract packing are often required by contract to keep process records for longer periods of time.

Electronic records must also be protected and should be subject to the same storage requirements as hard copies.

All these protocols that describe how records are stored must be documented in a procedure. The basic procedure must describe storage requirements, retention times, and mandate that the records be destroyed at the end of the retention time, and that records be maintained of this action. In addition, if a company elects to store records off-site, there should be a procedure that describes the basic requirements for an outside storage facility, how the storage firm is selected, and what is done to monitor their performance.

What is Required for Food Safety

As this article has shown, there are many different records that make up the food safety management system. These include:

- HACCP plan and support documentation used in developing the plan,
  - Meeting minutes,
  - Technical references,
- Records of CCP monitoring,
- Records of corrective actions,
- Records of verification activities,
  - Including validation data and internal audits,
- Calibration records,
- Prerequisite programs,
  - Cleaning and sanitizing,
  - Hand sanitizers,
  - Any that have been determined to affect product safety in the company's hazard analysis, and
- Training.

There must also be procedures or work instructions that describe how each and every one of these tasks must be done. Without road maps provided in these protocols, it is simply not possible to ensure that the work is done properly nor is it possible to properly train new employees.

Is this a great deal of work? Of course it is, but consider the alternative: food, ingredient, or beverage that makes someone sick or causes an injury. One such incident will cost more in terms of money, headaches, and loss of face than what went into developing, implementing, and maintaining the program.

See also: Food Safety Assurance Systems: Audits of Food Safety Management Systems; Essentials of Crisis Management; Hazard Analysis and Critical Control Point System (HACCP): Principles and Practice; Investigation of Incidents in Industry; Labeling and Information for Consumers; Management of Allergens in Food Industry; Microbiological Testing, Sampling Plans, and Microbiological Criteria; Recall Systems and Disposal of Food. Institutions Involved in Food Safety: International Organization for Standardization (ISO). Public Health Measures: Modern Approach to Food Safety Management: An Overview

Further Reading

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FOOD SAFETY ASSURANCE SYSTEMS

Labeling and Information for Consumers

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Glossary

Codex Alimentarius A collection of internationally recognized standards, codes of practice, guidelines and other recommendations relating to foods, food production and food safety adopted by the Codex Alimentarius Commission.

Control measure Any action and activity that can be used to prevent or eliminate a food safety hazard or reduce it to an acceptable level.

Food hygiene Comprises conditions and measures necessary for the production, processing, storage, and distribution of food designed to ensure a safe, sound, and wholesome product fit for human consumption.

Food safety control system The combination of control measures that, when taken as a whole, ensures food is safe for its intended use.

Monitoring The act of conducting a planned sequence of observations or measurements of control parameters to assess whether a control measure is under control.

Validation Obtaining evidence that a control measure or combination of control measures, if properly implemented, is capable of controlling the hazard to a specified outcome.

Verification The application of methods, procedures, tests, and other evaluations, in addition to monitoring, to determine whether a control measure is or has been operating as intended.

Introduction

Only if you are able to grow or rear your own food do you know exactly where it came from and what has happened to it before you eat it. Few consumers know much about the details of their food, what ingredients it was made from, let alone how it was processed, what benefits or risks it might have for health, or whether it was produced in accordance with religious codes or ethical values.

Food labeling is the key to unlocking information to meet consumers' various and varying needs. Whether living in a fast moving urban metropolis, or a remote community, in a rich or developing country, when purchasing prepackaged foods consumers need accurate food labeling to help make informed food choices – but this can be complicated. Food labeling needs to fulfill many varied functions to meet consumers' information needs, including:

- Who made the food and where?
- How much is in the pack?
- How long will it last?
- Is the food safe to eat as such, or are there any special conditions to follow?
- How should it be prepared, stored, and cooked to meet safety, nutritional, and organoleptic quality?
- Where was the food grown or reared?
- What processing has it undergone and how was it produced?
- What ingredients, additives, and processing aids have been used?
- What nutrients does it contain?
- Are there any restrictions for use, such as not being suitable for babies, young children, or pregnant women?
- Will the food provide any specific health benefits?
- Does it meet religious, moral, and ethical needs?

Food labels also provide food producers with the means to advertise and promote their goods. Exaggerated claims can mislead and confuse consumers about the true nature of the product within the pack, its composition, and particularly its nutritional value.

Legislation has developed over many centuries to protect consumers from misleading information. International food safety and labeling standards are agreed at the Codex Alimentarius Commission and form the backbone for food safety and information for internationally traded foods. Standards are implemented and enforced at the national level. National authorities may adapt food legislation to suit local needs to ensure good food hygiene practices throughout the food chain and to the eventual consumer.

Food safety standards and guidelines, particularly those of the Codex Alimentarius Commission pave the way for good food hygiene – meaning the conditions and measures necessary for the production, processing, storage, and distribution of food designed to ensure a safe, sound, and wholesome product fit for human consumption. Thus, the whole food chain should work to protect consumers and ensure safe food through a process of hazard analysis and critical control point (HACCP) guidance, right through to how the consumer will eventually use the product. Labeling plays an important link in this critical food chain.

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Producers and manufacturers are required to consider consumer practices in their HACCP plans, especially where the consumer is the final critical control point, for example, where cooking is necessary to ensure safety.

The validation of information on the package, both from a technical perspective, for example, time and temperature of storage and cooking, as well as clarity of the information, is mandatory within HACCP principles. Responsibility for food safety and hygiene can never be fully transferred to consumers. Where the implementation of safety measures by consumers is not feasible, and product safety cannot be assured, the product should not be marketed – it would be unsafe and unethical to pass food hygiene and safety controls directly to consumers.

Food must be safe to eat and will not cause harm to the consumer – when it is prepared and/or eaten according to its intended use. Companies are obliged to clearly identify 'intended use' and make sure that labeling provides the necessary information to consumers to ensure that it is safe, if used according to the 'intended use' instructions.

Food labeling legislation protects consumers but deciphering the complexity of information takes knowledge and determination: many consumers admit to finding difficulty in deciphering food labels and many do not have the time or inclination to pay much attention to food labels. A worldwide online survey of 25 000 consumers carried out in 2012, by market research company Neilson, found that more than half found food labels difficult to decipher.

Truth in Labeling

Ever since food and beverages have been sold, there has been the potential for unscrupulous traders to make 'extra profit' by adulterating, substituting, or diluting expensive ingredients. Foods and ingredients were sold over the counter, often unwrapped. Customers had to make a choice based on what could be seen – the only quality checks were to prod, sniff, or if allowed, to taste; none of which provided much of a guide to the exact composition or provenance of the food, especially not to its safety. The latter was only determined after the food was consumed.

Nowadays with vigilant quality assurance and food inspection checks during processing and producing, the risk of adulteration has been significantly reduced. Compositional standards have been set and quality assurance methods test for contamination. Ingredient listing is expected, as is nutritional information. Yet there is still room for consumers to be misled about the nature and quality of foods offered for sale by clever advertising and marketing. Strict rules about nutrition and health claims have been agreed to combat product hype and help consumers find their way through the complexities. Labeling legislation has a hard job to keep ahead of consumer trends, especially for affluent consumers in developed countries who are concerned about every aspect of their foods and how they were produced.

Consumers have legal rights of redress and protection against foods that are not what they thought they were, or worse, cause a food safety problem, be it foodborne disease or an allergic reaction.

Whatever information is provided on foods presented for sale, be it on the packet or at the point of sale, it must describe the food accurately, must not be false, misleading, or deceptive, or likely to create the wrong or misleading impression about the character of that food, in any respect, and this applies to text and graphs.

This is the fundamental basis of all food legislation be it local, national, or for international food trade – food labeling legislation should deliver foods that consumers can trust to meet their expectations, and importantly eat safely.

When reading this article you might find it helpful to take a look at the food labels in your store cupboard. Find the information that must be present by law, and review the additional voluntary labeling and information presented. Try to assess whether this additional information is essential, helps you make an informed choice, or is merely marketing hype. Evaluate how the information is presented in terms of visibility, for example, is a magnifier needed to read the text and for clarity of the communication, and the legitimacy of the images presented.

Legal Requirements of Food Labeling

There is common and international agreement about the essential information that should be provided on all food labels. These are mandatory in many countries and also for foods traded internationally.

Name

This sounds very basic and not something that needs to be prescribed by law. However, brand or trade names can obscure the true nature of the food, particularly if it is highly processed. Ideally the name of the food should be unambiguous but we have moved a very long way from this with food processing wizardry and marketing hype – especially where there is fierce competition for sales. Consumers can be misled by the name of a food and their gullibility exploited.

What sort of meat is it if it has been packaged, processed, smoked, and reformed? Is it pork or is it something with a fancier brand or trade name? Is it a 'secret formula' drink that looks like fruit juice, but has none in it?

If there is a brand or trade name then in close proximity on the label there should be a clear description of the food, explaining exactly what it is. For example, if it looks like a dairy butter product but has no dairy content, it should be described as something like 'nondairy spread made from a blend of fats and oils.' This description should be in the same field of view, close to the brand name.

In addition, if a product has undergone a processing treatment, such as dehydration, curing, or smoking or been previously frozen and defrosted this must clearly be labeled within the same field of view as the name, so as not to mislead the consumer about the true nature of the food product.

Weight and Volume

Prepackaged foods must declare a weight or volume and in the units of measurement commonly used in the country where they are sold.

Some foods are exempt from the weight declaration, such as a loaf of bread (this can often be prescribed in specific national bread regulations), or where food is sold by quantity – six bread rolls.

Manufacturer or Seller's Details

The name and address of the food business operator that made or sold the product must, by law, be on the label. In the event of any problem the responsible company can be contacted.

Some companies also provide a telephone contact number. It is in the companies' interest to provide direct contact information and the necessary infrastructure so that consumers can contact them in case of a problem. The sooner a problem is reported the faster the company can take corrective actions and contain any problem.

A website address is often provided by larger food business operators where more information can be found about the product, company, production process, recipes, etc. Some companies use such websites for the declaration of allergens. This can be particularly useful for food service establishments as it allows for a detailed, up-to-date investigation of the ingredients in a product, where the packaging has been removed.

Consumers who experience any problem with the safety or quality of food purchased have legal rights of redress from the seller or producer. Local and national food law enforcement responsibilities vary, but essentially customers have the right to complain if the product is not what was expected, or caused any food safety problem.

Instructions for Safe Use

To get the best from any food product it is essential to exactly follow all instructions to ensure the food is prepared, stored, cooked, and served safely.

Foods unsuitable for particular groups, such as babies or the elderly or those with a chronic illness due to the potential food safety risk must be clearly labeled. For example, there is a mandatory requirement to label untreated 'raw' milk. It must be labeled prominently as such, and that it should not be consumed by children, pregnant women, older people, or those who are unwell or have a chronic illness.

Instructions should not make any assumptions about the knowledge or skill of the user and should consider the risk perception of consumers and/or their habits and behaviors, as well as the feasibility of the recommended measures. This should be done through detailed investigation during product and labeling development, by direct surveys with potential consumers of the product. Everything about the product, its preparation, cooking, or storage should be specified to ensure it delivers the safe, quality product that the manufacturer intends and the consumer expects. If food is sold loose, information should be available at the point of sale.

Foods must be labeled with 'any special storage conditions or conditions of use' otherwise there could be an increased risk when eventually eaten. Instructions should include food safety warnings, such as 'once opened keep refrigerated' and 'consume within 3 days of opening,' 'not suitable for freezing,' or if foods are not suitable for reheating or refreezing once thawed.

Because of the increased food safety risk from inadequate or inappropriate cooking or storing, consumers must follow these specific instructions for use, especially for foods that have to be cooked to ensure their safety; instructions for correct storage and cooking can be the last control point in the food chain, to prevent the risk of foodborne diseases. Such labeling or warnings may provide a manufacturer's defense should any subsequent problem occur, nevertheless there can be unforeseen consequences, as some foodborne outbreaks demonstrate.

There have been high-profile cases where confusion, misinterpretation, lack of clear instructions, and a failure to follow instructions have sadly led to major food safety incidents. Raw or undercooked foods can cause serious food safety alerts, such as in the US when raw cookie dough was contaminated with *Escherichia coli* O157 and caused severe renal failure to some 70 consumers. Thorough cooking of the dough would have ensured the cookies were safe to eat, but some consumers were in the habit of eating raw cookie dough (which in this case was contaminated). The manufacturer's label did not specifically communicate the importance of cooking the dough, and not to eat the product raw. After the incident, the label was revised.

Raw chicken nuggets and chicken strips have also been associated with *Salmonella* infections such as in a 1998 Australian outbreak and when stuffed chicken products were implicated in five outbreaks in Minnesota from 1998 to 2008. Consumer confusion regarding whether these products were raw or cooked was documented in the outbreak reports. Products were not clearly labeled as containing raw poultry ingredients; since the products were breaded and prebrowned, consumers were confused and thought that the food had been precooked.

All warnings, including specific food safety control information should be verified by the producer to ensure that they meet food safety standards. For example, cooking the product according to the instructions is essential to ensure that any potential foodborne disease organisms are destroyed and that the food is safe to eat.

All instructions for consumers on the safe use of products, for example, storage temperature and instructions for heating should be validated for both scientific and technical accuracy, as should be the clarity of the communication so that consumers are able to understand the significance of these instructions.

Food safety warnings on labels need to be validated by checking that consumers, typical of those who would use the product, can understand, interpret, and follow the instructions correctly. There are internationally agreed guidelines for the validation of food safety control measures, and food business operators producing high risk foods must ensure appropriate hazard analysis and critical control to ensure consumers' safety from their products.

Minimum Durability or Shelf Life

Consumers should be informed about how long a product will stay fresh and more importantly how long it will remain

safe to eat. The shelf life will depend on the quality of raw ingredients, processing, packaging, and storage. Ultimately shelf life is determined by a combination of microbial, sensory, and chemical methods to ensure that the food is safe, palatable, and acceptable to eat at the end of its shelf life. Consumers are rarely in a position to assess the true shelf life, freshness, or safety of a food product, and must rely on accurate, clear labeling information from the manufacturer.

There is no absolute for the durability of a food product, because at any stage the integrity of the food can be compromised by mishandling. Manufacturers should give consumers best guidance, based on evidence, and stress the importance of complying with storage instructions, especially for perishable food where temperature control is critical.

A range of terms can be found on foods and there are differences worldwide, for example, expiration date, sell by, and use by. There are no internationally agreed terms but in the European Union use-by dates for perishable foods are mandatory. In other countries manufacturers' shelf life labels are advisory and not regulated. Baby and infant formula are required to be labeled with expiration/use-by dates to ensure that the nutritional value has not been compromised.

Best Before

Foods with a long shelf life carry a 'best before' date which is a quality or freshness indicator. 'Best before' dates indicate when foods will be at their freshest and best quality.

If stored correctly, usually in a cool, dry, dark place there is unlikely to be a food safety risk from such foods. Eating foods beyond their 'best before' date means there will inevitably be some deterioration in quality, particularly in taste or texture. Foods where best before labels can be found include dry goods, flour, dried pulses, dry pasta, and dried herbs and spices.

Use By

Highly perishable foods will have a 'use by' warning indicating the date by which the food should be used, when stored according to the instructions. This date should be established based on a rigorous examination of the potential growth of pathogens, and potential safety implications if consumed after that date.

Use by dates are essentially a food safety warning since after that date, even while the food may not have visibly deteriorated or 'gone off,' it could carry a higher risk of foodborne disease.

It is unsafe and unwise to eat foods beyond their 'use by' date and especially so for vulnerable groups including babies and young children, the elderly, and those whose immunity is compromised.

Foods with 'use by' labels can usually only be stored for a few days, such as fresh dairy products or ready prepared meals; they will also need to be kept refrigerated and the temperature should be specified.

Some foods such as fruit and vegetables, which are intended to be used fresh may not have a date mark; it is assumed that such foods will be eaten shortly after purchase, while still visibly fresh.

Manufacturers and retailers may put additional dates, such as 'display until' on some foods: these are for stock control purposes and consumers should always refer to the 'use by' date for safe use.

Storage

When offered for sale, be it at a market stall, street vendor, or supermarket – food must be safe to eat. Once purchased, whether sold loose or prepackaged, consumers must store food as instructed, to ensure it remains safe until consumed.

Storage and transport instructions must be specified to maintain food safety and quality. Correct and precise information about where to store a food, at what temperature, and for how long, before and after the pack has been opened must be given. The importance of following storage instructions for consumers can be illustrated by the botulism case linked to refrigerated carrot juice, which occurred in the US and Canada in 2006. The implicated products were pasteurized but were not heated to a temperature that would eliminate spores of proteolytic (the most heat-resistant type) *Clostridium botulinum*. Subsequent testing of leftover carrot juice recovered from the home of one of the affected persons found botulinum toxin in the juice. The carrot juice products involved in these illness cases were distributed under refrigeration, and were labeled with one or more of the following statements 'Keep Chilled,' 'Keep Refrigerated,' 'Perishable Keep Refrigerated,' or 'Extremely Perishable Keep Refrigerated.' Because proteolytic *C. botulinum* spores are known to grow and produce toxin only when there is severe temperature abuse, it was suspected that this particular juice may have been left unrefrigerated for an extended period, either during distribution, or once purchased by consumers, thus allowing *C. botulinum* spores to grow and produce toxin.

There is great confusion over where and how long to store foods yet this is crucial information. A good clue to storage at home is to store food as it was stored at the point of purchase – be it in the freezer, fridge, or at ambient shelf temperature.

Preparation

Prepackaged foods that are 'ready to eat' and do not undergo further safety treatment, such as cooking, should be safe to eat at the point of sale.

Prepacked food that is to be further prepared and cooked should have clear instructions about how to render it safe and/or maintain its integrity and safety until eaten. Instructions about how to prepare food should be explicit, for example, does the food need to be washed? What further preparation is necessary? Are there any special instructions to follow for safe food preparation to avoid cross contamination? This is especially important because no fresh foods are sterile and high risk foods always pose an increased risk of cross contamination and subsequent foodborne diseases from incorrectly prepared foods.

Workers in food production and food service have to undertake a 'Food Hygiene Training Course,' which ideally should be a mandatory requirement. Such courses stress the rules of basic food hygiene, how to avoid cross contamination, how to store and cook food safely to the correct temperatures. Domestic consumers may be unaware of the importance of good food hygiene and how to keep food safe and to prevent a foodborne disease, hence the need to have clear precise

information, instructions and where appropriate warnings on all food labels.

Foods which naturally contain bacteria, such as minced meats and chicken must be treated carefully during preparation to ensure there is no cross contamination to other foods, particularly ready cooked foods, such as meats and dairy products, which should always be stored separately. Good food hygiene practices are essential to reduce the risk of cross contamination.

Cooking

Cooking instructions should give the cooking temperature and time to ensure that the food is served safely and at its best: there should be no risk of a foodborne disease however the food is to be cooked.

When using different appliances the variations in time and temperature can be considerable and specific details should be given for each method.

It is important to ensure that food is cooked according to the correct instructions, not only for it to look and taste good but also to ensure that it is safe to eat. Some foods need thorough cooking to destroy any potentially harmful bacteria they may contain: this is particularly important for chicken, ready or preprepared meals, and eggs.

For some foods cooking is the final stage of food safety control to ensure food safety. Consumers must follow instructions to ensure that this control point is carried out as required. Food business must validate instructions and ensure that this final control measure is clear and easy to follow.

Providing clear instructions can be a due diligence defense should any subsequent foodborne disease occur through incorrect cooking. Specifically to reduce the hazard of *Salmonella* found in eggs, labeling is the final control measure among several, beginning on-farm right through to storage and use by the consumer: correct cooking will ensure safety against contamination of the raw egg. Raw eggs can pose a high risk to consumers, and particularly to vulnerable groups, specific warnings must be given on the label – Do not eat raw – Cook thoroughly until hard – Do not eat soft boiled eggs. Thorough cooking is necessary to destroy any potential food hazards, likewise for minced beef, which can be susceptible to contamination with *E. coli* and if not cooked correctly can cause severe renal problems and death in children.

Raw, ready to cook foods have been identified as the cause of several foodborne disease outbreaks. For example, in the US in 2007 'pot pies' caused a foodborne disease outbreak from *Salmonella*, and similarly frozen, not-ready-to-eat microwavable meals have been implicated in other salmonellosis outbreaks. The pot pies associated with this outbreak had a raw flour crust and were 'not-ready-to-eat.' Consumers could cook according to their preference. Even so, consumers were required to ensure that minimum cooking temperatures were reached to control microbiological hazards. In addition, because raw frozen poultry pastes had been used to make the liquid portion of the chicken and turkey pie fillings, the pies might have contained undercooked poultry, or been cross contaminated from these raw poultry pastes, which often harbor *Salmonella*. This outbreak report identified labeling concerns, specifically, recommended microwave cooking times on the pot pie packaging were based on wattage categories, but most consumers (who became patients) were unaware of microwave wattage. In this case improper microwave cooking could not account for the entire outbreak, however, given the limited knowledge of consumers about microwave wattage, and the frequency of deviating from microwaving instructions, microwaving probably led to inadequate cooking.

Inadequate microwave cooking has been thought partly responsible for other previous outbreaks of *Salmonella* infections. Industry and regulators should consider examining the manufacturing processes for frozen not-ready-to-eat foods to determine the extent to which microwave cooking is safe for these products. Labeling and cooking instructions on 'not-ready-to-eat' frozen foods should be clear to ensure that consumers are aware of health risks, and to facilitate compliance with validated cooking methods.

Clear and prominent listing of output wattage on microwave appliances might improve consumer adherence to manufacturer's cooking instructions. Consumers should follow cooking instructions specific for an oven's wattage.

Although often foods designed to be cooked in a microwave oven will give a warning to follow the specific wattage of the oven being used, many consumers, as noted above, are unaware of the wattage of their appliances. And even this instruction is no guarantee of correct heating. The only accurate way to ensure that the correct internal temperature has been reached is to use a food thermometer. Although these are common in food service, they are not so common in domestic situations.

Given the limited use of food thermometer in the home, some manufacturers use specific instructions on microwave foods, particularly to cook foods until 'piping hot throughout,' with instructions to stir during cooking, and to allow standing time to equalize the temperature throughout before serving. This is very important because hot and cold spots can hide, especially in frozen or chilled foods; the whole food product must reach the required high temperature. 'Piping hot' is not a specified temperature and can be ambiguous, but it provides general advice for consumers to ensure that a high enough temperature has been reached.

If the product needs to be defrosted before cooking, this instruction must be followed to ensure safe time–temperatures are reached during cooking.

The importance of correct cooking temperatures cannot be underestimated for food safety. All of this information and instruction seems obvious. Often, the specific details can be overlooked or ignored and consequently can contribute to foodborne illness if cooking temperatures have not been high enough to destroy harmful microorganisms that can cause foodborne diseases.

Composition

A list of all ingredients and additives must be declared in descending weight order under the heading 'Ingredients.'

Compositional standards for some staple products are prescribed in national regulations, but increasingly these have been withdrawn.

The quantity of the main ingredients must be declared when featured in the name of a product: a quantitative

ingredient declaration. This enables customers to make an informed value for money choice and compare products, such as beef burgers, to find the percentage of the key ingredients in similar products.

When listing smaller amounts of food ingredients there are many specific labeling standards and guidelines about how these should be labeled to ensure consistency and convenience, for shoppers and producers alike. It can be impractical to label every specific addition by its chemical name, yet a full declaration needs to be made to ensure that consumers can make appropriate choices; especially if they wish to avoid certain additives or ingredients due to allergies or hypersensitivity. Any product which has been made from biotechnology (or genetic modification (GM)) where there is any likelihood of transfer of an allergen must be labeled.

Permitted food additives must be declared, generally according to their function, such as color or emulsifier, and/or according to their specified name or classification, such as 'e' numbers within the European Union or the Codex International Numbering System for foods traded internationally. Functional classes include a long list of generic terms, such as flavor enhancers and sweeteners, and processing aids such as antioxidants, firming agents, and stabilizers, and the generic term can be used.

Compound ingredients should also declare ingredients (in brackets) after the main name. Any food additives in a compound ingredient must be specified in the full ingredients list.

When making declarations of ingredients some shortcuts to the full names and groups of ingredients are permitted, by the use of 'general class names.' For example, refined oils and fats of any type can be labeled in the ingredients list as 'oil' or 'fat,' likewise for 'starches' and 'sugars.' However, because foods, such as pork or beef, are prohibited in religious dietary rules they must always be specifically declared on the label, including pork or beef fat.

Added water should always be declared in the list of ingredients. Added water resulting from processing must be declared with its percentage, alongside the name of the product, for example, ham with 10% added water.

Hypersensitivity and Allergens

Some food ingredients and additives are known to cause hypersensitivity and an allergic reaction and should always be declared, especially if included as part of a compound ingredient. The list includes gluten containing cereals, crustacean, eggs, fish, peanuts, milk, etc. This list is regularly reviewed as more evidence emerges. National food control authorities keep a full list updated and published for food business to provide consumers with the most up-to-date information about ingredients that may cause a hypersensitive reaction.

National regulations may require more specific warnings where there is a particular risk or high level of sensitivity in a population group, such as, for peanuts where there is a high risk of an anaphylactic shock reaction.

High risk foods that could cause hypersensitivity and/or an allergic reaction must be labeled with a clear warning, such as – contains nuts, contains sulfites, according to the specific current list of foods that can cause hypersensitivity.

Origin

The place of origin must be declared, particularly if not doing so would deceive or confuse the consumer.

Consumers like to know where their food has been sourced, and many wish to support local or national producers, or alternatively boycott foods from certain countries for political, moral, or perceived food safety problems such as during the bovine spongiform encephalopathy epidemic in the UK and Europe.

Origin labeling is increasingly used to inform purchases. There must be a clear declaration of the country or region where the food originated, and where any additional processing or packaging occurred. National flags or logos can often be used in addition to identify the country of origin.

Within Europe there are three additional specific 'origin' labeling schemes. These ensure that food products genuinely produced in that region are protected, the name is protected for that food alone, and this is clearly and distinctly labeled. Thus protected designation of origin, protected geographical indication, and traditional specialty guaranteed labels are regulated and increasingly known in Europe and beyond. While originally devised to protect the reputation of the regional foods and help producers obtain a premium price (by eliminating unfair competition), nowadays these schemes are useful shortcuts for consumers seeking out genuine products, produced in specific areas, to specific quality standards and flavors. Protected names include Champagne and Roquefort cheese from France, Melton Mowbray pork pies from England, and Parma ham from Italy.

Clarity and Readability

It is self evident that food labels need to be clear and easily readable. Food regulations have evolved to set standards for clarity to guide producers and to meet consumers' needs.

Initially there were different voluntary schemes about the format of labeling information and differing codes of practice developed, but gradually harmonization is emerging at national and international levels. This is much appreciated because many consumers, and especially older people, complain about the clarity of labels, the small size of text, and difficult to read colors and fonts.

Wherever a product is sold the label must be written in the main language spoken – some internationally traded foods can have several different panels for different languages but they can often be so small that whether they can be read by anyone anywhere is doubtful.

A minimum size font is necessary for all food labels – there is no point in providing all the labeling information if it is too small to be read easily. Specifications are being agreed in Europe to ensure readability and clarity through the European Food Information for Consumers Regulations, due to be implemented at the end of 2014. Further guidance on clarity, visibility, and indelibility of labels will be provided.

Claims

Food labels provide an ideal mechanism for advertising and promoting the benefits of a particular food, ingredient, or

nutrient. As such all manner of claims evolved to promote special benefits. The validity of many claims was in question and it was impossible for consumers to ascertain the true nature and properties of the food. Regulation of such claims has become necessary because consumers were undoubtedly being confused and misled.

Nutritional content, functional and health claims are now highly regulated at the international level by Codex Alimentarius, the international standards setting body. Many national authorities follow the Codex guidelines on claims and transpose these into national regulations, but there can be considerable national variations and specific requirements for additional information and presentation formats to meet the specific needs of populations.

As a general principle nutrition and health claims must be consistent with national nutrition policies, be supported and validated with sound evidence, provide truthful and nonmisleading information to help consumers choose a healthful diet.

There are specific detailed regulations and guidelines for the main categories of regulated claims – nutrition declaration and nutrition, comparative, and health claims: these categories are briefly outlined below. Specific rules apply in different national legislation and should be consulted for more detailed reference and current requirements.

Nutrition Claims

Nutrient Function

For each nutrient a function that describes the physiological role of the nutrient in growth, development, and normal functions of the body is prescribed. These claims can be simply stated as the physiological role of a nutrient in the body and that the food is a source of or is high in that particular nutrient.

Nutrient Content

A nutrient content claim is a nutrition claim that describes the level of a nutrient contained in a food, such as, 'source of calcium' and 'low in fat.' Reference levels at which a content claim can be used are specified in international and national legislations. For example, to make a claim of low fat, the table of conditions for nutrient content claims applies. In this context it means that not more than 3 g fat per 100 g solids can be present. For each nutrient content claim the conditions are strictly prescribed.

Nutrient Declaration

If any type of nutrition claim is made on a food label, be it for the function of a nutrient, presence or absence of a nutrient in the food, or for the product or ingredients' health benefit then a nutritional declaration must be made.

The main nutrient groups to be declared are generally for energy, fat including saturates, carbohydrates, sugars, protein, and salt. Exactly how the declaration is made varies considerably between countries and could be presented as a list, a detailed nutrition panel, or a table, with legal specifications for how each nutrient should be declared, the unit to be used, along with specifications about per portion or per 100 g.

In some cases there is national guidance about how this information should be displayed, the format, and whether this should be on the front of the pack to guide food choice, by a pictorial representation, pie chart, or traffic light representation for each nutrient. In some countries voluntary codes have been agreed with the food industry to declare daily guideline amounts of nutrients and comparisons are made for a portion of the food and its contribution to the daily guideline amounts for specified nutrients.

Comparative Claims

A nutrient comparative claim compares the nutrient levels and/or energy value of two or more foods and will claim, for example, to be 'reduced fat' and 'less than.' The comparative food should always be declared.

Health Claims

Health claims are only allowed on a permitted basis, and on condition that they meet specific strict conditions, particularly for substantiation and proof of a positive effect on health. A health claim means any representation that states, suggests, or implies that a relationship exists between a food or a constituent of that food and health. Claims that imply they could prevent, cure, or treat a disease are prohibited.

The legal requirements permitting regulated health claims have significantly reduced the number of spurious, ambiguous health claims: consumers should now be more confident about the particular health benefits a food can confer, as part of a normal diet, and be better able to assess whether a particular health claim would be beneficial to their individual dietary and health needs.

The conditions that apply to health claims are detailed, and vary between countries, but essentially health claims must ensure that the claimed benefit is significant and appropriate for the population group being targeted and also available from eating normal quantities of the foods in the diet.

Health claims must be scientifically validated by the competent national authority and be relevant for the target population group.

Prohibited Claims

The following claims in the labeling or advertising of a food are prohibited – a claim that a food has tonic properties or that it has the property of preventing, treating, or curing a human disease.

Permitted Claims

Some claims relating to foods for particular nutritional uses are permitted but are very specifically regulated, such as for infants and baby foods, or those for particular nutritional use or population groups.

Misleading Claims

Some terms, logos, brand names, and pictures on food labels and advertisements can be misleading, and some maybe even deliberately so. When a product shows an idyllic country scene with cows grazing in green fields and hens being fed by the

farmer's wife, the expectation is that the product was made from animals reared under those same conditions – this is rarely the case given that most foods, including animal products, are likely to be mass produced, maybe under conditions more like a factory than the rural idyll of bygone eras.

Terms such as healthy, natural, farm fresh, freshly made, are meaningless and should not be used unless specific validation and explanation is given. These terms are often marketing hype: many are now prohibited in national regulations.

Additional Labeling for Consumers

Legal labeling requirements fulfill consumers' essential information needs but increasingly as food production becomes more sophisticated and prepackaged food more complex. Sometimes more information is needed and certainly much more is provided than most consumers ever read when making a purchase. This information is provided on a voluntary basis and, as such, there are no internationally agreed standards or guidelines. Notwithstanding this the general tenet that food labeling should not be misleading still applies.

Many consumers seek particular information to meet their individual health, religious, lifestyle, ethical, or moral needs and the sections that follow outline the particular categories of such additional information provided on food labels.

Religious Dietary Codes

Religious food codes and rules are strictly adhered and consequently methods of production, particularly slaughter, are strictly controlled, particularly for Halal and Kosher foods. Certain foods, ingredients, and production methods are prohibited and conformity to the rules usually overseen by the religious authorities. Products are certified to guarantee compliance to the strict dietary rules, and both Kosher and Halal foods are thus guaranteed worldwide for Jewish and Islamic followers, respectively.

Some labeling terms can cause particular concern and ambiguity for those trying to avoid certain animal products. For example, use of the term gelatin does not specify from which animal it was produced – be it beef or pork. More specific detail is essential for those who wish to follow Halal and Kosher dietary laws.

Vegetarian

The term nonvegetarian is applied to foods made from or with products derived from animals, for example, red meat, poultry, game, fish, shellfish, and other seafood.

To conform to religious and/or moral codes, the eating of meat, poultry, fish, and/or animal products such as milk, dairy, cheese, and eggs is restricted in the diets of many groups. Hindus maintain a strict vegetarian diet often eating no animal produce whatsoever, restricting especially beef and eggs. Others might refrain from eating meat and poultry for health or moral reasons yet still accept animal produce such as milk and eggs.

Dietary rules about what can and cannot be eaten are varied and yet precise. For this reason declarations and definitions of 'vegetarian' and 'vegan' vary between different cultures. While ingredient listings is a legal requirement, this puts the onus on consumers to check that the food is acceptable To help those wishing to avoid animal produce, many variations of labels and logos announcing 'suitable for vegetarians' can be found. Other labels and logos specify 'no beef' or 'no pork' to meet specific Hindu and Muslim dietary requirements, respectively.

The term vegetarian can mean different things to different cultures. For example, the acceptability of eggs as part of a vegetarian diet for some is contrary to others, especially Hindus. Hence, for this reason there is no internationally agreed definition of vegetarian. In some cultures the term vegan is familiar to denote free from any animal or animal derived products, but in other cultures this term is unfamiliar.

Consumers who have dietary restrictions are always advised to check the ingredients list and not to presume that logos or labels of 'vegetarian' will meet their particular needs.

Negative Labeling 'Free From'

Those with particular dietary needs are catered for by premium special ranges often sold and marketed as 'free from.' These can include wheat and gluten for celiac sufferers, sugar free for diabetics, or dairy free for lactose intolerance.

Regarding allergens or ingredients that might cause hypersensitivity guaranteeing that a product is 'free from' must be absolute. Product specifications and manufacturing processes must be accurate because there could be liability for any illness or reaction experienced from cross contamination. In general it is better not to make such claims for allergens because there can always be the risk of cross contamination, which could give a false sense of security to the customer. If there is any possibility that the product could be contaminated with an allergen then consumers must be warned to this effect and it is more common to see such allergen warnings on labels.

'Free from' products that guarantee to be free from an allergen are generally much more expensive as a result of the additional quality assurance to deliver the claim.

In addition, some food processing techniques are controversial and there have been calls for negative labeling, such as 'free from' or 'contains no' genetically engineered organisms, hormones to increase milk production, monosodium glutamate. Although such claims are not specifically regulated, they should all be verifiable and conform to the general principle of food labeling, which is not to mislead the consumers.

May Contain

Where a manufacturer cannot guarantee the absence of ingredients, such as peanuts, which might inadvertently cause an allergic and life threatening anaphylactic reaction, a 'may contain' warning should be given on the label – 'may contain nuts.' Sometimes wording such as 'made on the same

processing line as…' is used to better inform susceptible consumers of the potential risks.

Although every effort is made to ensure that cross contamination does not occur with high risk foods or ingredients during production, in some cases this cannot be guaranteed: For example, during chocolate or biscuit manufacture where nuts are used for other products manufactured in the same factory. Manufacturers may prefer to make the positive declaration that the product may contain (say nuts or a potential allergen) to warn susceptible consumers of the risk from cross contamination during production.

Production and Processing

How food and ingredients have been produced is of keen interest to some consumers who wish to make particular purchasing choices, often associated with meat and animal products, but not exclusively.

Many food claims and labels about food production cause confusion, but there are specific regulations and labeling prescriptions for some methods of production, usually those wishing to differentiate and command a premium price in the marketplace. For other newer techniques food labeling lags behind the technology and there is fierce debate about whether or not mandatory labeling should be provided. All novel foods and processes are scrutinized and approved to ensure the foods are safe to eat but consumers should have the right to full and accurate information to make an informed choice about the foods offered for sale.

Although labeling legislation will never be able to keep ahead of new technologies, consumer groups insist that it is a fundamental right to have full declaration of all such new technologies, especially novel processing techniques such as those produced from genetic modification, biotechnology, or nanotechnology – these have yet to be appropriately labeled for some consumers: Without the basic tenet of full declaration and truth in labels the consumer could be misled or worse deceived about the true nature of foods and their production methods.

Many voluntary, private labeling schemes have evolved to meet consumers' needs for specific information about production processes: these can address particular, legitimate consumer concerns, such as animal welfare, environment, eco credentials, hormone free, etc.

Few production standards and claims are regulated, but organic food production and Halal food labeling are examples where there is international agreement to labeling and production standards.

Organic

Within international food trade, certain production methods now have clearly defined standards of what can and cannot be done during production and processing and specific rules of how this can be labeled. Notable is organic (biological or bio) food production which is internationally regulated under Codex Standards because much organic food is traded internationally. These production and labeling standards provide the common baseline for trade. Private or national standards can make specific additional requirements over and above internationally recognized standards and guidelines.

Organic food production differentiates itself from more mass food production by limiting specific inputs to the soil and animal treatments. The certification and labeling of organic food and ingredients is highly regulated. Such products are certified as organic by regular inspection and certification or licensing of producers.

Organic labeling requirements are specific and need to include a regulated organic logo and a declaration of organic certifier.

Free Range

The rearing practices for food animals and their products can vary drastically and consequently have very different costs of production and welfare implications. Being able to differentiate premium production standards and premium products from imitations is essential for consumers and producers alike.

Free range eggs are a classic example – how does a consumer know if an egg came from a chicken able to roam freely, or from one confined to a battery cage? Consequently production methods and labeling regulations for eggs are regulated and marketing terms have to a large extent been simplified to help consumers in their choices.

There has been a backlash against intensive 'battery' egg production methods and battery egg cages have been banned in some countries. Eggs are still produced from caged birds but where there is more room for the chickens to move around, some have 'enriched environments' with more room to roam. Production methods dictate costs yet it is impossible at point of sale for consumers to detect a visual difference.

Much emphasis is placed on egg production standards and assurance schemes, which are regularly inspected. Clear definition for labeling ensures that consumers are aware of how eggs have been produced, be it free range or from caged birds.

Formed or Reformed Meat

Meat is often the most expensive food and many different quality manufactured products are available. Where meat is formed from prime cuts or reformed into 'authentic' looking joints of meat, food labels must make clear that this has taken place. Clear descriptions using the terms formed or reformed should be used on the front of the pack, in the same field of vision as the name of the product, such as ham made from formed cuts of pork.

Irradiated

Any food or ingredient that has been treated with ionizing radiation should, by law, be labeled and additionally can carry the internationally recognized logo (see **Figure 1**). Such a declaration should always be made next to the name of the food or ingredient even when a small part of a compound ingredient – there is no exception to this labeling requirement for food traded internationally.

Genetically Modified

Foods and food ingredients made through the process of biotechnology, with genetic material from another species, have gradually been introduced to the marketplace in some countries, whereas in other countries they are yet unfamiliar: their labeling has proved controversial.

Figure 1 Internationally recognized irradiated food logo.

Food ingredients and processing aids such as enzymes and yeast have been modified but no labeling declaration to this effect is mandatory.

Consumers have legitimate rights to be able to make an informed choice about their foods and to make a selection according to their own particular needs. Argument continues about GM or biotechnology – are the products same as those conventionally produced and therefore need no labeling or is this such a fundamental difference that by law all such foods and ingredients should be labeled? The jury is out on this matter.

There is international agreement that should a product carry any new material that is likely to cause hypersensitivity when transferred to a product that previously did not have this genetic material, then mandatorily it must be labeled.

Alcohol

The labeling of alcoholic drinks is relatively new. Familiar alcoholic drinks were originally sold on their brand name with emphasis on secret, age-old recipes, and until relatively recently in France ingredients listing for wine was not permitted, but things are changing, particularly in Europe.

The amount and percentage of alcohol must always be declared and in many countries this was initially introduced as a means of taxation. Consumer information about units of alcohol and safe levels to drink are relatively recent and still vary considerably from country to country, and can be voluntarily agreed with the drinks industry.

There are several important reasons to label alcoholic drinks: for the payment of duty, to protect those who may have a hypersensitivity to common ingredients such as sulfites, to indicate the strength of alcohol in the drink, and to give public health messages.

In some countries warning 'not to drink when pregnant' are prominent and in others, vague statements to 'drink responsibly or sensibly' are required.

Private Labeling Schemes

Retailers, trade bodies, environment groups, assurance, certification bodies, and consumer lobby groups can set up their own voluntary schemes according to their own particular criteria, be it for food safety, animal welfare, environment, fair trade, or food chain assurance. There are probably hundreds of such schemes worldwide.

Each private scheme will have its own standards and methods of verification, ideally with clear published information for consumers explaining how the scheme is managed, how standards are determined and assured.

Most often there will be a unique trade mark or logo which is the main marketing tool for consumers to identify foods belonging to a particular scheme. Each scheme will market and promote its own 'brand' and the consumer logo, according to its own rules.

The myriad of private label schemes tend to follow and also tap into current consumer trends be it for eco-labels, sustainable production, residue free foods, pest management, animal welfare, social responsibility, fair trade, or GM free products. Industry private schemes for meat, poultry, or vegetables tend to set quality assurance and standards for production.

Private label schemes can be an important shortcut and guide to making appropriate food choices and can have considerable loyalty amongst consumers. However, it is crucial for consumers to interrogate each scheme's detailed information, standards, and whether or not there is independent verification of the claims by appropriate certification schemes to ensure that expectations are actually delivered. Private label schemes are not controlled by the national food authorities, they are voluntary schemes managed by their own boards and standards setting body.

Concerned consumers will need to interrogate the specific criteria for each private labeling scheme to ensure that it meets their particular needs, rather than presuming that these claims deliver all that they imply.

Welfare Friendly

Animal welfare in some cultures is a major concern and ensuring that animals reared for food are treated well during their life and, at time of slaughter is a particular concern. This includes how the animal was fed, reared, whether it was allowed to display its natural behavior, with the right to roam or forage, or if it was restricted, being more confined and 'factory farmed.'

Differentiation in the marketplace is important and consumers are often willing to pay extra for foods that have been produced to higher welfare standards, but how those standards are set and enforced needs to be considered when making this choice.

To meet this legitimate consumer demand many schemes have been developed, each with their own specific parameters to meet different cultural and moral requirements: terms such as higher welfare, cruelty free, naturally reared, free range, outdoor reared, etc. Likewise for other labeling schemes such as dolphin friendly, quality assured.

Animal welfare schemes and labels are not defined in food law, but the basic rule of food labeling applies – that the product must be described accurately, must not be false, misleading, or deceptive, or likely to create the wrong or misleading impression about the character of that food.

Eco or Sustainability

Knowing where food has come from and how its production has impacted the environment is of keen interest for some consumers: food labeling and information has responded and many private schemes address issues of food miles, rainforest friendly, green, and sustainable production.

Exported Foods

Foods have always been traded from one side of the globe to the other, and all places in-between, many are prepackaged or will end up as ingredients in packaged foods.

Wherever the food is eventually sold there must be assurance that it is safe, and that it is accurately labeled.

It is mandatory to ensure that food labels are displayed in the main language of the country where it is sold. Some foods have labels in several languages to meet this requirement; others can have the essential legal information translated and be overstuck with a translated label to meet this requirement.

All exported and imported foods must meet minimum food safety standards. There are strict food safety and production standards at the national, regional, and international levels, as well as product-specific standards of quality and composition. The Codex Alimentarius Commission is the international food standards body where member countries agree on standards and guidelines for international food trade, to ensure it is safe and that, providing the Codex standards are followed there should be no barrier to trade for exported foods.

Eating Out

As eating away from home, be it at work, socially, or 'on the move' becomes ever more widespread, consumers with particular needs will also need particular information when eating out, to ensure their health and dietary codes are met.

Restaurant menus will of course give a detailed description of the food served, but detailed ingredient lists are not usually available. Claims are more about the flavor and quality of the final dish rather than about specific ingredients, the health and nutritional qualities of the food, or the allergens it might contain.

There are moves to introduce more specific labeling and information for consumers where there is no choice of food – on the move – for example, on airline foods especially for long distance flights. Here specific details of what is included or not are needed to meet the needs of different religious and cultural codes for consumers traveling beyond familiar territory.

Fraudulent practices where cheaper, substandard ingredients or worse, stale or contaminated foods are served are illegal. But it is difficult for consumers to tell exactly what is in foods served when eating away from home. Although there have been campaigns for 'truth in menu' to confirm exactly what is being served, such as the type of meat, for the most part the rule of '*caveat emptor*' – buyer beware – is paramount when eating out.

Quality Assurance

Traceability

Many of our commonly used foods contain an infinite number of ingredients from all over the world: international food trade is the norm, especially for commodities such as meats, cereals, fruits, vegetables, herbs, and spices.

Traceability of ingredients and food batches is essential with such extensive global and national food trade: manufacturers need to know exactly where and when a particular batch or lot was produced, and will label it accordingly. These batch or lot numbers are often indecipherable codes for consumers, but they contain key information for producers and retailers.

Should a subsequent problem arise, be it a food safety alert or be it from contamination with a foreign body, foodborne disease, or even a rouge ingredient that could cause hypersensitivity, then being able to trace a specific batch or lot of food by its unique number is essential for the effective and safe recall of the product.

Consumers can be alerted to such food recalls by national food agencies and also by retailers and manufacturers.

Quality Control

With keen emphasis on food safety, hazard analysis, and control at every stage of production, processing, manufacture, and retail, and with vigorous quality assurance, food business operators should be confident that the food products they offer for sale are safe. Yet as we know, problems are identified – through human error, manufacturing defects, and the like.

International and national rapid food safety alert systems can swing into action to withdraw suspect foods from sale, and recall any that have already been sold.

Some multinational retailers with sophisticated customer loyalty schemes can identify exactly who has bought the offending products from the scanning of the product bar code, at point of purchase, to pin point batch details and customers' receipt information. It is even possible to contact customers directly to alert them of the problem. This is truly comprehensive traceability from farm to fork, and something that goes on behind the scenes, to protect customers who remain oblivious, unless of course there happens to be a problem.

Much information can be obtained from manufacturers' bar codes, where pricing information can be scanned at point of sale, and stock control monitored.

Additional Information Provision

Many foods will now display contact details, for additional information sources, be it a website address for the producer, manufacturer, retailer or the food brand, or an internet, social media campaign.

Website information can be a very useful source of additional information, but it does not replace the need for clear, legally compliant information on labels: Obviously much more information can be provided on the internet.

For consumers who have particular dietary or food safety needs brand websites are a useful way of checking that a product meets specific needs and many manufacturers will disclose more information about their products for groups with health conditions such as celiac, diabetic, or those with particular hypersensitivities, especially to nuts. This is invaluable additional information and assurance for these particular groups of consumers.

Websites can give customers assurance about production methods, how ingredients were sourced or animals reared. Such is the thirst for knowledge from some consumers about particular moral issues, such as about meat production and animal welfare that live web cameras on farm, and at slaughterhouses, can also be provided via dedicated websites.

Food businesses now actively encourage customers to engage with them via social media and indeed this is now a significant way of advertising new food products through 'Facebook,' 'Twitter,' and the like. Major food brands encourage 'followers' and are then able to target advertising more closely to customers' likes and dislikes.

With new media it is likely that the Internet will become an important additional source of information but it does and cannot replace the legal requirement to provide accurate and informative food labels.

Disclaimer

This article is a general overview of food labeling and information for consumers: it is not a definitive legal guide.

National regulations vary: Food business operators, to meet their legal obligations, should seek formal legal advice about current applicable labeling requirements from the relevant national food authority.

See also: Disciplines Associated with Food Safety: Food Microbiology; Food Safety Toxicology. Food Safety Assurance Systems: Audits of Food Safety Management Systems; Food Safety and Ethics; Investigation of Incidents in Industry; Tampering. Food Technologies: Chilling; Food Irradiation; Freezing; Microwave Heating; Packaging. Foodborne Diseases: Foodborne Diseases and Vulnerable Groups; Overview of Biological Hazards and Foodborne Diseases; Overview of Chemical, Physical, and Other Significant Hazards; Overview of Emerging Food Technologies. Hazards of Food Contact Material: Food Packaging Contaminants. History of Food Safety and Related Sciences: History of Foodborne Disease – Part IV – Modern Times (CE 1900–Present Day). Institutions Involved in Food Safety: FAO/WHO Codex Alimentarius Commission (CAC). Mycotoxins: Aflatoxins; Mycotoxins – General. Organisms of Concern but not Foodborne or Confirmed Foodborne: Spoilage Microorganisms. Other Significant Hazards: Food Allergies and Intolerances. Public Health Measures: Foodborne Disease Outbreak Investigation; Fundamentals of Food Legislation; Health Education, Information, and Risk Communication; Modern Approach to Food Safety Management: An Overview. Safety of Food and Beverages: Halal Food Requirements; Kosher Food Requirements; Promotional Material

Further Reading

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FOOD SAFETY ASSURANCE SYSTEMS

Audits of Food Safety Management Systems

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Glossary

Adulteration A fraudulent action that is intended to: (i) omit a valuable constituent or substitute another substance, in whole or in part, for a valuable constituent; (ii) conceal damage or inferiority in any manner; or (iii) add any substance to increase its bulk or weight, reduce its quality or strength, or make it appear bigger or of greater value than it is.

Audit A systematic and functionally independent examination to determine whether activities and related results comply with planned objectivities.

Benchmark A reference point or standard against which performance or achievements can be assessed. A benchmark refers to the performance that has been achieved in the recent past by other comparable organizations, or what can be reasonably inferred to have been achieved in the circumstances.

Compliance The products and/or practices meet regulatory requirements (to be differentiated from conformity which means that activities are carried out according to the established procedures).

Control measure Any action and activity that can be used to prevent or eliminate a food safety hazard or reduce it to an acceptable level.

Corporate governance A system of law and sound approaches by which corporations are directed and controlled, focusing on the internal and external corporate structures, with the intention of monitoring the actions of the management and directors and thereby mitigating agency risks which may stem from the misdeeds of corporate officers.

Critical control point (CCP) A step at which control can be applied and is essential to prevent or eliminate a food safety hazard or reduce it to an acceptable level.

Good hygienic practice A system of management controls that need to be adopted at production, processing, storage, distribution, and preparation to ensure safety and suitability of products of consumption.

Hazard analysis and critical control point (HACCP) system A preventive system which identifies, evaluates, and controls hazards which are significant for food safety.

Validation Obtaining evidence that a control measure or combination of control measures, if properly implemented, is capable of controlling the hazard to a specified outcome.

Verification The application of methods, procedures, tests and other evaluations, in addition to monitoring, to determine whether a control measure is or has been operating as intended.

Introduction

Audits (food safety audits) refer to an industry activity to verify that the food safety management system is implemented correctly and effectively, and is maintained. The primary reason for auditing food safety management systems is to establish whether a food business has the ability to consistently produce, manufacture, or distribute 'safe' food and to ascertain that the food safety management provides adequate assurance. Audits may be carried out by a business itself as part of self-control in which case the audit is called internal audits. It can also be carried out by an external body such as the customer or a certification body, which is then referred to as 'third party audit.'

In the framework of the enforcement of laws and regulations, governmental authorities also verify the compliance of industry practices with laws and regulatory requirements. This activity is usually referred to as 'inspection.' Today, inspections follow a similar process as audits and have become very comprehensive; although they are carried out for a different purpose. For a more thorough review of inspection, the readers are referred to a specific article on this subject. The focus of this article is on the industry audit of the food safety management systems.

Definition and Purpose

An audit is an evaluation to verify that actual practices match set standards and codes and determine gaps, if any. With advances of the hazard analysis and critical control point (HACCP) system and the integrated food safety management system, the scope and purpose of audits in the industry have shifted from a snapshot examination of good hygienic and manufacturing practices to a more general audit of the food safety management systems. Thus, the primary purpose of audits is no longer for controlling hazards, but for confirming that control/preventive measures are implemented correctly and are effective.

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However, there are multiples reasons for which audits may be carried out. These include:

1. Confirming the compliance (or identifying the divergence) with the internal rules and/or regulatory requirements. This is perhaps one of the most frequent objectives of audits.
2. Evaluating the ability of a supplier or a contractor to produce, manufacture, or transport a food according to the set requirements. This can happen when choosing a supplier, a contract manufacturer, or even purchasing a new business.
3. Investigating violations or incidents, for example, investigating a recurring critical control point-related violation, employee complaints, alerts by internal whistleblowers, frequent consumer complaints, or a full-fledged incident.
4. Obtaining a certificate of assurance for the customers that their requirements are met. This may be with customers nationally or internationally.
5. Benchmarking or analyzing gaps in view of identifying the need for improvement, including the need for technical assistance, training and guidance on competences, and/or improving the infrastructure (equipment, design of premises), etc. This can happen when a new factory or business is purchased, or when companies are merged. Experience has shown that small- or medium-size businesses are often not resourceful enough to know the regulations that they often learn about these when they are visited by an inspector or assessed by a customer or the representative of a certification body. In such a situation, to avoid conflict of interest it is important that those involved in guiding the business are not the same individuals who will also assess for compliance.

Scope and Frequency of Audits

The scope and content of audits have also evolved with time. Some years ago, depending on the stage of the food chain, such audits were limited to verifying compliance with good fishery, agriculture, farming, manufacturing, transport, or hygienic practices. Later, they were developed to include audit of HACCP. Today, with the advance of an integrated approach to food safety management, particularly the development of ISO 22 000, audits include a variety of elements, such as:

1. Management commitment, organizational structure, corporate governance and its impartiality and resources.
2. Management of people (including training).
3. Awareness and infrastructure for monitoring the regulatory requirements of the country where products are produced and/or marketed.
4. Product traceability, recall, and incident and crisis management.
5. Product development.
6. Raw materials and supplier management; this should also include any possibility of fraud or adulteration.
7. Good hygienic practice.
8. HACCP system and implementation.
9. Verification activities (consumer complaints, pathogen and environmental monitoring, review of noncompliances, and their root cause analysis).

The decision on the scope and frequency of audits will depend on a number of considerations, in particular whether the audit is a first audit or a follow-up audit. Whether a full or a partial audit is carried out will depend on the original purpose of the audit. For example, partial audits might be appropriate for closing out noncompliances, for investigatory purposes after an incident, or where a previous audit has confirmed that a sound system is in place.

Classification of risks is an important criterion for prioritizing and deciding on the frequency, i.e., having more frequent audits at higher-risk premises or suppliers of high-risk material. The following information can be considered in the classification of risks and in deciding on the frequency and scope of the audits:

1. the potential hazards known to be associated with the product and/ or process, including the previous records of safety of the product;
2. the history or level of previous compliance;
3. the state of the food safety management systems and other management systems that may be in place, for example, ISO quality management systems and certification, total quality management (TQM), as well as the level of in-house expertise; and
4. other considerations such as processing methods, intended use and population at risk, size of operation (e.g., number of employees, volume of production, and turnover), type of products and processes, complexity of operation, quantity of product affected by the raw material used, market, or trade requirements.

Similarly, the following could be considered in deciding the scope of an audit:

1. whether it is an initial audit or a follow-up;
2. size of operation, for example, number of employees, volume of production, and turnover;
3. type of products and processes;
4. complexity of operation;
5. level of in-house expertise;
6. amount of available resources;
7. presence of management systems, for example, ISO quality management systems, and TQM;
8. results of previous audits; and
9. population at risk.

A change in the system (process, formulation, etc.), or the aftermath of a natural accident or disaster, for example, fire, flood, etc., can also justify an audit. As mentioned previously, audits may also be triggered as results of a previous food safety incident.

Subsequent frequencies for audits and their scope can be considered in the light of such findings.

Competence of Auditors

The validity of audits depends to a great extent on the competencies of auditors and their integrity. Food safety being a multifaceted subject, a carefully selected team of experts will be required. The composition of this team and the expertise of the members will be all the more important as the

responsibility for protecting public health is significant. In any case, for a full scope audit, the following competences, skills, and qualifications need to be considered:

1. the technical competence,
2. the skills in assessing and investigating (audit skills),
3. the people skills, i.e., independence of judgment, integrity and objectivity, and finally, a good audit requires, and
4. the cooperation and openness of the audit entity in providing truthful information.

Other factors such as time and financial constraints, availability of documents, also play a significant role.

With regard to technical knowledge, the following are needed at the very least:

1. Understanding the basic hygienic requirements, their relevance in supporting safe food production, and experience in assessing them.
2. Knowledge of laws, regulations, standards and general codes of hygiene, and/or criteria for the specific category of products.
3. Knowledge of relevant industry products and processes (including past failures in the category).
4. Knowledge of the HACCP system and its application, including:
   a. The identification and audit of potential hazards which may occur during food production, handling, preparation, storage, and transportation, including biological, chemical, and physical hazards.
   b. The ability to assess the effectiveness of control measures (validation) of the HACCP plan and its verification.
5. Understanding the role of the human factor and of company culture in food safety.

Reporting Structure

Another important factor in the validity of the audits is the organizational structure and the management reporting system. To prevent conflicts of interest, and ensure that corporate governance is functioning in all independence, it is important that audits are carried out by teams of professionals who are structurally independent from those who are designing and implementing the food safety management system.

The Procedure and Methodology

The procedure for an audit must be defined and carried out in accordance with a set format. Auditors should ensure that they plan the process properly, i.e., that:

1. The scope of the audit is predetermined and sufficient time is allocated.
2. The required skills are available within the team.
3. Tools needed are made available.
4. Arrangements are communicated and agreed upon with the site being assessed.

The procedures for audit will need to include the following stages:

1. a planning process to prioritize establishments, operations and their frequency, and scope of audits;
2. a desktop audit;
3. an on-site audit;
4. an evaluation process to analyze findings, determine compliance, and decide corrective actions and follow-up requirements; and
5. reporting and follow-up.

The Planning Process

Initial planning is important to clarify the scope of the audit and the approach that will be taken on-site. It helps to ensure that auditors have the necessary information and tools to complete an effective audit. Information that will help in this planning process includes:

1. relevant company documentation;
2. previous file records, data on premises and products; and
3. results from previous visits or audits.

The Desktop Audit

The audit itself is best carried out in two steps. The first stage, desktop audit, consists of the initial review of documentation, which may be carried out on- or off-site.

A desktop review also has the advantage of enabling auditors to plan their work, for example, to judge how the CCPs have been established, check the personnel required for detailed discussions, the specific questions to be asked, draw a list of priorities to focus on, and/or areas to visit during the on-site audit.

Examples of documents to review:

1. The food safety policy.
2. The organigramme, the responsibilities of the managers and food safety management team, and their respective technical expertise and competences.
3. The operation, and the type of products produced.
4. The range and number of raw materials used and their origin.
5. A site layout plan may give an idea of the flow of products through the site, the scale of the operation and the products produced.
6. The HACCP-related documentation, including:
   a. a process flow diagram and specifications relating to it,
   b. the HACCP study (showing how potential hazards have been identified and on which basis they are considered as nonsignificant if this is the case),
   c. an HACCP plan, including the monitoring plan and the validation of the control measures,
   d. records of CCP monitoring and corrective actions following the violation, and
   e. verification data, for example, consumer complaints, monitoring data for raw material, environment or end products, and reports of incidents and root cause analyses.
7. Training programs, for example, the manual or other tools used for training.

8. Incident and crisis management procedures.
9. Reports of management review of food safety and quality.

On-Site Audit

The second stage is the on-site audit.

The on-site audit will normally start with an initial or opening meeting to confirm, with the key people being assessed, the audit scope, timetable, facilities, and personnel required and in general to ensure cooperation.

The purpose of this step of the audit is to confirm that procedures and practices described in the food safety management system of the company or the regulatory requirements to ensure food safety are properly implemented in practice.

During the on-site visit, specific attention should be given to HACCP and prerequisite programs, including:

1. Confirmation of the accuracy of the process flow diagram(s). This is facilitated by an initial walk through the site. The auditor will subsequently need to engage in a range of questioning and investigative activities to assess the efficacy of the HACCP system.
2. Evaluation of the hazard analysis based on the state of the prerequisite programs mentioned above.
3. Confirmation of the suitability of CCPs, critical limits, and corrective actions.
4. That monitoring schedules are established and operating correctly.
5. Confirmation that persons responsible at CCPs perform activities correctly, understand the importance of the step for safety, and their responsibility in case Critical Limits are violated. This will require specific interviews with the personnel.
6. Establishing whether effective verification procedures are carried out.
7. Reviewing monitoring data of raw materials, products, environment, CCPs, as well as reports of internal audits, supplier's audits (inclusive of supplier monitoring programs), consumer complaints, personal reports, and complaints. It is particularly important to corroborate these results with the hazard analysis (for instance, if a contaminant is considered as not significant in the raw material, this is confirmed through the monitoring carried out for verification).

During these activities, the auditors will need to keep sufficiently detailed records and to collect supporting evidence to enable conclusions to be made. Use of checklists together with a narrative, notebooks, or where appropriate, tape recorders, will assist this process. Depending on the judgment of the auditors, checks might be made on items of equipment, on-site measurements may be carried out, or product or environmental samples may be taken for subsequent laboratory analysis.

Evaluation Process

The auditor (or the team) will need to identify and analyze all information obtained during the audit in order to draw up preliminary conclusions of deficiencies found, if any, and their effect on food safety, regulatory compliance, or other trade-related concerns. They should also advise on their severity and the speed with which they would need to be rectified.

Reporting and Follow-Up

The format of auditor reports varies according to company policy and prior agreements with auditing bodies. However, it is essential that the results of the audit be communicated to the management of the company and to all relevant persons within the organization (i.e., with responsibility for safety) in a timely manner.

Where an audit report indicates critical or serious gaps, these need to be followed up rapidly, and root cause analyses of these gaps are also made to identify the latent cause of the failures.

Conclusion

Audit of food safety management systems is an opportunity to improve food safety management and close the gaps. They should be carried out with objectivity and integrity. An unsatisfactory audit report should not always and necessarily be a reason for reprimanding the managers; rather over and above closing the gaps, a root cause analysis of the situation should be made and short- or long-term corrective action should be made. Not infrequently, the root of the problem may be in the management.

Reports of audits and food incidents have shown that some of the major source of food safety problems are:

1. Raw material and supplier management.
2. Failure in the design of equipment and their maintenance.
3. Good hygienic practice violation.
4. Failure in hazard identification.
5. CCP monitoring failure.
6. Failure in corrective actions.
7. Human negligence or error.

Acknowledgment

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See also: Food Safety Assurance Systems: Documentation and Record Keeping; Investigation of Incidents in Industry; Personal Hygiene and Employee Health. Public Health Measures: Food Inspections and Enforcement Systems; Modern Approach to Food Safety Management: An Overview

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FOOD SAFETY ASSURANCE SYSTEMS

Quality Assurance and Good Laboratory Practice

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Glossary

Accreditation The granting of approval to a laboratory by an official review board after it has met specific requirements.
Federal regulatory agency An agency that functions under the executive branch of the US federal government established to generate and enforce rules.
Good laboratory practice A system of management controls for laboratories and research organizations to ensure the quality, integrity, consistency, and reliability of results.
International Organization for Standardization (ISO) It is responsible for development of voluntary international standards for products, services, and practices.
Laboratory quality system A continuous process consisting of planning and documenting to control laboratory functions culminating in the generation of reliable data.
National Environmental Laboratory Accreditation Conference (NELAC) It is sponsored by the US Environmental Protection Agency and accredited under the NELAP. Established performance standards for environmental laboratories, to produce environmental laboratory data of reliable, documented quality.
Quality assurance A system for evaluating adherence to, and performance of, quality control (QC) in a laboratory, for the purpose of ensuring demonstration of competence and generation of defensible, reliable data.
Quality control Laboratory QC is performance of standard operating procedures (SOPs) and control activities to ensure standardized analysis. The control activities are designed to detect, reduce, and correct deficiencies in a laboratory's internal analytical process before the release of results.
Reliable data Data derived from laboratory results that are valid and credible.
Scope Testing areas (procedures, assays, and SOPs) that have been evaluated and conformed to standards as established by the accreditation body.
Standardization The process of developing standards that are universally applied to ensure consensus and compatibility.
Validation Practice undertaken to substantiate or confirm methods or procedures perform as expected and in a reliable manner and consistently meet expectations.

Introduction to Quality

Elements of a Quality System and Generation of Reliable Data

The term 'quality' has relative meaning in general conversation, but in a laboratory setting quality is specific, as in a plan employed to ensure production of legally defensible, reliable data that can be used to support well-informed decisions. For many food testing laboratories this plan, often referred to as a quality assurance (QA) plan, compiles specific and prescribed series of documents that collectively support accurate and defensible results. Defensibility of results demands laboratory competency, which is founded on verified compliance with documented procedures and proven proficiency, and thus, ability to generate valid and credible data.

So how does a laboratory ensure generation of reliable data? Competent laboratories often voluntarily obtain accreditation from a recognized, third party accreditation body that verifies procedural compliance and technical competency. There are myriad written standards for quality system evaluations depending on the type of lab; however, a common standard of evaluating laboratory competence is the International Organization for Standardization/International Electrotechnical Commission (ISO/IEC). ISO develops and publishes international standards for many sectors that are derived by experts in their fields. There are also standards for accreditation developed for specific commodities directly by processors and/or those providing oversight and governance. Often, measurably verified standards are mandated for laboratories generating data that are deemed reliable and defensible for specific commodities.

Quality Accreditation Types and Jurisdictions

Independent accrediting bodies are utilized to lend credence to data generated by both foreign and domestic accredited laboratories. The accreditation a laboratory holds often acts as an assurance that a minimum standard has been achieved and will be maintained. Though it is no guarantee, independent accreditation assures that a quality system exists and the

analysis the lab includes on its scope of accredited test methods has been validated. Part of the accreditation process includes an independent examination of the laboratory scope, which includes what type of analysis was performed, what type of matrix the lab analyzed, the target of the analysis, and the technique used for analysis, including the range or limit of detection or quantitation. Laboratories may also perform analysis not listed on their scope; however, the accreditation imparted on the lab will not cover the analysis. Many private laboratories, either research or third party contract testing labs, will have a 'special projects' or research division, which will not maintain the same level of standardization. They should, however, maintain the same level of QA and quality control (QC), in the form of compliance verification.

Laboratory accreditation is directly related to the type of testing it performs. There are accrediting bodies for clinical, calibration, environmental, and food laboratories. For example, a laboratory that performs drinking water analysis might obtain a National Environmental Laboratory Accreditation Conference Drinking Water Lab Certification. A food testing laboratory might acquire an ISO/IEC17025:2005 accreditation. This provides general requirements for the competence of testing and calibration laboratories, and is universally recognized. The standard is used by laboratories developing a system of QA. The US Food and Drug Administration's (FDA) recent passage of the Food Safety Modernization Act (FSMA) required laboratories testing FDA-regulated food commodities, under section 202 – Laboratory Accreditation, to be accredited. As a result, many labs maintain QC/QA programs that adhere to ISO quality guidelines and standards. FSMA was developed to ensure the US food supply and public health were protected by shifting the focus to prevention and strengthening regulatory authority.

Similarly, the US Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) recently issued a policy guidance document entitled, "Establishment Guidance for the Selection of a Commercial or Private Microbiological Testing Laboratory" to provide criteria to meat, poultry, and processed egg processors for selecting laboratories to analyze establishment samples. The purpose of this document is to clarify key considerations and responsibilities of the establishment in verifying the QA/QC practices of their laboratory provider. Issuance of this guidance and communication of responsibilities places a greater level of emphasis on the importance of verified laboratory QA/QC practices as part of the overall food manufacturing process and detection of foodborne pathogens. Such activities will continue to increase awareness, improve transparency, and increase stringency of monitoring laboratory compliance. Overall, such activities support a better avenue for detection of potential food safety hazards in a preventative manner.

Oversight of Accreditation

Part of the strategy for prevention of chemical, physical, and biological food safety hazards is detection of the hazardous food product before its entry into commerce. A laboratory must be capable of generating reliable data if public health decisions will be determined based on results. Though not a guarantee, an accreditation acts as a formal acknowledgment for customers to consider when they identify and select a reliable testing laboratory. It assures a quality system is in place, which is a necessary component in any competent laboratory; however, a quality system without daily implementation serves no purpose. The laboratory quality system is only as good as the staff adherence and support it garners. The elements of a comprehensive quality system must be more than a paperwork exercise. Laboratories with strong quality programs have several drivers including bench-level analysts with an inherent aptitude and commitment to the laboratory quality system, the commitment of time and resources from management for implementation, and recognition and value placed on successful QC. If the commitment to quality is as powerful a motivator as profit margins and return on investments it will be valued by all laboratory staff, and rewarded by management. This will ensure that effective implementation will not require constant policing. It will be evident to the auditor, by the lack of careless errors such as data obliterations or inadequate daily record keeping and poor organization. For the laboratory quality system to be truly effective, it must be adopted into the culture of the laboratory from management to analyst level, and palpable by the external auditors and customers of the lab.

Development and implementation of a comprehensive laboratory quality system, required for defending technical competence of laboratories, will be founded on good laboratory practices (GLPs), quality management (QM), QA, and QC. These often exist in most regulatory or contract testing laboratories but are not usually points of focus in research laboratories. Clinical laboratories have undergone a similar transition in an effort to bridge the quality gap between applied and theoretical research, and food laboratories are no different. The development and implementation phase of laboratory quality is time consuming, expensive and often requires external consultation. In the end, data are only as reliable as the lab in which they were generated. Research methods may indeed become validated methods utilized in food laboratories, thus quality systems should exist as the methods are being developed. Adherence by a laboratory to a quality system is a step toward the goal of generation of reliable data. Having a culture of quality in the lab is a means to that end.

Evolution of the Quality Concept

The culture of quality has evolved over many years and will continue to progress to meet the needs of analyses as they advance, and of analysts as they continue to increase in technical proficiency. When the level of sophistication increases, the quality system of a laboratory must address possible deviations and necessities of standardization. Even under the best of circumstances, variations will exist, between reagents and supplies and even between analysts. A quality system must aim to minimize these events and account for an uncertainty in reported results. To determine the future of quality systems and how they must adapt, an overview of past principles is beneficial. As with many scientific disciplines, the field of quality evolves and builds on past successes.

To continue to maintain a quality system that meets the needs of a laboratory, quality managers and staff must understand the foundation from which the quality system is created.

The Past: GLP

Only as Good as the Oversight

For nonclinical laboratories, in this case food testing laboratories, the Code of Federal Regulations Title 21 Part 58 (21 CFR 58) is the regulation that dictates guidance for quality. This was drafted specifically for all laboratories not involved in performing clinical studies or field trials with animals or humans. GLP was proposed and first adopted by FDA, then Environmental Protection Agency (EPA) in response to an incident of research and development fraud in the late 1970s. In 1981, the Organization for Economic Cooperation and Development also published GLP principles, prompting international adoption. As a result of the adopted legislation, laboratories are allowed flexibility in conducting nonclinical studies while still guaranteeing a quality program is in place with an inspection component to maintain existing systems. The desired outcome of the regulation was to protect public health and ensure satisfactory laboratory compliance during agency inspections. GLP is often equated with technical skill. This is not the case. Technical skill and technique are obviously important facets of generating reliable data, but they are not the whole story. Consistent production of reliable data requires another level of diligent evaluation, even if GLPs are in place. Although some guidance is provided in determining qualifications of key personnel based on education and training, determination of the ability of a laboratory staff to conduct a procedure with technical accuracy can rely heavily on third party audits and specifically on the experience level of the auditor.

GLPs: Introduction to Standardization and Transparency

GLP is an overarching set of practices that play a part in all aspects of laboratory function. GLP is far more than standard operating procedures (SOPs). GLPs may be considered a 'what if' plan of action. What happens if equipment fails? What are the criteria for samples to be accepted in the laboratory? Is there a rejection criterion? How can the laboratory prove the analysis was accurate and minimize analyst error? The time to figure out how to proceed is not in the middle of the laboratory anomalous event. GLPs are in place to ensure sample integrity and method validity. They are standards that keep the lab running and ensure an event will be handled in a way that is defensible, standardized, and controlled. Having established GLPs in a food testing laboratory ensures an analyst will know what to do when the unforeseen inevitably presents itself, in the areas of lab function, safety and health, data handling, as well as in the routine aspects of the lab function.

GLP Highlights

21 CFR 58 addresses routine maintenance and calibration, sample traceability records, and development and implementation of quality systems. According to the World Health Organization, all GLP texts, irrespective of their origin, stress the importance of the following fundamental areas:

1. *Resources*: Organization, personnel, facilities, and equipment. A clear understanding of resources will allow laboratories to contract and perform analysis while adhering to quality framework.
2. *Characterization*: Test items and test systems. Validation of laboratory testing options and troubleshooting food matrices is a challenging endeavor and integral to successful data generation. Which analyses can a laboratory reliably perform?
3. *Rules*: Study plans (or protocols) and written procedures. These documents are part of QC in any laboratory and should be controlled. 'Tweaks' are not permitted! Changes increase variability and method uncertainty.
4. *Results*: Raw data, final report, and archives. Data are only as reliable as the laboratory from which they were generated.
5. *QA*: ISO 4.1.5 (a) is specific in ISO/IEC17025, that a laboratory shall have personnel with the authority and resources needed to implement, maintain, and improve the QM system (QMS), and to identify the occurrence of departures. They also should have the ability to initiate actions to prevent or minimize such departures from the established system QC.

Guidance for Laboratory Quality and Its Evolution

The modern concept of QM and QA is directly related to GLP; however, modern interpretation tends to be more prescriptive. The quality manual can be traced to GLPs, which require laboratory manuals and SOPs. As laboratory functions are so varied, there is no standard format for a quality manual across laboratories, although accrediting bodies will often provide a guide. The QM aspect of the overall laboratory quality system in a lab is typically GLP-based and includes a specific set of goals. The guidebook for the laboratory quality system is the quality manual, which should make clear the goals and requirements of how the quality system is managed. It should describe in details, the policies and procedures the laboratory has in place to assure the quality system is functioning as intended.

Only as Good as the Analyst

QA and QC support the management structure and theoretically ensure that the laboratory produces defensible, reliable data. However, the execution of the established system is what ultimately determines reliability of the data. The analysts carrying out each step of the quality plan are a variable, and clearly not all variables can be controlled. This has less to do with analyst skill and more to do with the prescriptive and standardized practice of laboratory quality. Blatant disregard for GLP guidance or laboratory accidents can be discovered and dealt with; however, variation based on human error or interpretation may be the root cause of laboratory issues. Although GLPs were an excellent start to managing quality in nonclinical laboratories, the dynamic nature of food analysis

has demanded that more be done to minimize variations in analysis and results.

The Present: QM, QA, and QC

QM

The next iteration of GLP is the QMS. Though directly related to GLPs a QMS is very specific. QMS is the system in place to ensure the laboratory not only develops but also adheres to guidance in the form of quality manual, quality procedures, technical procedures, administrative procedures, work instructions, and requirements for record keeping. A QMS is only as effective as the QA and QC within each laboratory (Figure 1). Collectively, each component is essential to the production of reliable data.

Most laboratories display a unique quality statement that essentially imparts the same message: The quality system in this laboratory assures the data are reliable. But is that really the case? QM, though similar in scope to GLP, is specific and all encompassing. Documentation is the key to any quality system. The 'golden rule' of quality is this: If it is not written down, it did not happen! The QMS must be adopted from the top down and must be practiced by all. The QMS is the encompassing system by which the lab functions, but it is supported by the following Sections QA: The Rule Book for Reliable Data and QC.

The quality system sets the tone for everything that happens in the laboratory. If there is no acceptance from laboratory management to the analyst level, the efficacy of the laboratory's quality system may be in question. This is where the concept of transparency becomes critical. The transparency of laboratory quality systems and the procedures in place to support the system are key to successful implementation.

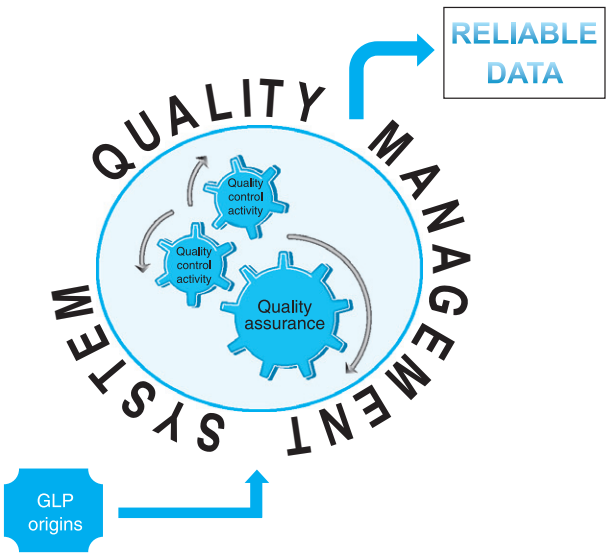

Figure 1 Quality structure in the laboratory. The QMS is only as strong as the supporting concepts of assurance and control. All the cogs must function to produce reliable data.

Performance assessments, both internal and external, are invaluable for laboratories to monitor the success or failure of their quality systems. The structure for monitoring should be clearly explained in the QA plan in any laboratory generating data.

QA: The Rule Book for Reliable Data

The term QA is exactly what the name implies: procedures in place to assure that the data generated by a laboratory are reliable. QA plans should contain explicit requirements to produce reliable data. These will include specific requirements for documentation of organization of the laboratory, staff training requirements, standard operating procedures (SOPs), QC measures for equipments, reagents, calibration, maintenance, repair and performance, laboratory safety, and testing methodology. The organizational chart of the lab may seem to be an insignificant aspect of the QA; however, chain of command is critical. Customers must know to whom they can address questions or complaints. Analysts encountering any errors or issues must know who to call and who has the authority to make an executive decision. Staff training requirements are an area that is often glossed over and satisfied by a checklist, which does not adequately address training. The purpose of staff training is to develop a metric to defend analyst competence. Without the ability to demonstrate analysts are proficient in performance of an assay, any unexpected results may be called into question as lab error.

QA is not just SOPs, though it is often the part labs spend the most time preparing. SOPs define how to carry out protocol-specified activities. Most often they are written in a chronological listing of action steps. Labs should have SOPs for all activities relating to quality and analysis. These should be controlled documents to ensure standardized practices throughout the lab. Multiple copies or a lack of document control leaves a lab open to method changes and nuances that are not applied universally. Often two other aspects of the QA plan, internal and external performance assessments, are under emphasized. External performance assessments are fairly straightforward; most laboratories participate in proficiency and/or split sample testing in one form or another. Internal performance assessments tend to be slightly more subjective. ISO requires that the laboratory shall periodically conduct internal audits of its activities to verify compliance with the management system and the ISO standard. This is a powerful tool that many laboratories fail to utilize. Nonconformities and errors cannot be found unless there is consistent review and auditing. This should be considered a process of continuous monitoring for the laboratory and a critical part of any quality system.

Putting the Assurance in QA

QA is the policy and paperwork aspect of the QMS. If followed it will, in theory, allow the lab to demonstrate competence and generate defensible reliable data. To ensure the quality system is intact, a third party should be available to check results and QA paperwork. This is another example of a process in place to assure the quality system is in place and

followed. Depending on the size of the laboratory this is not always possible. In this case, someone who is capable of affecting change and someone who has not performed the work should be chosen to review paperwork and results. QA is not one size fits all and often dependent on the types of products and analysis performed. Thus, critical review of program details on a case-by-case basis may be needed to ensure results are defensible and valid.

An appropriate analogy for a laboratory QA is a bonsai tree (yes, a bonsai tree)! A bonsai requires constant cultivation techniques like pruning, root reduction, defoliation, and grafting to produce the characteristic small trees. If the bonsai is ignored, you end up with a planter full of sticks, or a full-size tree. If the laboratory QA is ignored, a breakdown in quality systems will occur. This will ultimately lead to data that are neither reliable nor defensible. This, like a dead tree, is not the desired outcome!

QC

QC, the other side of the QMS, is primarily aimed at the documentation of specific actions performed to ensure quality and the prevention of errors that could affect the analysis or results a laboratory provides. Where QA is an implementation process that may be very similar in labs that perform similar functions, QC is very specific to the laboratory. Documentation and procedures may vary, however, QC should act as a safety net to ensure the QMS is in place and reliable data are generated. Yet, analysts are human and inevitably errors occur; however, if a lab has functional QC, errors will be detected and allow an avenue for process improvement.

Root Cause Analysis: Laboratory Detective

As with any good investigation, the lab will try to discover the root cause for an error. This will involve an investigation, or root cause analysis, into what happened, how and where did the error occur, why did it happen, and who was involved? Once these questions are answered, corrective action can be taken and steps can be implemented to prevent the same mistake from being repeated. Sometimes finding the root cause itself can be a challenge, which is usually reflected by repeated issues of the same type. The investigation for the correct root cause will continue until the appropriate corrective action can be taken. An important element of this process is the cooperation of all staff. If this process is conducted in an accusatory manner, it may not provide the desired results. If this process is conducted with a spirit of continuous improvement overall quality system, the lab can work in cooperation to improve. If the lab understands the error may have occurred at the hand of one or a few, but the ultimate responsibility lies with the plan, the individual blame and associated morale issues may be mitigated.

Documentation: The Key to Quality

QC, as with all aspects of the laboratory quality system, hinges on adequate documentation. Documentation in the form of written logs or documentation via a laboratory information management system must be in use and able to be retrieved. To achieve traceability of managed or controlled documents, QC logs for media preparation should be maintained and must include several pieces of information, including date made, name of media, lot number, expiration date, amount of media weighed, and volume prepared. Several areas are integral for proper QC. The first is maintaining proper chain of custody throughout the evaluation process. Once samples are obtained, a laboratory code for identification must be assigned. It is a critical step that ensures the results from the analysis of a sample actually are from that sample. The identification number should follow the sample throughout its time in the laboratory from the front door to the release of the result. Another area of critical QC is purchasing, receiving, and storing consumable materials (i.e., disposables, media, and reagents). The laboratory should maintain a QC log that includes item name, manufacturer, lot number, amount, date received, and expiration date. The person receiving or opening the consumable must also initial the physical container. This ensures materials used in analysis are in date and any error may not be attributed to reagents, consumables, or any materials used in the analysis.

Calibrations

It is also expected that a food testing laboratory will have written documentation of calibrations and verifications for reagent preparation, which must be accessible for laboratory evaluation. There are also several areas of QC that will have a critical impact on analysis and defensibility. These general areas of documentation are water quality, media and reagent sterilization, calibration (thermometer, balances, pH meter, etc.), productivity and process controls, and all quality-dependent actions as listed in the quality manual. The concept of calibration of staff via training and proficiency may sound abstract; however, staff training and monitoring are designed to achieve just that. Each analyst shall perform the same method the same way, culminating in the same result, at least in theory. Most of these activities are fairly intuitive for the seasoned analyst; however, controls are often a source of consternation and debate regardless of the assay.

Analytical QC samples and control charts are another tool in the QC toolbox that have a critical impact on analysis and defensibility. This method of statistical control, traceable to the analyst level, is often common practice in both chemical and microbiological food analysis laboratories. It is a continual check of analyst precision and critical component of many laboratory accreditation programs. Precision is often utilized to determine measurement uncertainty (MU). Though there are courses and manuscripts devoted to this principle alone, briefly, the estimation of uncertainty based on reproducibility estimates and control charting is often implemented in laboratories as opposed to complex statistical calculations. Use of suitable control samples or secondary reference materials to measure reproducibility and repeatability allow laboratories to determine MU, which accredited laboratories, under ISO/IEC17025 (section 5.4.6.3), are required to determine and make available to clients and auditors when requested.

Controls for Productivity of Media and the Process

Including positive and negative controls with samples for analysis is a common practice and an established example of GLP. When interpreting sample results, the analyst or supervisor uses control results to confirm the validity of positive or negative findings. For example, in microbiological analysis, a positive control confirms that the target bacteria the media supports were able to grow; bacteria that were not desired to grow were in fact inhibited and that the uninoculated media, buffers, or water used during the analysis were sterile. These control results support the assumptions that any bacteria found must have originated from the inoculated environmental sample or any lack of bacteria found was not due to sampling or lab error.

Productivity controls serve to verify the media is capable of performing its function. For example, does a Gram-negative media support growth of a Gram-negative organism? If the answer is yes, the media is productive. The inverse of this is also important to verify. Will organisms that are expected to be inhibited fail to grow? Productivity controls determine if the media is made correctly and if it is suitable for use. It is optimal for media productivity to be confirmed before release for use by analysts.

Process controls determine if specific environmental conditions exist at the appropriate intervals to support growth of the target organism(s) while inhibiting competing or background flora. These controls are prepared at the time of testing and ideally should represent the levels expected in the sample for analysis. Continuing to use the example of microbiological analysis, if an analysis target is detection of <100 colony forming units (CFU), controls of 10 000 CFU may not be adequate to ensure a sample containing fewer organisms had the necessary components for growth and proliferation. The intent of a positive control is to produce a positive response when actual conditions or levels of samples are replicated.

Particularly in a laboratory with a chemistry focus, certified reference materials are an integral part of laboratory QA/QC. The use of certified or standardized reference materials (both in solution and matrix) is important in establishing accuracy in chemical analysis, internally validating a method for the laboratory, and provide a tool to determine traceability and MU. Organizations such as the National Institute of Standards and Technology and the National Research Council, Canada provide standard reference materials, standard reference data, and sometimes proficiency evaluation materials, so labs can determine the efficacy of their QA programs and assist a customer in establishing traceability of measurement results. This is a critical component of any quality system, continual commitment to the culture of quality and data defensibility.

QC is typically the element of laboratory quality analysts are most familiar with, and often times these actions are performed by rote. An understanding of the importance of each control and what it is aimed at preventing is a significant step in minimizing errors and creating a culture of quality in the food testing laboratory.

Future

The future of laboratory quality is greater transparency, oversight, and standardization. Third party audits have become an accepted and common occurrence by the food industry in an effort to improve food safety, and the laboratory is no exception. An independent laboratory must trust that the sample provided by the customer was gathered properly. Other than offering guidance and rejection criteria, there is little that the lab can control before sample receipt. There are two elements that laboratories have entirely within their control that impact the reliability of their data that have in the past been left up to the individual laboratory to oversee.

1. How the sample is prepared: Has the laboratory optimized preparation for efficient delivery of the target for detection?
2. How testing was conducted: Were the methods fit for purpose and performed appropriately?

The concept of 'fit for purpose' validation is important in terms of making sure a test method is applied according to the manner in which it was validated. In the past Association of Official Analytical Chemists (AOAC, now AOAC International) would validate a method based on Official Methods of Analysis and a method was used. This process has undergone standardization and is now streamlined in what is known as Standard Methods Performance Requirements. This process was extensive and involved a multiple laboratory validation protocol. The FDA's Bacteriological Analytical Manual and FSIS's Microbiology Laboratory Guidebook both list validated microbiological methods for food matrix analysis, including sample preparation, isolation, and identification of the major foodborne pathogenic microorganisms and their toxins. Recently method validations have become more varied, and many commodity producers have self-governed the validation process in which new detection methods are determined to be fit for purpose. For this reason, technical evaluations have become an even more crucial part of a program evaluation or audit.

Audit Changes and Auditor Qualifications

The changes in validation and selection of methods may necessitate a shift in how audits are conducted. In the future, reviewing protocol and practice along with a critical review of key areas that could affect outcome of the test may be crucial to ensuring reliable data. A focus on technical audits will highlight the importance of the auditor and his/her qualifications. The evaluator may be required to not only observe the method as performed per the SOP but also s/he may be called upon to make determinations about method selection and analytical proficiency. The level of sophistication of analysis and the commitment to QA may also require the training needs of bench-level technician to increase. Both auditor and technician will have to understand not only how to perform the method but also the reasoning behind the SOP.

The Global Food Supply and Changes to Quality Systems

As we move toward a more global food supply, greater importance is placed on accreditation program standardization and method validation. When there is a worldwide call for an

ISO or ISO equivalent auditing system, two questions come to mind.

1. Even with increased transparency in lab methodology and auditing procedures, how do we guarantee international harmonization for our food/raw materials that are imported?
2. Who decides what is ISO equivalent?

The truth is, at this point, the answer is not clear cut. FSMA has stressed the importance of harmonization for testing, especially for imports. However in 2000, the Global Food Safety Initiative (GFSI) was launched at the retail level on an international scope. GFSI has recognized several schemes, based on the food safety standards of the Codex Alimentarius for the purpose of common requirements universally implemented in audits of food establishments. This is a global endeavor, but of the accepted programs, British Retail Consortium (BRC) and Safe Quality Food (SQF) have been widely adopted in the US. The concept of food product testing being a component of the manufacturing process is one that has been embraced by several large retailers. As SQF explains, one link in the food chain does not get rattled without it affecting the next link. The need for standardization is ever increasing; it is imperative to utilize properly validated systems that employ continuous monitoring.

Balancing Act

The widespread acceptance of BRC and SQF is just one example of industry changes affecting laboratory testing. There is also a concerted effort to take successful manufacturing principles and implement the ideals in the laboratory. It is naïve to think that a laboratory is not a business, clearly it is and pressures to drive profits can lead to compromised quality systems. There will inevitably be laboratories that make profit-driven decisions, potentially compromising the quality system. The concept and practice of continuous quality system monitoring may identify areas of system weakness. This benefits the industry as a whole, both the testing laboratory and the industry contracting the analysis for their product.

So How Does This Affect Laboratory Quality?

Changes at the retail level affect the laboratory. In Edition 7 of the SQF Code, dated 1 July 2012, it states: "Where external laboratories are utilized to conduct input or product analysis, the laboratories shall be accredited to ISO 17025 or an equivalent national standard."

As an 'equivalent national standard' is unclear at best, the quality system in laboratories of the future must be founded on core quality principles but flexible enough to adapt to changing demands. The QA must be living documents, capable of rising to the challenge of changing technology, tighter budgets, and transparent global standards. The evolution of GLP and QA will continue to grow and change to meet the needs of an ever changing global food supply. These changes have required both regulatory bodies and private industries to strive for a standardized and prescriptive metric, with greater transparency and oversight. As we move down a path toward a culture of quality and reliable data production, our food supply will be safer and public health will be protected.

See also: Food Safety Assurance Systems: Food Safety and Quality Management Systems

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Investigation of Incidents in Industry

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Glossary

'At risk' period The period of time during which a batch or batches of food are suspected of being affected by a failure in manufacturing.
Consumer complaint system A system that records information related to consumers complaints.
Crisis An incident that is out of control.
Direct cause The cause that directly resulted in the incident occurring, e.g. failure of a sterilization process due to faulty steam supply.
Disease surveillance The monitoring of foodborne disease cases to establish patterns of progression of disease.
Hazard A biological, chemical or physical agent in, or condition of, food with the potential to cause an adverse health effect.
Incident A 'loss of control' situation, where a company accidentally produced or distributed product that may endanger consumer's health or the Brand's or company's reputation.
Incident investigation The investigation of an incident to identify the direct and underlying causes with the purpose of identifying corrective actions to prevent recurrence.
Risk A function of the probability of an adverse health effect and the severity of that effect, consequential to a hazard(s) in food.
Underlying cause The basic cause that if corrected, would prevent the incident occurring, e.g. underlying cause of steam failure in sterilization may be due to inadequate equipment maintenance linked to poor training of personnel.

Introduction

Foodborne disease continues to be a major concern for public health worldwide. Food businesses are responsible for the safety of food that they manufacture, source, pack, transport, or market, and it is a fundamental principle that those businesses must not place unsafe food on the market. Nevertheless, incidents involving food manufacturers continue to contribute to foodborne disease to varying degrees, with some of these involving large volumes of food, affecting large numbers of exposed consumers. The continuing occurrence of these incidents is disconcerting given the efforts made to improve food safety over the past 40 years or so, where this period has seen the introduction of food safety standards, practices, procedures, and food safety management systems, such as the hazard analysis and critical control point (HACCP) system that are designed to reduce the likelihood of these events occurring. Moreover, many of the incidents are recurring events caused by the same or similar failures where it would be expected that lessons would already have been learned. These failures are often associated with poor implementation of 'prerequisites' such as cleaning and disinfection, and good manufacturing practices and poor hazard analysis or in general implementation of the HACCP system.

The predominant cause of incidents in many regions continues to be presence of biologic hazards with the majority of these incidents being caused by microbial agents. This article focuses on the principles to be used in the investigation of incidents with the primary purpose of identifying underlying causes that, if addressed appropriately, should prevent recurrence. It does not provide detail of the different methods that should be used for analysis of different pathogens/toxins as these are being developed on a continuing basis and they are too numerous to cover here.

The other potential causes of incidents include presence of allergens, chemical contaminants, naturally occurring toxins, foreign bodies, or other physical hazards such as glass or metals, incorrect labeling, and other regulatory noncompliance issues. These are not considered in this article, although many of the principles described below will be relevant for these other causes. For further information on this aspect, the reader is referred to Motarjemi and Wallace (2013).

Impact of Incidents

The impact of these incidents can be significant, both from a public health perspective and also from an economic point of view, and a number of these are identified in Table 1. The scale of these various costs varies according to the nature of the incident, the amount of product affected, the amount of product released to the market, the reaction to the incident from consumers, retailers, and the media. In recent years, due to large scale manufacturing and distribution of products, there have been a number of incidents affecting large numbers of individuals spread over wide geographic regions. In some

Table 1 Company costs associated with incidents

Internal costs	*External costs*
Scrap/waste and safe disposal	Loss of sales
Reworking of product	Complaint adjustment
Retesting of product and manufacturing environment	Recovery/return of affected product
Failure analysis	Warranty charges
Downtime in manufacturing	Liability costs
Yield losses	Loss of contracts/shelf space
Reconfigure/redesign	Loss of reputation
Recommissioning	Loss of brands
Verification	Loss of market share
	Loss of business

cases, the damage caused by the incident has resulted in prosecution and in extreme cases, bankruptcy for the business affected, and there is often a knock-on effect for businesses marketing similar products. For example, an outbreak of *Escherichia coli* O157:H7-related foodborne disease attributed to contaminated spinach in the US in 2006 resulted in losses of US$12 million worth of farm sales of spinach and a retail loss of over US$63 million. In outbreaks where the vehicle responsible has not been properly identified, losses can be equally serious for foods that may only be suspected, erroneously, as a cause. For an outbreak of salmonellosis initially linked to fresh tomatoes in the US in 2008, tomato prices decreased and the farm-level loss was estimated to be US$25 million and retail loss was US$89 million. It was not until later that jalapeño peppers were identified as the true source of the outbreak. The figures estimated for public health costs will depend on the severity of illness caused and the health care required for the individuals affected. On average, estimated costs in the US are approximately US$1000 per case of illness. Some microorganisms cause serious life-threatening disease, whereas others may cause self-limiting illness that may only last for a short period. In some cases, incidents are caused by microorganisms that do not cause illness or disease but will cause deterioration in product quality and these can also have a significant impact, particularly on brand image and reputation.

The Incident Process

The first step in the incident process is receipt of information alerting the company or an external body (e.g., public health agency) that substandard product has been released to the market. These indications or signals can vary in nature and reach the company affected through different routes, from internal sources (e.g., quality records) or external sources, and it is important that companies have effective ways of capturing this information and that they have systems in place to react quickly.

The routes include:

- Consumer complaints – logged, trend analysis, assessment of changes;
- Information from sales force – sales representatives collect samples in case of suspected product defects;
- Complaints from the trade or other professional customers;
- Information about other manufacturer's problems – this requires an assessment of relevant overlap/structural similarity in the supply chain;
- Media – media monitoring and a triggering system for relevant products, brands, issues, and special consumer groups;
- Internal quality records;
- Trend analysis and reporting of noncompliances within the company, for example, using an internal system where manufacturing operations report noncompliances to those responsible for quality and safety; and
- Notification by authorities and rapid alert systems – early warning may depend on establishing and maintaining good working relationships and proper internal communication.

Preparedness Plans

It is critical that food businesses have systems in place that monitor for the signals associated with incidents and trigger initiation of the incident management and investigation processes, which should be regarded as two separate, but interdependent activities. The key components of incident management or preparedness plans include

- An assessment of vulnerability to incidents;
- Details of roles and responsibilities of personnel who will be involved in the response to the incident;
- Documented incident investigation and management procedures, including product withdrawal/recall procedures;
- Effective surveillance systems that are closely monitored and raise alerts as soon as out-of-normal patterns are evident;
- Traceability system for suppliers and customers;
- Capacity and capability (internal or external) to verify and investigate incidents; and
- Links to relevant regulatory and public health agencies and other bodies that are involved in the management of incidents.

An incident management team should include senior managers with a detailed knowledge of various functions within the food business, including safety/quality, sales, marketing/public relations, buying, production, distribution/logistics, regulatory affairs/legal, and consumer services. If in-house expertise is not available, it is essential that external bodies/organizations are identified that have the relevant skills, experience, and competencies necessary.

Supporting Systems

Two important aspects of incident investigation are rapid access to information/data and recording actions taken. These require supporting systems that are ideally integrated using computer systems and networks allowing easy and rapid access. The type of information that is often required in the early stages includes traceability records, stock inventory, process and quality assurance (QA) and quality control (QC) records.

It is essential that all the necessary information is available to allow detailed interrogation of these data. If such systems are not in place, the same information should be made available through alternative means, such as paper records.

Indicators of Incidents

Consumer Complaints

Collection, monitoring, and analysis of consumer complaints is an important activity for identifying problems associated with products. Consumer complaints are one of the primary sources of information indicating that an incident may be taking (or has taken) place. With microbiological hazards, the obvious signs of microbiological contamination may be noticed by consumers before or upon opening the affected packs. These signs include visible damage to the pack itself, presence of off-odors, and gas production (e.g., swelling of packs or containers), deterioration in product structure, visible growth of microorganisms (e.g., molds) on the food surface, and presence of off-flavors/tastes. In some cases, there may be no visible sign or noticeable sign of contamination, and in the case of some ready-to-eat foods, these may be consumed directly from the container where the visual cues are not perceptible before consumption. Contamination with pathogenic microorganisms, if present at sufficient levels, can often result in acute illness, either as a result of infection, toxicoinfection, or intoxication. In the case of intoxication, toxins from commonly reported pathogens such as *Staphylococcus aureus* and *Bacillus cereus* strains producing emetic toxin will cause illness in a matter of hours, whereas for infectious agents, the time to onset of symptoms may be matter of a few days to many weeks. All of this information is important for the investigation of the incident, particularly for aiding in the identification of the microorganism(s) associated with the incident. It is therefore essential that there is an effective consumer complaints system in place to capture and record this type of information.

Consumer Complaint System

The consumer complaint system should incorporate a number of features that include:

- Ability to search across different fields, such as name of complainant, nature of complaint, geographic region, time/date of the complaint, action taken in response to the complaint, and the product(s) and code, if available, associated with the complaint;
- A monitoring process that allows trending of data and assessment in a timely manner;
- An alert system to rapidly identify out-of-norm situations, for example, multiple complaints, including those of a different nature, from one food type;
- Integration with consumer complaint systems in other regions where affected products may have been distributed.

Poorly worded questionnaires or untrained personnel recording information may lead to problems going unrecognized or being misdiagnosed. The staff who are involved in consumer complaint systems, such as customer care lines, should be trained such that they are able to gather all the relevant information and are able to ensure they have the relevant contact details in case it is necessary to gather more details at a later date.

Consumer Complaint Data

To ensure that the information gathering process is effective, it is advisable to use a template that can be used by the complaint handler, prompting for the relevant information. The type of information collected is as follows:

- Contact details of the complainant;
- Nature of the complaint, including visible/sensory signs of contamination or pack damage and if any illness occurred;
- If illness occurred, the predominant symptoms and when these occurred (time and date), number of people ill, their symptoms, and others who may have been exposed;
- Details of physician or other medical personnel, if consulted and details of tests and test results, if carried out;
- Details of suspected food product consumed, including pack date code, reasons why particular food is suspected, unusual signs upon opening the pack, how the food was prepared and handled before consumption, unusual taste/flavor and how much food was consumed by each person exposed;
- Whereabouts of remaining product/container and same products purchased at the same time as the implicated product;
- Details of other foods consumed within the previous 72 h.

Complaints from the Trade or Other Professional Customers

Occasionally, defects in packs or packaging are detected during distribution, for example, when products are repacked into secondary packaging or when packs are stored in retail, on the shelf. Pack defects can include swollen or 'blowing' packs or blown packs, where the pressure of gas formation leads to the packs releasing their contents. Where pin holes are the cause of microbiological contamination postprocess, these are often difficult to detect.

Information about Other Manufacturers' Problems

It is important to monitor incidents that may be occurring with similar products on the market or with products that may be using common raw materials/ingredients. Trade associations and regulatory agencies can provide valuable information in this respect, where commonly used materials are identified and then companies potentially sourcing these same materials are alerted to the problem. It can take some time for the implicated material(s) to be identified and it may also be the case that different materials processed on the same manufacturing lines will be affected. Only through detailed investigation will these other materials be identified.

Notification by Authorities and Rapid Alert Systems

Disease Surveillance

Methods for investigation and data collection related to cases of foodborne illness vary from country to country. In some

countries/regions, methods are relatively sophisticated with coordination being carried out at a national level, whereas in many other countries, collection of data and reporting of illness are more limited and often nonexistent. The tools with which public health professionals can identify and track foodborne disease outbreaks have been improved dramatically in recent years but only a few countries are able to take advantage of these developments. The development of 'fingerprinting' (typing) methods and national and international databases can allow cases of illness to be linked and the vehicle of infection to be identified much more easily than has been possible in the past, over wide geographic areas. These surveillance activities rely on good reporting systems, central coordination of data collection and analysis, and good communication between local and national authorities.

Outbreaks of foodborne illness in developed countries are usually followed up by local authorities. In these investigations, basic clinical and demographic details are obtained, together with food histories and other exposure details (e.g., foreign travel and exposure to recreational water) from each case. In some cases, analysis of clinical samples provides additional microbiological information on the causative agent. Much of the data collected is related to bacterial foodborne infections/intoxications, where isolation/characterization/typing methods are more readily available, compared with viral and parasite infections. It is not unusual for infectious disease information to be collected separately from toxinogenic-related illness data. Requirements for reporting/recording illness related to bacterial pathogens differ from country to country.

In case–control studies, cases are interviewed to develop a hypothesis and then cases and controls are interviewed using a tailored questionnaire. Differences between the case and control groups are used to identify the cause of the outbreak. Cohort studies are used to investigate illnesses among people who attended a particular event and everyone involved is asked about what they ate to see if links can be made between a food and the illness.

Very often, clinical samples taken from cases of infectious intestinal disease are negative for pathogens. Sentinel surveillance programs use more general information from general practitioners/physicians, which record the total number of patients presenting with intestinal disease symptoms. Syndrome (such as bloody diarrhea or vomiting) surveillance is another method that can be used for investigation of foodborne illness where organisms are not recovered.

Sporadic cases of illness are much less frequently investigated. The follow-up of sporadic cases of foodborne illness is a form of active surveillance that extends information gathered through passive surveillance systems. Data are gathered from postal questionnaires, personal interviews, or telephone questionnaires. These additional data-gathering exercises could help determine modes of transmission, risk factors associated with the disease, and detect outbreaks.

Laboratory surveillance is actually one of the key methods for the detection of isolated cases of foodborne disease being part of the same cluster, that is, related to the same source. Such a system is particularly important in detecting outbreaks related to industrially produced foods. This was the method that led to the detection of several major multistate or international outbreaks (Borgdorff and Motarjemi, 1997).

Surveillance of Foods

Public health authorities occasionally carry out microbiological surveys of foods, looking for the presence of particular pathogens. These surveys provide a 'snapshot' of the situation with the particular foods targeted. Some public health authorities use the information collected in these surveys to identify particular foods that may pose a greater risk to human health. In rare cases, these surveillance studies have resulted in incidents being identified, where presence of pathogens at harmful levels is reported.

Rapid Alert Systems

In some regions, rapid alert systems can be an important initial vehicle for identifying an incident. For example, the Rapid Alert System for Foods and Food Products in Europe provides information on incidents reported in different member states. For incidents that involve more than one country, the International Food Safety Authorities Network coordinated by the WHO and Food and Agriculture Organization aims to promote the rapid exchange of information during a food safety-related event.

Incident Investigation

The main purposes of an incident investigation are to define the problem and then decide what actions are needed in relation to the affected product in the market, or in distribution and what action is needed for the company to resume manufacture. The company contact should obtain information before any further data are generated. It is important to know what actions have been taken so far, and the basis of these actions should also be known. It is also important to focus on the facts and not to jump to conclusions at an early stage.

In most countries, there are procedures that must be followed in the event of incidents and these will often involve notification to relevant authorities as early as possible, even if this is only a potential incident. The company must be prepared to share all relevant information in an open and timely manner. Where these hazards have the potential to cause serious harm to consumers, the affected products should be withdrawn from sale and where necessary, recalled from the homes of consumers as quickly as possible.

For microbiological incidents, key information is required to estimate the risk to consumers. If there is an immediate threat to consumer safety, action should be taken immediately to minimize the risk to consumers. The information required to estimate risk includes the nature of pathogens/toxins involved, the rate or frequency of contamination, the subpopulations exposed to the hazards, vulnerability of these subpopulations, the quantity and distribution of the particular food affected, other foods that may be affected (e.g., produced in the same factory as the implicated food), food preparation instructions, and thus potential for consumer illness. Incident investigations generally provide a much clearer picture of the scale of a problem and can also point to likely or possible causes. For example, initial information concerning swollen packs will provide data on the rate of contamination from visual examination of packs but does not necessarily provide a true account of this. If packs are contaminated with different

types of microorganisms or if packs are exposed to different storage conditions, then some packs may appear to be unaffected. The actual rate of contamination (e.g., % packs contaminated) in these circumstances can only be determined through more detailed examination of pack contents, which will often involve opening packs and carrying out further tests, including physical measurements, such as pH and microbiological tests. Following such examinations, it is often the case that the frequency of contamination is greater than initially thought and this can have a significant impact on the risk to consumers or to the brand. The type of contaminants can also provide valuable information. If, for example, there are only spore-formers growing in a contaminated product, this may point to a thermal process failure or contamination source inline following application of a sterilization treatment. If there are nonspore-forming microbes growing in a contaminated inline heat-processed product, this is indicative of postprocess contamination and may point to a failure in pack decontamination, a problem during filling or a failure in pack integrity caused by poorly manufactured packs or damage during distribution/storage.

Gathering Initial Company Information

The first step of the incident investigation process is to gather information relating to the affected product for further testing purposes, such as where this is, in what state this is in, how it is stored, is it all under control, and if there is any third party interest in the material, for example, public health authority or independent testing laboratory. Concerning any samples tested so far, it is important to understand how these samples were drawn, how big the samples are, how these were handled, where these samples are stored, how they are stored, what analyses have been carried out, what methods were used to carry out the tests, and by whom these were carried out. This gives an indication of how reliable the initial information may be and how much confidence there may be in this information. For example, there may be potential for contamination and there is also a possibility that storage conditions may impact on the results. It is good practice to store some portion of affected product frozen, for independent analysis. The methods of analysis used should be thoroughly validated for the food tested before use and preferably should be standard methods. On some occasions, particularly when microbiological testing is carried out, testing laboratories will apply rapid alternative methods that may not have been properly validated for the product tested. These rapid methods include polymerase chain reaction tests where the gene targets selected may be shared by a wider group of organisms than the intended pathogen. The laboratory carrying out the tests should be applying sound QA principles.

At this stage it is possible that the problem has already been defined (initial hypothesis) by other bodies and if this is the case, then this need to be verified. To do this, it is often necessary to design an action plan. The purpose of the action plan needs to be defined, timings need to be clearly understood, protocols, responsibilities, and methods of interpretation all need to be agreed before any further testing takes place. To reiterate, it is important not to jump to conclusions on the basis of limited information. For example, the cause of swollen packs may be assumed to be microbiological (gas production by contaminating microorganisms) but gas production may also be caused by chemical reaction within the pack, for example, from pack contents coming into contact with defective inner layers of packaging material, from release of dissolved gases in the pack contents, or change of temperature leading to expansion of gas.

In the design of the action plan, the following should be considered:

- Define options,
- Build a decision matrix,
- Agreement on sampling protocol,
- Agreement on analytical protocol(s),
- Agreement on handling protocol (from sample unit to analytical unit),
- Agreement on responsibilities,
- Agreement on timing, and
- Agreement on interim actions for 'at-risk' material.

Analysis of Initial Information

The next step is to analyze existing data and this may include QA or QC data, data from the market place, and data from the supply chain. For QA/QC data, it is important to acquire all available data and then analyze historical and current data relevant to the 'at-risk' period. The analysis should look for trends/abnormalities in the data and it is essential that data analyzed go back far enough to a period of 'normal' performance so that the baseline can be established. It is sometimes the case that a failure in manufacturing has been continuing for a long period of time and that analysis of the data does not show any 'abnormality' simply because the true background or baseline has not been properly established. To analyze these data, a number of tools are available. These tools are commonly used during manufacturing to trigger alerts when processes deviate to ensure that they remain in control. Such tools can also aid in incident investigation to identify when processes develop to 'out-of control' situations and are important for correctly identifying the 'at-risk' period and product manufactured during this period. These are commonly referred to as Statistical Process Control tools and include histograms, check sheets, Pareto charts, cause and effect diagrams, defect concentration diagrams, scatter diagrams, Shewart charts, and Cusum analysis. Cusum is particularly useful for identifying small but significant shifts in data, such as pH of a product (where pH is a critical control point), where cumulative sums of deviations of sample values from a target value are estimated.

Data from the market place includes complaints, rejects from retail or distribution, storage controls, information from sorting, resorting, and repalletization activities, customer/distribution patterns, and seasonality. Data from the supply chain includes obtaining an 'incident log' for manufacturing over the 'at-risk' period and previous times, checking line performance data (e.g., line efficiency, raw material changes, rework activities, and rejection rates) and preparing a log of corrective actions for the same period. Important information

that is not formally recorded and that can help identify unusual occurrences during production can be gleaned by interviewing operators and other plant personnel.

All of these data should then be assembled. At this stage, it is relevant to consider the following:

1. If the data are consistent with the initial hypothesis;
2. Whether the data are consistent with each other;
3. Whether an 'at-risk' period can be defined; and
4. If corrective action be specified for any retained material and for future production.

Sampling Protocol for New Data

For new data that will be generated, it is necessary to consider lot integrity, sample types, sample handling, and sample coding. For lot integrity, this refers to the relationship between lot, batch, and coding; understanding how to interpret all codes; and deciding how lot integrity will be maintained. For sample type, this considers random versus nonrandom samples, use of multiple level samples, the role and source of controls, and commercial impact of the sampling protocol. For sample handling, speed and cost of investigation and storage and handling are key considerations.

Analysis

New data can include sensory data, results from simple physical measurements and specialist testing that is targeted. Sensory information covers all observations using sight, odor, touch, and (on occasion) sound, for example, tap tone for canned foods. Taste is rarely an option where microbiological contamination or chemical contamination is suspected. For sensory analysis, all investigators must agree a common sensory vocabulary and (where appropriate) the investigator should be trained. For simple physical testing, such testing can be carried out first, for example, weight, color, pressure, pH, viscosity, and microscopy. The investigation procedure should seek interpretation of data at this stage and check if the working hypothesis is still relevant. In addition, the relevance of the proposed analytical method(s) should also be checked and the results obtained so far are reasonable. The working hypothesis is formed using available information, such as the nature of the defect, nature of complaints (if any), nature of symptoms reported, and so on. As mentioned in Section Incident Investigation, it is important not to jump to conclusions too quickly where a potential cause of a problem may get overlooked. For example, some symptoms of illness such as vomiting may be caused by chemical hazards, such as phytohemagglutinins present in uncooked kidney beans, although presence of microbiological hazards such as staphylococcal enterotoxins may be initially suspected. There are a number of sources available describing typical symptoms associated with both chemical and microbiological hazards (see Further Reading).

Where specialist analysis is required, the following are key considerations:

- Identify analyses that require specialist intervention
- Where can the analyses be done?
  - Competence of the testing facility,
  - Facilities,
  - Trust, and
  - Security

  Is there knowledge to interpret the data?

For targeted testing, the following considerations are important:

- Choose analyses to test the hypothesis;
- It is rarely efficient to start with a 'scatter gun' approach to the investigation;
- If a hypothesis cannot be formed then either there is a lack of expertise or more time needs to be spent on understanding before further analysis should be undertaken;
- The limitations of the analysis should be understood.

For recording new data, there should be an agreement on how records/results will be maintained, who is responsible for recording this information, how and where all material under analytical investigation will be retained, and rules for disposal of material under analytical investigation.

For interpretation, it is critical to ensure that the implications of the result to the analytic unit are first understood, and then move on to putting this into the context of the issue, for example, implications of finding a contaminant. The analyst is often not the person to put the finding into context and interpretation involves intimate understanding of the sample (physical and mathematical characteristics). Controls are paramount.

Other Outputs

Other outputs that can provide useful additional information for an incident include QA/QC and supply chain data and information from external agencies, such as regulatory bodies and public health authorities. The proper role of QA/QC (if any) needs to be defined and should consider its effectiveness, use for trend analysis or lot definition, and cost effectiveness. For supply chain–related information, the supply chain manager should be supported to design the most cost-effective and safe management system – never assume that this involves offline analysis.

Regulatory agencies can be a valuable resource and source of expert knowledge and skills. For regulatory-related information, this may include knowledge of incident impact on consumers, specialist sources of information or expertise, or specialist analytical capabilities (e.g., reference laboratories). Importantly, regulatory bodies in different countries may have a particular view on risk posed by hazards in food products, where a hazard-based approach is taken rather than a risk-based approach, for example, presence of mold or bacterial spores in dry products.

Actions to be Taken

It is important that the appropriate action is taken to reduce any risk that is posed by product in the market. These actions include public recall, which is normally the default action taken for consumer safety issues, but is also an option for significant quality issues. Alternatively, silent or trade recalls may be appropriate where relatively minor quality noncompliances occur. In some cases, no further action may be

required, for example, where there are minute deviations, or very small quantities involved (e.g., at the end of shelf life) or where there are no consumer complaints.

Communication with the Consumer and Other Stakeholders

Communication with the media and public must be carried out in an effective manner and must be well managed. Risk communication is one of the key elements of risk analysis, together with risk assessment and management. This needs to consider perceived risks and real risks. Any communications must be properly prepared and the company should stand by any statements made. It is often useful to anticipate the reaction from consumers and prepare questions and answers. Any communications must include a clear description of:

- the action being taken (e.g., product recall);
- the product(s) affected;
- what the problem is;
- what the consumer should do, for example, check dates on packs of affected product and instructions to return packs;
- information related to other products not affected;
- an apology;
- further information, such as a toll-free careline phone number.

Questions and answers should anticipate on a broad variety of questions coming from different stakeholders. These stakeholders include consumers (and their organizations), trade partners, authorities, the media, and the workforce of the company.

Identification of Direct and Underlying (Root) Causes

The direct causes of the incident may already be identified in the early stages of an incident or may become apparent during the investigation. The direct causes can be categorized according to where the failures occur, such as raw material supply (supplier), product/process design, manufacturing, or distribution/storage. In some cases, the direct cause may not be immediately obvious or clear and it may be necessary to gather more information, for example, through interviews of personnel who may have knowledge of what occurred, and whether anything changed to cause the incident. For processed food products, the primary causes tend to occur during manufacturing, raw material supply and, to a lesser extent, in product/process design. The direct causes can be subdivided into substandard acts or substandard conditions, and these are often attributed to human error. Substandard acts include failure to follow procedures, policies, or good practice; failure to identify hazards and risks; and failure to check/monitor. Substandard conditions include defective or poorly maintained equipment, defective materials, inadequate instructions or procedures, inadequate information or data, inadequate preparation or planning, inadequate support or assistance, and inadequate communication or systems/processes.

The major underlying or root causes for food incidents involving microbiological hazards may be classified according to the following:

1. Quality management system failure;
2. Hygiene management system failure;
3. Plant design standards;
4. Product and packaging design;
5. Cleaning and disinfection; and
6. Raw material supply.

These underlying causes may be linked to lack of knowledge, skill, or experience; inadequate leadership or supervision; inadequate maintenance; or other factors. It is important that all of the underlying causes are properly identified so that effective and appropriate corrective actions can be taken. Tools that can aid in the identification of direct causes and link these to underlying causes include the systematic cause analysis technique, 'fishbone' analyses, fault tree analysis, and failure modes and effect analysis. Although many of these tools were designed to identify underlying causes of engineering failures or occupational safety incidents, their principles can be applied in the context of consumer incident investigations.

See also: Food Safety Assurance Systems: Essentials of Crisis Management; Recall Systems and Disposal of Food; Root Cause Analysis of Incidents. Foodborne Diseases: Foodborne Diseases and Vulnerable Groups; Overview of Biological Hazards and Foodborne Diseases; Overview of Chemical, Physical, and Other Significant Hazards; Overview of Emerging Food Technologies. Public Health Measures: Foodborne Disease Outbreak Investigation; Modern Approach to Food Safety Management: An Overview; Surveillance of Foodborne Diseases. Risk Analysis: Risk Communication; Risk Communication: Biological Hazards

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Relevant Websites

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International Food Safety Authorities Network.

http://ec.europa.eu/food/food/rapidalert/index_en.htm
Rapid Alert System for Foods and Food Products in Europe.

Recall Systems and Disposal of Food

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Background and Scope

As food farming, manufacturing, retailing, and distribution become more mature around the world, consumers increase their knowledge of both of 'what can go wrong' in food products and their awareness of their personal rights. At the same time, the technical ability to detect noncompliance improves. When something does go wrong and a product is defective, it is often necessary to remove the item from sale. These two conditions have led to food recalls becoming more common in many countries in recent years. For example, the European Union Rapid Alert System for Food and Feed, known as RASFF, records and communicates several thousands of cases every year – with an upward trend – and in America both the US Department of Agriculture's Food Safety and Inspection Service (FSIS) and the Food and Drug Administration (FDA) maintain an ongoing summary of current recalls. There are still countries that are far less familiar with recalls, but the practice of informing customers and the general public about safety or legal concerns involving specific products – with the request not to use the product and return it or dispose of it – has spread very rapidly during the past decades.

In this article, the principles and organizational aspects of recall and disposal of food, from the perspective of a food manufacturer, food service provider, or retailer, will be covered, illustrated with some examples where appropriate.

Withdrawals and Recalls

Even with an efficient quality assurance (QA) system, there may be times that a company's products do not meet quality and safety criteria and the company has to take actions, so all QA systems will need to include an effective Incident Management System.

Below, the organization and decision process around recalls and withdrawals will be discussed, but at this stage, it is helpful to clearly distinguish between the two.

- A withdrawal involves the removal of product from that part of the supply chain that is within the company's direct control (definitions may be used differently in different countries, see e.g. the Australian/New Zealand document on recalls: http://www.foodstandards.gov.au/_srcfiles/Food%20Recall_WEB.pdf). That may include in-house storage, own trucks or warehouses, or even (from a retailer's perspective) store shelves. A withdrawal does not necessarily mean that no product has reached customers or consumers, it means just that it is not deemed necessary to inform the general public. A withdrawal may always be sufficient if there is a certainty that all products can be accounted for (nothing out of the company's control, meaning nothing could have reached the customer and cause harm) or when the defect is considered to be minor (small misprint on the label or a situation that might cause a somewhat higher rate of complaints but no more) and a recall is seen as disproportionate. Where there is any danger to public health, a withdrawal can only be justified if the product is entirely within the company's control and is fully accounted for; in other words, it is carried out as efficiently as a recall, with enough resources dedicated by the company to ensure total product removal in a timely period.
- Instead of going public, companies sometimes consider a 'silent recall,' which means that product is recalled from professional customers only (e.g., a manufacturer requests retailers to take products off the shelves but does not inform the general public), but this form has become rarer in recent years. Authorities in many countries need to be informed about product defects, and where there is sufficient cause to withdraw products from shelves, they will normally conclude that there is sufficient cause to inform the public.
- A recall (sometimes known as a 'public recall' or 'consumer recall') is then the most radical step to regain control over a product issue, informing the general public and the competent authorities and providing instructions on how to handle the situation. Typically, recalls are indicated in situations where products (are expected to) give rise to unacceptable risks to users, constitute major legal nonconformities, and/or where a recall is mandated by authorities.
- For products with undeclared allergens, some governments may accept a public information rather than a public recall.

The Elements of an Incident Management System

Structural Elements

Effective handling of a food safety or compliance situation requires systematic preparation. Companies will, therefore,

need to develop a standard procedure, which will typically involve the following elements:

- A company policy may typically state the intention to protect their customers, employees, and the general public against negative impact on their health, safety or security, the environment against pollution, their brands, operations, and the company as a whole against legal liabilities and/or damage to property or reputation, and in order to assure business continuity and limit financial damages where possible. This covers the scope and the order of priorities of the incident management system.
- Organizational principles and responsibilities: Individuals and functions carrying specific responsibilities in an incident situation need to be defined. This normally involves nominating a focused team, often called an Incident Management Team (IMT) or similar, with a small number of core members (for our current purposes that would be QA, Legal Counsel, and Communications) and additional support roles where needed. For companies with multiple sites, the structure at sites or country organizations need to be defined, as well as the relative responsibilities (Head Office may be in the lead, or incidents get managed primarily locally, or the location of the responsibility will depend on the scope and seriousness of the issue.).
- Facilities: the IMT and its members must have adequate meeting and communication facilities. This includes: a meeting room that is/can be made permanently available and accessible also outside office hours, with TV, a telephone with telephone conference capabilities (ideally with a 'mute all' button), and computer network/internet connections (also for social media tracking). Individual members (or their appointed backups) need to be reachable at all times through telephone and e-mail.
- A notification procedure, assuring that the appropriate information is forwarded to the IMT as early as possible. The notification procedure needs to include some simple escalation rules in order to assure the necessary information – and only that – getting notified immediately to the IMT. Escalation rules may include triggers like: two or more complaints of illness relating to the same product/batch code, one very serious complaint (clear danger to health and hospitalization), one case of allergen mislabeling, report of product tampering, etc. Experience indicates that no escalation system works perfectly; there will always be 'false positives' (notifications to the IMT that on balance did not require IMT attention) and 'false negatives' (issues that should have been notified but were not). It is the responsibility of the IMT to monitor and manage the balance on an ongoing basis. Maintaining a low but steady level of false positives is probably where the optimum lies in practice. In other words, it is better to overcommunicate – even in the case of a false positive, advice can be invaluable and trust develops between business units and the IMT. The wise and experienced counsel of a central 'node' of communication can often be used to reduce panic and disseminate best practices in a complex organization.
- Training needs to be organized for all involved, in order to assure an up to date understanding of the internal procedure and its specific requirements for every individual's position. Typically included in training are: (1) awareness for all employees, including their responsibility to alert the IMT (directly of otherwise, as appropriate in the specific company environment) of any situation that has the potential to require a withdrawal or recall, (2) specialized instructions for the 'first line responders,' i.e., company telephone, reception, or customer service personnel, and (3) satellite IMTs (if any).
- Communication within the different divisions of a company is important because a recall decision in one country may occasionally differ from a decision in another. This is often a contrast between developed and developing markets, certainly where traditional trade is involved. Clearly, this will not apply in the case of FDA Class 1 type situations. However, qualitative issues require awareness of local market sensitivity, the local legal environment, and the awareness of what a recall is for.
- Product testing during incidents needs to be very carefully managed. In the context of incidents, testing should exclusively be carried out where it is necessary, conclusive, and will uniquely and decisively enable the company to take and/or – where necessary – explain and defend decisions. The testing methodology used must be recognized as valid (i.e., it actually measures the parameter of interest), sufficiently sensitive (able to detect/quantify the parameter at levels relevant to the issue), and reliable (repeatable, reproducible, and unlikely to give false positives or negatives), and approved testing facilities must be available. Furthermore, the company should be prepared for the full range of potential outcomes of the analysis before having their products tested. Typical pitfalls include:
  - Testing of unrepresentative or uncontrolled samples (testing leftovers from a restaurant may reveal the occurrence of a pathogen but is unlikely to decisively point to a root cause).
  - Testing aimed to clear product that has failed a test before ('you cannot test your way out of a positive'), which may only lead to confusion and delays.
  - Testing in the absence of a realistic concern (e.g., bovine spongiform encephalopathy (BSE) testing of beef material originating from a certified BSE-free country), which only incurs the risk of false positives.
  - Testing without a clear objective or specific purpose, which may end up raising entirely unrelated uncertainties/suspicions. (It is wise to pose the question in advance – 'what will we do with the results?')
  - Testing at nonapproved labs and in the absence of clear confidentiality agreements. In many countries, laboratories are obliged to inform authorities if they find evidence of unsafe or noncompliant product, but it must always be clear what a lab may communicate to others (nonauthority) and what will be subject to confidentiality.
- Communication between manufacturers, food service providers, and retailers: in any recall situation, removing noncompliant product from distribution requires teamwork between the makers and the distributors of the product. It is helpful to have this agreed in advance of

any cooperation; key contacts should be agreed, obligations for communication defined (often contractually), and standard statements and templates prepared in advance. Some retailers, particularly in traditional trade environments in developing countries, may not be completely diligent in removing offending product from their shelves, and it requires active engagement from the manufacturing company to physically remove product, using the sales force on the ground ('putting sales into reverse'). Indeed, anecdotal evidence suggests that with a 'public recall,' the percentage retrieval of noncompliant product is surprisingly low (perhaps 10%?), whereas with an engaged 'reverse sales' team, much closer to 100% of implicated lots maybe removed from sale.

- Incident classification systems are used in two ways: (1) a priori systems, where an incident is classified according to its potential to do harm, and the incident management is based on this classification. This approach may provide firm guidance in specific situations ('if the situation has *xyz* characteristics, a recall will always be done'), but the downside is that classifications may change over time, and some companies are uncomfortable with a priori limiting their options. Many companies, therefore, use (2) an a posteriori system, whereby the incident only gets classified after the active phase is over, on the basis of its characteristics (microbiology related, allergy, etc.) and the impact it has had (actual harm caused, damages, media involvement, authority reaction, etc.). The purpose of the a posteriori system is then to facilitate 'lessons learned' exercises and enable trend analyses over time. The FDA has developed a widely used system, primarily for a priori classification, which then guides the:
  - Depth of recall, i.e., extending to the consumer or user level, the retail level, or the wholesale level.
  - Public warning; local, national, targeted to specific groups, etc.
  - Effectiveness check (an assessment of how effectively the recall was communicated, understood, and actioned by relevant stakeholders).

The above topics all need to be discussed, defined, and communicated – and the relevant staff trained – before and separately from the management of an actual incident in order to give clear guidance and prevent time consuming internal discussions at the most inappropriate moment.

Early Warning

Complaints

It is clear that the earlier a company is informed about a problem, the more it can do to prevent harm and damage, and the better are its chances to limit the scope of a recall or withdrawal. Therefore, the company will want to start with putting an effective customer inquiry and complaint system in place. To these authors' experience, food complaints typically range from less than one per million units sold to a hundred or sometimes more than that, for processed food, depending on the geography (complaint culture) and the type of food (coated nuts typically contain more foreign material objects than refined sugar). Complaints are an invaluable source of early warning that something may be amiss and they need to be recorded and (trend) analyzed very carefully. Being able to pick up unusual complaints and investigate them as soon as they occur may save lives (as these authors have witnessed in a 'may contain' allergen case). Not every complaint needs to lead to a recall or withdrawal, and not every recall is preceded by a complaint, but in a very large number of cases, there are warning signals that can be used to prevent things from escalating. Operating an effective complaint system will in most cases imply having a call service available, with clear instructions on how to recognize and escalate serious or suspicious complaints, covering the appropriate opening hours (which may be somewhat outside normal working hours) and managing the percentage of 'missed calls' to a minimum.

These days, the challenges of social media mean that the definition of a 'complaint' is blurring. In the past, companies could glean information on the 'stock-keeping unit' (SKU), lot code, nature of the issue, etc., directly from the complainant, whereas now a serious issue may be posted in real time via Facebook, YouTube, or Twitter without the opportunity for a producer or retailer to respond appropriately. The potential impact to brand integrity and consumer perception of the company is significant. Nevertheless, any challenge brings with it opportunities: if a company is in doubt on the seriousness or scope of a problem, a search of social media sites is useful in establishing whether consumers are chattering or even aware of the issue.

External Information

Incidents may be homegrown, but they may also be imported. Companies have the possibility to scan a wide range of news media for signs of trouble, which may involve, for example, a source of raw materials getting associated with an issue (e.g., various dioxin contamination cases over the past few years in Europe). Other sources of information could be, for example, a recall being announced by a supplier with whom they do business (or a competitor who sources their product from the same supplier), news about professional partners losing their food safety or quality certificates, or trends communicated by authorities and established laboratories.

Having an early warning system in place, continuously monitoring internal and external signals, is an essential element in an incident management system, which can facilitate a damage limiting early response. Trade organizations such as FoodDrinkEurope (previously known as CIAA, *Confédération des Industries Agro-Alimentaires de l'UE*) in Europe and in the US, the Grocery Manufacturers Association (GMA) can have a very useful role in this.

Traceability

Food law in many countries requires traceability systems to be in place – generally on the 'one step forward, one step back' principle. Where decisions need to be made regarding withdrawal and recall, having exact traceability in place – with all information readily accessible – is a decisive factor in determining the nature and scope of the retrieval action.

For manufacturers, it is important to define upfront for all their raw materials and ingredients as well as for their internal processes, what the appropriate definition of a 'batch' is. Formally, a batch represents a quantity of materials produced under the same conditions, but in practice, things may be quite complex and batches may not always be as homogeneous as expected. Root cause investigations may be facilitated by production time coding, which can then be correlated with other timed events (equipment or power breakdown, incidents on the line, changeover to another batch of ingredients, etc.), but for retrieval purposes, a date code is normally used. When a failure is detected in time, the availability of complete and reliable traceability data may make the difference between a withdrawal and a recall (is all affected product still under our control?) and between recalls in one country or more (do we know exactly where this product was shipped and whether it is already on the market in each of these countries?). Linking internal traceability to root cause elucidation may make the difference between recalling one date code and 'everything on the market.'

Where an EU rapid alert has been issued, the competent authorities may request to be informed about both the whereabouts of the product (sometimes well beyond the on-up-one-down requirement) and the actions taken. Up to three countries may often be involved, including:

1. where the product defect was originally found and communicated,
2. where the product was made, and
3. where the multinational sales company has its legal residence.

Traceability in all these cases then explicitly includes the ability to account for the entire affected volume. Where products have been sold to known individual customers – as is the case with professional wholesalers – the integrity of the traceability system may allow for the above mentioned 'silent recall,' where all customers are informed, but the general public is not. In most cases, however, there is a possibility that the products were sold on to the general public and these cases generally end in doing both: informing the customers directly and informing the general public through the media.

Traceability requirements, however, are not restricted only to materials, products, production conditions, and distribution. Effective retrieval management also requires a 'traceability' of key stakeholders. For the incident management procedure, the company will need names, addresses, contact numbers, and clear agreements on accessibility at all times for:

- Incident management team members and internal support functions.
- All other functions within the company that need to provide information and/or take action.
- External support resources (laboratories, medical, and toxicological expertise).
- Competent authorities (food and health authorities and sometimes also police and emergency services).
- Business partners (suppliers, customers, and logistics providers).
- Media.

Managing Recall, Withdrawal, and Disposal of Food

Communicating and implementing a decision to retrieve product will involve a number of elements:

- What to retrieve – this will involve a very clear and precise description, preferably accompanied by pictures of the product(s) involved: brand, type of product, type of packaging, volume/weight, and date (production or best before). Trade companies typically have a standard form for this purpose, assuring completeness of the information and supporting a standardized process. When communicating with the general public, the emphasis needs to be on how people can recognize the product in question, involving usually front/back of pack pictures. Communication routes include traditional media such as newspapers, radio, and TV, but increasingly, shopper loyalty scheme information together with internet or phone campaigns may be used.
- Why it is being retrieved – the relevant detail here may depend on the audience. Communication with authorities may involve a greater level of technical detail than an internal retrieval order at a retailer or a media release intended for the general public. Specific warnings for target groups (allergic, immunocompromised, etc.) can be part of this communication.
- What people who already have used (eaten) the product should do – this depends entirely on the nature of the issue. Companies should normally not provide medical advice (they are not medical institutions) but refer concerned consumers to available medical services. This may include a brief summary of realistic expectations such as a description of illness symptoms and advice to see a doctor if these occur. Where the situation demands and geographical distribution permits, companies may organize medical support for their customers. Care must be taken to respect privacy and medical confidentiality in these cases.
- What the public should do with the product – when the public is informed, the advice is always not to use the product but to either discard it (if this can be safely and definitively done without harming the environment) or return it to the point of sale or any other facility mentioned, for a full refund. When the advice is to destroy the product and not return it, the mechanism for obtaining the refund should also be made clear.
- What people should do if they have questions – normally, this will involve a telephone service, with a standard list of frequently asked questions and their answers. Experts (e.g., medics) should be available to deal with questions outside the standard list and update/refine the list as needed.
- How the product should be collected and stored. This may be different for withdrawal and recall situations. Withdrawals may be carried out on a purely precautionary basis, with the expectation that subsequent investigation may clear the product and it may be returned into circulation. In those cases, withdrawn product must be separated, marked, registered as 'on hold, not to be distributed' and monitored for integrity on a regular basis. Where retail organizations issue a withdrawal notification internally, they typically require both a confirmation of reception

from their stores and a return notification of the withdrawal having been completed, including quantities. Recalls, however, are normally seen as irreversible – once a product is recalled, and the general public is informed about the issue and the reasons not to use the product, the batch(es) can never get back into circulation. For internal handling purposes, they, therefore, need to be completely separated as quickly as possible, and using off-site storage space that is not used for distribution purposes and is sufficiently guarded is recommended. At the same time, the product still needs to be clearly labeled as 'on hold, not to be distributed' and stock counts should be carried out at regular intervals until final disposition. Over the years, there have been several cases of recalled product that inadvertently got back into circulation, posing a risk to consumers, damaging the brand, a situation deeply embarrassing for all involved.

- How the recalled product should be destroyed. Destruction needs to be:
  - Irreversible – there have been cases where product that went into landfill was dug up and redistributed later. It also means that not only the content but also the packaging material must be put beyond salvage – there have been cases where glass jars and caps, with their labels and prints, have been salvaged from general waste dumps and reused for counterfeit purposes.
  - Complete. Destruction of the entire recalled product needs to be assured and recorded. That may also involve affected raw materials which formally were never part of the recall. Also, in some cases, a recall will mean the commercial end of a product and there may still be unused packaging – sometimes at a supplier's site. In case the packaging material and labels will no longer be used, they also must be destroyed in this context, along with the specific tools or printing matrices used for their production.
  - Safe for the people involved and for the environment.
  - Witnessed and recorded – fraud in these circumstances may be a sudden and relatively large opportunity. Depending on the local level of confidence, a company may want to take very specific measures to assure effective and complete destruction. This applies especially when the product in question remains visually acceptable.
  - Timely. The longer the defective and recalled product is stored, the higher the chance that it will find its way to the market (within the country or elsewhere).
  - In accordance with local authorities' requirements. Authorities in various countries have strict regulations on the destruction of food products, and they may set time limits, require specific methods, and may want to supervise destruction directly.
  - Officially terminated. When all products and materials have been accounted for – or when the recall can reasonably be considered to have achieved its purposes, i.e., to inform all stakeholders, prevent them now and in the future from using this particular batch of product and retrieve and secure (destroy) all products that were originally released – the recall must be formally terminated. Terminating a recall does not involve a notification to the general public, but it does require a close-out notification to those who have been professionally involved (warehouse, logistics, call center, etc.).
  - Evaluated and reported. After a recall, the Incident management team must assess the handling of the situation and lessons learned, including root cause and improvement actions. The evaluation may also typically include a product recovery percentage as demanded by authorities in some countries. Recovery rates for recalls will rarely, if ever, reach 100%. Some products will have been consumed, some consumers will not be aware despite all communication efforts, and some may not be interested. A common cause of recall is allergen-related mislabelling, and nonallergic consumers are likely to simply ignore those recalls, thereby driving down product recovery rates. As mentioned above, those companies that have defined an a posteriori incident classification scheme will conclude their final rating at this stage.

In summary, preparing for withdrawals and recalls is an integral part of any quality management system. A basic purpose of the quality assurance (QA) system is to avoid these situations, but in practice, any organization will at some point in time face the need to make rational, science-based decisions, and act as pragmatically as possible in order to protect the general public and their own reputation. An effective system will not only require preparation, training, and clear structures, roles and responsibilities, but also a company culture that is ethically strong. Companies with robust practices and discipline will be rewarded with the trust and confidence of all stakeholders across the value chain.

See also: Food Safety Assurance Systems: Essentials of Crisis Management. Public Health Measures: Alerts and Early Warning Systems; Modern Approach to Food Safety Management: An Overview

Further Reading

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FOOD SAFETY ASSURANCE SYSTEMS

Tampering

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Introduction

By dictionary definition, tampering could be considered as any act of meddling or interfering with any given item. However, within the food industry, the word has come to have a particular association with extortion, blackmail, and deliberate attempt to damage the safety or palatability of a food product, and/or to undermine the reputation and commercial viability of the food manufacturer.

The act of tampering is generally seen as something done to the finished product, perhaps following legitimate purchase, with the product being returned to the shelves for sale to an unsuspecting victim. That said, there is no reason to dismiss the possibility that someone, such as a disgruntled employee or disaffected supplier, might also tamper with raw ingredients or even the machinery on the production line, in order to create similar problems for the manufacturer.

Such acts should be seen as being different from deliberate adulteration of ingredients, both in scale and motivation. In the case of adulteration, a cheaper ingredient is usually substituted for a more expensive one (e.g., adding rape oil to olive oil). Alternatively, attempts are made to disguise the inferior quality of an ingredient by addition of an illegal and/or undeclared substance (e.g., melamine in milk and Sudan Red I in spices). In neither instance of adulteration is the perpetrator likely to draw attention to his crime. In the case of tampering, the perpetrator will certainly seek or threaten publicity, and may even eschew carrying out the act of tamper in favor of merely threatening or claiming to carry it out. Whether the tamper actually happens or not may be of little consequence if the motive is to create fear and suspicion of a product in the hope of achieving some financial gain.

Of course, for the manufacturer to make the appropriate response, it matters to know whether the tampering has occurred or not, and whether the threat is as great as the perpetrator intends to imply. After all, the perpetrator may threaten something that is not possible in practice, or that may not be especially serious, as will be discussed below.

The Extent of the Problem

It is difficult to obtain exact figures on the extent of the tampering problem within the UK, never mind the whole world. It is undoubtedly one of those crimes where fear of the consequences exceeds the likelihood of an incident. This is understandable given the unpredictable nature of the crime, the entirely random choice of the victim (at least in terms of the ultimate consumer), and the disproportionate extent of the commercial damage that can be caused. It might only need one actual incident of tampering to result in thousands of products having to be recalled.

There are no reliable statistics available to indicate the number of incidents that occur. The Food Standards Agency (FSA) in the UK advises that in the last 5 years (2005–10), there has only been one case where the risk to consumers was assessed to be significant and required action by the Agency at the national level. This was the case of Allied Bakeries receiving five complaints after fragments of glass or sewing needles were reported in Kingsmill sliced bread.

In a press statement issued by Allied Bakeries at the time, the foreign bodies were described as being clearly visible once the bread bag was opened and were not embedded in the bread. Therefore, in agreement with the FSA, the company decided to alert the public and urged consumers to be vigilant. The company also worked with the police to increase security at its Orpington site "in order to minimize the risk of further tampering", clearly indicating that the incidents were viewed as possibly occurring at the factory. Unfortunately, the incidents continued with further alerts being issued in October and December 2006. At the time of writing, no public announcement has been made concerning identification or arrest of the perpetrator(s).

The Office of Criminal Investigation, a division within the US Food and Drug Administration (FDA), was invited to offer their own statistics on the tampering issue but did not do so. However, the office did refer to an arrest in January 2010 of a man who had tampered with the labels of Gatorade, although apparently not with the contents.

Some indication of the scale of the problem might be derived from statistics released by one of the laboratories in the UK that investigates contamination incidents on behalf of food and drink manufacturers. Reading Scientific Services Ltd (RSSL) estimates that in the past few years, approximately ten of its Emergency Response Service (ERS) investigations have arisen due to actual or threat of tampering, out of a total approaching nearly 1000 investigations. If this experience is in anyway typical, it suggests only 1% of product contamination emergencies (foreign body and chemical) are due to tampering. The logical conclusion is that accidental contamination,

factory problems, packaging problems, adulteration, environmental contaminants etc. are much more common.

A review of incidents has indicated how difficult accurate data is to come by, but some companies report hundreds of cases of tampering. Many of these cases go unreported for fear of copycat issues. The majority of these cases are not conceived with the intent to cause harm rather to extort restitution or merely to gain free products from the manufacturers. The review also established that the majority of incidents actually occur at the end of the supply chain, and it is these incidents that have the potential to cause the greatest impact to public health. Of those cases that occurred postdistribution (89 cases over 40 years) 52% were aimed at extortion from either manufacturer or retailer.

Table 1 lists some examples of tamper incidents in different parts of the food chain through recent history. There has been an increasing trend in reporting of incidents. This is thought to be linked to a range of factors including an increase in copycats, technology to track and report such events, and increased recording in China.

Although highly feared and often linked to potential terrorism, only a limited number of cases involve biological agents. This is thought to be partly because of the difficulty in obtaining agents. Generally, perpetrators have ready access to pathogens through work in hospitals or laboratories. The most renowned incident is that of the Rajneesh religious cult tampering of salad bars with *Salmonella*. The majority of the US casualties from biological tampering relate to this instance that was actually thought to be a trial run to see if the group could incapacitate voters.

Radiological contaminants, such as polonium, are also highly feared as potential terrorism agents but are very rare and so far unlinked to true tamper issues, but have been used as an alleged assassination tool (e.g., the Litvenenko case, 2006).

Two Textbook Cases

There are two key cases of tamper from the past 30 years that demonstrate the importance of the issue, and the risks that the food industry must consider seriously.

Hence, the cases affecting Tylenol (not a food product, but relevant nonetheless) and Heinz baby food are offered as examples of deliberate tamper, as well as being cases that changed the way food manufacturers address the issue and try to mitigate the risks.

Tylenol

In 1982, Johnson & Johnson's Tylenol medication, manufactured by its subsidiary, McNeil Consumer Products Co., commanded 35% of the US over-the-counter analgesic market. This represented something like 15% of the company's profits.

During 1982, seven people died after taking Tylenol tablets, which were subsequently found to have been laced with cyanide. The associated publicity is believed to have knocked $1billion off the company's market value, and led to the FDA issuing its tamper-resistant packaging requirements in 1982, and the introduction of the Federal Anti-Tampering Act in 1983 in the USA.

The prime suspect was a certain James William Lewis, an unemployed accountant at the time of the killings, who was sentenced to prison in June 1983 for demanding US$1 million from Johnson & Johnson, "to stop the killing." It transpired that Johnson & Johnson was the former employer of Lewis's wife. However, although Lewis admitted sending the letter demanding money, it must be noted that he has consistently claimed he had nothing to do with the product tampering, and never meant to collect the money.

The incident led to the US FDA introducing the tamper-resistant packing regulations. The Federal Anti-Tampering Act passed in 1983 made it a crime to tamper with any packaged consumer products.

Unfortunately, Johnson & Johnson was targeted in a similar way in 1986. This time the company reacted differently, ordering that Tylenol should be recalled from every outlet and not just those in the state where tampering had been confirmed. The company also decided the product would not be reestablished on the shelves until something had been done to provide better product protection.

As a result, Johnson & Johnson developed new 'tamper proof' packaging for Tylenol, and within 5 months or so, it had recovered 70% of its market share for the drug.

By acting quickly and putting consumer safety ahead of any other consideration, Johnson & Johnson's approach is widely seen as the model for handling all similar product crises.

As a footnote to this case, the Chicago Tribune reported on 05 February 2009 that the Federal Bureau of Investigation (FBI) had reopened the investigation into the seven unsolved murders, and referred to the original investigation as having involved more than 6500 leads, and 400 possible suspects. Almost a year later, in January 2010, Lewis and his wife attended a closed hearing in a Woburn courtroom to determine whether the couple must provide fingerprints and deoxyribonucleic acid (DNA) samples to authorities, presumably to provide evidence that can be analyzed by techniques unavailable in 1982. No further details have thus far been made public, but in May 2011, the FBI is reported to have also sought DNA from so-called 'Unabomber' Theodore Kaczynski as part of this investigation. It could be that the case has a new twist to come.

Heinz Baby Food

The UK's most notorious, and influential, case of tampering was carried out by a former police detective, Rodney Witchelo. His acts included spiking a jar of Heinz baby food with caustic soda, two drawing pins, and a note that threatened more to come. The amount of caustic soda used was sufficient to kill 27 babies, and fortunately, when the purchaser opened the jar (to feed it to her dog), it made her eyes water and alerted her to a serious problem with the product.

Witchelo demanded £3.75 million from Heinz and Pedigree Pet Foods and having demonstrated his ability to tamper products successfully, threatened to flood the shelves with poisoned product.

Table 1 Examples of cases of tamper throughout the food supply chain

Supply chain			
1978, Europe	Oranges	Mercury	Tamper occurred at distribution port, by a Palestinian militant group, tainted citrus fruit showed up in Germany, Spain, Belgium, England, and Morocco
1989, USA	Grapes	Cyanide	A warning call claimed that Chilean fruit had been laced with cyanide, only 2 grapes tested positive. Suspected anti-Chilean government protestors, no injuries but serious economic implications
2006, Australia	Cereals	Glyphosate weedkiller	Spiked in crop spray, visual differences in products, testing carried out, and product recalled before reaching consumers
Retail			
2010, USA	Jell-O pudding	Product replaced with sand and salt	Elderly couple were indicted on multiple counts of petty larceny and tampering, no injuries
2003, USA	Ground beef	Nicotine (pesticide) in ground beef	Supermarket employee tamper, several hospitalizations, no fatalities. Culprit jailed and fined
2003, Italy	Bottled water	Acetone, bleach, or ammonia in bottled water	30 hospitalizations, no culprit identified, suspected anticapitalist activists or eco-terrorists
1984, USA	Girl Scout cookies	Pins, needles, and other foreign objects in boxes of cookies	Resulted in tamper-evident boxes, the FBI concluded the 800 reported incidents were false alarms or copycat cases
1986, UK and USA	Baby food	Glass, razor blades, pins, and caustic soda	An extortion case which resulted in several copycats. Companies involved learned lessons about handling the press relations and regulatory authorities
1976, USA	Foods and pharmaceuticals	Various agents	One culprit part of a larger part of larger campaign designed to cause extortion and terror
1984, Japan	Candy	Cyanide	Complex organized attempt to extort money from the manufacturer, no arrests
Restaurants/food service			
1984, USA	Salad bar	*Salmonella*	Rajneesh incident, up to 751 cases reported
1992, China	Flour	Arsenic	Zhengzhou, 788 cases of arsenic poisoning in a college canteen
1998, Japan	Curry	Arsenic	4 deaths and 63 hospitalized after cook-poisoned food at a summer festival
2002, China	Various hospital foods	Rat poison	Tangshan, 42 deaths and 300 hospitalized in a feud between restaurateurs
2002, China	School food	Arsenic	China, two caterers laced school food injuring 193 in a feud with authorities
2005, Philippines	Cassava fritters	Coumaphos insecticide	Canteen worker laced fritters during cooking to serve to school children, 28 died and 130 injured
2006, Australia	Soup and salad bars at different restaurants	Rat poison	No injuries but the restaurant chain delayed notifying authorities. The case prompted Queensland lawmakers to draft a law requiring that all food establishments report suspected tampering immediately, or face a fine of US$15 000

One characteristic of this case, and one that should worry every food manufacturer, was the number of 'copycat' incidents that it spawned. These included individual consumers chancing their arm in the hope of receiving significant compensation, to other more serious criminals copying the *modus operandum* of Witchelo. RSSL's laboratories were involved in several of these investigations, and noted a very definite peak in foreign body investigations in the period that Witchelo was working.

Sadly, Witchelo's influence continued long after his arrest and conviction in 1990. A letter bomber who tried to blackmail the Tesco supermarket chain, approximately 10 years

later, claimed at his trial that he had been inspired to commit his crimes after reading an account of the police investigation into Witchelo in a magazine. Although his strategy was very different, the basic idea was the same, i.e., to extort money from a well known, profitable, and major business.

His influence may also have extended to the USA. On 27 February 2004, the Office of Criminal Investigations was advised by FDA Emergency Operations of a tampering and extortion complaint received in Cincinnati, OH, USA. Ultimately, a British citizen was convicted of trying to extort $180 000 from a supermarket chain by threatening to place contaminated baby food on store shelves.

Copycats

A direct correlation between the amount of publicity and number of copycat incidents is questionable. Different commentators present different views. However, it is certainly the case that publicity of a specific incident has 'inspired' copycat incidents and prompted false claims from consumers. It is also the case that any such activity merely obscures and confuses the picture concerning the original incident, potentially wasting police time and providing false leads that require investigation.

Prevention and Control Measures

Prevention measures in terms of tampering are not easy to put in place. While tamper proof packaging can protect consumers and manufacturers alike, they can be circumnavigated. Governments and manufacturers have emergency systems or crisis plans designed to protect food, plants, and animals from accidental or intentional events. Such plans will rely in part on surveillance programs and food recall systems. Despite such systems, a product may still be contaminated at any point in the food supply chain, which extends from farms to stores. Food retailers play a vital role in securing the supply chain. They need to be aware of the food purchased, its supply chain, and storage security. For example, the city of New York recommends that even small food retailers and restaurant facilities follow the simple rules in Box 1. So vigilance is perhaps the key, retailers and also consumers should be aware of how to recognize the signs of food tampering, which can be seen in Box 2.

Box 1 Retailers prevention advice:

- Purchase only from reputable vendors. Keep an inventory, know who delivers your food.
- Be familiar with the foods you purchase, prepare, and serve.
- Schedule deliveries when staff are present to inspect and secure the delivery.
- Examine foods before use. Do not use foods with foreign objects or an unusual odor, texture, or appearance.
- Know your employees, run background checks on new employees and keeping them supervised during training, which should include an element of how to recognize signs of tampering.

Several governments and regulatory authorities have recognized the risk to consumers, the increased threat of the use of tampering as a terrorist approach has resulted in the development of advice and guidance for consumers and retailers. One such system is the INK approach shown in Box 3.

Manufacturers need to be prepared, having an effective crisis plan with procedures and systems to deal with product tampering. This will involve access to a multifunctional team that includes personnel from scientific and regulatory affairs, manufacturing, public affairs, sales and marketing, and legal counsel. Key responsibilities such as investigation and reporting to the authorities and press should be defined. The plan should also give ready access to manufacturing and supply chain information to expedite investigations. Most companies now have crisis management plans prepared in advance so that they are better able to cope with this kind of incident. They should also have procedures and systems in

Box 2 Recognizing signs of tamper:

- Packaging that has been opened and resealed;
- products that have damaged or missing safety seals or tamper-evident seals;
- products or packaging that is cut, torn, punctured, or discolored;
- products that are dirty or damaged;
- products with strange odor or flavor;
- cans or jars with signs of leakage, spillage, or corrosion;
- vacuum-packed products with no vacuum seal;
- packaging that has been altered, including labels, product lot codes, and other identifying information; and
- the presence of a foreign object or nonfood item in the product.

Box 3 The INK approach:

- Isolate the food (for retailers or manufacturers investigate – gather evidence)
  - Do not eat any of the food or feed it to livestock or pets.
  - Get medical attention right away if anyone who has had contact with the food feels sick.
  - Do not handle the food more than you need to.
  - Carefully place all the food in a sealable container (even if it is half-eaten, partially-cooked, and so on). Close the container. Write 'DO NOT EAT' on the container.
  - Keep any unopened containers of similar product, but do not open them.
  - Keep the food away from your regular food supply and away from your family.
- Notify authorities or the retailer:
  - If there is an injury, call the police or regulatory authorities right away to report the incident.
  - Call the retailer.
- Keep information:
  - Keep the container, packaging, label, receipt, and grocery bag that came with the food.
  - Write down any information you remember about the product, such as product codes, 'best before' dates, and where and when product was purchased.

place, which are designed specifically to reduce the risk of tampering within the production environment. Readers are referred to useful 'checklist' documents produced by the US FDA, which deal with prevention.

Manufacturers will have systems in place adhering to hazard analysis critical control point guidelines, which will assist in managing these issues. Automated weight and visual surveillance systems will often detect foreign body issues and reject products, making tamper with some items easy to rule out as a factory issue. Retailers and manufacturers also need to be able to give detailed information on the scope and distribution chain of products to aid the regulatory authorities in their risk assessments.

However, no amount of planning and preparation can remove the tamper risk entirely, and should the unthinkable happen, the crisis management plan should direct staff through the appropriate steps to at least lessen the impact of the incident.

Investigating the Incident

From the perspective of a laboratory that routinely investigates cases of food contamination, the immediate investigation of each and every incident is the crucial first step in deciding how to react. It is worth remembering that when a customer first reports that they have found something in their food, or noticed a tear in their tamper-evident packaging, it need not be the case that tampering has occurred. There is a myriad of other potential explanations, possibly relating to problems in the factory or supply chain, which the food manufacturer and their suppliers may need to address as a matter of urgency.

Similarly, when the extortion letter arrives, but there have been no complaints from consumers or retailers, it is by no means clear that the tampering has taken place, or ever will.

In either event, it is important to gather as much information as possible, and as soon as possible, so that better decisions can be made. There are many things that need to be done beyond a laboratory investigation of course, not least of which will be to involve the relevant authorities in the country where tampering has occurred or been threatened. Most of what happens thereafter will be determined by the police and relevant food authorities, but as noted above, when a case of contamination is first reported, it may be by no means clear that it is due to tamper. At that point, the food manufacturer is in charge of its own investigation.

There are, of course, limits to what laboratory analysis can tell you. Sadly, a real laboratory has none of the magic boxes that are prevalent in television dramas and films. However, as the following intends to demonstrate, the sophistication of modern scientific instrumentation does make it possible to glean far more information than many people realize, and at the very least, this may provide useful corroborating evidence should there ever be an arrest and trial of the tamperer. At best, it may even provide evidence that leads to the arrest.

Foreign Bodies

Foreign bodies might seem to be an obvious 'weapon of choice' for a tamperer. Almost anything will do. It requires no particular expertise to break a bottle to obtain fragments of glass, pick up a handful of nails, or find a dead fly.

It is however, much more complicated to put any such items into a product in such a way that it appears undisturbed, so that some other person will buy it and suffer some consequential damage.

Complicated should not be taken to imply impossible, as much depends on the type of packaging, the nature of the foreign body and the ingenuity of the tamperer. However, it should perhaps persuade a food manufacturer always to question the validity of a consumer complaint that "someone has put glass in the product I bought." It is always easier for a consumer to contaminate the product in this way themselves than it is for an extortionist to do so.

It is for the food manufacturer to decide how best to respond to the foreign body complaint, so it is worth knowing the kind of information that laboratory analysis can reveal.

The first thing to note about any foreign body (never mind the customer's account of how it was found) is that it may not be as obvious as it appears at first sight. For example, there are naturally occurring ingredients that have the appearance of glass, detritus from factory equipment that can look like rodent fecal matter, and ingredient reactions that result in strange precipitates and color changes.

So, no foreign body should be dismissed or accepted without some investigation. For the most part, that investigation will begin with close examination of the foreign body by stereo or compound light microscopy. The techniques used are generally nondestructive and are applicable to very small samples.

In some cases, microscopy will be sufficient on its own to identify the contaminant. In others, the results of microscopy will suggest the most appropriate course for subsequent chemical or microbiological analysis to take. The most commonly encountered foreign bodies and techniques for their analysis are outlined below.

Glass Fragments

Microscopy of any original surfaces of a glass fragment can provide important information on its mode of manufacture, whether, for example, the original glass article was molded (e.g., milk bottle) or spun (e.g., light bulb). Surface interferometry gives information on the curvature of a fragment, distinguishing between flat (e.g., window) and curved glass (e.g., tumbler and milk bottle). Using this technique it is possible to estimate the radius of curvature of a minute glass fragment and thus form a conclusion about the diameter of the region of the item from which it originated.

More information is available from X-ray microanalysis, a technique used in conjunction with scanning electron microscopy. This technique relies on the fact that different elements emit X-rays of characteristic energies and wavelengths when irradiated with an electron beam. Detection of the emitted X-rays reveals the elemental composition of the glass fragment and allows it to be compared with reference samples, either from the factory or from the laboratory database that can have more than 400 samples. Using this technique, it is possible to differentiate between sheet glass,

lighting glass, containers (bottles and jars), lead glass, borosilicate (i.e., heat resistant glass), and domestic glass (tumblers, dishes etc.).

Of course, knowing that a glass fragment came from sheet glass rather than bottle glass, may not be that helpful in deciding whether one is dealing with a case of tampering or not. However, suppose a consumer claims that their fragment relates to a known case in the public domain, but their glass fragments can be clearly shown to be of different origin from those known to be from a tampering incident. This might at least cast doubt on the validity of the claim, and help the understanding of the extent of the problem.

Metal

Like glass, metal fragments and objects may arise from a variety of sources other than tamper, such as factory machinery, packaging (laminated foil), and even dental fillings. Most factories have metal detection facilities online, which helps limit the problem of metal fragments reaching the consumer but none of this equipment guarantees an end to the issue.

The origin of a tiny metal fragment or dust can only be determined once its elemental composition is known. This may be achieved by X-ray microanalysis, which allows distinctions to be made between different base metals, steels, and other alloys. The same analysis can be used to determine any match between samples and reference materials.

In the case of deliberate tamper, one might expect to see whole objects rather than metal dust, especially items that are sharp or rusty.

Plastics

Polymers and plastics have replaced metals and glass in many industrial applications. As a result, plastics present an increasingly common foreign body problem. It is probably fair to suggest that plastics are less likely to be used in tamper cases than glass or metal, because they are generally softer and less likely to cause damage. Few criminals would be likely to choose plastic if they have the option of glass or metal.

However, for completeness, it is noted that a combination of microscopy and spectroscopy techniques can be used to identify plastic materials. Fourier Transform Infrared (FT-IR) spectroscopy or microspectroscopy can be used to characterize the chemical structure of the sample. The spectrum obtained by FT-IR can be compared with reference spectra from a polymer library. A second technique, known as differential scanning calorimetry can also be used to characterize plastics in terms of their melting point, degree of crystallinity, and glass transition temperature. In some cases this will differentiate between different forms of the same polymer.

X-ray microfluorescence spectrometry is another powerful technique for elemental analysis of all kinds of solid fragments and is especially valuable in the analysis of coatings, paint flakes etc. The technique is nondestructive and permits fast, simultaneous, multielement detection for all elements in increasing atomic number from sodium to uranium. It also permits the gathering of qualitative, quantitative, and spatial distribution information. Again, such information can be especially useful in matching samples from different incidents, and forming a chain of evidence that links one incident to another.

Other Foreign Bodies

There are many other potential foreign bodies that the criminally minded consumer might use, especially to contaminate their own product. These include items such as hairs and fibers, insect parts, and animal droppings, all of which can be investigated using a combination of microscopy, microbiology, and chemistry techniques.

It is usually possible to determine the species or family of insect, giving a good clue as to its country of origin and association with specific raw materials. This may help in assessing whether it is likely that the insect arrived in the product through natural means or whether it has been placed there by the consumer. It is sometimes possible, in addition, to use a test to determine the activity of the phosphatase enzyme to verify whether the insect has been heat treated or whether it gained access to the product after processing had finished. This is because the enzyme is heat sensitive and loses its activity on heating.

DNA and protein methods may be applied to animal body parts and to fecal matter to suggest species of origin, or to discount animals entirely. Black bits that resemble rodent droppings often turn out to be balls of congealed fat, either burnt and/or colored by oil and metal dust.

Chemicals

There are thousands of chemicals that might be used for tampering food and drink products, ranging from the obvious rat poisons and strong bleaches legally on sale to the general public, to the narcotics and recreational drugs that require a little more effort to obtain. Then there is the multitude of industrial chemicals that only certain people might have access to, as well as the potions that a handful of committed criminals have been known to concoct in their own homes.

The issue of analysis is therefore hugely difficult to address in a general article. Any analytical approach depends very much on the nature of the product that has been contaminated, the first best guess as to which chemical has been used, and whether known methods exist for extracting and identifying the suspected contaminant. Sometimes methods have to be developed 'on the hoof,' presenting particular challenges to the chemist involved.

Clearly, the sophistication and sensitivity of modern techniques such as gas chromatography–mass spectrometry (MS), liquid chromatography–MS, inductively coupled plasma–MS, nuclear magnetic resonance, and atomic absorption spectroscopy do give the chemist every chance of isolating and identifying any contaminant. Nonetheless, a successful conclusion does largely rely on the expertise and experience of the individual analyst, simply because a food matrix generally contains thousands of other chemical entities that have the potential to interfere with the analysis and obscure any results.

Also, from the chemist's perspective there is a big difference between cases that present like the Tylenol and baby food incidents mentioned. In the former, the tamperer used a strong poison, at levels that were undetectable to the unfortunate consumers. In the latter case, the caustic soda used was so strong smelling that it caused the consumer to cry, and of course, the drawing pins and extortion note were obvious added signs that all was not well!

Any given tampering incident might present to the laboratory at any point on a scale between these two examples. Where no other indication exists, the best one might hope for is that the product smells, looks, or tastes sufficiently strange that the purchaser is persuaded to consume no more before reporting the problem to the manufacturer.

In the subsequent analysis, it is these early sensory signals, often refined and defined by a trained sensory analyst, that give the chemist the best start in identifying the nature of the contamination.

Of course, not every chemical contamination results in a bad smell or taste, and tampering incidents involving chemicals of this kind will only come to light when a consumer is harmed, or the extortion note arrives. Indeed, it may be the case that only the extortion note arrives without any tampering taking place. After all, it is far easier to threaten to poison a product than to actually go out and do it. When the criminal is only interested in easy money, why go to the trouble of working out the best way to tamper with a product and run the risk of a shop's closed circuit television filming the moment that he returns it to the shelf?

That is not a flippant observation. Our laboratory has worked on many projects where a food manufacturer wishes to know if a threat is feasible in practice or too ridiculous to take seriously. By recreating the intended threat it can quickly become apparent if the chemicals to be used are likely to destroy the packaging or product to such an extent that no consumer is at risk of buying it, or perhaps that the chemicals to be used represent no serious threat to anyone's health at the quantities at which it is practically possible to add them.

It is not only the practicality or danger of the chemical that can be assessed but also the vulnerability of the packaging. Obvious questions to ask in such assessments are whether it will be clear to consumers that the product has been tampered with (i.e., effectiveness of tamper-evident packaging). There are many well-designed tamper-evident seals that can be bypassed quite easily by hyperdermic syringe, for example, or packets that are tricky to open from the top, yet surprisingly vulnerable at the bottom.

The vulnerability of packaging leads back to the application of microscopy techniques, because close visual assessment is often required to examine if and how packaging has been compromised by the tamperer. It is often possible to tell, for example, whether a packet has been cut or torn, whether a puncture hole is from a needle or some other implement.

Integrity of Evidence

For any investigation of suspected tampering, or indeed, any other issue of product contamination, it is important that the integrity of the food product and packaging are preserved as much as possible before these items reach the laboratory. No one would want any additional contamination to occur, or any existing contamination to be lost during transit between consumer and analyst. Similarly, it is important that the laboratory retains such evidence as securely as possible in case it is requested by a police forensic laboratory.

Conclusion

Although the food industry must take every possible step to minimize the risk of tamper, through use of tamper-evident/resistant packaging, and by preventing unauthorized access to production facilities, it is clearly the case that tamper cannot be completely prevented. The only 100% tamper proof packaging would also be impossible to open.

It is also clear that tamper is neither the most common crime nor indeed the issue that poses the greatest risk to a food company. There are many other issues that are more likely to compromise the safety of a food product and damage the reputation of the manufacturer. Tamper can occur throughout the supply chain, and the increasing complexity of this chain could impair investigations if tamper occurs at an early point. However, most tamper events occur during retail or the latter stages of the supply chain and are rarely undertaken by organized criminals.

Tampering is therefore one of those issues that must be taken seriously, and that must be considered in any risk management program and in any crisis management plan, but with any luck, it will also be one of those issues that the majority of food companies will never actually encounter.

See also: Food Safety Assurance Systems: Essentials of Crisis Management; Investigation of Incidents in Industry; Recall Systems and Disposal of Food. Other Significant Hazards: Physical Hazards in Foods. Public Health Measures: Food Defense: Prevention of Sabotage and Bioterrorism; Modern Approach to Food Safety Management: An Overview

Further Reading

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FOOD SAFETY ASSURANCE SYSTEMS

Essentials of Crisis Management

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Glossary

Crisis A predicted or unpredicted event which represents an immediate or future significant threat to an organization, its employees, consumers, and the public at large.

Focus group A form of qualitative research in which a group of people are asked about their perceptions, opinions, beliefs, and attitudes towards a product, service, concept, advertisement, idea, or packaging.

Food industry The term includes primary, manufacturing, and processing industry as well as some other establishments involved in the food chain.

Food safety Assurance that food will not cause harm to the consumer, when it is prepared and/or eaten according to its intended use.

Management commitment Direct participation by the highest level executives in a specific and critically important aspect or program of an organization. In quality management it includes (1) setting up and supervising or serving on a quality committee, (2) formulating and establishing quality policies and objectives, (3) providing necessary resources and training, (4) overseeing implementation at all levels of the organization, (5) evaluating and revising the policy in light of results achieved, and (6) ensuring that internal or external complaints, in particular reports by whistleblowers are thoroughly examined and adequately addressed.

Risk communication The interactive exchange of information and opinions throughout the risk analysis process concerning risk, risk-related factors and risk perceptions, among risk assessors, risk managers, consumers, industry, the academic community and other interested parties, including the explanation of risk assessment findings and the basis of risk management decisions.

Traceability The ability to follow, forward as well as backward, the movement of a food through specified stage(s) of production, processing and distribution.

Whistleblower A person who tells the public or someone in authority about alleged dishonest or illegal activities (misconduct) occurring in a government department or private company or organization.

Introduction

In any organization dealing with a risk-prone subject such as food, chemicals, drugs, health, transport, finance, a crisis is an almost unavoidable situation; any organization with a professional management should be prepared for it. Organizations operating in the food sector, be they a food business or agencies responsible for overseeing food businesses, are by nature of their work exposed to such an eventuality.

The vulnerability of the food sector to experiencing a food crisis comes from the fact that over and above human errors there may be other totally unexpected event or finding (the Black Swan Effect) such as the emergence of a new biological or chemical agent. This was the case with epidemic of cholera in Latin America in early 1990s, bovine spongiform encephalopathy (BSE) in UK (1996), acrylamide (2002), semicarbazide (2003).

Factors that can trigger a crisis situation are summarized in Table 1.

It is to be stated that what is or not a crisis depends also on the reaction of the public, industry, and governments, but it also depends on the geographic location where the event takes place and the perception of risk, culture, and societal values. There may be issues that would generate a crisis in North America and not in Europe or vice versa. For instance, the information on health risk associated with acrylamide did not cause the same reaction in North America than in Europe.

The consequences of a crisis can be disastrous for an organization as well as for the society. For consumers, a food safety crisis situation means that they may potentially be exposed to unsafe products and lose their trust in the food supply. For businesses, consequences are economic and affect their image, i.e., product recall and waste of produced food; loss of reputation; loss of market shares; and loss of the trust of consumers, customers and regulatory authorities. Additionally, they may be subject to further or more stringent regulatory measures. Loss of trust by customers may also trigger more stringent requirements, for example, provision of a certificate of analysis. Trust of consumers, customers is one of the most important assets of a business or an organization. It takes many years to build trust, but it can be destroyed with one single incident, particularly if it is poorly managed. When lost, its impact is often long-term and will take many years to rebuild.

For public health and regulatory authorities, even when a crisis is initiated in the industry, the good management of a

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Table 1 Factors that trigger a crisis

Triggered by	*Examples of crises*
New scientific findings	• Acrylamide (Worldwide, 2002) • Semicarbazide (Worldwide, 2003)
Emergence of new hazards	• Bovine spongiform encephalopathy (Worldwide, since 1986) • *Escherichia coli* O157 (US, Japan, Europe, etc. since 1980s) • *Vibrio cholerae* (Latin America, 1993) • Avian influenza (2004)
Human error: Scientific, technical, managerial Operational error, or violation	• *Salmonella* in chocolate (UK, 2006) • Vitamin B1-deficient infant formula, ex-Germany, Israel 2003 • Isopropylthioxantone (Worldwide, 2005) • *Salmonella* Saintpaul (US, 2008) • *Salmonella* Typhimurium (US, 2008–09) • *E. coli* O104:H4 (Germany, 2011)
Fraud or malicious acts of sabotage, for example, tampering, terrorism	• Dioxin in animal feed (Belgium, 1999) • Wheat gluten in pet food adulterated with melamine, ex-China (North America, 2007) • Adulterated sunflower oil ex-Ukraine (Europe, 2008) • Infant formula adulterated with melamine (China, 2008) • Beef adulterated with horse and pork meat (Europe, 2013)

crisis is crucial, because consumers consider their government as the guardian of the safety of the food supply and as ultimately responsible for food safety.

Where governments fail to manage a food safety crisis, they may also lose their image, reputation, and the trust of the general public in their capabilities to ensure safety of the food supply. In such a scenario, the trade in food can collapse. Such situations were experienced with meat and meat products in the BSE crisis in the UK and other European countries, and with fruits and vegetables in the case of *Escherichia coli* O104:H4 in Germany. Failures in managing an incident or a crisis have also been the cause of political turmoil. Following the 1999 dioxin crisis in Belgium, the Belgian Ministers of Agriculture and Health had to resign. The ruling Christian Democratic government was also voted out of office. In China, following the melamine crisis, the governmental officer in charge of food safety was executed.

The loss of trust of consumers in their authorities following the BSE crisis and a plethora of small- or large-scale incidents which occurred is one of the factors leading to the mistrust of consumers for new technologies like genetically modified food, food irradiation, and application of agrochemicals in the food productions. In Europe, many consumers turned toward organically produced food.

The consequences of a crisis for an organization or a society depend on how well the organization or the society is prepared for a crisis situation. As for a boat whose survival through a storm depends on its solidity, the training of the sailors and the skills of the captain, the outcome of a crisis depends on the infrastructure in place, the training of the staff, and the management skills of the manager.

From the above, it can be understood that the importance of crisis management, and preparation for it, cannot be overemphasized. Provided that crises:

i. do not occur too frequently,
ii. are not a consequence of obvious or gross negligence,
iii. do not involve unethical or malicious malpractices, and
iv. health and concerns of the general public are given priority.

A good crisis management can to some extent reinforce the trust of consumers and trading partners or improve the reputation of the affected organizations, or at least limit the damage. Consumers who would observe that, in case of any adverse event, the business or the government will take necessary measures to protect them will have increased trust in the business and governments. As mentioned above, in a crisis situation, both governments and the industry may be implicated and will have to bear the consequence of a crisis; therefore, interactive communication and full transparency between the two parties is essential. Additionally, both may be brought to explain why their preventive measures failed.

Whereas collaboration and communication between governments and industry is important to manage a crisis, it is also important that the process of decision and implementation are not biased. Therefore, the principles for risk management and the process of risk analysis developed for the management of food safety in normal times applies also in a crisis situation, except that decisions have to be taken under time constraint, with incomplete data, and often under media scrutiny. The more the food safety management in a country is solid in terms of organization, for example, definition of responsibilities, values, standards (existence of legislation and enforcement mechanism) and procedure, and skilled and competent managers the better the country will be prepared for managing a crisis situation. The same applies in a food business. In the dioxin case in Belgium in 1999, the lack of norms for dioxin at the European level and of traceability were gaps that, among others, negatively impacted the management of the crisis. In a similar dioxin crisis a few years later in Ireland, the existence of a norm was one of the factors that eased the decision making process.

As for food safety management, crisis management requires a structured, systematic, and consistent approach and includes four stages:

1. Crisis prevention.
2. Crisis preparedness.
3. Crisis management.
4. Recovery and rebuilding after a crisis.

The following principles and recommended practices for crisis management are formulated in a general manner. With some adaptation to the circumstances, they can be applied both in governments and in industry.

Crisis Prevention

It has to be recognized that a crisis situation starts when food safety management has failed. Therefore, regardless of how well it is managed, a crisis is often an indication of failures in food safety management and will have some negative consequences, particularly if crises occur too frequently or past errors are repeated.

Management of a crisis situation mobilizes a lot of resources within the organization and disrupts the normal operations. Therefore, the repetition of a crisis situation not only will erode the trust of the trading partners, customers, and the public at large, but also undermine the routine of the operations and will wear out the staff. Subsequently, these may be more disposed to human error. A vicious cycle of vulnerability sets in. Hence the importance of preventive measures, as the better we manage food safety, less likely we are to have a crisis. Thus, paradoxically and ironically, the best crisis management becomes its prevention.

The preventive measures are not any different than those that are necessary for food safety management; crisis management should be seen as a continuum of food safety management. For an overview of food safety management, the reader is referred to the article on food safety management. However, some aspects of food safety management find particular importance during a crisis situation, these are described below.

During a crisis, trust becomes a very important asset, as people operate under acute conditions requiring real time and strategic decisions taken at high level. Owing to the urgency of the situation, there is no time for checking the validity and thoroughness of the data as in a normal situation. Many decisions are to be taken based on the trust in the integrity and competence of staff or experts and on their respect of the values and policies of the organizations. However, trust is not built or achieved in one day; it takes years of good practice and of responsible and transparent behavior and management. Although, as mentioned above, the practices during a crisis management can enhance and reinforce trust, its foundation is to be built during normal periods, i.e., during the day-to-day operations. An organization that behaves responsibly will not have difficulty in transparency and giving the truth about the cause of its incident. Therefore, values such as open organizational culture, transparency, prioritizing the safety of products over economic considerations should be spelled out in the policy of organizations and actively supported by the Management. In other words, policies should not be a declaration of good intentions but practiced by the leaders on a daily basis.

As part of management, the definition of policies, processes, responsibilities, and the provision of logistic support all are important for a good management of food safety. Additionally, in a food business, the food safety assurance system, including good manufacturing practice, the hazard analysis and critical control point system and various verification measures, will need to be implemented in a flawless manner. Regulatory authorities should also monitor the food supply for safety and have an efficient foodborne disease surveillance program to depict any problem at an early stage.

An important and integral part of the food safety management system is management of human resources, as no matter how many principles, systems, and tools are innovated for managing food safety, it is finally the staff who have to implement these. Human resource management is often a neglected area in food safety management, whereas it is fundamental to an efficient food safety management and should be considered at the heart of the system. Experience from various crises shows that very often failures leading to an incident or a crisis are known by the staff, or could have been predicted; however, due to fear for repercussions on their career, they fail to report or do not bother to report as they do not believe in the fair evaluation of their information.

Therefore, over and above their knowledge and skills, staffs need to be motivated and encouraged and most importantly, not fear for their career or potential repercussions when reporting potential gaps or malpractices. Any gap or malpractice which is addressed at an early stage will decrease the risk of an incident and eventually of a crisis. Employees need to believe in the commitment of their Management in the true sense of the word. Hence the importance of credibility of the Management and their walking the talk on a consistent and continuous basis, as any noncompliance or complacency at the higher level of management will set a bad example for the entire organization and have serious repercussions on the entire organization. The importance of management commitment, having an open culture, promoting the reporting of problems, and their investigating and closing the gaps for prevention of crisis cannot be overemphasized. Naturally, governments have the leading role in protecting the right of the staff and whistleblowers.

As part of food safety management, but of particular importance to crisis management, are of course traceability, recall procedure, and the procedure for crisis management itself. The latter will be described below. With regard to traceability, without such a system, in case of a nonconforming ingredient or a product, it will not be possible to make a selective recall of products, and all products suspected to be potentially affected will have to be recalled and destroyed. A case in point is the incident with dioxin in Ireland (1999) where, due to the absence of traceability, all pork products were recalled, whereas for beef, as a traceability system was established following the BSE incident, a selective recall, i.e., a recall of contaminated products, was possible. The same applies for an incident affecting a food business. The finer the traceability system, the more likely it will be possible to narrow the recall of products that are affected by a contaminant. For instance, a company

that can trace a contamination to the precise time of production can limit the recall to those specific products. In absence of such a system, unless the nature of the contamination is such that it is possible to segregate affected products by testing, all the production of a day, week, or longer period may need to be blocked and withdrawn.

Many organizations may also benefit from an active early warning system or may even do research on potential emerging issues. Such a system is at the frontier between prevention and preparedness for crisis management. Depending on the nature and size of the organization, the system could include monitoring, surveillance, and analysis of:

1. literature and scientific data,
2. the regulatory development and alert networks,
3. incidents and experience of other companies and countries,
4. consumer complaints,
5. foodborne diseases, animal diseases, and monitoring of contaminants,
6. postlaunch of new products,
7. audit and/or inspection reports, reports of compliance of products, and
8. internal account of staff reporting noncompliance or mismanagement.

Crisis Preparedness

One of the key principles for crisis management is the speed of action, be it investigation into the case or informing the general public. In all incidents where there has been a delay in action, it has caused outrage and hard judgment by the public. In the dioxin incident in Belgium (1999), melamine in China (2008), *Staphylococcus aureus* in Japan (2000), and *E. coli* O104:H4 in Germany (2011), one of the main failures for which the responsible authorities were severely criticized was the delay in removing products from the market or in informing the public; a similar experience was observed with Toyota (2011) and Sony who were slow to inform the public about their defective cars or their security system that was hacked. Therefore, to ensure a rapid course of action in such a situation, a certain number of actions and activities have to be carried out in advance to actively and specifically prepare for a crisis situation. To this end, it is important to consider:

1. Infrastructure and resources that may be required during a crisis include:
   - Developing a network of collaboration and alliance with various stakeholders, as during a crisis there will be a need for a rapid exchange of information. Examples are media, other food companies or industry associations, regulatory authorities, and consumer organizations.
   - Being aware of the regulatory requirements in relation to: (1) the procedure for reporting an incident (i.e., who should be informed, at which point and what kind of information needs to be provided), (2) legal requirements for withdrawing or recalling products, (3) eventually for disposing of contaminated products in a safe manner, and (4) penalties or penal actions for the responsible person, in case of consumer injuries.
   - Being informed of the requirements of the Codex Alimentarius Commission and the International Health Regulation in case an incident or an outbreak takes an international dimension or affects foods entering the international trade.
   - Foreseeing the scientific support (e.g., access to experts) and additional logistic support.
   - Organizing a database on products and their traceability records, i.e., their destination and/or the source of the ingredients.
   - Establishing a contingency plan, for example where the work or operations can be transferred, or how raw material can be sourced (alternative source).
   - Definition of roles and responsibilities in times of crisis and the network of people who should be informed.
   - Additional administrative support and infrastructure, such as designation of a specific meeting room, extra lines for telephone, mobile phones, etc.
   - Good organization (responsibilities, network) and written procedure, for example, first minute actions.
   - Red folder: Data needed in case of accidents: organization chart, emergency telephone numbers, phone numbers of key partners, governmental agencies, food companies, customers, scientific experts, and specialized laboratories.
2. Principles and procedures. As for defining procedures and principles, it is important to also define the specific procedures for crisis management, i.e., how the early warning system should work, who decides, implements, and communicates during a crisis situation. Often these may be the same as in normal circumstances; however, each organization has to give this matter specific consideration and take a conscious decision, as the same infrastructure and setup may not be suitable for all types of conditions and organizations. The types of questions that should be considered are:
   - The line of reporting of information.
   - The type of information (consumer complaints, regulatory actions, media, disease surveillance, food ban, etc.) that should be reported as part of early warning.
   - The crisis manager and the skills required for this position.
   - Composition of the core crisis management team, competence needed, and responsibilities.
   - Those who should possibly be informed internally or externally (regulatory authorities, medical community, consumers/general public, suppliers, customers, media, food companies, trade or international organizations, and police in case of tampering).
   - Define the principles of decision making and the authority, i.e., who would need to approve a decision.
   - The person who will be responsible for implementing decisions and for following up.
   - The spokesperson.
   - The procedure for preparation of the communication.
   - How the issue will be coordinated nationally or internationally.
3. Defining a crisis manager. It is to be noted that the crisis manager does not always need to be the same as the food

safety manager. A crisis manager should have specific skills, such as:

- Leadership;
- Good technical knowledge (scientific, product, supply chain, and regulatory information);
- Organizational management skills;
- Public relations and communication skills;
- Recognition and trust of stakeholders;
- Experience;
- Emotional intelligence (empathy); and
- Pragmatism and common sense.

Finally, as part of crisis preparedness, it is important to communicate to all stakeholders, or all potentially involved parties, the flow of reporting, i.e., the communication plan, and the principles and procedures; it is also important to train the crisis team members in crisis management, and periodically perform a crisis management exercise to ensure that the procedures and principles are correctly understood, feasible, and complied with. It is clear that in the light of the outcome of the exercises or in case of any new internal or external experience, the crisis management procedure is to be reviewed and improved. An important element for training is, of course, skills in crisis communication. As will be seen in section Crisis Communication for this purpose, specific skills are required.

Crisis Management

Then comes the time when the house is on fire, i.e., a crisis hits. The procedure, for example, convening the crisis management team, informing the management, is to be put in place. It is important to act swiftly, but calmly. Speedy and timely decisions and actions are key, but it is equally important not to take decisions in a panic mode, and as far as possible to take the decisions in consultation with the crisis management team, and/or depending on the case, with the support of the management of the organization. Where applicable, external bodies, for example, other industries, or industry associations, or government of other countries or international organizations, may need to be consulted.

In the eye of the storm, a number of decisions are to be taken and implemented. To this end, a few principles are to be observed:

1. To get the facts right and be aware of uncertainties. Examples of information which would be required are:
   - What has happened?
   - What level of contaminants was found in the food, which method was used and its validity, sensitivity of the method, possible product variation or limitation of the analytical techniques, or the competence of the laboratory.
   - Range of products that are affected, the time the affected products have been on the market, i.e., how far back a product may need to be recalled if necessary, their expiry date, their distribution, and products which are in the warehouse at the time of the crisis.
   - What was the possible cause of the problem? This information will be essential to determine which products, and up to which time, need to be pulled out of the market.
   - Who is informed about the subject?
   - Evaluating risks and possible management options. In evaluating the risks, the health consequences for consumers should be the primary concern and the priority. As part of this, different types of risks need to be considered, for example, safety risks versus nutritional risks. Safety risks can also entail microbial versus chemical risks. It is to be borne in mind that a rapid change in a product or food consumption pattern without taking the necessary precautions may also lead to exposing consumers to new risks. Other types of factors to consider in the decision making process are regulatory and legal aspects, potential environmental risks, reputation risk, economic and financial implications, social consequences, perception issues. Decisions are to be taken based on the above consideration and considering the pros and cons of different management options, including feasibility and possible time frame.

To ensure that the intentions with decisions are understood and the decisions are followed, it is important to explain the basis for decisions and to keep records on the reason for the decisions, those who participated in the decision making process, as well as the data that were considered. The implementation and the outcome of the actions are to be monitored and evaluated at all times. Where necessary, for example, in the light of new information, the course of action or decisions may require amendments. In taking decisions, it is important to consider both short-term as well as long-term consequences, and also to think globally as today food safety is global and decisions can have broad consequences. Experience in other countries may also be beneficial. Finally, to ensure a rapid course of action, it is important to have a plan of action (including a contingency plan).

Crisis Communication

During the last two to three decades, there has been an increasing recognition of the importance of risk perception and risk communication, in particular during the period of a crisis. It has, among others, been realized that perception of the general public, although not always based on science or in line with the view of scientists, is the main driver in the acceptance of products and/or technologies and influences the food market. A huge amount of research has been carried out in recent years. It has been found that the perception of consumers is influenced by a number of factors such as:

1. Prospects of significant benefit for 'me'.
2. Whether the risk is voluntary (consent) or involuntary.
3. Whether the risk is familiar.
4. The 'dread' factor in the risk.
5. Whether the risk and benefit are 'fairly' distributed.
6. Whether the risk is part of an unethical activity.
7. Whether the risk assessor and risk manager are trustworthy.
8. Whether the risk is natural or unnatural.

Consideration of these factors is important, as much in the risk communication as in the decision making. For instance, in an incident where infant formula was contaminated with traces of photoinitiator in ink in 2005, the affected food

companies decided not to recall their products on the grounds that the contamination did not represent a risk to health, whereas many consumers would not accept to buy such a product and as the risk was unnatural, involuntary, and the risk benefit was not equally distributed. With regard to risk assessment and risk communication, the fact that initially, the risk assessment was carried out by the infant formula manufacturers and not by the authorities undermined the validity of risk assessment and communication. However, in an incident which occurred two years earlier in 2003, where traces of semicarbazide were found in baby food, the rapid risk assessment by the European Food Safety Authority and its communication to the general public led to a more peaceful resolution of the crisis.

Consumers and interested parties get their information in different ways. Therefore, different methods and means of communication need to be used to reach the target audience as widely as possible. These include: press release and/press conference, TV interview, website, podcast, telephone voice messages and hotline for specific consumer queries, alert networks such as the European Rapid Alert system, the World Health Organization/Food and Agriculture Organization of the United Nations, International Food Safety Authorities Network (INFOSAN), etc. A rumor hotline can also help the crisis management team understand if any erroneous message needing correction or explanation is circulating.

Experience from a few crises shows that in the heat of the management of a crisis, a few groups of people are forgotten in the line of communication; they are mentioned here as *aide-mémoire*, as depending on situation, it is essential to keep them informed. Some may need assistance with a brief, a draft declaration, or a Question and Answer, in case they are contacted by the public or the media. These are:

1. the Chief Executive Officer or the Director General of the organization;
2. trade associations/regulatory authorities, or international organizations;
3. stakeholders of the food chain, for example, retailers;
4. employees; and
5. the switchboard or consumer services on possible answers to the general public or consumers.

The exact choice of the method and the mechanisms of communication depends on the case, and the strategy for communication needs to be examined very carefully so as not to create undue panic in the population, yet to inform them as needed. In the 2002 acrylamide crisis, the Swedish authorities decided to hold a press conference. According to communication experts, this method of communication created a big communication crisis and media attention, although contaminant was not new (humans have been exposed to it as far back as the Paleolithic period when food was cooked over fire), its risk were not yet known, and its content in the food could be reduced only after extensive research, meaning that an alarming communication would only create panic without providing consumers with a solution.

A few additional principles need to be considered in crisis communication:

1. Speed: A communication should be made within 24 h.
2. Accepting blame for death or injury, if the product is proven to be implicated, even if the cause is still unclear.
3. Coordinate all communications through one person.
4. Depending on the situation, the communication could include: the facts, i.e., what is known and what is not known, the decisions, actions, and the basis for the decisions.
5. Communicate in such a way as to avoid any misunderstanding by the audience, i.e., the potential for being understood in different ways than what was meant. This can be done by testing the communication on a focus group or people representing the target audience.
6. Confirmation of receipt of important messages.
7. Full transparency and consistency, in particular to be aware that any attempt to downplay or hide facts will cause more damage to the reputation.
8. Having and expressing empathy with the victims and affected people.
9. Frequency, mode, and content should be culture-specific and effective for the specific target consumers.
10. Avoiding terms that would amplify the scare or unduly minimize and mislead the target audience.
11. In communicating with the media, any gap in information can lead to misinformation.

Documentation and Records

Documentation and records are important means of communication and these are an equally important task in the management of food safety.

In a crisis, which is often a situation where the safety of products have gone out of control, having records of events, decisions, and actions, as well as supporting documentation becomes even more important. As part of this, all facts and decisions are to be recorded in a logbook. The logbook should contain records of the events, who decided what and who was informed. When meetings have taken place, it should also include minutes of the meetings. These should also record reservations made by any member of the crisis management team and should also be disseminated to all attendees and other interested parties. The preparation of a case report on the event can help in communicating the event to stakeholders in a consistent and transparent manner, and also facilitate the identification of any discrepancy and/or uncertainty that may be detrimental to the process of decision making. It can also support the development of consensus and the later evaluation of decisions and of the crisis management.

Another important document is a statement or the position of the organization for communication to stakeholders (authorities, industry, media, public, or internationally).

Recovery and Rebuilding after a Crisis

Management of a crisis is often so exhausting that once the storm is over, members of the crisis team tend to return to their normal duties. However, the evaluation of the crisis management and determination of the root cause of the incident, its consequences, lessons learned, and corrective

Table 2 Summary of lessons learned from various incidents and crises

Incidents	*Lessons learned*
S. aureus intoxication, milk (Japan, 1955)	• Importance of rapid action (halt of sale, recall of products, and public apology) increases trust
Mineral water contaminated with benzene (France, 1989)	• Rapid product recall increases trust • Importance of valid information for decision-making • Attempts to downplay the extent of an incident will damage the reputation of a company • Importance of consistent communication • Importance of international coordination
BSE/vCJD (Worldwide, (Europe, 1996–2000))	• Importance of transparency and ability to communicate uncertainty to public • Importance of prioritizing public health over economic considerations • Importance of consumer/public perception (e.g. dreadful nature of disease) • Need for separation of risk management from risk assessment • The social dimension of food safety • Importance of traceability and farm-to-fork approach • Role of media • Importance of risk communication by a global public health authority
Animal feed contaminated with dioxin (Belgium, 1999)	• Importance of speed of action • Demonstration of the complexity of the food chain and the need for traceability • The need for farm-to-fork approach • Role of media • Importance of risk perception • The need for resources (e.g. laboratories)
Soft drink allegedly contaminated with pesticides (Belgium, France, 1999)	• Importance of communication • Importance of considering the context of an incident • Voluntary recall can increase trust • Role of media
S. aureus intoxication, milk powder (Japan, 2000)	• Speed of action • Priority to public health • Communication: empathy with the victims • Full transparency: any attempt of denial, or minimizing the impact (false or partial information, partial product recall) will damage the reputation more • Preparation: clear procedures and training • Mechanism for reporting problems to management • Beware of culture of fear!
Mislabeling of beef product (Japan, 2001)	• A good crisis management does not always help! • Fraud and ethical malpractice will not be forgiven
Packaging contaminant: semicarbazide (Worldwide, 2003)	• Early and transparent communication of the food industry increases trust • A rapid risk assessment and communication by trusted sources prevent escalation of a crisis and ensure coherent actions across Europe
Packaging contaminant: isopropylthioxanthone, (Worldwide, 2005)	• Importance of risk assessment and communication by competent authorities • Alignment with government views • Coordination of government agencies • Importance of risk perception • Financial consequences can also create a crisis • Importance of documentation and records • Media plays an immense role
Salmonella/peanut butter (USA, 2006 and 2008/9)	• Importance of root cause, corrective actions based on understanding the underlying factors for malpractices
Pet food adulterated with melamine (North America, 2007)	• The need for additional resources (e.g. to handle consumer queries) • Early reporting to public health authorities to minimize damage • Importance of coordination and communication between authorities • Importance of considering the fate of disposed products • Root cause analysis of incidents and dissemination of our experience can prevent future crises
Sunflower oil adulterated with mineral oil (Europe, 2008)	• International coordination to prevent dumping of contaminated food to other countries or food sectors • Difference between being allowed to keep a contaminated product already in the market and being allowed to release that product
Melamine/infant formula (China, 2008)	• Importance of root cause, corrective actions based on understanding the underlying factors for malpractices • Risks associated with fear culture • Control of contaminated products and their safe disposal
Enterohemorraghic *E. coli* (EHEC O104:H4) and fenugreek (Germany, 2011)	• Importance of speedy action • Validation of information before communication to the general public

(*Continued*)

Table 2 Continued

Incidents	*Lessons learned*
Horsemeat (Europe, 2013)	● Coordination among authorities ● Impact of public fear on food market ● Lack of ethics in food businesses ● Complexity of the food chain, limitation of traceability ● Where there is no ethic, all kind of unpredictable problems can happen

Abbreviations: BSE, Bovine spongiform encephalopathy; vCJD, variant Creutzfeldt–Jakob disease.

actions are very important for preventing future incidents. In a case when an infant had died (Belgium, 2001) where the contamination of infant formula with *Cronobacter sakazakii* (previously *Enterobacter sakazakii*) was considered as a possible cause of the incident, the authorities questioned the manufacture on the corrective actions that the company had taken since its previous *C. sakazakii* incident. Thus, the outcome of the evaluation can be instrumental in improving the crisis preparedness and procedures, or the food safety management system in order to prevent or minimize future problems. A final report on the case, including the root cause analysis and the lessons learned, needs to be communicated widely to prevent future similar cases. Lessons learned from some of the major food safety crises are presented in Table 2 as examples. Public health authorities need to also report the results of their investigations. The repetition of several important incidents, for example, melamine, salmonella in chocolate, salmonella in peanut butter, tends to indicate that the causes of incidents are not always fully investigated and the lessons learned are not widely communicated. Finally, the roles and responsibilities, including those of the members of the management, in the incidents need to be clarified. A major mistake would be to fire the personnel who *a priori* is viewed as responsible before the investigation is finalized and the root cause of the incident is identified. A critical review of the root cause of incidents can show that not infrequently, the failures can be traced to the management's practices and commitment.

Conclusion

In life, the unthinkable can happen. In food safety, every detail may be the occasion for an incident or mishap with the potential for causing a crisis. A proactive approach to the management of food safety can minimize the likelihood of an adverse event leading to a crisis. A crisis is never good and will cause damage; it should by all means be prevented; however, when it occurs, how it is managed can be the opportunity for demonstrating the management capabilities and the values of an organization. In the management of a crisis, the objectives should be to maintain the trust of the stakeholders, in particular the public, authorities, and trading partners. Decisions should be based on facts, including consideration of the uncertainties, and putting the health of consumers as a priority. The perception of consumers is also an important consideration. Speed of action, a consistent and transparent approach, and empathy for the victims are some of the key values for which an organization would be scrutinized. In a crisis situation, the media plays an important role and the communication with the media, or through the media with the public, is key for the management of a crisis. The values and the culture that an organization promotes and actively implements is a determining factor for the early identification and management of potential issues and for the prevention of a crisis.

See also: Food Safety Assurance Systems: Investigation of Incidents in Industry; Root Cause Analysis of Incidents. Other Significant Hazards: Physical Hazards in Foods. Public Health Measures: Food Defense: Prevention of Sabotage and Bioterrorism; Foodborne Disease Outbreak Investigation; Health Education, Information, and Risk Communication; Modern Approach to Food Safety Management: An Overview. Risk Analysis: Risk Analysis of Hazards in Food: An Overview; Risk Assessment: Microbiological Hazards; Risk Communication: Biological Hazards; Risk Communication: Chemical Hazards; Risk Communication: Novel Foods and Novel Technologies; Risk Communication; Risk Management: Application to Biological Hazards

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http://www.who.int/foodsafety/fs_management/infosan_brochure_en.pdf
The International Food Safety Authorities Network (INFOSAN).

Root Cause Analysis of Incidents

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Glossary

5-Whys A problem-solving technique for identifying the root cause of a problem.
Failure mode and effect analysis A problem solving method for investigating possible causes and effects of failures in systems.
Hazard and operability study (HAZOP) A structured, systematic technique for examining potential faults in system.
Incident A deviation from standard practice leading to sub-standard product or dissatisfaction of customers/consumers or regulatory authorities, for example, due to injury or perceived food safety problems.
Influence diagram A pictorial method for portraying the causal factors in an incident happening at different levels within an organization.
Ishikawa cause and effect analysis A pictorial method for grouping information and understanding the relationships between cause and effect.
Root cause analysis A method for tracking back to the root or originating causes of an incident or problem.

Introduction

One of the fundamental expectations of consumers is to be able to trust the safety of food supply. This means that consumers should

1. be able to rely on food businesses and a responsible management of the safety of their products and
2. be confident that regulatory authorities are competent to oversee the safety of the food supply, in particular, the operation of food businesses.

It is also recognized that in spite of all efforts, incidents – be they due to human error or other reasons – may occur and substandard products may reach the market. Experience has shown that although consumers and the general public may tolerate incidents, they will not accept repetitive incidents resulting from negligence or complacency, or deliberate violation of the law. Therefore, in the case of a failure, a responsible management must not only take immediate corrective action but also ensure that the failure does not reoccur. To this end, in the case of any incident, or a near-miss situation, a root cause analysis of the causes of the failures needs to be carried out.

A root cause analysis should not be mistaken with the investigation of the primary cause of an incident, which must be carried out as part of the management of an incident. Rather, a root cause analysis is a postmortem exercise for better understanding the underlying factors leading to an incident or a near-miss situation.

Concept of Root Cause Analysis

To understand the concept of root cause analysis, examining the way an incident occurs is important. This has been described by James Reason, and his approach to organizational incidents is used here.

In food safety assurance, a series of measures are foreseen to control hazards. These measures can be grouped under basic good practices, hazard analysis and critical control point (HACCP), and verification measures. When an incident occurs, usually it is the result of a gap or failure, or rather a series of gaps or failures, in these measures. A gap or failure in any of these measures creates a weakness or vulnerability in the food safety management system and causes the threat situation, which, if investigated and corrected immediately, prevents an incident from occurring or recurring. However, if a gap is not addressed, with time, combined with some other gaps, it may potentially lead to an incident, and if this incident is not managed effectively, it may escalate to a crisis situation. Such situations where gaps of different level and nature can combine to cause an incident are referred to as the 'Swiss cheese model' (Figure 1). An example of the additive effect of gaps in systems is the crisis where chloramphenicol was found in honey imported from China. In 2002, honey, or products containing honey, manufactured by a large number of food companies were contaminated with the illegal antibiotic chloramphenicol. A root cause analysis of the incident showed that at that time China did not have legislation against the use of chloramphenicol, and the food manufacturers in Europe were importing honey without verifying the product for chloramphenicol. The situation led to a major crisis in Europe, with loss of millions of euros (Figure 2(a)). In another incident in Israel in 2003, some 15 babies suffered from damage to the nervous system and 2 died. The incident was due to infant formula that was deficient in vitamin B1 (thiamine). In this incident, the cause of the incident was an error in product formulation, but a second failure was in the

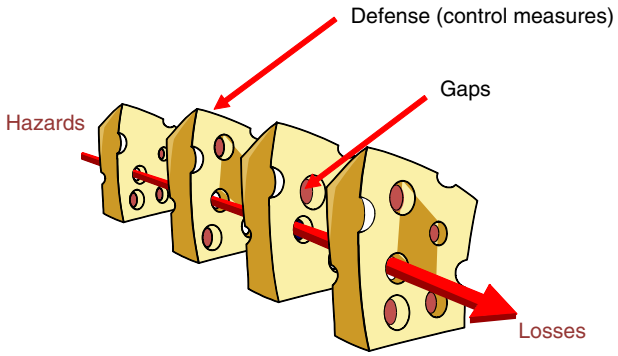

Figure 1 Swiss cheese model. Reproduced from Reason JT (1997) *Managing the Risks of Organizational Accidents*. Aldershot, UK: Ashgate.

verification of the composition of the product before its release (Figure 2(b)).

A second concept that must be understood is active and latent failures relating to management of people (Figure 3). Behind any control measure, there are people who have to implement the measures or verify that they are correctly implemented. These can, for instance, be a worker on the line or in the farm, an operator monitoring the temperature recorder, a truck driver who has to manage the temperature during transportation, a food handler who has to wash his hands before preparing food, etc. Failures to perform these tasks are referred to as active failures because their actions will have a direct and immediate bearing on the safety of products (Figure 3). These are the types of failures that are typically investigated in case of an incident or near misses. Often, as a result of the investigation, an employee is blamed, or may even be fired. Usually the investigation ends at this point. The same process and relationship also exist between regulatory authorities and food establishments that are implicated in an incident.

However, in a root cause analysis the task is to go deeper in the investigation and understand the conditions that have led to the noncompliance, i.e., understanding the reason for the implicated person to commit the so-called active failure. Worldwide, studies indicate that factors that lead to active failures are often related to the working conditions, for example, time constraint, lack of clear instructions, failure in defining the responsibility or authority of the person or in providing adequate training and coaching, creating a culture of fear or demonization, etc. Such situations are latent conditions that result from management decisions and management culture. Failures of the management in creating conditions that are optimal for managing food safety are referred to as latent failures (Figures 2(a), (b), and 3). Latent failures may not have an immediate impact, but they weaken the food safety management system and increase its vulnerability. They create opportunities for active failures and incidents. Latent failures have been the cause of numerous accidents in the petrochemical, transport, and food industries and in financial institutions.

To recapitulate, a root cause analysis requires a truthful and objective investigation of an incident at several levels, i.e., understanding

1. the primary cause of the incident: often a technical mistake, equipment failure, or human error/violation. Examples are errors in the technical parameters of a product or processing, a broken sieve, or a staff using a wrong thermocouple;
2. the conditions leading to the noncompliance of the person in charge of implementing the control measures, such as lack of training, time constraint, or difficulty to understand an instruction; and
3. the managerial decisions that have led to those working conditions, for example, failing to provide the necessary policies, to appoint a competent manager or personnel, to plan an optimum reporting and organizational structure, or to provide adequate financial or human resources, or adequate equipment, and a management behavior in contradiction, or in violation, with the instructions, or requiring impossible tasks and forcing staff to take risky shortcuts or violate the rules. Worst would be a management that promotes a fear culture or violates its own policies. This will have repercussions for the entire company.

Therefore, to ensure that root cause analyses are carried out objectively and in-depth, it is important to ensure the independence of investigators. A responsible business will accept the investigation of the root causes of incidents up to the management level, as frequently, but not always, the incident is due to shortcomings at the management level or due to a lack of management commitment.

Tools for Root Cause Analysis

Root cause analysis is used quite widely in healthcare and business settings, but as yet, it has not really been adopted by the food industry to any great extent, although the concept is identified as necessary in some Food Safety and Quality Certification Standards, for example, the BRC Global Standard for Food Safety, Issue 6. However, some of the tools of root cause analysis, notably structured failure mode and effect analysis (FMEA), have been used for some time in food companies for various applications. For example, Mortimore and Wallace advocate the use of FMEA to challenge the controls within an HACCP plan before it is implemented within a food operation, the idea being that by understanding the likely causes of failure in the control systems, the controls can be strengthened further, delivering additional confidence of food safety assurance.

As discussed earlier in this article, root cause analysis needs to investigate an incident in detail to gain an understanding of all the conditions that have led to the incident. It is necessary to consider all possible contributing factors, and this requires both a structured approach and the ability to 'think the unthinkable.'

Root Cause Analysis Teams

Like many aspects of food safety management systems, root cause analysis is best performed using a team approach rather than by an individual or individuals working alone. The team needs to include personnel from within the business who have knowledge of key areas of investigation. These include personnel who have knowledge of and responsibility within technical/quality, manufacturing, and engineering functions

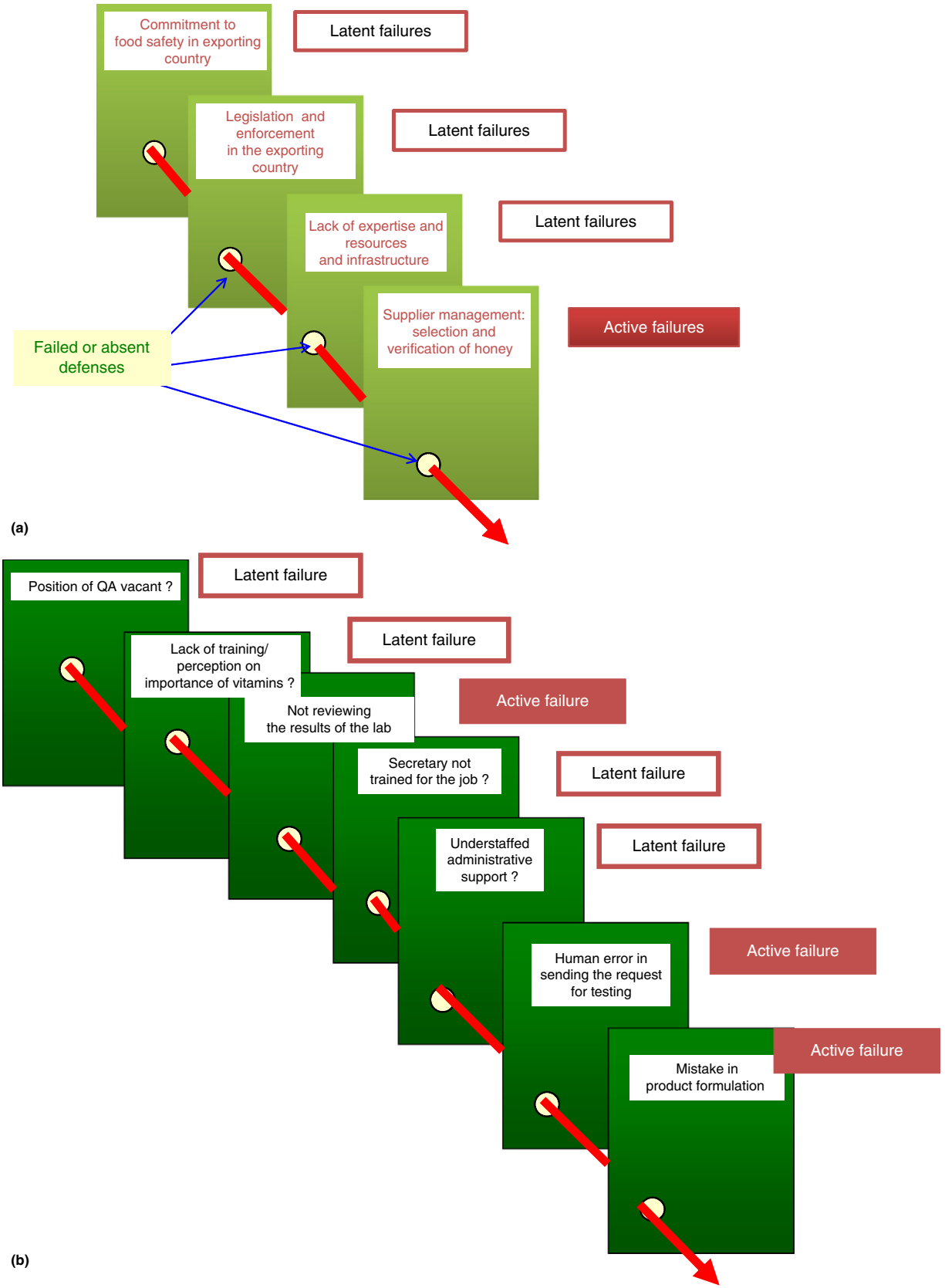

Figure 2 (a) Root cause analysis of the food safety crisis associated with chloramphenicol in honey imported from China or products containing honey. (b) An analysis of the root cause of an incident related to thiamine-deficient infant formula (Israel, 2003) based on information reported from unofficial sources. Some of the failures are mentioned for didactic reasons to illustrate the concept of root cause analysis. QA, Quality manager.

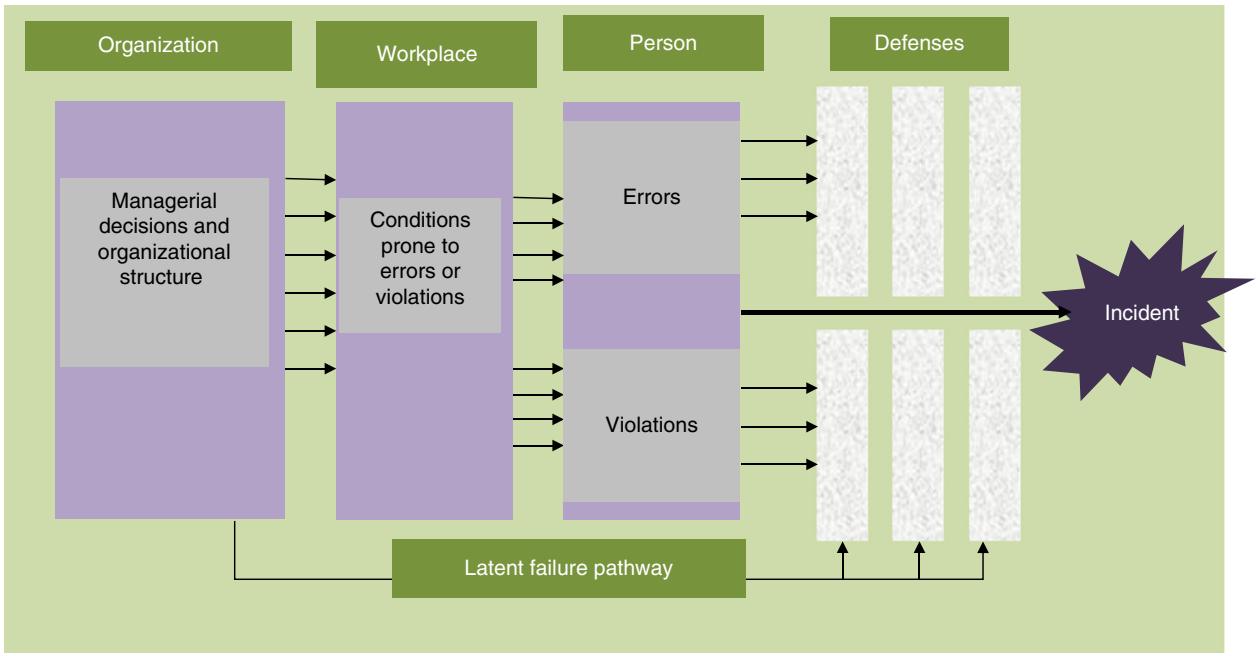

Figure 3 Levels and types of failures leading to an incident. Adapted from Reason (1995) Understanding adverse events: human factors. *Quality in Health Care* 4: 80–89.

plus additional relevant personnel, for example, human resources, purchasing, warehouse, and transport managers and so on, depending on the nature of the incident.

Structuring the Root Cause Analysis

To perform an effective route cause analysis, it is important to use a stepwise approach and take the time to gain a detailed understanding at each stage before moving on. Figure 4 shows the steps of the structured approach to root cause analysis. Although there is general agreement on the necessary actions, various texts on root cause analysis use different numbers of steps within their models. A seven-step process is used here because it covers both the analysis and the implementation and verification of corrective actions.

Step 1 Define the Fault/Incident

Members of the root cause analysis team first need to understand what has gone wrong. At this stage it is helpful to compile as much information as possible about the fault or incident, i.e., a summary of what has gone wrong, including as much as possible the sequence of events and what has been done so far in terms of immediate corrective action and incident management. This information is useful as background to the team, allowing everyone to gain an appreciation of the incident situation.

Step 2 Collect Data

Next, it is important to collect further, more detailed information that will assist in evaluating the problem. For example, this might include the following:

- Product test results.
- Lists of implicated products or processes.

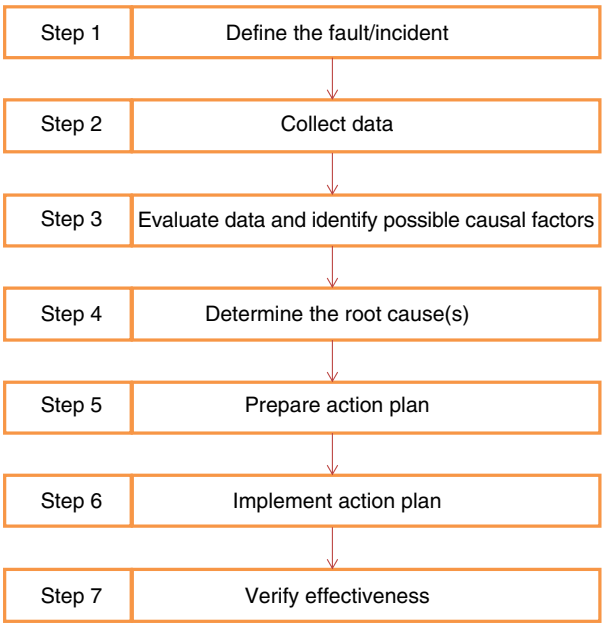

Figure 4 Root cause analysis – seven-step process.

- Lists of raw materials associated with implicated products and processes.
- Lists of packaging materials.
- Monitoring results covering dates and times thought to be implicated (the results sample(s) should be large enough to capture all relevant data around the suspect dates, i.e., building in a margin of error).
- Corrective action records covering the dates and times thought to be implicated.

- Engineering and maintenance records.
- Pest management records.
- Complaint records and customer contact information.
- Any other relevant information, for example, interview information from staff, etc.

Brainstorming will be a useful tool to make sure that all the necessary information sources can be identified and then members of the team can be allocated particular records to obtain and review on a preliminary basis.

Step 3 Evaluate Data and Identify Possible Causal Factors

To identify the possible causal factors, you need to evaluate and discuss all the data you have collected so far and also identify any further information required. This is best done by the team working together to discuss the information found and by bringing in additional personnel as necessary to help understand the situation, for example, factory floor staff who are familiar with the ongoing processing situation and additional experts (possibly external) who can advise on specific issues. Further discussion and brainstorming will help to elucidate the possible causal factors, and these all need to be recorded by the group or its appointed secretary/scribe.

Step 4 Determine the Root Cause(s)

The list of possible causes needs to be considered further by the team, evaluating how each one may have contributed to the problem. The use of tools from the root cause analysis toolbox (see section Root Cause Analysis Toolbox) will help the team understand how the possible causes may be interrelated and will assist in tracking backward to the root cause(s). Grouping techniques such as the Ishikawa Cause and Effect Analysis and questioning techniques such as the 5-Whys are particularly helpful in this context, although teams may also find some of the other tools helpful in prioritizing possible causes from their list.

The team should agree on the root cause or root causes (likely if there are distinctly different gaps or causal factors involved in an incident). The discussion can then progress onto what needs to be done to address the root cause(s). Additional tools from the root cause analysis toolkit can be helpful at this stage, such as the FMEA, which considers the current controls and then identifies recommended new controls for each cause of failure.

Step 5 Prepare Action Plan with Timescales and Responsibilities

The team's recommendations for new controls, systems, personnel, and infrastructure actions need to be built into an action plan with appropriate timescales for completion/implementation. Appropriate responsibility from the management hierarchy should be defined for each action point and personnel should be advised accordingly.

Step 6 Implement Action Plan

Individual actions on the action plan need to be implemented and signed off as complete. Depending on the nature of the actions and the timescales involved, this will need close management to make sure the plan stays on track; this can be led by members of the root cause analysis team.

Step 7 Verify Effectiveness

Verification of effectiveness is the final step in the root cause analysis process, and this is done to check that the necessary changes identified in the action plan are actually working in practice and are effective at addressing the root cause of the problem. It is also important to check at this stage that the changes have not introduced any other problems that were not foreseen. Verification can be done using audit techniques, and following verification it is likely that the business will wish to implement additional monitoring around the changes within the normal scheduled monitoring activities.

Root Cause Analysis Toolbox

A wide range of management problem-solving tools may be used in root cause analysis, and companies will find their own preferences with the experience of trying different approaches. There are no precise rules for this; it is all about getting to understand all the possible contributing factors to gain an understanding of the likely chain(s) of events leading to the incident. This will allow prioritization of necessary changes to control systems, infrastructure, and/or management practices. The following short notes are intended to help businesses understand the strengths of a selection of tools used in root cause analysis within different industries. Further, more detailed discussions on the different tools can be found in other management and problem-solving handbooks. Trial of some of these techniques within the business outside of an incident situation, perhaps as part of a business improvement project, will allow identification of preferred tools that can be used when an incident occurs.

Following is a menu of possible tools:

Brainstorming

Brainstorming is an established management tool used to capture ideas from the individuals within a group. It is particularly useful because it allows for a large number of ideas to be generated in a short time and the lateral thinking involved means that initial ideas spark off other ideas and contributions from other group members. Ideas are never criticized or commented on during the brainstorming session because this may influence or even stifle subsequent suggestions. The key point is to get as many solutions down as possible for later evaluation and it is normally necessary to allocate the role of scribe to one team member in order to record the ideas effectively.

Failure Mode and Effect Analysis (FMEA)

FMEA is well known as one of the systems that helped to originate the HACCP approach to food safety management. Its method of considering the causes and potential effects of failure is useful in looking at prevention of problems, but it can also be employed when investigating all the potential causes of an issue in an incident. Table 1 shows an example of FMEA being used to explore the causes of metal complaints due to metal detection failure.

Some FMEA methods include a risk-scoring approach although this is not often used in food manufacturing. However, it can be seen from the example in Table 1 that the sheer number of possible causes might mean that there is a need for prioritization of the recommended solutions/controls.

Table 1 Challenging metal detection failure using failure mode and effect analysis

Issue (outcome of failure)	*Failure*	*Current control*	*Possible causes of failure*[a]	*Recommended controls*
Complaints of metal in product from customers. This could result in lost credibility, lost customers, and bad publicity. Worse still, metal in product could cause customer injury and may result in prosecution.	Failure to detect metal in products[b]	Check metal detector hourly with test pieces and record result	Metal detector breakdown Metal detector not properly calibrated Wrong sensitivity check pieces Incorrect metal detector in use – wrong sensitivity Metal detector in wrong place in line Rejection mechanism faulty Rejection system not synchronized with detector Rejects not controlled Metal detector checks not done Metal detector checks done incorrectly Metal detector check reveals failure but this is not recorded Metal detector check reveals failure but no corrective action taken Staff not trained to perform metal detector checks Effectiveness of training not verified in terms of practice Workplace culture issues result in staff not taking responsibility for necessary checks	A range of controls will need to be considered around: ● Appropriate sensitivity and calibration ● Set-up verification at start up – correct sensitivity ● Maintenance systems Lockable receptacle needed that will accommodate all rejects A range of controls will need to be considered around: ● Appropriateness and coverage of training – are enough people trained and can they actually do the checks? ● How can training effectiveness be verified? ● What supervision is needed? ● Are the checks allocated within appropriate job roles and instructions? ● Management systems and commitment issues need to be investigated

[a]This will be a brainstormed list of ideas from the root cause analysis team.
[b]This is likely only one failure mode associated with the issue. Other failure modes to consider will include how the metal got into the product, for example, consideration of raw material streams and processing/equipment maintenance issues on site or possible damage of the products in distribution.
Source: Adapted from Mortimore SE and Wallace CA (1998) *HACCP: A Practical Approach*, 2nd edn. Gaithersburg: Aspen Publications.

This can be done using a simple likelihood of occurrence scheme, for example, high, medium, and low likelihood. Severity may also be considered, although it is likely that severity may be relatively similar in some cases, for example, in Table 1 the possible causes may all result in undetected metal in product.

5-Whys

The 5-Whys is a simple problem-solving technique that helps users to get to the root of the problem quickly. Made popular in the 1970s by the Toyota Production System, the strategy involves looking at any problem and asking 'Why?' and 'What caused this problem?' Normally the answer to the first 'Why' will prompt another 'Why' and the answer to the second 'Why' will prompt another and so on. It is thought that at least five questions need to be asked to track back to the root cause, hence the name the 5-Whys strategy. In reality, more than five questions may need to be asked depending on the complexity of the situation.

5-Whys helps the root cause analysis team to start at the end result and work backward toward the cause by continually asking 'Why?' until the underlying cause of the problem becomes clear. In addition to its use in root cause analysis, it is useful at the start of a remodeling or change process and is a recognized lean manufacturing technique, challenging those working on an issue to analyze any problematic situation in a logical manner, thus enhancing change and continuous improvement.

A number of benefits of the 5-Whys approach have been recorded.

- Simplicity: It is easy to use and requires no advanced mathematics or tools.
- Effectiveness: It helps to quickly separate symptoms from causes and identify the root cause of a problem.
- Comprehensiveness: It aids in determining the relationships between various causes of the problem.
- Flexibility: It works well alone and when combined with other quality improvement and troubleshooting techniques.
- Engaging: It fosters and aids teamwork and teambuilding within and outside the organization.
- Inexpensive: It is a guided, team-focused exercise. There are no additional costs.

Ishikawa Cause and Effect Analysis

This approach (also known as Fishbone Diagram) is a pictorial method (Figure 5) of organizing information about

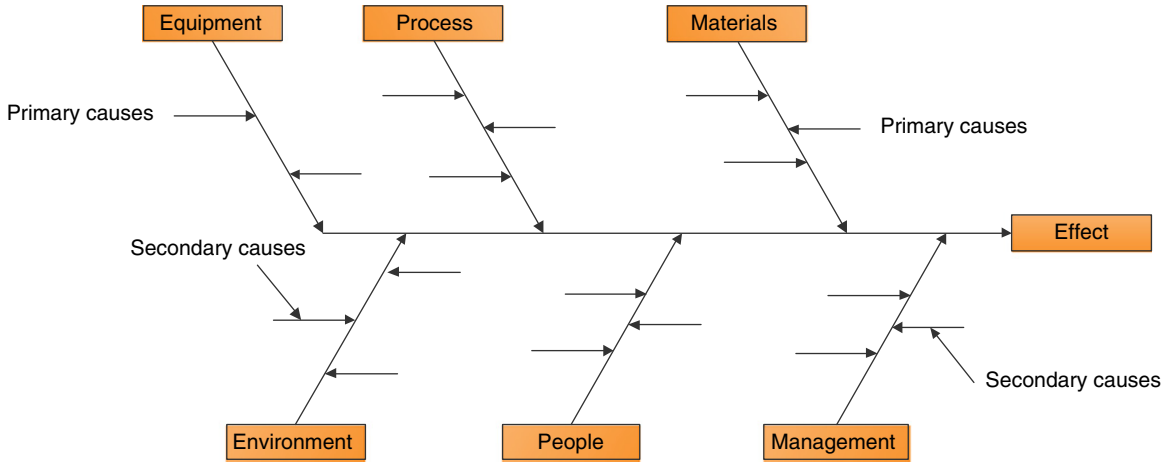

Figure 5 Example Ishikawa cause and effect analysis diagram.

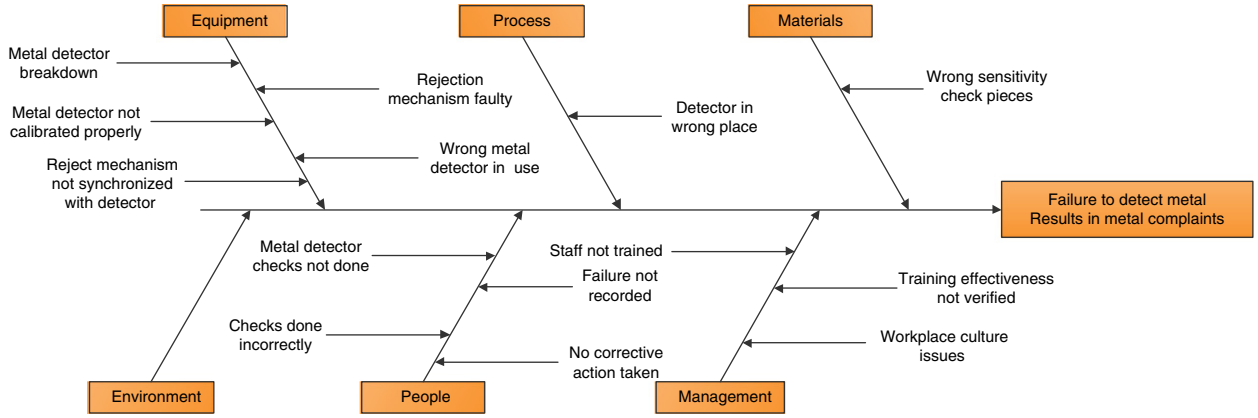

Figure 6 Ishikawa cause and effect diagram for metal contamination example.

causes and understanding the relationships between cause and effect. It is a widely used technique in problem solving, and seeks to understand the possible causes by asking questions such as 'What happened?,' 'When?,' 'Where?,' 'Why?,' 'How?,' and 'What was the impact?' The Ishikawa analysis is useful in evaluating complex situations where there may be many potential causes.

In Figure 5, it can be seen that causes are grouped into six categories of equipment, process, materials, environment, people, and management. These are commonly used category groupings in manufacturing situations; however, the categories in the Ishikawa analysis are not predetermined, so it is possible to choose your own groupings. The diagram also shows how primary and secondary causes are portrayed and in this way the causes and causes of the causes can be identified, helping to work back to the root cause. Figure 6 shows how this method can be applied to an incident, based on the metal complaints issue from Table 1.

This example (Figure 6) shows one way of grouping the possible causes identified; however, it is important to note that some causes could be grouped under more than one heading. Also in this case only the primary causes are shown; these would need to be followed up with consideration of the secondary causes and it is possible that, with further consideration, some of the points listed under the 'management' grouping might be the secondary causes affecting other groups within the diagram. There is no right or wrong way here – it is up to the team to decide how best to portray the data in their unique situation.

Hazard and Operability Studies (HAZOPs)

This is another structured and systematic technique for examining potential faults in systems. Like HACCP, HAZOP is often used as a technique for identifying potential hazards in a system, but it also focuses on identifying operability problems that are likely to lead to nonconforming products. In HAZOP, faults or incidents are thought to be caused by deviations from design or operating intentions.

In HAZOP, the identification of deviations from the design intent is achieved by a questioning process using predetermined 'guide words.' The role of the guide word is to stimulate imaginative thinking, to focus the study and elicit ideas and discussions, thereby maximizing the chances of study completeness. Further detailed guidance on how to use HAZOP,

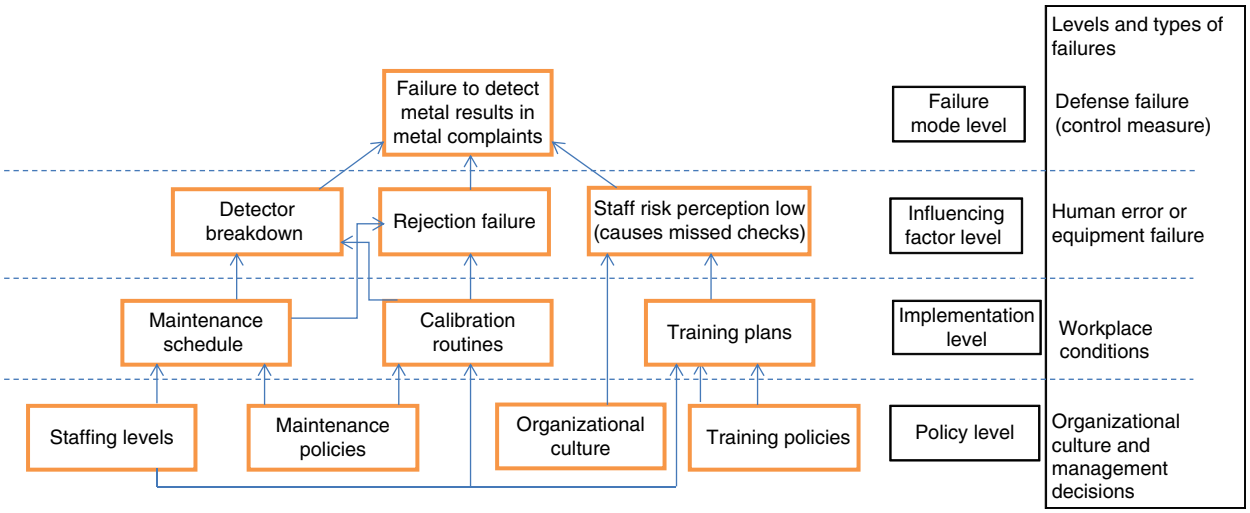

Figure 7 Example influence diagram for metal complaints (selected causes only).

including lists of typical 'guide words,' can be found in the International Electrotechnical Commission's guideline Hazard and Operability Studies (HAZOP Studies) Application Guide (BS IEC 61882:2001).

Influence Diagrams

The influence diagram approach is another technique for visual portrayal of causal factors involved in an incident. The outcome diagram is derived in similar ways to the other tools already discussed, in that expert input, group discussion, and brainstorming techniques are used. The technique differs in that it considers the possible causal factors occurring at different levels in the organization. According to Reason, the levels to be considered are as follows:

- Influencing factor level: The unsafe acts or technical failures immediately responsible for the event.
- Performance-influencing factor level: The immediate workplace conditions that shape the occurrence of human or technical failures.
- Implementation level: The underlying organizational factors that create the workplace performance-influencing factors.
- Policy level: Policy and regulatory factors that determine organizational processes occurring at the implementation level.

An example influence diagram is shown in Figure 7. In this diagram the levels and types of failures that can result in an incident that were previously outlined in Figure 2 are also highlighted on the right-hand side, indicating the practicality of application of the influence diagram approach to food safety incidents.

Additional Specialist Tools

A variety of other tools are used for problem solving in different industries. Root cause analysis teams may wish to consult the 'problem solving,' 'risk management,' and 'error avoidance' literature to identify techniques that could be trialed for suitability in the analysis of incidents. Some of these examples are more quantitative and involve risk-rating categories, which might be more difficult to apply in a food-manufacturing scenario. Further tools used in other sectors include Fault Tree Analysis, the Human Error Assessment and Reduction Technique (HEART) and the Maintenance Error Decision Aid (MEDA).

As can be seen from the list, the root cause analysis toolbox contains a plethora of techniques that will assist when faced with an incident to investigate. Using any of these tools does require practice so there is no substitute for trialing chosen tools when not in the middle of an incident; the majority of these tools are also useful in preventative improvement projects, which would be a much more suitable time to try them out. A further important point is that there is no substitute for involving the correct people in root cause analysis, so it is important to consider carefully who can contribute to the understanding of the incident and its causes.

Summary

The management of a company bears the ultimate responsibility for incidents. They are responsible for creating an organizational culture that allows employees to openly report issues and provides them with the opportunity to see that their concerns are adequately addressed. An open and fair organizational culture is fundamental for the motivation of staff and is at the core of food safety management. In the case of an incident, they have to not only follow best practice in managing the ongoing incident but also be candid with analyzing the root cause of the incident, such that they can redress the situation in a fundamental way to prevent recurrence of incidents in a long-lasting manner.

As for crisis management, the lessons learned from incidents need to be reported in a final report and disseminated both internally in the organization and externally with the food safety community at large in order to prevent the recurrence of incidents in the society.

See also: Public Health Measures: Modern Approach to Food Safety Management: An Overview

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FOOD SAFETY ASSURANCE SYSTEMS

Food Safety and Ethics

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Glossary

Ethics Ethics is the philosophical study of the moral value of human conduct and of the rules and principles that ought to govern it.

Precautionary principle The precautionary principle states that if a product, an action, or a policy has a suspected risk of causing harm to the public or to the environment, protective action should be supported before there is complete scientific proof of a risk.

The common good approach The common good approach links ethics to social responsibility and calls attention to the common conditions that are important to the welfare of everybody.

The fairness approach The fairness approach treats all human beings equally based on some standards that are defensible.

The right approach The right approach states that humans have a right to be treated as ends and not merely as means to other ends.

The utilitarian approach The utilitarian approach deals with consequences, and tries to increase the good done and reduce the harm done.

The virtue approach The virtue approach emphasizes dispositions and habits that enable humans to act according to the highest potential of their character.

Food Safety and Ethics

Food production is a complex matter, affecting people' s life, organizations profits, and the well-being of the whole planet. It is not always straightforward to say what is right and what is wrong when it comes to production of food. Many ethical questions can be raised. Climate change, animal welfare, fair trade, health and safety of employees as well as consumers, fair treatment of employees and their social rights, economic sustainability, and use of natural resources are all important dimensions within the system of production, processing, and trade, and where every food item often includes value conflicts.

Ethics is defined as the philosophical study of the moral value of human conduct and of the rules and principles that ought to govern it. Simply stated, ethics refers to standards of behavior that tell us how human beings ought to act in the many situations in which they find themselves – as a friends, parents, children, citizens, businesspeople, teachers, professional food producers, consumers, etc. Some ethicists emphasize that the ethical action is the one that produces the greatest good and does the least harm for all those who are affected – customers, employees, shareholders, the community, and the environment 'the utilitarian approach.' The utilitarian approach deals with consequences; it tries both to increase the good done (e.g., ending hunger) and to reduce the harm done (e.g., environmental and social destructions). Other philosophers and ethicists suggest that the ethical action is the one that best protects and respects the moral rights of those affected 'the rights approach.' This approach starts from the belief that humans have a dignity based on their human nature per se or on their ability to choose freely what they do with their lives. On the basis of such dignity, they have a right to be treated as ends and not merely as means to other ends. According to the Rome Declaration, everybody has a right to adequate and safe food and a fundamental right to be free from hunger. The list of moral rights – including the rights to make one's own choices about what kind of life to lead, to be told the truth, not to be injured, to a degree of privacy, etc. – is widely debated; some now argue that nonhumans like animals and plants have rights, too. Also, it is often said that rights imply duties – in particular, the duty to respect others' rights. Aristotle and other Greek philosophers have contributed the idea that all equals should be treated equally 'the fairness or justice approach.' Present day, this idea is used to say that ethical actions treat all human beings equally, or if unequally, then fairly based on some standard that is defensible. The power distribution between retailers and suppliers have led many producers to state that multiple food retailers are abusing their position of power and engaging in practices that adversely affects the competitiveness of suppliers. To address these adverse effects it has been recommended that a code of practice be introduced to govern retailer–supplier relationships. The Greek philosophers have also contributed the notion that life in community is a good in itself and our actions should contribute to that life 'the common good approach.' This approach links ethics to social responsibility and calls attention to the common conditions

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that are important to the welfare of everyone. Companies have a duty to be good citizens including their own workers and staff and 'to do the right things.' A very ancient approach to ethics is that ethical actions ought to be consistent with certain ideal virtues that provide for the full development of our humanity 'the virtue approach.' These virtues are dispositions and habits that enable us to act according to the highest potential of our character and on behalf of values like truth and beauty. Honesty, courage, compassion, generosity, tolerance, love, fidelity, integrity, fairness, selfcontrol, and prudence are all examples of virtues. Virtue ethics asks of any action, 'What kind of person will I become if I do this?' or 'Is this action consistent with my acting at my best?'

Each of these approaches mentioned above helps us determine what standards of behavior can be considered ethical. Different actors may not agree on the content of some of these specific approaches. They may not all agree to the same set of human and civil rights or on what constitutes the common good. They may not even agree on what is good and what is bad. Nonetheless, each approach gives us important information with which to determine what is ethical in a particular circumstance. And much more often than not, the different approaches do lead to similar answers.

So what are the ethical dilemmas in food safety? Is it reasonable to forbid sales of cheese made with unpasteurized milk? Here, food safety is set up against people's pleasure of eating tasty cheese, traditional food cultures, and the food industries economical interest. Is unpasteurized cheese safe enough? Similar questions can be asked about raw minced meat (steak tartar) served in some restaurants, raw milk sold in some countries, or worse, when raw milk is given to children. In theory, a fully informed consumer might decide which food-related risk to take and which to avoid. But what does it mean to be fully informed? Can we expect that all consumers are able to collect the detailed information about the wide array of food safety issues and make their own decisions? Are there any good strategies for providing relevant information in such a way that consumers understand the risk? Is it the correct thing to delegate this responsibility to responsible authorities? What does it mean in terms of responsibilities for authorities? And what if the consumers do not trust these authorities? Finally, who becomes responsible when illness occurs: Industry which has provided a contaminated product, authorities who have failed to educate consumers, or consumers themselves who have not taken the necessary precautions?

Another key question is when a food industry transfers responsibility of safety to consumers without providing proper warnings, crystal clear instructions on safety measures, or even worse, market and promote a product in a society when it knows that consumers would not be able or have means, to ensure its safety. The latter issue was raised with the question of breast milk substitute in the developing countries, until WHO established the Code of Breast milk substitute that responsible companies comply with. The issue of warnings on food package is still not well addressed in most legislation and the clarity with which drug providers or electrical equipment or aviation companies provide information has not yet been established in the food industries. This has made some companies to write an ambiguous text that do not raise concern of consumers, but in case of an incident, the company is covered and can decline any responsibility.

Food safety management relies very much on a fair and professional management of people, including ensuring that employees are competent for their job, have received the proper training and briefing about their responsibilities. They are given the means and authority to do a professional job, and also setting the right reporting structure minimizing conflict of interest in audits and investigation of incidents, and most of all a company culture which fosters reporting and openly discussing problems, protecting and rewarding whistleblowers, and a management which walk the talk and follow its own policies. Unfair practices cannot only lead to discouragement and an uncaring attitude of employees, but occasionally has also been the source of outrage and sabotage of the food (i.e., tampering), thus a risk for consumers. The company culture is an area which is not legislated and enforced by law, but is one of the most important aspects of the ethical practice of a company as it impacts on all aspects of operations, including safety and health and the social right of employees, as well as safety of products and health of consumers.

Finally, one of the most fundamental aspects of ethics in food industry is the commitment and 'real will' of the company to build a solid food safety assurance system or just to do the minimum necessary to meet the requirements of legislation and certification bodies. As safety of food products is the outcome of the food safety assurance system (including the professional management of its staff) of food companies, no matter how comprehensive and strong the regulatory system of a country may be, it cannot replace the everyday vigilance of managers, workers of a company who have to oversee the safety of products.

The Precautionary Principle

The precautionary principle states that if a product, an action, or a policy has a suspected risk of causing harm to the public or to the environment, protective action should be supported before there is complete scientific proof of a risk. In the absence of scientific consensus, the principle implies that there is a social responsibility to protect the public from potential harm. This is a 'better safe than sorry' or 'caution in advance' principle that applies both to human health and to environmental protection.

One of the primary foundations of the precautionary principles, and globally accepted definitions, results from the work of the Rio Conference, or 'Earth Summit' in 1992. Principle #15 of the Rio Declaration notes:

"In order to protect the environment, the precautionary approach shall be widely applied by States according to their capabilities. Where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation."

The application of the precautionary principle has been made a statutory requirement within the European Union (EU), and according to a Food and Agriculture Organization of the United Nations Expert Consultation report on food safety

international, food safety organizations must make clear that science, although an important tool, is not sufficient in itself for food risk analysis and that it needs to operate within an ethical framework. The problematic cases are the ones where there are disagreements about the value judgments made in the risk assessment. For Novel Food, new technologies, or newly identified hazards, the answer to what is 'safe' may not be the subject of a consensus. When both the likelihood of damage and the consequences of damage are unknown, then risk assessment becomes difficult and the assessors face ethical dilemmas. Genetically modified organisms (GMO), is one example of a new technology which has created a lot of debate. The presence of different interest groups and diverse citizens' values in different political arenas triggered a range of policy responses to GMOs in the 1990s. Although GMO was strongly supported by the scientific and biotech industry in the USA, and lead to a flourish of GM crops, European citizens were strongly opposed by GM plants. Consumers were skeptical and talked about 'unnatural Frankenstein's food.' They lacked confidence due to their governments' response to a series of food scares in the 1990s. Today, the GMO regulatory framework in the EU is different from the one in US and more in line with the public's perception of risk than with the scientific definition of risk. The precautionary principle is applied in the EU and is constantly challenged. A main ethical question which raised a controversy with regard to GM foods was few years ago when EU countries would, as part of food aid, propose GM foods to the developing countries (Africa). In 2002, Zambia announced it would not accept GM food aid in any form. So the question was, why a technology and product rejected by the European population will be good for the African population? Most African countries approach GM technology applied to crops with caution. "Why shouldn't we be wary of this technology and its possible long-term health impacts, if the EU is? If it is not good for them, why should it be good for us?" said Tewolde Egziabher, Ethiopia's director of the Environmental Protection Agency. Positions were polarized to a great extent after a quote from a US state department official, "Beggars can't be choosers," hit the headlines. It prompted the then president, Levy Mwanawasa, to say hunger was no reason for feeding his people "poison."

The evolutionary aspect of the food system, influences risk assessment of food safety and triggers some ethical dilemmas. Although, consumers' food variety increases and the food industry potentials for new business grow, there are food safety issues to consider. The food market is becoming more and more global. Not only food, but also food pathogens are distributed around the world. In Norway, sugar peas from Kenya lead to an outbreak of dysentery in 2009, probably due to consumption of raw products. In Kenya, people boil or fry vegetables before consumption. This is not the case in Norway where unpeeled fruit and raw vegetables are consumed frequently. In many ways Norway is a food safety oasis in Europe, with a livestock population virtually free from Salmonella and where only one out of nine national outbreaks of infectious intestinal diseases linked to lettuce, sprouts, sugar peas, and basil in the past 20 years came from Norwegian produce. The problem is that consumers' food safety habits and routines, inherited from parents, are not always adapted to handle new food scares from imported products. When food and food pathogens change, whereas food preparation routines stay the same, then food safety becomes an issue. The solution to this food safety problem raises ethical questions related to freedom of choice, economic prosperities for developing countries, distribution of pathogens into clean areas, etc.

A related issue is the question, to what extent food safety should be regulated and harmonized across the world? On the one hand harmonization of regulation is important from a human rights perspective as all human beings, regardless of their color, race, gender, and culture should have the right to the same level of health protection and thus food safety, and products rejected in one country should not be dumped in another. However, for various reasons, presently the same level of safety is not achievable in all countries and trying to meet a high standard of food safety in some countries may be to the detriment of causing hunger and malnutrition. On the other hand, failing to harmonize food safety regulations has lead to a double standard in some countries, i.e., foods produced for export have a higher standard of quality and safety, whereas foods of inferior safety and quality standard, possibly with higher level of contamination, is directed to the domestic market and local people. However, can one deny a higher level of health protection to countries which can meet these?

Ethical Decision Making

Making good ethical decisions requires a trained sensitivity to ethical issues and a practiced method for exploring the ethical aspects of a decision and weighing the considerations that should impact our choice of a course of action. Having a method for ethical decision making is absolutely essential. The more novel and difficult the ethical choice we face, the more we need to rely on discussion and dialog with others about the dilemma. Only by careful exploration of the problem, aided by the insights and different perspectives of others, can we make good ethical choices in such situations.

In a company where ethics is a valued principle, the management ensures that food safety is managed professionally and objectively. However, the ethic of its management becomes conspicuous particularly in time of an incident or a conflict. This will be demonstrated by questions such as to what extent the company will:

1. voluntarily acknowledge a contamination or and if necessary recall its products,
2. investigate the root cause of the incident up to the management level,
3. accept loss of benefits to protect consumers,
4. act transparently and reveal information on the incident and its cause, and also,
5. take punitive actions against those who have knowingly and irresponsibly violated the policies (note: Errors are different from violations and should be not subject of punitive actions, see other articles).

In the health care or aviation sectors, reporting of noncompliance or problems, and independence in investigations of incidents is much more advanced and can be a model for the food sector if food safety is to be strengthened.

At the national level, ethics of the regulatory authorities is proven by the system of decision making that they put in place to ensure:

1. independence of health sciences and institutions generating scientific and technical data,
2. an unbiased decision making process with separation of risk assessment and risk management function,
3. priority to consumer health over economic considerations, and
4. mechanism for reporting malpractices and noncompliances, for example, protection of whistle-blowers.

Other Aspects of Ethics of Relevance to Food Industry

Over and above aspects of ethics in relation to food safety, few words should also be said on other practices in the industry that are unethical. For instance, abuse and mistreatment of animals, child labour, negligence leading to environmental damages, or fraud and deceitful practices such as the recent horse and pork meat scandal, which was revealed as this article is going into publication.

In the latter mentioned case, the deceitful practice of the food industry was perhaps not affecting safety of products *per se* but in other cases, such as the incident of melamine in USA in 2007 and repeated again in China in 2008, it did have serious health consequences for consumers.

Although the fraud of replacing beef with horse and pork meat took place at the suppliers of minced meat products, the responsibility of the processors and manufacturers who were manufacturing and marketing ready-to-eat foods is not less, as they were responsible for verifying their suppliers. The gravity of the scandal does not only lie in the fact that some people unknowingly and against their personal values ate horse or pork meat, but the fact that so many people were aware of the fraud, yet they turned a blind eye. Under such circumstances the door is open to any wrongdoings and consequently there cannot be a guarantee for food safety as what cannot be anticipated cannot be prevented. This scandal also revealed the poor conditions in which horses were maintained and transported.

To promote ethical practices in companies, the public at large should value ethic and be outspoken about it, for instance by the choice of the products they buy. The experience from the bovine spongiform encephalopathy crisis showed the power of consumer pressure in promoting health considerations in the decision making process. Subsequently, giving priority to health over economic considerations has today become a key principle in risk management. Similarly, in a national referendum in 2013 in Switzerland, referred to as the Minder Initiative, the Swiss public expressed its dismay with disproportionate high salaries of managers in companies, and forced politicians to take legislative measures to limit such practices, showing the power of public and consumers in changing practices and promoting ethics.

However, over and above that of the general public, the responsibility of poor ethical practices in any company, or any other organization, falls on the staff of the organization, who should also react and challenge the decision-makers to force them to behave ethically and respect the international standards and charters in the areas of human rights, environment, animal welfare, consumer health, etc.

Conclusion

The likelihood of becoming sick from the next meal has probably never been less than it is today, but the long-term consequences of today's food production is less known. The production process has increasingly become complex and less transparent and consumers are no longer in control of the production system. They need to trust food producers, food manufacturers, and retailers. Consumers are worried, some of these worries are directly linked to the risks involved, be it real or perceived. Other worries are more linked to ethical questions related to well-being, free choice (autonomy), and fairness (justice). Availability of safe food needs to be addressed in relation to factors, such as respect for consumer choice, right to information on safety, universally affordable food, adequate income, and working conditions for employees and workers, fair practice in trade, animal welfare, and sustainability of biotic populations. Also, consumers are worried about new technologies and if these new techniques take into consideration their health and safety, or if they merely are developed for business interest and the benefits of producers. Some consumers wonder to what degree science is being developed impartially, and if governments and public health authorities give priority to consumer's health in their opinion on risks and risk management options, and if incidents are investigated independently and transparently.

A dialog about the ethical implications of food production, processing, policy, supply, and consumption, may help involved partners making better decisions. The discussion needs to be lifted up to a level above what each company at any given point in time feels is best for them.

Aristotle says that identifying the good with pleasure is to prefer a life suitable for beasts.

"It is better to be a human being dissatisfied than a pig satisfied; better to be **Socrates** dissatisfied than a fool satisfied. And if the fool, or the pig, are of a different opinion, it is because they only know their own side of the question… "

Every company has a social responsibility. Not behaving according to accepted norms for ethical behavior may not only have consequences for food safety, but also on a company's image, reputation, and performance. Ethics is not a question of thoughtless and slavish worship of rules, and to scrupulously checking every action against a table of do's and don'ts. The fundamental question of ethics is not 'What should we do?' but 'What image would we like for our company?' Will we accept compliances, deceive full negligence or noncompliance as long as we are not caught, or will we be vigilant in any condition?

"Integrity is doing the right thing, even when no one is watching (Clive Staples Lewis,1898–1963)," and ethics is to the industry what integrity is to a person.

See also: Food Safety Assurance Systems: Investigation of Incidents in Industry; Root Cause Analysis of Incidents. Public

Health Measures: Modern Approach to Food Safety Management: An Overview. Risk Analysis: Risk Management: Application to Biological Hazards

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INSTITUTIONS INVOLVED IN FOOD SAFETY

Contents

FAO/WHO Codex Alimentarius Commission (CAC)

T Heilandt, FAO, Rome, Italy
CA Mulholland and M Younes, World Health Organization, Geneva, Switzerland

Glossary

Contaminant Any substance not intentionally added to food, which is present in such food as a result of the production (including operations carried out in crop husbandry, animal husbandry and veterinary medicine), manufacture, processing, preparation, treatment, packing, packaging, transport or holding of such food or as a result of environmental contamination. The term does not include insect fragments, rodent hairs and other extraneous matter.

Food Any substance, whether processed, semi-processed or raw, that is intended for human consumption, and includes drink, chewing gum and any substance that has been used in the manufacture, preparation or treatment of "food" but does not include cosmetics or tobacco or substances used only as drugs.

Food additive Any substance not normally consumed as a food by itself and not normally used as a typical ingredient of the food, whether or not it has nutritive value, the intentional addition of which to food for a technological (including organoleptic) purpose in the manufacture, processing, preparation, treatment, packing, packaging, transport or holding of such food results, or may be reasonably expected to result, (directly or indirectly) in it or its by-products becoming a component of or otherwise affecting the characteristics of such foods. The term does not include "contaminants" or substances added to food for maintaining or improving nutritional qualities.

Food hygiene Comprises conditions and measures necessary for the production, processing, storage and distribution of food designed to ensure a safe, sound, wholesome product fit for human consumption.

Hazard A biological, chemical or physical agent in, or condition of, food with the potential to cause an adverse health effect.

Pesticide Any substance intended for preventing, destroying, attracting, repelling, or controlling any pest including unwanted species of plants or animals during the production, storage, transport, distribution and processing of food, agricultural commodities, or animal feeds or which may be administered to animals for the control of ectoparasites. The term includes substances intended for use as a plant growth regulator, defoliant, desiccant, fruit thinning agent, or sprouting inhibitor and substances applied to crops either before or after harvest to protect the commodity from deterioration during storage and transport. The term normally excludes fertilizers, plant and animal nutrients, food additives, and animal drugs.

Risk A function of the probability of an adverse health effect and the severity of that effect, consequential to a hazard(s) in food.

Risk analysis A process consisting of three components: (1) Risk assessment that is a scientifically based process consisting of the following steps: (i) hazard identification, (ii) hazard characterization, (iii) exposure assessment, and

(iv) risk characterization. (2) Risk management, which is the process, distinct from risk assessment, of weighing policy alternatives, in consultation with all interested parties, considering risk assessment and other factors relevant for the health protection of consumers and for the promotion of fair trade practices, and, if needed, selecting appropriate prevention and control options. (3) Risk communication, which is the interactive exchange of information and opinions throughout the risk analysis process concerning risk, risk-related factors and risk perceptions, among risk assessors, risk managers, consumers, industry, the academic community and other interested parties, including the explanation of risk assessment findings and the basis of risk management decisions.

Traceability/product tracing The ability to follow the movement of a food through specified stage(s) of production, processing and distribution.

Veterinary drug Any substance applied or administered to any food producing animal, such as meat or milk producing animals, poultry, fish or bees, whether used for therapeutic, prophylactic or diagnostic purposes or for modification of physiological functions or behavior.

Safe, Good Food for Everyone – The Need for an International Food Code

Foodborne illness is a major cause of morbidity and mortality around the world. Outbreaks of foodborne illness can also damage trade and tourism and lead to loss of earnings, unemployment, and litigation. People have the right to expect their food to be safe, of good quality, and suitable for consumption.

In the beginnings of agriculture and food trade, traditional knowledge, based on experience and trial and error, directed what to eat and what not and how to keep food safe. People grew their own food or bought it in exchange for services, goods, or money from someone they knew, which ensured the link between them for safety, quality, and fairness. Although some may still live that way, for most people food comes from producers who they have never met and from places they have never seen. Wholesale markets that supply local sellers, source food from anywhere around the world and buyers order items that they have not seen, touched, or smelled, thus, they have to rely on the product descriptions and guarantees that the sellers provide.

The need for rules and codes for safety and quality in food production, transport, and trade grew over time as the local food chain, involving a handful of actors, transformed into a global chain involving millions of people. National food laws exist in most countries and in order to facilitate trade and ensure harmonization, the idea of a global food code (*Codex Alimentarius*) was born.

Codex Alimentarius and Codex Alimentarius Commission

The Codex Alimentarius Commission (the Commission) – jointly sponsored by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) – held its first session in 1963. The Codex Alimentarius is a collection of international food standards, guidelines, and codes of practice whose main purpose is to protect the health of consumers and ensure fair practices in food trade. The Codex Alimentarius thus covers food safety matters (residues, hygiene, additives, contaminants, etc.) and quality matters (product descriptions, quality classes, labeling, and certification). Its internationally agreed texts allow harmonization of national requirements and, in this way, facilitate international trade.

The Codex Alimentarius serves as the basis for many national food standards and related regulations. All Codex standards and related texts, as well as all meeting information, are in the public domain and available at: www.codexalimentarius.org.

The Role of Codex Standards in International Trade

The Codex Alimentarius is a recommendation to its members. Application of Codex standards, guidelines, and codes of practice is voluntary until implemented in national or regional law. However, since 1995, after the establishment of the World Trade Organization (WTO) following the Uruguay Round of Trade Agreements, Codex standards and related texts have become international references for food safety under the WTO's Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement). Similarly, the standards of the World Organisation for Animal Health (OIE) became the reference for animal health and those of the International Plant Protection Convention (IPPC) became the reference for plant health.

For WTO members this means that, as long as they apply Codex standards for food safety measures, no other WTO member can challenge such measures as unjustified barriers to international trade. If a country wants to set stricter standards, this needs to be justified scientifically through a risk analysis process. In case of a dispute, the WTO provides the relevant dispute settlement process.

Codex texts are also recognized as international standards by the WTO Agreement on Technical Barriers to Trade (TBT) and have been used in dispute settlements. Contrary to the SPS agreement, the TBT agreement does not mention any specific standard setting organization. However, Codex texts have been used in TBT dispute settlement procedures.

How the Codex Alimentarius Commission Works

The Commission and its specialized technical subsidiary bodies (Figure 1) provide structured, neutral meetings for discussion of all topics related to food safety and trade within its mandate. Representatives from governments, consumer groups, industry, and academia meet to exchange views

Figure 1 Joint FAO/WHO Food Standards Programme (status 2012).

about food safety and trade and to adopt standards or related texts.

Statutes

The statutes are the legal basis for the Commission's work and formally reflect the concepts behind and reasons for its establishment. Article 1 of the statutes contains purposes, terms of reference, and objectives. Article 2 defines eligibility for membership of the Commission, which is open to all Member States and Associate Members of FAO and WHO.

Rules of Procedure

The Rules of Procedure of the Codex Alimentarius Commission describe: Conditions of membership; appointment and responsibilities of Commission officers (chairperson and three vice-chairpersons); appointment and responsibilities of the other members of the Executive Committee (six regional coordinators and seven members elected on a regional basis); appointment and role of the Secretary; establishment of an Executive Committee to meet between Commission sessions, to act on behalf of the Commission as its executive organ; frequency and operation of Commission sessions; nature of agendas for Commission sessions; voting procedures; observers; preparation of Commission records and reports; establishment of subsidiary bodies; procedures to be adopted in the elaboration of standards; allocation of a budget and estimates of expenditure and; languages used by the Commission.

Representation of Members and Current Meeting Schedule

In June 2012, 99% of the world's population was represented in the Commission through 184 member countries and one Member Organization (European Community). Currently, the Commission meets annually at the headquarters of FAO or WHO. Special or extraordinary sessions can be scheduled if needed. Representation at sessions is on a country basis. National delegations are led by senior officials appointed by their governments. Delegations may include representatives of industry, consumers' organizations and academic institutes. Countries that are not yet members of the Commission sometimes attend in an observer capacity. To facilitate continuous contact with member countries, members have established country Codex Contact Points, and many member countries have National Codex Committees to coordinate activities nationally.

Observers

In June 2012, over 200 International Governmental Organizations and International Nongovernmental Organizations (NGOs) had observer status with the Commission. The Codex process allows such organizations to put forward their points of view at every stage except in the final decision, which is the exclusive prerogative of members.

Codex Strategic Plan

The Commission is guided in its work by a strategic plan, which is reviewed every 5 years.

The vision statement of the present plan (2008–13) reads: "The Codex Alimentarius Commission envisages a world afforded the highest attainable levels of consumer protection including food safety and quality. To this end, the Commission will develop internationally agreed standards and related texts for use in domestic regulation and international trade in food that are based on scientific principles and fulfill the objectives of consumer health protection and fair practices in food trade."

The plan contains the following five goals which have been broken down further into a number of activities:

- Goal 1: Promoting Sound Regulatory Frameworks.
- Goal 2: Promoting Widest and Consistent Application of Scientific Principles and Risk Analysis.
- Goal 3: Strengthening Codex Work-Management Capabilities.
- Goal 4: Promoting Cooperation between Codex and Relevant International Organizations.
- Goal 5: Promoting Maximum and Effective Participation of Members.

Codex Secretariat and Administration

The Codex Secretariat is a team of less than 20 professional and technical staff located in FAO headquarters in Rome. The Secretariat deals with all aspects of the functioning of the Commission and its subsidiary bodies (e.g., preparing agendas and distributing working documents and drafting the reports of the sessions) and with the updating and publication of the Codex Alimentarius.

Work on the Codex Alimentarius

Standard Setting Process

As stated in Article 1 of the Commission's Statutes, one of the principal purposes of the Commission is the preparation of food standards and their publication in the Codex Alimentarius.

The process for preparing standards is open and transparent and ensures that all interested parties can be heard and contribute. It consists of a number of steps that starts with the submission of a project document to the Executive Committee and the Commission either by a subsidiary body or a Codex member in line with the requirements set out in the Procedural Manual justifying the need for new work and setting out the time frame. This formal proposal is often preceded by informal discussions in subsidiary bodies where members inform others of their interest and reasoning and evaluate if there is support for the project.

Step 1: Decision by the Commission that a standard be developed as proposed based on the criteria in the Procedural Manual and the priorities in the strategic plan. The Commission selects the subsidiary body to be responsible for steering

the standard through its development. If necessary, a new subsidiary body may be created.

Step 2: Preparation of a proposed draft standard is arranged by the Codex Secretariat and circulated to members and observers for comments.

Step 3: Comments are considered by the relevant subsidiary body that may present the text to the Commission as a draft standard for adoption at Step 5, or send it back for redrafting. Where relevant, the draft will be referred to the Codex Committees responsible for labeling, hygiene, additives, contaminants, and methods of analysis for endorsement of any special advice in these areas.

Steps 6 and 7: A second round of comments and discussions takes place in the subsidiary body before the standard is recommended to the Commission for adoption at Step 8.

Steps 6 and 7 may be omitted if there is a consensus on this in the subsidiary body and the Commission (adoption at steps 5/8) or it may be decided when starting a project that it should be finalized in 5 steps (accelerated procedure, adoption at step 5A).

Most standards take a number of years to develop. Once adopted by the Commission, a Codex standard is added to the Codex Alimentarius. A recent study showed that the speed of standard setting in Codex was 4.2 years overall and 3.5 years for food safety standards. The minimum time needed for adopting a standard is presently 1 year i.e., from one session of the Commission to the next. For example, the maximum limits for melamine after the food safety crisis related to adulterated milk were adopted in 1 year. Faster adoption could be possible only through an extraordinary session of the Commission.

The Commission and its subsidiary bodies keep Codex standards and related texts up to date to ensure that they are consistent with current scientific knowledge and the needs of the member countries. Countries now require less prescriptive standards, especially for commodities, than those developed in the 1970s and 1980s.

In recent years, many older, detailed individual product standards have been consolidated into more general or group standards, which allow wider coverage and innovation in the development of new food products. The procedure for revision or consolidation follows that used for the initial preparation of standards, but can be shortened in the case of editorial or consequential amendments.

Decision Making

The Commission and its subsidiary bodies strive to find a consensus on all issues under discussion. Voting is possible in accordance with the procedures, but past experience has shown that standards adopted by a vote have a lower acceptance as the original controversy does not disappear with the vote. For this reason, some issues remain on the agenda for a significantly longer time than the average because it takes longer to come to a consensus.

Subsidiary Bodies

The Commission may establish three kinds of subsidiary bodies.

- Codex Committees – which are of a standing nature (but may be adjourned or abolished) prepare draft standards for submission to the Commission or deal with Codex procedures;
- Ad hoc intergovernmental task forces – which are established for one specific purpose and are dissolved when their task is completed;
- Coordinating Committees, through which regions or groups of countries coordinate food standards activities in the region, including the development of regional standards.

There may be as many as 20 Codex Committee meetings in any 12-month period. With the exception of the Executive Committee and Coordinating Committees, Codex Committees and task forces are hosted by member countries, which are responsible for the cost of the committee's maintenance and administration and for providing the chairperson. Sessions are usually held in the host countries but recently an increasing number of industrialized country hosts have made use of the possibility to hold sessions in developing countries under cohosting agreements. The designation of host countries for the committees is a standing item on the agenda for the Commission.

The Executive Committee as well as the Commission meetings alternate between the headquarters of FAO and WHO (Rome and Geneva), and all costs are covered by the Codex budget. Coordinating Committees are usually hosted in the country of the coordinator (but may be cohosted in other countries) and the main costs (interpretation and translation) are funded by the Codex budget which is covered entirely by FAO and WHO.

General Subject Committees

General Subject Committees (horizontal committees) work on issues that can apply to any commodity or groups of commodities. They develop concepts and principles applying to foods in general, specific foods, or groups of foods, and endorse or review relevant provisions in Codex commodity standards. Based on the advice of expert scientific bodies, they develop major recommendations pertaining to consumers' health and safety.

Six of the General Subject Committees have the responsibility of ensuring that specific provisions in Codex commodity standards are in conformity with the Commission's main general standards and guidelines in their particular areas of competence. They are the committees on Food Additives; Contaminants in Foods; Food Hygiene; Food Labeling; and Methods of Analysis and Sampling.

These committees also develop standards, maximum limits for additives and contaminants, and codes of practice or other guidelines for either general application or in specific cases where the development of a complete commodity standard is not required.

For example, the Committee on Food Hygiene has developed a Code of Hygienic Practice for Spices and Dried Aromatic Plants, and the Committee on Food Additives and Contaminants (divided into two committees in 2006) has developed a Standard for Maximum Levels of Lead in Foods.

The committees on Food Labeling and on Nutrition and Foods for Special Dietary Uses have worked together to prepare the Codex Guidelines on Nutrition Claims.

The Committee on Pesticide Residues and the Committee on Residues of Veterinary Drugs in Foods prepare Maximum Residue Limits (MRLs) for these two categories of chemicals used in agricultural production. The MRLs are based on scientific advice regarding the safety of the residues that remain after the substances are used in accordance with defined good agricultural or veterinary practices.

The Committee on Food Import and Export Inspection and Certification Systems deals with the application of standards to foods moving in international trade, in particular to the regulatory measures applied by governments to assure their trading partners that foods and their production systems are correctly regulated to protect consumers against foodborne hazards and deceptive marketing practices. The guidelines developed by the Committee include advice on how governments should respond to emergencies in the food safety system, including channels of communication to the public and to other governments by means of the International Food Safety Authorities Network (INFOSAN) emergency information system operated by WHO and FAO.

The Committee on Nutrition and Food for Special Dietary Uses has a double role of serving as a general committee for questions of nutrition, and as a commodity committee for special dietary foods such as baby foods or gluten free foods.

The Committee on General Principles advises the Commission on such basic matters as definitions, the Rules of Procedure, rules and working procedures for the establishment and operation of Codex Committees and task forces, relations with other organizations, and the general principles that underlie the preparation of all Codex standards, codes of practice, and other texts.

Commodity Committees

The responsibility for developing standards for specific foods or classes of food lies with the Commodity Committees (vertical committees). Commodity Committees convene as necessary and go into recess or are abolished when the Commission decides their work has been completed.

There are currently four Commodity Committees that meet regularly: Fats and Oils, Fish and Fishery Products, Fresh Fruits and Vegetables, and Processed Fruits and Vegetables.

The following Commodity Committees work through correspondence or are adjourned: Milk and Milk Products; Cereals, Pulses and Legumes; Cocoa Products and Chocolate; Meat Hygiene; Natural Mineral Waters; Sugars and Vegetable Proteins.

Host countries convene meetings of Codex subsidiary bodies at intervals of between 1 and 2 years, according to need.

Ad hoc Intergovernmental Task Forces

In 1999, the Commission realized that its committee structure was too inflexible to cope with the demand for standards and guidelines across an ever-widening range of subjects. It decided to create a third type of subsidiary body the ad hoc Intergovernmental Task Forces, which are Codex Committees with very limited terms of reference established for a fixed period of time. To date, the Commission has established the following ad hoc Intergovernmental Task Forces:

- Task Force on Animal Feeding – 1999–2004, 2012–present;
- Task Force on Foods Derived from Biotechnology – 1999–2003 and 2005–09;
- Task Force on Fruit and Vegetable Juices – 1999–2005;
- Task Force on the Handling and Processing of Quick Frozen Foods – 2006–07.
- Task Force on Antimicrobial Resistance – 2006–11.

Coordinating Committees

Coordinating Committees ensure that the work of the Commission is responsive to regional interests and to the concerns of developing countries. They meet at 2-year intervals. The country that chairs the Coordinating Committee is also the Regional Coordinator for the region concerned. These Committees have no standing host countries but usually meet in the country that is the Coordinator as nominated by the relevant Coordinating Committee and confirmed by the Commission. There are six Coordinating Committees, one each for the following regions: Africa; Asia; Europe; Latin America and the Caribbean; Near East; and North America and the Southwest Pacific.

Contents of the Codex Alimentarius: Standards, Guidelines, and Codes of Practice

Codex Standards

By far, the largest number of specific standards in the Codex Alimentarius is the group called 'commodity standards.' The major commodities included in the Codex are:

- Cereals, pulses (legumes), and derived products including vegetable proteins.
- Fats and oils and related products.
- Fish and fishery products.
- Fresh fruits and vegetables.
- Processed and quick-frozen fruits and vegetables.
- Fruit juices.
- Meat and meat products; soups and broths.
- Milk and milk products.
- Sugars, cocoa products, and chocolate and other miscellaneous products.

Commodity standards follow a common format set out in the Procedural Manual consisting of the following categories:

- Scope – includes the name of the food to which the standard applies and, in most cases, the purpose for which the commodity will be used.
- Description – includes a definition of the product or products covered with an indication, where appropriate, of the raw materials from which they are derived.
- Essential composition – includes information on the composition and identity characteristics of the commodity, as well as any compulsory and optional ingredients.

- Food additives – contains a reference to the General Standard on Food Additives and any specific provisions needed.
- Contaminants – contains a reference to the General Standard on Contaminants and Toxins in Food and Feed.
- Where appropriate, reference is also made to the Codex MRLs for pesticide residues and for residues of veterinary drugs in foods.
- Hygiene – makes reference to relevant Codex Codes of Hygienic Practice for the commodity concerned. In almost all cases, it is required that the product shall be free from pathogenic microorganisms or any toxins or other poisonous or deleterious substances in amounts that represent a hazard to health.
- Weights and measures – contains provisions such as fill of the container and the drained weight of the commodity.
- Labeling – includes provisions on the name of the food and any special requirements to ensure that the consumer is not deceived or misled about the nature of the food. These provisions must be consistent with the Codex General Standard for the Labeling of Prepackaged Foods. Requirements for the listing of ingredients and datemarking are specified.
- Methods of analysis and sampling – contains a list of the test methods needed to ensure that the commodity conforms to the requirements of the standard. References are made to internationally recognized test methods that meet the Commission's criteria for accuracy, precision, etc.

MRLs for residues of pesticides or veterinary drugs in foods are examples of numerical standards.

Codex general standards for food additives and contaminants and toxins in foods contain both general and numerical commodity-specific provisions.

The Codex General Standard for the Labeling of Prepackaged Foods covers all foods in this category.

Codex methods of analysis and sampling, including those for contaminants and residues of pesticides and veterinary drugs in foods, are also considered Codex standards.

Codex Guidelines

Principles that set out policy in certain key areas are:

- Addition of essential nutrients to foods.
- Food import and export inspection and certification.
- Establishment and application of microbiological criteria for foods.
- Conduct of microbiological risk assessment.
- Risk analysis of foods derived from modern biotechnology.

Interpretative Codex guidelines include those for food labeling, especially the regulation of claims made on the label. This group includes guidelines for nutrition and health claims; conditions for production, marketing, and labeling of organic foods; and foods that claim to be 'halal.' There are several guidelines that interpret the provisions of the Codex Principles for Food Import and Export Inspection and Certification, and guidelines on the conduct of safety assessments of foods from DNA-modified plants and microorganisms.

Codex Codes of Hygienic Practice

Codes of practice define the production, processing, manufacturing, transport, and storage practices for individual foods or groups of foods that are considered essential to ensure the safety and suitability of food for consumption. For food hygiene, the basic text is the Codex General Principles of Food Hygiene, which introduces the use of the Hazard Analysis and Critical Control Point food safety management system. A code of practice on the control of the use of veterinary drugs provides general guidance in this area. A series of codes of practice gives indications to producers on the prevention or reduction of specific contaminants in specific foods for example, aflatoxins in tree-nuts.

Science base of Codex and Risk Analysis

Science Base

The SPS Agreement requires that health and safety requirements for food should be based on sound scientific assessment of risk. In 1995, the Commission adopted four Statements of Principle Concerning the Role of Science in the Codex Decision-Making Process and the Extent to Which Other Factors are Taken into Account. These principles were supplemented by Statements of Principle Relating to the Role of Food Safety Risk Assessment (1997) and by Criteria for the Consideration of the Other Factors Referred to in the Second Statement of Principle (2001). The Comprehensive Working Principles for Risk Analysis in the Framework of the Codex Alimentarius was adopted by the Commission in 2003 and incorporated into the Procedural Manual of the Codex Alimentarius Commission.

Other Codex Committees (Additives, Contaminants, Hygiene, Pesticide residues, Nutrition, and Residues of Veterinary Drugs in Foods) have since then adopted specific risk analysis principles for their fields of activity that have also been included in the Procedural Manual.

Codex has also adopted Working Risk Analysis Principles for Food Safety for Application by Governments, which have been included in the Codex Alimentarius.

Risk analysis in Codex

Risk analysis in Codex follows a structured approach comprising the three distinct but closely linked components of risk analysis (risk assessment, risk management, and risk communication); each component being integral to the overall risk analysis.

The three components of risk analysis are documented fully and systematically in a transparent manner and effective communication and consultation with all interested parties is ensured throughout the risk analysis process.

There is a functional separation of risk assessment and risk management, in order to: Ensure the scientific integrity of the risk assessment; avoid confusion over the functions to be performed by risk assessors and risk managers; and reduce any conflict of interest. However, it is recognized that risk analysis is an iterative process, and interaction between

risk managers and risk assessors is essential for practical application.

Risk Assessment and Scientific Advice for Codex

The scientific advice for Codex is provided by expert bodies convened by WHO and FAO. These expert bodies are independent of the Codex Alimentarius Commission (and its subsidiary bodies) in order to ensure that the provision of scientific advice remains independent of the pragmatism that is required of risk management. The main principles of developing scientific advice are as follows.

- Excellence: Use of internationally recognized expertise, supported by the creation of a platform for global scientific discussions based on best practices in elaborating guidance.
- Independence: Experts contribute in their own capacity and not on behalf of a government or institution; they are required to declare possible conflicts of interest.
- Transparency: Procedures and methods to ensure that all interested parties understand the processes for the development of scientific advice and have access to the reports, safety assessments and evaluations, and other basic information.
- Universality: A broad base of scientific data is critical for the elaboration of international standards-setting activities. Therefore, institutions and all interested parties throughout the world are invited to make data available.

FAO/WHO Expert Bodies

The expert bodies currently providing scientific advice to Codex are as follows.

- The Joint FAO/WHO Expert Committee on Food Additives (JECFA).
- The Joint FAO/WHO Meetings on Pesticide Residues (JMPR).
- The Joint FAO/WHO Expert Meetings on Microbiological Risk Assessment (JEMRA).
- An equivalent body for nutritional scientific advice (JEMNU) is under discussion.

JECFA and JMPR have been providing scientific assessments for more than 50 years. JEMRA began its work in 2000 and aims to optimize the use of microbiological risk assessment. In addition to providing scientific advice as basis for risk management decisions by Codex, the evaluations produced by these bodies are widely used by governments, industry, and research centers. These assessments contribute to the development of Codex standards, codes of practices, and other guidelines in the area of food safety. Ad hoc expert meetings to address specific topics that either require a rapid assessment or are outside the terms of reference of existing expert bodies are also organized by FAO and WHO.

Alternatives to Standards

When there is evidence that a risk to human health exists, but scientific data are insufficient or incomplete, the Codex Risk Analysis process provides that Codex should not proceed to elaborate a standard but should consider elaborating a related text, such as a code of practice, provided that such a text would be supported by the available scientific evidence.

Collaboration with Standard-Setting and Other Organizations

The Codex Alimentarius Commission works closely with the other standards-setting bodies mentioned in the SPS Agreement (OIE and IPPC) The Commission has strengthened collaboration with the standard setting activities of OIE relative to animal production food safety, in recognition that effective management of food safety risks demands a food chain approach from primary production to consumer. Codex and IPPC interact directly and inform each other through mutual participation in governing bodies.

FAO and WHO collaborate with other intergovernmental organizations to provide the best possible and most complete scientific advice. For example, the import_imagesInternational Atomic Energy Agency (IAEA) provides advice and support on levels of radionuclide contamination in foods and on food irradiation. The OIE provides advice on animal health, on animal diseases affecting humans, and on the linkages between animal health and food safety.

Ensuring Full and Effective Participation in Codex

Despite the importance of Codex in protecting health and facilitating trade, many countries have not been able to participate fully in the establishment of international food safety standards in Codex, mainly as a result of low income levels. To redress this situation, the Directors-General of FAO and WHO launched The FAO/WHO Project and Fund for Enhanced Participation in Codex (Codex Trust Fund) in 2003. The Codex Trust Fund is aimed at assisting developing countries and transition economy countries to enhance their level of effective participation in the Codex Alimentarius Commission. This is a vital step toward ensuring that the Codex system is inclusive, participatory, and equitable.

The Codex Trust Fund provides support to eligible countries to:

- Prepare for and participate in Codex Committees and related meetings;
- Participate in Codex training courses to enhance participation in Codex meetings;
- Prepare and present scientific/technical positions and data related to the work of Codex.

As of April 2011, 1418 participants from 139 countries have been supported to attend Codex meetings, task forces, and working groups, with the majority of support going to the least developed countries. A total of 336 participants from countries in all Codex regions had received Codex training to enhance their effective participation in Codex. This support was made possible with over US$ 11.7 million in contributions from 14 Codex Member States and 1 member organization.

FAO and WHO provide a range of capacity development activities aimed at ensuring that developing countries and transition economy countries participate effectively in Codex. Many of these training programs are based on an FAO/WHO training package for enhancing participation in Codex which is available on CD-ROM and the Internet.

The Codex Alimentarius Today and in the Future

Benefits of Codex for all Actors in the Food Supply Chain

Consumers

Today consumers enjoy a variety of food from all over the world. However, there is a risk that this food may be unsafe due to microbiological or chemical contaminants or additives, or may contribute to unhealthy diets due to nutrient content. The Codex Alimentarius rules for food labeling, food additives, pesticide residues, contaminants, food hygiene, and others provide a foundation for ensuring food safety and nutrient content of food. Therefore, consumers can be more confident about the safety and quality of the food they consume, regardless of its origin.

Food Exporters

The global market for trade in food continues to expand. In this growing and dynamic market, exporters need to be able to rely on a uniform and universally accepted set of standards such as those provided by the Codex Alimentarius. More and more countries participate actively in the standard-setting of the Commission and have adopted the standards on food production and processing, thereby facilitating food trade and contributing to the economic health of countries and regions around the world.

Food Producers

Food producers, including farmers and fishers, have a vital role in feeding the world. The Codex Alimentarius Commission assists them by developing standards that cover various types of food, including fats and oils, milk and milk products, fish and fishery products, and fruits and vegetables. Once producers meet these standards, they can be confident that their products are safe, of high quality, and are acceptable in export markets.

Developing Countries

FAO and WHO have capacity building programs to help developing countries comply with Codex Alimentarius standards and improve food quality and safety. Assistance includes helping countries to revise their food laws and regulations in accordance with Codex Alimentarius and strengthening their national food control systems (management, inspection, and laboratory services).

Keys to Success and Challenges

The keys to the success of Codex are that it is member driven, science based, transparent, inclusive, flexible, and based on consensus. This allows it to take on any new challenging issue brought up by a Member State.

The challenges facing Codex today include: The need to develop standards rapidly while remaining inclusive and transparent; finding consensus among members with different socioeconomic status and trade interest/needs; the need to increase the participation of developing countries and the increased use by businesses of private standards that may have different/stricter requirements than Codex standards.

See also: Institutions Involved in Food Safety: Food and Agriculture Organization of the United Nations (FAO); World Health Organization (WHO). Public Health Measures: Challenges of Developing Countries in Management of Food Safety; International Standards and Harmonization of Food Safety Legislation; Modern Approach to Food Safety Management: An Overview. Risk Analysis: Risk Analysis of Hazards in Food: An Overview

Further Reading

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http://www.who.int/foodsafety/codex/trustfund/en/
FAO/WHO Project and Fund for Enhanced Participation in Codex (Codex Trust Fund).

http://www.fao.org/ag/agn/agns/index_en.asp
Food and Agriculture Organization of the United Nations, Food Quality and Standard Service.

http://www.who.int/entity/foodsafety/chem/jecfa/en/index.html and www.fao.org/ag/agn/agns/jecfa_index_en.asp
Joint FAO/WHO Expert Committee on Food Additives (JECFA).

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http://www.who.int/foodsafety/en/
World Health Organization, Department of Food Safety and Zoonoses.

INSTITUTIONS INVOLVED IN FOOD SAFETY

Food and Agriculture Organization of the United Nations (FAO)

E Boutrif, Food and Agriculture Organization of the United Nations (FAO), Rome, Italy

Introduction

The Food and Agriculture Organization (FAO) is the principal United Nations' specialized agency whose mandate covers all aspects of food production, storage, transportation, processing, and marketing, and the development of agricultural and food-based programs to improve nutrition and enhance rural and national economies. Since its establishment in 1945, FAO has been leading the international community's effort to improve global food safety and quality through several major programs: (1) the provision of scientific advice to member governments, food industry, and consumers on food safety and quality; (2) the development of international food standards, guidelines, and recommendations; (3) the provision of policy and operational advice; (4) direct technical assistance to member countries in establishing or strengthening their national food control systems; (5) the emergency prevention system for food safety (EMPRES Food Safety), and (6) voluntary standards and schemes.

FAO's role in food safety and quality was further enhanced during the past decade through the emergence of the 'food chain' approach to food safety as the only effective way of ensuring the safety of the food supply at the global level. As a consequence, more attention is being given in FAO programs to food safety at different stages of the food chain, that is, primary production, storage, processing, distribution, etc. In addition to the central and longitudinal food safety work of the Food Quality and Standards Service, several units (animal production and health, plant production and protection, marine fisheries and aquaculture, food processing and marketing, and water for irrigation) have stepped up their work in food safety and quality assurance including the application of good practices.

FAO's Food Quality and Standards Service in FAO's Headquarters in Rome is the main central structure that deals with food safety matters. The Service benefits from the accumulated FAO technical and operational experience resulting from the interaction of normative and operational activities undertaken in cooperation with a wide variety of national and international partners at the global level. The Service also provides the Secretariat for the Joint FAO/World Health Organization (WHO) Food Standards Program (Codex Alimentarius). These facts, coupled with the interdisciplinary nature of FAO's work covering a wide range of policy and technical matters related to agricultural (including animal production and health) and fishery production ensure that quality and safety are considered throughout the food chain.

The technical assistance provided by the Food Quality and Standards Service also benefits from the extensive government and industry experience and expertise of its staff. The staff members have many years of experience in national and international food control programs and the food industry.

Provision of Scientific Advice

Risk Assessment: Scientific Basis of Food Safety Measures

FAO (jointly with WHO) promotes the application of risk assessment in all matters involving food safety. This must be based on sound scientific advice and evidence provided by panels of competent and independent experts. Risk assessment is one of the components of risk analysis – the other two being risk management and risk communication.

The Codex Alimentarius Commission (CAC) defines risk assessment as a scientifically based process consisting of the following four steps: (1) hazard identification; (2) hazard characterization; (3) exposure assessment; and (4) risk characterization. The risk assessment process is a means of providing an estimate of the probability and severity of illness attributable to a particular pathogen–commodity combination. The four-step process enables this to be carried out in a systematic manner, but the extent to which the steps are carried out will be dependent on the scope of the risk assessment. This can be defined clearly by the risk manager through ongoing dialog with the risk assessor.

Risk assessments provide information for identifying and characterizing food hazards. Risk assessment information is useful in determining which hazards are of such a nature that their prevention, elimination, or reduction to acceptable levels is necessary. The information is also useful in determining the most effective intervention strategies.

At present, there are two long-standing panels that provide advice to Codex, governments, and industry. They are the Joint FAO/WHO Expert Committee on Food Additives and Contaminants (JECFA) and Joint FAO/WHO Meeting on Pesticide Residues (JMPR). In addition, FAO and WHO convene ad hoc expert consultations whenever needed to address specific issues not covered by the permanent panels. In recent years, several expert consultations have been held on microbiological hazards in food, the risk assessment of foods derived from biotechnology and on animal feeding and food safety. FAO and WHO are currently studying the possibility of establishing an overall expert body on food safety risk

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assessment that would oversee the entire work in this field and ensure the necessary link, synergy, and harmony between the specific expert panels and consultations.

JECFA

The JECFA is an international expert scientific committee that is administered jointly by FAO and WHO. It has been meeting since 1956, initially to evaluate the safety of food additives. Its work now includes the evaluation of contaminants, naturally occurring toxicants, and residues of veterinary drugs in food. To date, JECFA has evaluated more than 1300 food additives, approximately 25 contaminants and naturally occurring toxicants, and residues of approximately 80 veterinary drugs. The Committee has also developed principles for the safety assessment of chemicals in food that are consistent with current thinking on risk assessment and takes account of recent developments in toxicology and other relevant sciences. As of June 2001 the Committee had met a total of 57 times.

JECFA serves as a scientific advisory body to FAO, WHO, and their member governments, and to the CAC. Advice to the CAC on food additives, contaminants, and naturally occurring toxicants is normally provided via the Codex Committee on Food Additives and Contaminants (CCFAC) and advice on residues of veterinary drugs via the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF).

All countries need to have access to reliable risk assessments of chemicals in food, but relatively few have the expertise and funds available to carry out separate risk assessments on large numbers of chemicals. JECFA performs a vital function in providing a reliable source of expert advice, and some countries use information from JECFA in formulating their own regulatory programs. In the same way, CCFAC and CCRVDF develop standards for chemicals in food based on JECFA evaluations.

For food additives, contaminants, and naturally occurring toxicants, the Committee does the following:

- elaborates principles for evaluating their safety;
- conducts toxicological evaluations and establishes acceptable daily intakes (ADIs) or tolerable intakes;
- prepares specifications of purity for food additives; and
- assesses intake.

For residues of veterinary drugs in food, the Committee does as follows:

- elaborates principles for evaluating their safety;
- establishes ADIs and recommends maximum residue limits (MRLs); and
- determines criteria for the appropriate methods of analysis for detecting and/or quantifying residues in food.

For food additives, JECFA normally establishes ADIs on the basis of available toxicological and other relevant information. Specifications of the identity and purity are also developed for food additives, which help to ensure that the product in commerce is of appropriate quality, can be manufactured consistently, and is equivalent to the material that was subjected to toxicological testing.

For contaminants and naturally occurring toxicants, levels corresponding to 'tolerable' intakes such as the provisional maximum tolerable daily intake or provisional tolerable weekly intake are normally established when there is an identifiable no-observed-effect level. When a no-observed-effect level cannot be identified the Committee may provide other advice depending on the circumstances.

In the case of veterinary drugs, data on good practice are evaluated and corresponding MRLs in animal tissues, milk, or eggs are recommended. Such MRLs are intended to provide assurance that when the drug has been used properly, the intake of residues of the drug present in food is unlikely to exceed the ADI.

More detailed information on JECFA's work can be found on the dedicated website, available on both FAO and WHO websites.

FAO/WHO JMPR

The JMPR is comprised of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and in the Environment and the WHO Core Assessment Group. JMPR carries out toxicological evaluation of pesticide residues, normally resulting in an estimate of the ADI. In addition, JMPR proposes MRLs for individual pesticides in or on specific commodities. These MRLs are primarily based on the residue levels estimated in supervised field trials when the pesticide is used according to good agricultural practices (GAPs). In cases where initial estimates indicate that the ADI may be exceeded, more refined intake calculations are performed using national food consumption data and information from pesticide residues monitoring programs.

These Expert Committees establish chemical safety standards based on a review of toxicological studies in the more sensitive test animal species. They factor in an adequate level of safety, use risk assessment procedures, consider use and consumption patterns, and define the specifications of the identity and purity of food grade chemicals to be used.

Microbiological Risk Assessment

Since 1999, and at the request of the CAC, FAO and WHO have initiated a series of joint expert consultations to assess risk associated with microbiological contamination of foods (Joint FAO/WHO Expert meetings on Microbiological Risk Assessment). This followed the adoption by the CAC of the Principles and Guidelines for the Conduct of Microbiological Risk Assessment (MRA).

The aim of these joint expert consultations is to provide a transparent review of scientific data on the state of the art of MRA, and to develop the means of achieving sound quantitative risk assessments of specific pathogen–commodity combinations. The work includes an evaluation of existing risk assessments; a review of the available data and current risk assessment methodologies, highlighting their strengths and weaknesses and how they may be applied; provision of examples; and identification of data and information needs/GAPs. A further aim of these consultations is the development of guidelines relating to the different steps of risk assessment,

such as hazard characterization and exposure assessment. The purpose of such guidelines is to help the risk assessor, the risk manager, and other interested parties to understand the principles and science behind the risk assessment steps.

A series of such consultations has already been organized. They have dealt with the risk assessment of *Salmonella* spp. in broilers, *Salmonella* Enteritidis in eggs, *Listeria monocytogenes* in ready-to-eat foods, *Campylobacter* in broiler chickens, and *Vibrio* spp. in seafood. The work plan and priorities for MRA are established in close collaboration with the Codex Committee on Food Hygiene.

Ad hoc expert advice: FAO frequently carries out expert consultations and meetings, often jointly with the WHO, on a range of issues related to quality and safety of foods. Examples include the FAO Technical Consultation on Food Allergies, the Joint FAO/WHO Consultation on Biotechnology and Food Safety, the Joint FAO/WHO Consultation on Food Safety Risk Management, the FAO Expert Consultation on Animal Feeding and Food Safety, and the FAO/WHO Expert Consultation on Nanotechnology and Food Safety.

Example of Ad hoc Expert Advice: Genetically Modified (GM) Food Safety Risk Assessment

The use of modern biotechnology for the genetic modification of plants, microorganisms, and animals for the production and processing of foods poses additional concerns to certain consumer groups. FAO recognizes that modern biotechnologies have potential to raise agricultural productivity, reduce dependence on harmful chemicals, and increase the nutritional value of foods. However, FAO also acknowledges that there are possible risks to human and animal health and to the environment, which require a case-by-case assessment.

FAO, jointly with WHO, organized a series of expert consultations to consider general safety and nutritional aspects of foods derived from modern biotechnology. The consultations addressed 'Strategies for assessing the safety of foods produced by biotechnology' in 1990, 'Biotechnology and food safety' in 1996, and 'Safety aspects of genetically modified foods of plant origin' in 2000 and 2001. The latter consultations addressed questions specifically on safety that were raised by Codex Intergovernmental ad hoc Task Force on Foods derived from Biotechnology. The 2000 Consultation reframed the concept of substantial equivalence and identified a set of priority issues that are to be addressed in future FAO and WHO consultations. The 2001 consultation revised the international guidelines on the assessment of potential allergenicity of novel recombinant proteins to address broader concerns or critics of the previous approach. A second consultation in 2001 was convened to consider the criteria essential for the risk assessment of food and food ingredients produced with the aid of or containing viable or nonviable GM microorganisms.

The recommendations of Joint FAO/WHO expert committees and ad hoc consultations provide valuable guidance to member countries and also form the basis for intergovernmental discussions within the CAC. The resulting Codex standards, guidelines, and recommendations provide the basis for harmonization of national food safety regulations.

Development of International Food Standards, Guidelines, and Recommendations (Codex Alimentarius)

FAO and WHO are cofounders of the Codex Almentarius Commission, an intergovernmental body entrusted by the FAO Conference (1962) and the WHO World Health Assembly (1963) to develop international food standards, guidelines, and recommendations with the double objective of protecting consumer's health and ensuring fair practices in food trade. FAO hosts the Secretariat of the CAC and provides the necessary technical and administrative support for its smooth running. More details on the role of the FAO/WHO Codex Alimentarius Commission in food safety is provided elsewhere in this encyclopedia.

Provision of Policy and Operational Advice

FAO provides policy orientation and advice on the organization, management, and operation of national food safety and control systems. This is provided either directly to member countries on request, or indirectly through FAO's authoritative publications and reports. Examples of such work include:

- *Food contamination and residues monitoring*: Food contamination by chemical and biological agents continues to be a serious problem around the world. Surveillance and monitoring of contaminants (chemical and biological) in foods is therefore important not only for the protection of public health but also because of its negative economic impact. Excessive levels of aflatoxin or pesticide residues in food raise consumer's concern about their health implications, and are often a cause of food import rejections in international food trade. FAO advises member countries on the establishment and operation of national food contamination monitoring programs to allow a continuous assessment of the food safety situation at national level and enable food safety authorities to take timely, corrective measures whenever a food safety problem is identified. FAO also assists in the analysis of the causes of the contamination problems and in formulating and implementing preventive actions.
- *Food control management, inspection, analysis, and quality control*: Adherence to Codex standards, guidelines, and recommendations and achieving the appropriate level of sanitary and phytosanitary protection related to food safety can only be assured by establishing effective national food control systems. Therefore, technical assistance provided by FAO is directed toward establishing or strengthening the necessary elements of such systems. FAO's technical assistance aims at creating the political will for, and technical understanding of, these elements to enable proper institutional building process.

FAO provides expert advice required for the development and operation of effective national food control systems including food inspection and sampling programs. Advice is also given on food laboratory management and on accurate, reliable, and cost-effective methods of analysis. Organizing

simple but effective systems of food quality control based on the Codex General Principles of Food Hygiene throughout the food chain improves food quality and safety, reduces food losses, and facilitates international food trade.

FAO's manuals and tools related to food safety and quality provide practical guidance for the development and operation of food quality and safety systems. These are aimed primarily at providing advice to developing countries, and document modern approaches for the development and implementation of risk-based food safety programs throughout the food chain. Such an approach is instrumental in facilitating international trade in food. Key titles in the series include: Food Safety Risk Analysis – A Guide for National Food Safety Authorities (2006), Assuring Food Safety and Quality: Guidelines for Strengthening National Food Control Systems (2003), Manual on the Application of the HACCP System in Mycotoxin Prevention and Control (2001), Animal Feeding and Food Safet, (1998), Imported Food Inspection (1993), and Management of Food Control Programmes (1991).

Direct Technical Assistance to Developing Countries

Direct technical assistance, in the form of project formulation, implementation, consultation, training, and/or other advisory services, is provided, at their request, to many developing countries and countries in transitional economies through the FAO Technical Cooperation Program or through other financing sources (FAO/Government Cooperation Program, Trust Fund arrangement; UNDP, and others). The trade in food is, for many countries, a prime source of foreign exchange. The challenge is to meet the appropriate level of sanitary and phytosanitary protection of importing countries. This requires the establishment of effective export food control systems that provide assurance of adherence to Codex/importing country's standards, guidelines, and recommendations by the exporting country. FAO technical assistance is provided to countries in developing effective food import and export controls thereby increasing confidence in the countries' food products and reducing the number of shipments rejected by importing countries. The Codex Committee on Food Import and Export Inspection Certification Systems provides valuable advice and recommendations in this area.

The following are some highlights of a few of these activities that are being carried out at present and which provide a general idea of FAO's work, which is carried out to meet general or specific food control requests from developing countries.

- *Development of national strategies*: Developing an effective national strategy on food quality control and safety should take into account the development needs of the country and contribute to national development programmes, particularly those related to food security. To provide coherence in national food control strategies, FAO assists member countries in reviewing their food control strategies and infrastructure, usually by convening national workshops involving all those directly and indirectly concerned with food safety and quality. More than 100 such workshops or reviews have been held in developing countries, with the participation of Ministries of Agriculture, Health, Commerce, and/or Industry, as appropriate to the existing infrastructure, as well representatives of the industry, consumers, and other interested parties. Weaknesses and strengths are identified and measures are proposed to strengthen the national infrastructure, often with the help of FAO-executed projects.
- *Advice on food legislation*: FAO assists countries in the review and updating of their national food legislation so as to take into account recent developments, including the World Trade Organization Agreements on Sanitary and Phytosanitary Standards (SPS) and Technical Barriers to Trade (TBT). The Model Food Law developed jointly by FAO and WHO and the Codex Alimentarius standards, guidelines, and recommendations constitute unique references in the development and harmonization of food legislation worldwide.
- *Training and manpower development*: Training of governmental and food industry personnel in all aspects of food quality and safety is a major aspect of FAO technical assistance and capacity development work. Emphasis is placed on food inspection, food analysis, certification requirements and procedures for export, application of HACCP systems in food processing and handling establishment, effective participation in Codex standard setting process, food safety management programs at various levels. These training activities are carried out as part of Regular Program activities or under FAO-executed projects.
- *Export and international trade*: Special emphasis continues to be given to improving national export food inspection and certification programs so that exporting countries have confidence that their products are acceptable to import authorities. Projects dealing specifically with this matter have been implemented in many countries including countries with emerging economies.

National and regional workshops on export food control have been organized for many countries to assess the existing situation and develop strategies and actions for the future.

EMPRES Food Safety

The FAO EMPRES Food Safety contributes to global effort for the prevention and control of food safety risks with the double aim of improving food security and protecting public health. EMPRES Food Safety complements and enhances FAO's ongoing work in animal and plant health emergencies. It serves FAO member countries through the three pillars of early warning, emergency prevention, and rapid response.

Early warning is provided through the FAO/WHO International Food Safety Authorities Network operated by WHO. It involves activities in incident scanning, identification, and verification. EMPRES Food Safety is developing an international network to gather information and intelligence data to conduct horizon scanning, involving other FAO units and decentralized offices, other UN agencies, national and regional governmental bodies, universities, research institutes, and related groups.

FAO, in collaboration with WHO, has developed a series of technical tools that provide countries with practical

guidance on food safety emergencies and reinforce emergency preparedness.

In case of a food safety incident, EMPRES Food Safety works with national food safety authorities to conduct an urgent appraisal of the event and mobilize the needed experts. Wherever needed, the input of experts in public health is sought through WHO. Together with national food safety authorities, EMPRES Food Safety determines the best possible emergency response, and assists in its implementation.

Voluntary Standards and Schemes

In recent years, there has been growing consumer interest for food and agricultural products that have specific characteristics regarding the final product or production system. This has led to the development of a number of voluntary standards, certification systems, labels, and regulatory instruments that relate to factors such as preservation of environment, social welfare and equity, traditions and geographical origin, nutritional aspects, etc. FAO's work on voluntary standards and schemes aims at providing the necessary support to relevant institutions value chain actors in interested countries, in setting up and implementing voluntary standards and schemes, taking into account their needs and constraints as well as the international regulatory framework and standards.

See also: Institutions Involved in Food Safety: FAO/WHO Codex Alimentarius Commission (CAC). Public Health Measures: Challenges of Developing Countries in Management of Food Safety; Evaluation of the Efficacy of National Food Control Programs; Fundamentals of Food Legislation; Modern Approach to Food Safety Management: An Overview

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FAO: Food Safety and Quality: Microbiological Risks and JEMRA.

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FAO: Food Safety and Quality: Voluntary Standards and Schemes.

INSTITUTIONS INVOLVED IN FOOD SAFETY

World Health Organization (WHO)

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Glossary

Hazard A biological, chemical, or physical agent in or property of food that may have an adverse health effect.
Risk A function of the probability of an adverse effect and the magnitude of that effect, consequential to a hazard in food.
Risk analysis Formalized process to assess, manage and communicate risk.
Risk assessment Scientific formalized process to estimate risk and its attendant uncertainties.
Risk communication An interactive process of exchange of information and opinion on risk among risk assessors, risk managers and other interested parties.
Risk management The process of weighing policy alternatives to accept, minimize or reduce assessed risk and to select and implement appropriate options.

Food Safety as a Health Issue

Foodborne disease (FBD) continues to be a major public health issue. Food safety and FBDs have implications both on the health of individuals and the development of societies. Concerned by this, the Sixty-Third World Health Assembly (WHA), the governing body of the World Health Organization (WHO), in May 2010 reminded the Director-General of WHO of the need for efficient gathering and exchange of information in and among countries; and adopted a resolution 'Advancing Food Safety Initiatives (WHO, 2010).' The resolution asked Member States to establish disease burden estimation and surveillance and to contribute to the timely conduct of international risk assessments through the provision of relevant data and expertise. And specifically the resolution called upon the Director-General of WHO to help countries build relevant capacity to improve cross-sectoral collaboration along the whole food production chain and to establish with the International Food Safety Authorities Network (INFOSAN) an international initiative for the collaboration of laboratory partners in support of surveillance of FBD, identification of food contamination, and emergency response, including outbreak investigation.

WHO's Role in the Conceptual Development of Food Safety

WHO's central role in food safety as well as in other public health areas is a normative one and includes the facilitation of risk assessment and international standard setting. The most thorough normative change over the past decades in the food safety area has been the introduction of a formalized system to prepare and utilize science-based risk assessments to improve the safety of food, both at international and national level. For almost 20 years, WHO has promoted the new concept of risk analysis as a framework for the management of food production and food safety. Developed by WHO in collaboration with the Food and Agricultural Organization of the United Nations (FAO), the risk analysis framework and principles are depicted in Figure 1. Within this framework it is important to achieve functional separation of risk assessment (the science) and risk management (the intervention). The final goal of assessment should be to inform risk managers and other stakeholders of the nature, occurrence, and size of the risk in order to improve the quality of risk management decisions.

New concepts were needed because the old food safety systems have failed in the sense that the incidence of FBDs seems to have increased over the past decades in most countries. One of the reasons for this has been the intuitive reliance on testing. It must be realized that safety cannot be achieved by monitoring the presence of pathogens in the end product because it is impossible to test sufficient samples to obtain the necessary degree of statistical power to detect all contaminants at levels that may create unacceptable health risks. Therefore, a proactive approach is required, starting with the producer, including in many cases the primary production sector, ensuring a safe product based on predictive risk assessment and, where relevant, implementation of action plans to lower the prevalence of relevant pathogens.

The main focus of WHO's work in the area has been the development of methods for quantitative microbiological and chemical risk assessment, FBD surveillance, and also the assessment of the safety of new products used in food, including genetically modified (GM) foods. The use of risk assessment to improve risk management decisions and risk communication to enable transparent and proactive interaction between all interested parties, constitute the two other components of the risk analysis framework.

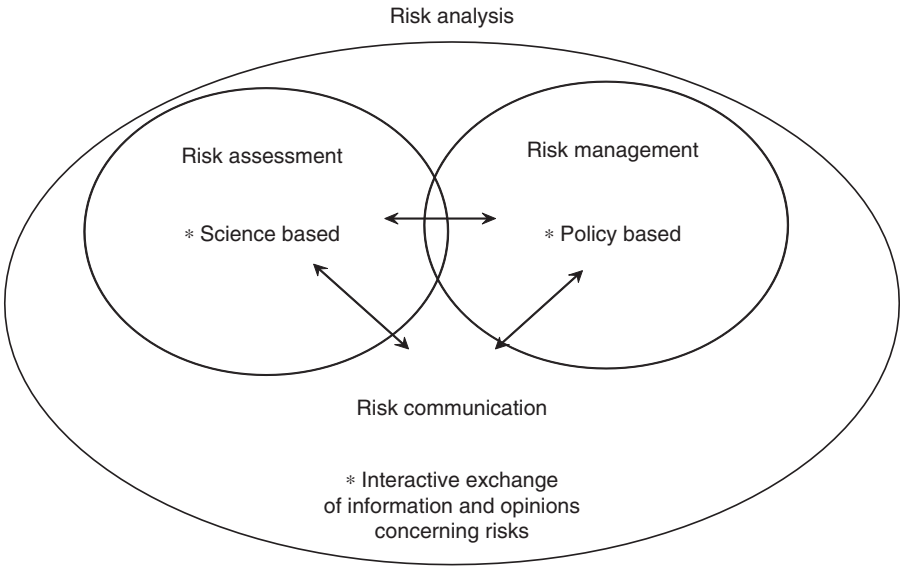

Figure 1 The WHO/FAO risk analysis paradigm, with risk assessment independent from undue influence from risk managers, and with everything floating in a sea of risk communication.

The Structure of WHO Relative to Food Safety Work

WHO's food safety activities extend from the WHO headquarters (HQ) in Geneva, Switzerland, often through six Regional Offices: AMRO (Regional Office for the Americas), AFRO (Regional Office for Africa), EMRO (Regional Office for the Eastern Mediterranean), EURO (Regional Office for Europe), SEARO (Regional Office for South-East Asia) and WPRO (Regional Office for the Western Pacific). The regional structure of WHO stems historically from the creation of WHO in 1948, where the preexisting Pan-American Health Organization was included in WHO as AMRO with an existing, independent governance structure. This independent structure was replicated in the other five regional offices, thereby creating an organization with not one, but seven politically appointed heads. In effect this means that each Regional Director is independently elected, and thus in effect can define policy course on his or her own. The regional offices receive approximately 75% of the WHO's budget and because regional directors are not appointed by or formally responsible to the WHO's Director-General, criticism has been voiced that some of the agency's activities are uncoordinated and not based on the best scientific evidence.

Over the later years, WHO has seen significant cuts in its budget. Therefore, a number of technical departments in the WHO HQ have seen cuts in technical staff. But even before these cuts the technical area of food safety was small in the WHO HQ as well as in regions. At its peak in 2008, the Department for Food Safety and Zoonoses at the WHO HQ included approximately 12–14 scientific staff and 6–8 other staff, whereas the regional offices typically had less than 1 scientific staff in this area. At the third layer of the organization, the country offices, food safety is typically covered by staff, who also cover a number of other technical areas. Nevertheless, the WHO has been able to support significant development in national food safety programs, and shoulder a very significant work load in the area, primarily through the active support of – mostly nationally funded – scientists from all over the world.

Most of the WHO work in the food safety area is conducted in some sort of collaboration with FAO. In the 1990s, the collaborative efforts between the two organizations were somewhat hampered by policy disagreement and to some degree by personal fights between WHO and FAO staff, including staff at senior level. Under the administration of WHO Director-General Dr Gro Harlem Brundtland, WHO in the late 1990s introduced the 'farm-to-fork' collaborative line with FAO, a policy line mirrored by FAO. This did not mean that the two organizations necessarily agreed in all policy areas, but a cooperative effort was underlined in most areas of work. Following the major zoonotic outbreaks in the first decade of 2000, including severe acute respiratory syndrome (SARS), avian influenza, and type H1N1 influenza, the collaboration between FAO and WHO was extended to also include the World Animal Health Organisation (OIE).

Scientific Advice

The collaborative efforts between WHO and FAO in the area of provision of scientific advice include three expert groups with relatively regular meetings. These groups are managed differently, but all in collaboration between WHO and FAO. They are the Joint FAO/WHO Expert Committee on Food Additives and Contaminants (JECFA), the Joint FAO/WHO Meeting on Pesticide Residues (JMPR), and the Joint FAO/WHO Expert Meetings on Microbiological Risk Assessment (JEMRA). For further description of the work of these joint committees, see the article on FAO, or visit:

1. http://www.who.int/foodsafety/chem/jecfa/publications/en/index.html (JECFA).

2. http://www.who.int/foodsafety/chem/jmpr/publications/en/index.html (JMPR).
3. http://www.who.int/foodsafety/micro/jemra/en/ (JEMRA).

In addition to these expert groups, FAO and WHO have hosted a number of ad hoc expert meetings in relation to food safety issues. An example of such an ad hoc expert meeting is the Ad hoc Expert Group on Food Safety Risk Assessment of GM Food, which is described in an other article.

Providing Scientific Advice That Has Not Been Asked For

Whereas the expert groups described above all typically respond to specific requests for scientific advice, in most cases from the FAO/WHO Codex Alimentarius Commission (CAC), WHO has in a number of cases initiated scientific work without such request. The examples are manifold, but three important examples shall be briefly described here.

1. Antimicrobial resistance (AMR) is a global public health issue that is impacted by both human and nonhuman antimicrobial usage. The continuing emergence, development, and spread of pathogenic microorganisms that are resistant to antimicrobials are a cause of increasing concern. WHO's involvement in the containment of AMR due to nonhuman antimicrobial usage dates back to the late 1990s, and includes the hosting of a number of expert meetings from 1997 to this day. Although the other relevant international organizations in this area – FAO and OIE – were not immediately supportive of the suggestions coming out of these expert meetings, through continued efforts from WHO the issue has since 2007 also been included in CAC work. These efforts have led to clear recommendations about prudent use of antimicrobials in animals as well as a description and selection of critically important antimicrobials. Whereas some recommendations have been implemented in some parts of the world, a very significant number of recommendations have not. In most countries, antimicrobials are still used as growth promoters, and veterinarians are still allowed to make profits from selling such drugs.
2. Acrylamide was until 2002 considered an occupational health issue only, as this substance had not been found in food. Therefore, it was considered dramatic news when Swedish researchers in March 2002 unveiled research showing very high concentrations of acrylamide in ordinary food items, such as French fries, bread, and coffee. Although some experts dismissed the importance of these findings – for example, with statements about how 'we have eaten these types of food for centuries' – WHO maintained a serious attitude toward these findings relative to a substance that had been proven carcinogenic in animals. Thus, a WHO/FAO expert consultation was undertaken less than 2 months after the Swedish news. The consultation considered that the available data suggested that toxicological findings in animals should be assumed to be relevant for extrapolation to humans. The consultation also provided a range of recommendations for further information and new studies to better understand the risk to human health posed by acrylamide in food.
3. In September 2008, the INFOSAN Emergency Surveillance System obtained information from the Chinese Ministry of Health (MoH) that a serious and widespread contamination event had occurred in China. The contamination of infant formula with melamine had been spurred by fraudulent use of melamine to disguise the dilution of milk with water. Through further interaction between INFOSAN and MoH the issue of potential other use of the contaminated milk powder as well as parallel (illegal) distribution of contaminated milk powder to other countries was investigated. An INFOSAN Emergency Alert was distributed to the network and subsequently updates were issued regularly during the following months. A WHO Expert Meeting was held in collaboration with FAO a few months after the event unfolded in an effort to elucidate the normal ('baseline') exposure of humans to melamine, as well as suggest relevant tolerable daily intakes of melamine through food.

International Standards and Guidelines

People in ancient times already understood they could get sick from consumption of infected meat, and that keeping their animals healthy and using dedicated methods of food preparation and conservation could improve their health. Maybe the oldest written document about this, 'On Airs, Waters, and Places' is by Hippocrates that describes how human health is influenced by its interaction with the environment. A clear understanding of the importance of food and food safety for health led to the creation of the FAO/WHO Food Standards Programme in 1963, the active arm of which is the FAO/WHO the CAC. The CAC now meets once a year and agrees on food standards, guidelines, and codes of practice, typically developed in one of its 16 committees and task forces. However, the CAC system had become a very heavy system with significant bureaucracy. Realizing this, WHO and FAO in 2001 initiated an evaluation, resulting in some, but not very profound changes, including a higher level of inclusiveness especially toward developing countries. However, the system has also proven a capacity to move fast, even when dealing with politically charged issues. For example, the development of guidelines for the assessment of GM foods was finalized within a time period of only 4 years. When considering that such standards are developed based on several FAO/WHO expert meetings, as well as several task force meetings, many times with the participation of 50–70 countries, this is actually an impressive international achievement.

Because the work of CAC is governed by the member countries, the system typically does not deal immediately with new or upcoming issues, unless key member countries take a direct interest in this. An example of an important food safety issue that has only been taken up reluctantly by CAC is the problem of AMR in microorganisms. Later in this article, forward-looking initiatives by the WHO in this area will be described, but it is noteworthy that it was only in 2007 that

CAC agreed to start an initiative in this area, i.e., the Codex AMR Task Force.

Although CAC standards and guidelines are in effect just voluntary guidance documents, the specific reference to CAC in the World Trade Organization agreements as the 'gold standard' in the food safety area has meant that most countries take CAC guidance seriously. Nevertheless, CAC standards and guidelines have no legal status above this. However, WHO has an international legal instrument that covers certain aspects of food safety, the International Health Regulations (IHR), which is a legally binding agreement for all 194 Member States of the Organization. The aim of these regulations is to help the international community prevent and respond to acute public health emergencies that have the potential to cross borders and threaten people worldwide. Such public health emergencies include risks related to food, because diseases can spread far and wide via international food trade. A health crisis in one country can impact the health, livelihoods, and economies in many parts of the world. The IHR aims to reduce unwarranted interference with international traffic and trade, while ensuring public health through the prevention of disease spread.

The present IHR, which entered into force on 15 June 2007, require countries to report certain disease outbreaks and public health events to WHO. Building on the unique experience of WHO in global disease surveillance, alert, and response, the IHR defines the rights and obligations of countries to report public health events, and establish a number of procedures that WHO must follow in its work to uphold global public health security. Thus, countries have an obligation to inform the global community, through WHO, about any public health risk related to food, which has the potential to cross borders or that are otherwise unique. WHO has created a specific system for the reporting of such food safety events: The INFOSAN, which operates in collaboration with FAO, is described in this article under the section 'Food Safety Emergency Action and Exchange of Experience over Borders.'

FBD Burden

Although important parts of international food safety work involves the collaborative WHO–FAO framework, a number of issues related more specifically to the occurrence, surveillance, and prevention of FBD are primarily supported at the international level by WHO. FBDs result from the ingestion of contaminated foods and food products and include a broad group of illnesses caused by biological and chemical agents, which contaminate food at different points in the food production and preparation process. In work sponsored by the WHO FBD Burden Epidemiology Reference Group (FERG), it was estimated that in 2008, 1.336 million children under the age of 5 years die every year from diarrhea caused by contaminated food or water. Consequently, diarrhea is the second leading cause of death among children after respiratory diseases. In 2009, another FERG-sponsored study estimated that diarrhea-related deaths among adolescents and adults were 1.15 million per year. The total mortality of 2.486 million deaths due to diarrhea is more than deaths due to AIDS, malaria, and measles combined.

As usual the poorest part of the population is at the highest risk: In general, malnutrition can result in a 30 fold increase in the risk for diarrhea-associated death. When considering these estimates of child deaths, it is important to realize that they do not include deaths in other age groups, deaths as caused by foodborne microorganisms not resulting in diarrhea nor the probably very significant disease burden caused by chemical substances, including naturally occurring chemical substances in food. Such chemical substances include aflatoxins caused by fungi growing in food as a result of poor storage conditions, or acrylamide formed in certain foods when heated $>120^{\circ}$C.

Although most diarrheal deaths occur in poor countries, FBDs are not limited to developing countries. It is estimated that, in 2011 in the USA, FBDs resulted in 48 million illnesses (one in six people), 128 000 hospitalizations, and 3000 deaths per year resulting in medical costs and productivity losses in the US$ billions. The full extent of the burden and cost of unsafe food is currently still unknown, but its impact on global health security, trade, and development is considered to be profound. Thus, valid estimations of the real FBD burden are basically nonexistent. Recognizing the current data gap, WHO has launched an Initiative to Estimate the Global Burden of FBDs from all major causes using summary health metrics that combine morbidity, mortality, and disability in the form of the disability-adjusted life year. This has been initiated through the establishment of the FBD Burden Epidemiology Reference Group (FERG). The FERG members are mandated to engage in assembling, appraising, and reporting on currently existing burden of FBD estimates, conducting epidemiological reviews for mortality, morbidity, and disability in each of the major FBD, providing models for the estimation of FBD burden where data are lacking, developing source attribution models to estimate the proportion of diseases that are foodborne, and developing user-friendly tools for burden of FBD studies at country level. Although the work of FERG was originally stipulated to result in (some) global FBD data by 2012, the work is still ongoing, and parts of the work seem hampered by recent funding trouble.

Food Safety Emergency Action and Exchange of Experience over Borders

In 2000, the Fifty-Third WHA recognized the serious threat to public health posed by foodborne illness and called for improved 'gathering and exchange of information in and between countries and regions on matters of food safety'. The Fifty-Fifth WHA in 2002 expressed serious concerns about health emergencies posed by natural, accidental, and intentional contamination, including food contamination, and requested WHO to coordinate the identification of and response to such emergencies.

In 2004, in response to the aforementioned resolutions and in reply to a specific request made by the FAO/WHO CAC, WHO established the INFOSAN, in collaboration with FAO. The network was built to help Member States deal with international food safety incidents and emergencies, and to facilitate communication and information sharing among all food safety stakeholders. Currently, 167 Member States are members of INFOSAN.

The INFOSAN network not only responds to the reporting of human FBD cases to WHO, but also provides information to countries when a food contamination event has the potential to affect human or animal health at a later stage. To promote seamless action throughout the food chain continuum, INFOSAN and the Global Early Warning System (GLEWS) for Major Animal Diseases, including Zoonoses exchange information directly. GLEWS is a confidential early warning network of WHO, FAO, and OIE used to track, verify, and analyze transboundary zoonotic diseases. This network brings together the expertise of the three different organizations to maximize prevention and control of zoonotic diseases. INFOSAN and GLEWS coordinate efforts relative to food safety events linked to animal health (e.g., avian influenza), animal feed (e.g., aflatoxin), or farm practices (e.g., AMR).

In addition to emergency information, INFOSAN facilitates the exchange of food safety information and experience among its members through the publication of INFOSAN Information Notes, in the six official WHO languages. These Notes provide INFOSAN members with summaries on relevant food safety issues. This INFOSAN function is supplemented with capacity building efforts aimed at the building of integrated food safety systems able to manage and monitor events with national or international implications.

Data and Laboratory Networks

WHO has for a number of years hosted the first ever global database for food contamination, focused on chemical contaminants. The Global Environment Monitoring System–Food Contamination Monitoring and Assessment Program (GEMS/Food) was established in 1976 to inform national governments, CAC, and other stakeholders, as well as the public, on levels and trends of chemical contaminants in food and their contribution to dietary exposure. The program operates through a network of WHO Collaborating Centers and national institutions located in approximately 70 countries around the world. National data are submitted to GEMS/Food to conduct international scientific assessments of exposure, as part of chemical risk assessment. In addition, WHO has developed an approach to describe the various diets around the world. The GEMS/Food Consumption Cluster Diets were updated in 2006 and are now used both nationally and internationally for exposure assessment of food contaminants and pesticide residues (Figure 2: WHO GEMS/Food Consumption Cluster Diets).

Recognizing an urgent need for building Member State capacity in surveillance of foodborne and other enteric infections from the farm to the table, the WHO in 2000 initiated the WHO Global Salm-Surv, now called Global Foodborne Infections Network (GFN). The network promotes integrated, laboratory-based surveillance, and fosters intersectoral collaboration and communication among microbiologists and epidemiologists in human health, veterinary, and food-related disciplines. The network has been created with the support of some of the most respected national laboratories in these areas from a long list of WHO Member States, including Australia, Canada, Denmark, France, Germany, Japan, Netherlands and the USA.

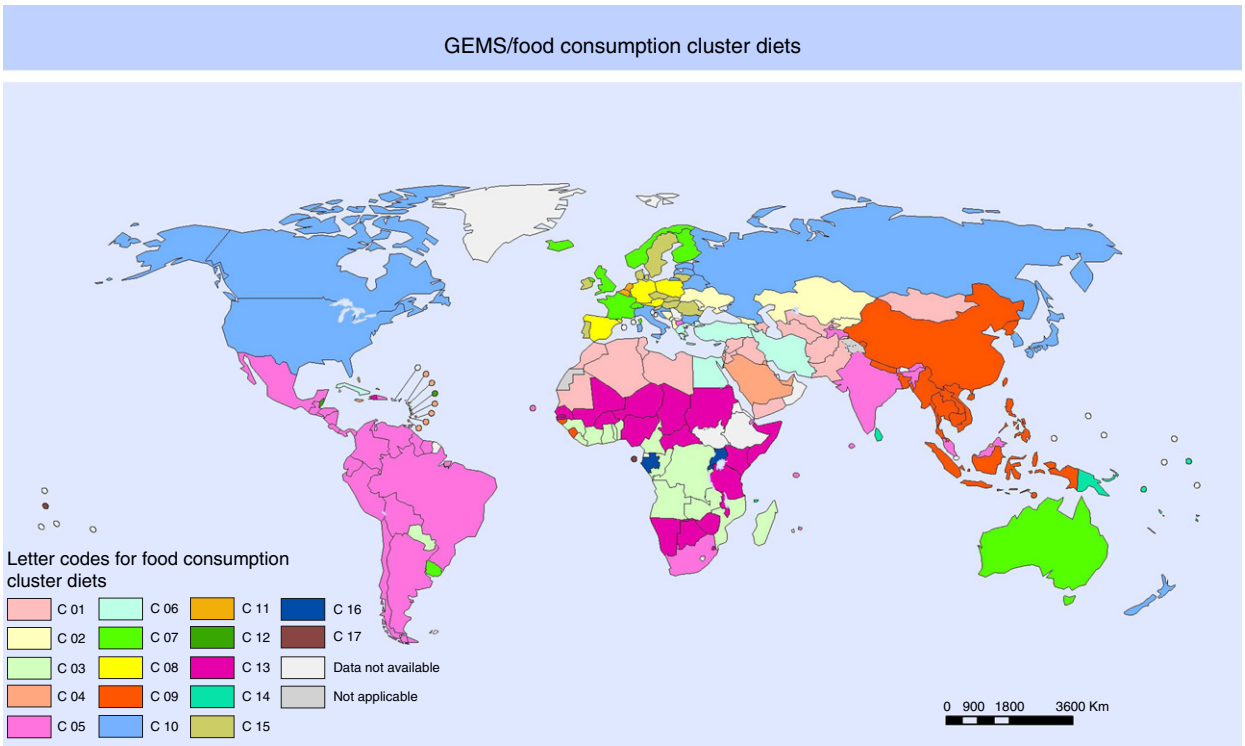

Figure 2 WHO GEMS/Food consumption cluster diets. Reproduced from http://www.who.int/foodsafety/chem/Global_GEMS_CLUSTERS_2012.jpg.

By May 2012 the GFN included 1062 members from 184 Member States, and continuous activities covering: regional training courses on FBD surveillance, risk assessment and epidemiology, external quality assurance programs on microbiology, reference testing of selected foodborne pathogens, and provision of technical information and methodological support for microbiological laboratories. The GFN also hosts a country data bank with data on national *Salmonella* isolates from more than 83 countries comprising approximately 1.5 million human and 360 000 nonhuman isolates.

WHO considers new activities linking databases covering microbiological and chemical contaminants in food in an effort to promote the sharing of food contamination data among countries.

The Future of Food Safety and WHO's Role – Promoting Safer Food and Better Health

The future food safety systems will most likely enable new ways of evaluating disease metrics and attribute such disease directly to food groups. It is likely that new genetic fingerprinting techniques will enable attribution for all pathogenic microorganisms related to food, and new chemical fingerprinting (metabolomics) will enable a significantly better understanding of the effect of chemicals in food. At the same time the new line of risk-based approaches will enable the setting of national – and international – targets for disease reduction, as well as provide the evidence base for such reduction efforts. These approaches will make their way into all parts of the global market, including the developing countries, which are likely to become more and more important agricultural producers and exporters. The introduction of a risk-based framework will enable developing countries to learn from mistakes (and successes) elsewhere. These countries have the potential to 'leap forward' into preventative systems focusing on risks. In addition, at a time where trade restrictions and national/regional protection of the agricultural production through heavy subsidies is likely to come to an end, it will be of paramount importance for the production sector also in developing countries to adapt to the new times. The benefits of improving food safety amount to a 'win-win' situation with improved national health as well as improved export potential. However, it will be crucial for these potential future developments that the developments in new food safety systems is clearly documented and communicated. Such communication has not yet commenced; national authorities as well as international organizations have an important future task in this area.

In supporting such developments, the WHO should focus on its core business. WHO is neither a funding agency nor an implementing agency like United Nations Children's Fund (UNICEF). Instead, it should aim to be the paramount knowledge organization in global health – gathering up the best technical, scientific, and practical information and making it accessible to all countries. This does not mean that WHO products must necessarily only be theoretical analyses aimed at scientific debate. A very significant amount of WHO documentation is directly applicable in national food safety efforts. The information material about GM food ('20 Questions on GM Food'), the exchange of national experience in risk mitigation activities through INFOSAN, or indeed the global spread of the simple WHO message on safe food handling ('Five Keys to Safer Food') all are clear testament to the fact that if the core remains sound science, the application of practical support to countries can take many forms.

WHO should continue to take bold action in support of continuous improvement of national and global food safety. This also means that WHO must remain an impartial and independent broker of sound science. Only thus can WHO contribute to a better – and safer – future for the world.

See also: Institutions Involved in Food Safety: FAO/WHO Codex Alimentarius Commission (CAC); Food and Agriculture Organization of the United Nations (FAO). Public Health Measures: International Standards and Harmonization of Food Safety Legislation; Modern Approach to Food Safety Management: An Overview

Further Reading

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Relevant Website

http://www.who.int/foodsafety/about/en/index.html
World Health Organization.

INSTITUTIONS INVOLVED IN FOOD SAFETY

World Organisation for Animal Health (OIE)

M Thibier and B Vallat, World Organisation for Animal Health, Paris, France

Glossary

Antimicrobial resistance The resistance of a microorganism to an antimicrobial medicine to which it was previously sensitive. Resistant organisms include bacteria, viruses, and some parasites. The World Organisation for Animal Health (OIE) recognizes that antimicrobial resistance is a global public and animal health concern that is influenced by the usage of antimicrobial agents in humans, animals, and elsewhere. Those working in the human, animal, and plant sectors have a shared responsibility to prevent or minimize pressures for the selection of antimicrobial resistance factors in humans and animals.

Emerging diseases A new infection resulting from the evolution or change of an existing pathogenic agent, a known infection spreading to a new geographic area or population, or a previously unrecognized pathogenic agent or disease diagnosed for the first time and which has a significant impact on animal or public health.

Food safety A scientific discipline describing handling, preparation, processing, and storage of food in ways that prevent foodborne illness. This includes a number of routines that should give assurance that food will not cause harm to the consumer when it is prepared or eaten according to its intended use.

Foodborne hazards A biologic, chemical, or physical agent in, or a condition of, food such as an animal product with the potential to cause an adverse health effect (OIE). Biologic hazards can be posed by parasites, viruses, or bacteria. Chemical contaminants in foods can come from industrial and agricultural sources, from food processing, or from the food itself. Toxic chemicals also come from biological sources such as molds and algae. Foreign objects present in food could constitute a physical hazard to the consumer.

Foodborne illness Also called as foodborne disease and colloquially referred to as food poisoning resulting from the consumption of contaminated food, pathogenic bacteria, viruses, or parasites that contaminate food, as well as chemical or natural toxins such as poisonous mushrooms.

Veterinary Services The governmental and nongovernmental organizations that implement animal health and 'welfare' measures and other standards and recommendations in the Terrestrial Code and the OIE Aquatic Animal Health Code in the territory. The Veterinary Services are under the overall control and direction of the Veterinary Authority. Private sector organizations, 'veterinarians,' 'veterinary paraprofessionals,' or aquatic animal health professionals are normally accredited or approved by the Veterinary Authority to deliver the delegated functions (OIE). The Veterinary Services protect and improve the health and welfare of animals, safe animal products, and veterinary biologics by preventing, controlling, and eliminating animal diseases, and monitoring and promoting animal health, animal welfare, and food safety. The OIE considers the Veterinary Services as a Global Public Good.

Introduction

The goal of Food Safety is that the consumers are able to trust that all food offered for sale is safe for its intended use. The last decades of the twentieth century have been the subject of increased concern in this area, related to numerous factors: changes in the production systems, much wider distances between the sites of breeding, production, and of consuming – particularly in industrialized countries – and occurrences of some new and emerging or reemerging diseases, such as the spongiform encephalopathies in ruminants. This period has stressed the critical importance of animal production and absolute necessity of a holistic approach to food safety and not only on the end products. This underpins the need for an integrated, multidisciplinary approach that covers the whole food chain and even beyond, such as feed for animal products, paradigm summarized with the famous 'from stable to table.'

OIE Involvement: The Animal Production Food Safety Working Group

Basic facts regarding Public Health show the following: 60% of human pathogens are zoonotic, 75% of emerging diseases are zoonotic, and 80% of agents having a potential bioterrorist use are zoonotic pathogens. Similarly, a great proportion of foodborne diseases is due to microbiologic pathogens of animal origin. It was hence recognized essential that the World Organisation for Animal Health (OIE) and the International InterGovernmental Organization (IGO) reference for animal

health and zoonoses gets involved in this area in close cooperation with other IGOs also working on Food Safety, such as the Codex Alimentarius, but from a different angle linked with its global mandate: "The improvement of animal health and welfare all around the world."

The Office International des Epizooties was created through the international Agreement signed on 25 January 1924 following outbreaks of devastating contagious animal diseases and in particular Rinderpest in Europe (nowadays totally eradicated worldwide – official recognition of the eradication in May and June 2011). In 1994, following the Marrakech agreement with the setting of the World Trade Organization (WTO) Agreement on the application of sanitary and phytosanitary measures (SPS), OIE was formally recognized as one reference organization by the WTO–SPS mandate to safeguard world trade by protecting human, animal, and plant health in conjunction with its two 'sister' organizations (Codex Alimentarius Commission (CAC) and International Plant Protection Convention). In 2011, OIE has a total of 178 Member Countries.

The third OIE Strategic Plan (2001–05) identified Food Safety as the first priority and at the 70th General Session in May 2002, the World Assembly of OIE delegates adopted resolutions defining the role of the OIE in this area aiming at strengthening its standard-setting activities in food safety through a new Animal Production Food Safety Working Group (APFSWG). The fourth OIE Strategic Plan (2006–10) supported the continuation of this mandate, recommending that the APFSWG "continue to work with other relevant organizations, especially the Codex Alimentarius Commission, in reducing foodborne risks to human health due to hazards arising from animals."

Hence, the OIE's goal for animal production food safety is to reduce foodborne risks to human health due to hazards arising from animals and hence provide better guarantee of safety of food of animal origin. It should be noted that these hazards include pathogens, which may not cause clinical signs in animals.

After adoption of the 2002 resolution, an ad hoc group of Experts chaired by the President of the CAC, was convened by the Director General in April 2002, and it pursued as a permanent working group of Animal Production Food Safety (APFSWG) in its series of meetings since then.

The terms of reference of this Working Group include among other items, the following:

1. Consideration of all foodborne hazards arising from animals before slaughter;
2. A primary focus on food safety measures applicable at the farm level;
3. Consideration of food safety measures applicable elsewhere, for example, during animal transport and harvesting of wild animals for food; and
4. Work criteria and priorities that take into account global food safety priorities and current work programs of relevant international organizations, especially the CAC.

Within these terms of reference, the role of this working group is to:

1. Provide advice to the OIE Director General on policy and strategic issues relating to the OIE's work on animal production food safety, which has the goal of reducing foodborne risks to human health by preventing, eliminating, or controlling hazards arising from animals before primary processing of animals and animal products and
2. Act in a steering group capacity, as required by the OIE Director General, regarding the work of OIE expert groups:
   a. Giving advice to the Director General on membership, scope, and terms of reference for expert groups and
   b. Reviewing texts arising from relevant expert groups for consideration by the relevant Specialist Commissions.

At its first meeting in November 2002, again chaired by the President of the CAC, the Working Group drew up a detailed work program for the OIE on the development of recommendations on animal production food safety covering pre-slaughter issues and those before the first transformation of the animal products, with the primary focus being on food safety measures applicable at the farm level. The Working Group also reviewed existing OIE Terrestrial Animal Health Code chapters dealing with food safety and zoonoses (brucellosis, tuberculosis, etc.), and recommended necessary changes in standards and additional work in accordance with the overall work program proposed by the Working Group.

A report of their work is delivered each year at the OIE General Session followed by resolutions giving the APFSWG its road book for the year ahead.

Priorities were given along this last decade and include the following:

1. Horizontal issues such as animal identification and traceability, good farming practices, guidelines for animal feeding, ante- and postmortem meat inspection standards, role of Veterinary Services in the reduction of public hazards;
2. Disease-specific OIE texts: Terrestrial Animal Health Code (Terrestrial Code) chapters on brucellosis, salmonellosis in eggs, etc.; and
3. Strengthening relationship between the OIE and the CAC.

Relevant documents were then drafted and later approved by the World Assembly of OIE delegates during various General Sessions this last decade, such as

1. The Guide on Good Farming Practices;
2. Guidelines for the control of hazards of animal health and public health importance through ante- and postmortem meat inspection;
3. Animal identification and traceability later followed by General Principles for identification and traceability of live animals incorporated in the Terrestrial Code (chapter 4.2);
4. Guidelines on the Detection, Control and Prevention of *Salmonella* Enteritidis and *S.* Typhimurium in Poultry Producing Eggs For Human Consumption;
5. Guidelines for the control of Hazards of Animal Health and Public Health importance in animal feed (chapter 6.3. of the Terrestrial Code);
6. Antimicrobial resistance (surveillance programs, chapter 6.7–6.11 of the Terrestrial Code) in coordination with the Codex Task Force;
7. Role and functionality of Veterinary Services throughout the food chain;
8. Review of a paper published on: Priority pathogens for standard setting by OIE.

In addition, the World Assembly of OIE Delegates, during the General Session of 2009 approved in its resolution XXIV, the expansion of the mandate of the Aquatic Animal Health Standards Commission to address Food Safety of products derived from aquatic animals. A first meeting of a working group focused on this issue had met in 2008. At its 2011 meeting, the group finalized the chapter 5.3 of the Aquatic Animal Health Code (Aquatic Code) dedicated to the "criteria to assess the safety of aquatic animal commodities."

Several ad hoc groups also tackle food safety issues in their meetings such as the OIE *ad hoc* groups on identification and traceability of live animal, on animal feeding, on pet food, on trade in animal products, on assessment of food safety related to the use of recombinant vaccines in food-producing animals.

Many seminars under the auspice of OIE and OIE publications, in particular the Scientific and Technical Review, were focused on the benefits for food safety issues to get the OIE and the Veterinary Services widely involved in prevention and control of foodborne diseases.

Among the key documents published by the OIE on food safety those last years, the following can be mentioned:

1. The Guide to good farming practices for animal production. This guide aims at describing a set of generic good farming practices intended to minimize hazards and to recommend good practices to address the listed hazards under six headings: general management, animal health management, etc. with a particular and original focus on animal feeding and watering. Its Appendix 1 is also most valuable as it draws a multientry table of "Hazards and their corresponding control points."
2. Priority pathogens for standard setting by the OIE related to animal production food safety. The authors used expert opinion and a literature review to identify the pathogens that were to be prioritized by the OIE for the development of future standards for animal production food safety. Interestingly, their conclusions were that *Salmonella* (from species other than poultry) and pathogenic *Escherichia coli* were considered as top priorities. *Brucella* spp., *Echinococcus granulosus*, and *Staphylococcus aureus* were also mentioned. The same article also lists the current coverage of farm level control of foodborne disease agents in the OIE Terrestrial Code or OIE guidelines.

OIE Involvement in Food Safety with the Veterinary Services

In addition to the extensive work of APFSWG, the OIE supports the improvement of food safety in the field by, the implementation of the rules to apply for Food Safety via the expertise of the national Veterinary Services. The latter are most often the national key focal points for all food safety issues as a great proportion of reported outbreaks of foodborne diseases is due to contaminations of foods with zoonotic agents, often during primary production. They act in their capacity to prevent and control foodborne zoonosis at the farm level or of other foodborne disease and chemical contamination of foods. They indeed exert their surveillance at all stages of animal production, on farm controls (animal health, feed, use of antimicrobial, identification, and traceability as well as animal welfare) and at the meat inspection level (ante- and postmortem inspections at slaughterhouses). They also provide certification of animal products for international trade.

In this context, the Director General of the OIE asked the OIE Delegates to nominate a national focal point for animal production food safety according to established terms of reference. The OIE also regularly organizes seminars for national focal points to provide information and contribute to capacity building of Veterinary Services.

Further to the implementation of the role of the Veterinary Services in the food chain from stable to table recommended by OIE, the OIE has put some emphasis both on capacity building and veterinary education. Wall stressed the big challenge for Veterinary educators to stimulate interest in public health medicine and make the curriculum relevant to those issues. The OIE in conjunction with the World Veterinary Year 2011 got also involved in trying to standardize the curriculum of veterinary education.

Close Cooperation with the CAC

Since its first involvement in this area, the OIE recognized that the goal to reduce foodborne disease due to hazards arising from animals can only be achieved in collaboration with the World Health Organization (WHO), the Food and Agriculture Organization (FAO), and their subsidiary body, the CAC. This is totally in line with the recent agreements signed between the Directors General of OIE, WHO, and FAO. The FAO–OIE–WHO tripartite strategic alignment recognize that addressing health risks at the human–animal–ecosystems interfaces require strong partnerships among players who may have different perspectives on some issues and different levels of resources.

The OIE has an SPS responsibility for elaborating international standards and related texts for the prevention, control, and eradication of animal diseases and zoonoses, whereas the CAC elaborates standards and related texts for both safety and suitability aspects of food control. CAC and the OIE have strategies and mechanisms in place to coordinate and integrate food safety activities across the production to consumption continuum and so enhance the safety of foods of animal origin on a worldwide basis. In terms of identification and traceability, for example, there is a clear need to strengthen bridges between animal and product traceability.

The terms of reference of the APFSWG include a link at the working level with the CAC (CAC, FAO, and WHO) and the working group has produced a guidance paper on the cooperation between the CAC and the OIE in food safety throughout the food chain. It identified as priorities an examination of the scope to develop joint OIE and Codex standards, address gaps and duplication in standards, and develop procedures for mutual recognition of standards where appropriate.

The cooperation between the CAC and the OIE currently include

1. Cooperation through mutual exchange of information and participation in meetings;
2. The use of a common text in the elaboration of a standard and harmonization of definition;
3. Cross-referencing to the other organization's standards; and
4. The construction of complementary texts taking into account the existing standards.

The next challenge in this cooperation approach is to set common working groups on specific topics, which would set the standards before following the respective procedures of these two organizations for respective official approval.

Conclusion

OIE plays a key role worldwide in Food Safety issues both at the starting points in elaborating and editing Codes of Practices or Standards for specified hazards of biologic origin and on the ground via its guidelines and capacity building to the national Veterinary Services. Through its specific working group APFSWG, the OIE has been an active partner of international organizations, and has been working in close cooperation with CAC, all having complementary functions and sharing the common approach of 'production to consumption' to food control.

See also: Institutions Involved in Food Safety: FAO/WHO Codex Alimentarius Commission (CAC); Food and Agriculture Organization of the United Nations (FAO); World Health Organization (WHO). Public Health Measures: International Standards and Harmonization of Food Safety Legislation; Modern Approach to Food Safety Management: An Overview

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Relevant Websites

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http://www.fao.org/
The Food and Agriculture Organization (FAO).

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The International Federation for Animal Health (IFAH).

http://www.who.int/
The World Health Organization (WHO).

http://www.oie.int
The World Organisation for Animal Health (OIE).

http://www.wto.org/
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INSTITUTIONS INVOLVED IN FOOD SAFETY

Consumer Organizations

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Consumer Organizations Involved in Food Safety and Food-Related Issues

Consumer organizations are a prominent sector of civil society; they speak for and represent consumers on a wide range of economic, safety, civil rights, and political issues. Consumer organizations have long been actively concerned with food safety, on scales ranging from local to global. Because they represent consumers and are independent from commercial interests, consumer organizations bring important and generally credible perspectives to food safety policy making.

What is a consumer organization? Consumers International uses this definition to determine eligibility for membership:

- The organization must have members drawn from the general public.
- It must be independent – that is, it should receive neither funding nor tangible support from any commercial entity with an interest in any issue the group addresses.
- It must be transparent – that is, the identities of those who direct and control the organization must be public.
- It must be democratically governed – by a board, a committee, or leaders chosen by its members – and should pursue issues and take positions supported by the members.

Consumer Organizations in a National Food Safety Context

Consumer organizations exist, to varying degrees, in virtually every country of the world. There are hundreds of consumer organizations in the world's 200 or so countries and their food safety interests span a wide spectrum of issues. On the one hand, consumer groups in Mali, Tanzania, and several other African nations have pressed local health authorities in recent years to oversee the safety of street-vended foods. On the other hand, consumer organizations in Europe and North America have lobbied their governments to regulate and label genetically modified foods.

Many consumer organizations, especially in developing countries, are essentially grass-roots community activists with very limited resources. Others, in both developed and developing parts of the world, have scientists and food-safety policy specialists in their staff, and bring professional expertise as well as a strong proconsumer perspective to bear on food safety issues. Some larger consumer organizations operate independent laboratories that test products for quality and safety, and publish magazines to inform consumers of their findings. Such organizations combine a scientific approach to testing, an educational outlook, and a commitment to improve the lot of consumers. This three-part mission is characteristic of consumer nongovernmental organizations (NGOs), both in countries where advanced consumer protection legislation and institutions exist and in nations where that protective framework, largely, still needs to be built.

For more than 50 years, consumer organizations have joined in international alliances, forging broad commonalities of outlook among consumer NGOs worldwide. At the same time, the consumer movement remains quite varied in its political outlook, its choice of issues, and the tactics it uses when pursuing its goals.

Most consumer organizations are concerned with food issues, given the fundamental importance of foods to all consumers. Consumer NGOs may address food sufficiency, nutrition and food quality, trade in foods and food safety; many work on all four. The full scope and diversity of consumer organizations' work on food safety issues cannot be covered in a brief article, but examples from two rather different countries may suggest both the variety of these efforts and some of their common themes.

In the USA, consumer organizations were instrumental in the campaign to pass the first national food safety law, in 1906, and were just as actively involved in the 2010 campaign that led to the passage of major new food safety legislation. One major US consumer NGO, Consumers Union, has worked for stronger food safety standards since 1936. Its recent priorities include regulation and labeling of genetically modified foods, safeguards to keep bovine spongiform encephalopathy out of US cattle herds, and better consumer information about methylmercury in fish. Another US consumer group, the Center for Science in the Public Interest, has created a database of outbreaks of foodborne pathogens for public use, filed numerous petitions with federal regulators to restrict potentially harmful food additives, and taken grocery chains to court for their failure to remove recalled foods from their shelves. Both organizations publish consumer magazines, each is engaged on a much wider range of food safety issues, and of course, these are but two of the many US consumer organizations that address some aspects of food safety.

In India, numerous consumer organizations work on food safety issues. The Voluntary Organization in Interest of Consumer Education (VOICE) has published a guide to food safety for consumers and advocates, tested soft drinks for pesticide residues, lobbied the Indian government for

restrictions on the use of genetically modified crops, and participated in the worldwide campaign for the safety of infant formulas. The Centre for Science and the Environment tested honey and found multiple illegal antibiotic residues. The Association for Consumer Action on Safety and Health (ACASH) promotes breastfeeding and lobbies to strengthen food safety regulations. The Consumer Education and Research Centre (CERC), which operates an independent product-testing laboratory, has found unacceptable levels of arsenic and fluoride in bottled water and widespread microbial contamination of processed foods, such as *Escherichia coli* and *Staphylococcus aureus* in milk and ice cream. These are just a few CERC food test reports available on its website, and these four organizations are but a small sample of the many Indian consumer NGOs working on various aspects of food safety.

Consumer Organizations in an International Food Safety Context

Just as foods and their associated safety issues cross national boundaries, many national consumer NGOs have joined with their sister organizations in other countries to work on food safety at the regional and global levels.

Very similar definitions of consumer organizations (comprised of ordinary citizens, independent, transparent, democratic, and international) are used by the World Health Organization (WHO), the Food and Agriculture Organization (FAO), and the Joint WHO/FAO Codex Alimentarius Commission to determine eligibility for observer status as a consumer international non-governmental organization (INGO). A relatively small number of organizations meet these criteria. Nevertheless, consumer INGOs have participated actively in international food safety deliberations for several decades. Codex has been the primary medium for this consumer involvement, but consumer INGOs have also engaged with WHO, FAO, and the World Trade Organization on food safety issues at the Global Forums on Food Safety, and in other contexts. For brevity's sake, the focus here is on consumer participation in Codex, but the consumer role at other food safety-related intergovernmental bodies has been similar to that in Codex.

In early 2011, there were 199 registered Codex observer organizations. Of those, eight were international consumer-oriented public-interest organizations. Each organization, and the role it has played at international food safety bodies, is described briefly below. The eight organizations are:

- 49th Parallel (49P)
- Bureau Européen des Unions de Consommateurs (BEUC)
- Consumers International (CI)
- Greenpeace International (Greenpeace)
- International Association of Consumer Food Organizations (IACFO)
- International Baby Food Action Network (IBFAN)
- National Health Federation (NHF)
- Pesticide Action Network (PAN)

Several of these consumer observers are essentially single-issue organizations; for example, 49P is concerned almost exclusively with genetically modified foods, and IBFAN works only on infant feeding issues. But at least two consumer observers, CI and IACFO, have broad food safety portfolios and have participated over the years at multiple Codex committees and at other food safety meetings. The breadth and depth of their interests and their highly professional interventions have enabled consumer INGOs to play a more significant role in international food safety deliberations than their small numbers might suggest.

Profiles of Individual Consumer Observer Organizations

Consumer-based Codex observer organizations represent a variety of perspectives, geographical regions, and issue interests. They are listed here in alphabetical order. Further details on each, including their national members where applicable, can be found on the websites listed at the end of this article.

49th Parallel (49P)

49th Parallel is a small organization with membership in the northwestern USA and southwestern Canada (where the 49P the parllel forms the international boundary). 49P's main interest is foods and food ingredients derived from genetically modified organisms. It has attended the Codex *Ad Hoc* Task Force on Foods Derived from Biotechnology, several meetings of the Codex Committee on Food Labeling where international standards for labeling genetically modified foods were being deliberated, and meetings of the Committee on General Principles and the Codex Commission when issues of interest to 49P were on the agenda.

Bureau Européen des Unions de Consommateurs (BEUC)

BEUC, known in English as The European Consumers' Organization, is an alliance of 44 consumer organizations from 31 European countries. BEUC is the European branch of CI. It participates at the Codex Regional Coordinating Committee for Europe, representing CI's European members. At all other Codex bodies and in other international food safety forums, BEUC and its members are usually represented by CI.

Consumers International (CI)

CI is the oldest and largest international association of consumer organizations, with some 220 member organizations in 115 countries in all regions of the world. CI first began participating at the Codex Commission in the 1970s, and has done so more or less continuously since then.

In the 1990s, CI expanded its international food safety work in response to growing member interest in the topic. CI members in the USA, the UK, and several European countries led the effort, assigning their own food safety experts to take part (as CI) at many Codex committees and other international food safety gatherings. At the peak of its activity, CI was engaged in the work of at least 17 different Codex bodies, including the Commission; all six regional Codex coordinating committees; subject committees on Food Labeling, General Principles, Food Hygiene, Food Additives and Contaminants, Pesticide Residues, Residues of Veterinary Drugs in

Foods, Nutrition and Foods for Special Dietary Uses, and Import/Export Certification Systems; and *Ad Hoc* Task Forces on Foods Derived from Biotechnology, and on Management of Antimicrobial Resistance. CI sent experienced delegates to meetings, often submitted detailed written comments on draft documents, and participated in voluntary working groups that met between meetings to draft and revise those documents.

CI also sent delegations to the WHO/FAO Global Forums on Food Safety in 2002 and 2004. Several individuals from CI member organizations have participated as invited experts in joint WHO/FAO expert consultations and similar scientific meetings.

Because of the breadth of its interests, and because its delegates brought expertise on issues, experience in international meetings, and a diplomatic attitude to the table, CI has the played a robust and effective role in international food safety forums for many years.

During the late 1990s, with grant support, CI expanded its presence to include delegates from its members in developing countries. These diversified delegations broadened CI's interactions with national delegations and gave many CI member organizations, new to the process, an opportunity to learn how to participate effectively in global food safety policy making. By building the capacity of its own members, CI also increased its presence at the regional codex coordinating committee meetings, especially those in Africa, Asia, and Latin America.

In recent years, CI's international food safety work has significantly diminished. Key experienced delegates left CI member organizations; grants ran out; and most CI members faced competing needs. As the food-related policy priorities of CI as a whole diversified, an intensive emphasis on Codex-work no longer seemed appropriate, and resources were redirected elsewhere.

Today, CI's involvement in Codex and similar international food safety activities, although still substantial, is a slim shadow of what it once was. CI tracks food safety issues that most concern its members (such as genetically modified foods, safety of infant formulas, and antimicrobial resistance) and attends meetings where those issues are addressed. In recent years, CI's strongest presence has been at the Committee on Food Labeling and the *Ad Hoc* Task Force on Antimicrobial Resistance. CI still attends Codex Commission and General Principles meetings, and keeps its members informed on the status of issues that interest them. CI nowadays is as likely to be represented by someone from Uganda as by someone from the UK, and rarely sends multinational delegations to meetings.

Greenpeace International

Greenpeace International has not often participated at Codex or in other global food safety forums. Greenpeace did join other consumer INGOs attending the 2009 Codex Committee on Food Labeling to lobby in favor of labeling of genetically modified foods.

International Association of Consumer Food Organizations (IACFO)

IACFO was established in 1997; in 2011, it had 12 member organizations in North America, Europe, Asia, and Africa. IACFO is interested in all aspects of international trade in foods, including food safety. IACFO has attended meetings of the Codex Commission and the Committees on General Principles, Food Labeling, Food Hygiene, Residues of Veterinary Drugs in Foods, International Import and Export Certification Systems, and Nutrition and Foods for Special Dietary Uses. IACFO also has participated in deliberations of WHO, FAO, the World Trade Organization, and the Organization for Economic Cooperation and Development (OECD).

In 2004, IACFO created a second global consumer food safety entity called Safe Food International (SFI), described as a project to empower consumer organizations to improve food safety on a global level. SFI developed a set of guidelines for consumer organizations wishing to pursue that goal, and in 2005, organized an international conference at which consumer organizations met with national food safety officials and experts from FAO, WHO, and other intergovernmental bodies.

IACFO's participation in international food safety activities has been led by experts at its US member, the Center for Science in the Public Interest. IACFO has been able to maintain a consumer presence at several critical Codex committees, including Food Hygiene and Veterinary Drugs, after CI could no longer do so, and is probably the most broadly involved consumer organization now working on global food safety issues.

International Baby Food Action Network (IBFAN)

IBFAN is, as its name indicates, concerned with infant feeding issues. Formed in 1979, IBFAN is an alliance of public-interest groups from Africa, Latin America, Asia, Europe, and other regions. As a single-issue organization, IBFAN has interacted with global and regional intergovernmental agencies (such as WHO, United Nations International Children s Fund (UNICEF), and the Pan-American Health Organization (PAHO)) that address infant nutrition policies. At Codex, IBFAN has been involved at the committees on Nutrition and Foods for Special Dietary Uses and on Food Labeling, and participated in the Food Hygiene committee's effort to set microbial safety standards for infant formulas. IBFAN has also attended meetings of the Codex Commission, the Codex Committee on General Principles, and on occasion, some Regional Codex Coordinating Committees.

IBFAN was instrumental in lobbying for the International Code on Marketing of Breastmilk Substitutes, adopted by the World Health Assembly in 1981, helped organize a boycott of Nestle over its infant-formula marketing practices in developing countries, and is currently active in Codex efforts to set standards for the nutrient composition and nutritional labeling of infant formula. Several of IBFAN's member organizations have formed a Codex Working Group to coordinate inputs by the global IBFAN network into the Codex process. The working group has submitted detailed technical comments on some of the Codex drafts, and sent delegates to make expert interventions at Codex meetings.

Because of its single-issue focus, its representation of lower-income consumers from all corners of the earth, the ethical leverage associated with representing babies, and the quality of its experienced, knowledgeable delegates, IBFAN has been one of the most effective consumer INGOs working on global food safety.

National Health Federation (NHF)

NHF is a global association of consumer health advocacy organizations with members in at least 20 countries. First established in 1955 as a national organization in the USA, NHF describes itself as a defender of health freedom. It has participated at the Codex Committee on Nutrition and Foods for Special Dietary Uses, chiefly to oppose standards that it perceives might restrict consumers' rights to buy nutritional supplements. NHF has also joined other consumer observers to support a Codex standard for labeling of genetically modified foods.

Pesticide Action Network (PAN)

PAN is an international alliance of environmental, farm-worker, and consumer advocacy organizations that promotes pesticide safety in a variety of contexts. PAN has been actively involved with the FAO's Agriculture Committee and with the United Nations Environment Program. Pesticide residues in foods are one of PAN's many concerns, and PAN has attended occasional past sessions of the Codex Committee on Pesticide Residues.

Conclusions

The consumer movement is actively engaged in the food safety policy process at all levels, from strictly local to national and global scales. This participation has benefitted consumers and enriched the process. The consumer perspective is independent and often valuable, and sometimes brings substantial insights and expertise to bear on issues that might not have been presented by other stakeholders. Although the intensity of their participation has ebbed and flowed over the years as resources wax and wane, consumer INGOs are a fixture in the world food safety system and are likely to play a significant role in the future.

See also: Institutions Involved in Food Safety: FAO/WHO Codex Alimentarius Commission (CAC)

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Centre for Science and the Environment (India), Tests of Honey.

http://www.cspinet.org/foodsafety/
Center for Science in the Public Interest (USA), Food Safety.

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http://www.codexalimentarius.org/members-observers/observers/en/
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http://www.consumersinternational.org/who-we-are/about-us
Consumers International Organization Website Home Page.

http://www.consumersunion.org/food.html
Consumers Union of USA, Food Safety.

http://www.greenpeace.org/international/en/campaigns/agriculture/problem/
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http://www.cspinet.org/reports/codex/iacfosum.html
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INSTITUTIONS INVOLVED IN FOOD SAFETY

National Industry Organizations – Case of UK Food and Drink Federation

K Chinyama, Food and Drink Federation, London, UK

Glossary

Codex Alimentarius The Codex Alimentarius is a body of standards decided by the Codex Alimentarius Commission to protect the health of consumers and to ensure fair practices in international food trade. The Codex Alimentarius Commission is a joint FAO/WHO organization.

Contaminant Any substance not intentionally added to food, which is present in such food as a result of the production (including operations carried out in crop husbandry, animal husbandry, and veterinary medicine), manufacture, processing, preparation, treatment, packing, packaging, transport or holding of such food, or as a result of environmental contamination. The term does not include insect fragments, rodent hair, and other extraneous matter (definition adopted by Codex Alimentarius Commission).

Genetic modification (GM) A process of altering the genetic makeup of an organism by techniques of modern biotechnology.

Hazard analysis critical control points (HACCP) A system that identifies, evaluates, and controls hazards that are significant for food safety.

Incident Any event which, based on the information available, causes concern about actual or suspected threats to the safety or quality of food that could require intervention to protect consumers.

Rapid Alert System for Food and Feed (RASFF) A notification system for incidents in the EU, which is coordinated by the European Commission.

Overview of the Food Industry Organizations

The Food and Drink Federation (FDF) is the UK representative of FoodDrinkEurope, the confederation of the European food industries (the umbrella trade association for the European food and drink industry). As of January 2010, membership of FoodDrinkEurope was made up of: 26 national federations, including 3 observers, 26 EU sector associations, and 20 major food and drink companies. FoodDrinkEurope aims to promote and represent the interests of the food and drink industry in the EU and beyond. It covers issues such as food quality and safety, nutrition and health, novel foods, labeling, agricultural policy, international trade matters, sustainable development, respect for the environment, and enlargement of the EU. The food and drink industry is one of the Europe's most important and dynamic industrial sectors.

More information on the European food associations and federations that are part of FoodDrinkEurope can be found on the FoodDrinkEurope website. A list of the major associations can also be found on the European Commission, Enterprise, and Industry website.

Below are a few examples of some of the sectoral associations:

- Association of the Chocolate, Biscuit and Confectionery Industries (CAOBISCO) – represents approximately 2000 companies of the chocolate, biscuits, and confectionery sectors of the EU.
- European Sugar Manufacturers Committee (CEFS) – represents all European sugar manufacturers and refiners.
- European Dairy Association (EDA) – represents the European dairy industry.
- European Snacks Association (ESA) – is Europe's trade organization dedicated to advancing the savory snacks industry on behalf of member snack manufacturers and suppliers.
- The Federation of European Union Manufacturers and Suppliers of Ingredients to the Bakery, Confectionery, and Patisserie Industries (FEDIMA) – represents the intermediate products industries for the bakery and confectionery trades.
- Union of European Beverages Associations (UNESDA) – represents the nonalcoholic beverages manufacturers in the EU.

The food industry organizations are structured in a similar way. The common feature of these trade associations is that they have a policymaking body in their structure as well as committees or working groups that discuss specific issues and oversee the day-to-day management of issues. *Ad hoc* groups or task forces can also be set up within a working group or committee to address specific issues. All the businesses are governed by EU legislation. The sectors/associations influence the environment in which their members operate. There are horizontal legislations formed in Brussels and vertical legislations covering products in different sectors. In addition to

legislation, below are some of the aspects addressed by sectors or associations:

- Representing, promoting, and protecting members' interests.
- Policy developments on food safety and quality, nutritional health, sustainability and market management as part of the EU Common Agricultural Policy (CAP), as well as guaranteeing the right to be competitive and innovative.
- Promoting dialogue and exchange of information at technical and legislative levels, for example, on additives, labeling, food quality, environment, nutrition and diet, with the European Commission, European Food Safety Authority (EFSA), national regulators, and other international bodies like Codex.
- Keeping members informed of EU developments.
- Working with legislators and regulators with the aim of improving the workability, proportionality, or enforceability of legislation by consulting and contributing on behalf of members to the setting up or review of European legislation directly affecting members.

An overview of how the national federations within the EU food industry work is reflected in the structure and function of the description given for FDF and its sector associations. The details and exact structures or membership may differ with each national federation, association, or sector within the EU Member States. However, the overall objective is to represent, promote, and protect the particular interests of the members of the concerned sector, association, or federation.

Food and Drink Federation

FDF is the voice of the UK food and drink industry. FDF is a trade organization representing, promoting, and safeguarding the interests of the UK food and drink manufacturing industry in relation to scientific issues and regulatory systems and associated developments which impact on the production and sale of food and drink products.

FDF represents the interests of the UK's food and non-alcoholic drinks manufacturing industry, the largest manufacturing sector in the UK, employing more than 400 000 people. The industry has an annual turnover of £72.8 bn accounting for 15% of the total manufacturing sector. Exports amount to almost £10 bn of which 79.2% goes to EU members. The industry buys two-thirds of all UK's agricultural produce. FDF membership comprises manufacturers of all sizes as well as trade associations dealing with specific sectors of the industry.

Member Services and Sector Groups

FDF is a membership-based organization. Its policies are developed through industry-wide consultation channeled through its governing Council, advised by specialist committees/panels, and informed by sector networking groups. Current FDF membership comprises of 13 associations, 7 sector groups, and other membership organizations (See annex) (Figure 1).

In Scotland, Wales, and Northern Ireland, some government policies and public services are different from those in

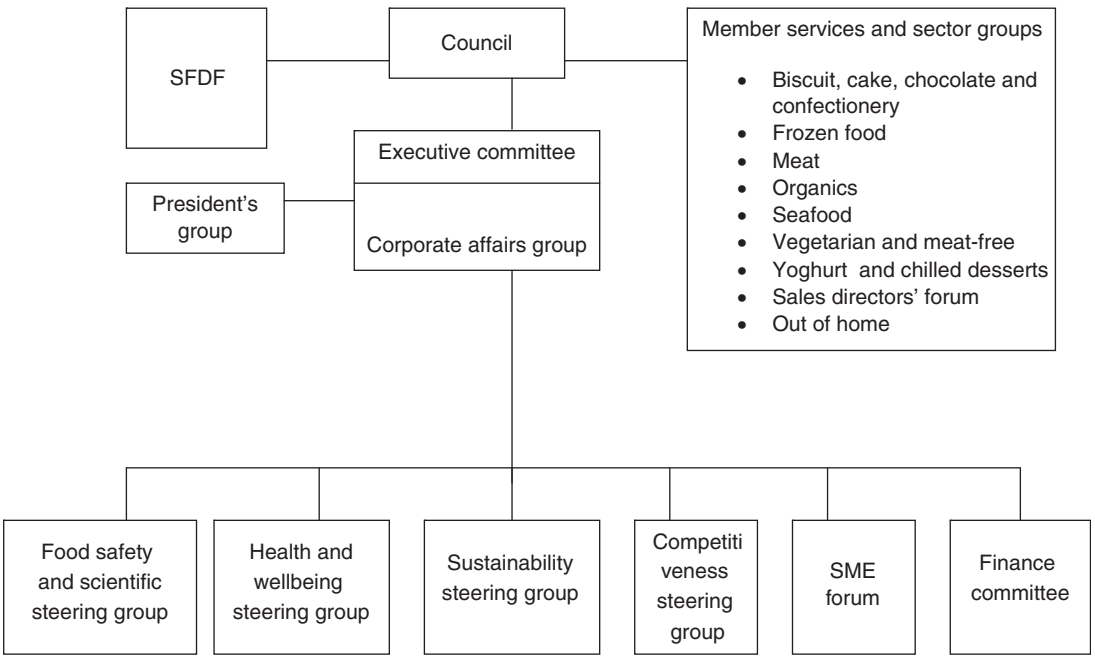

Figure 1 FDF organizational structure. SFDF, Scottish Food and Drink Federation; SME, small- and medium-sized enterprises.

England. Since 1999, the UK central government has given certain powers to devolved governments, so that they can make decisions for their own areas.

Scottish FDF (SFDF) is a devolved division of FDF and is 'the voice' of food and drink manufacturers in Scotland, ensuring the Scottish food industry is heard where it matters in the Scottish, UK, and EU contexts.

Each sectoral organization represents the specific interests of its group. For example the Biscuit, Cake, Chocolate, and Confectionery (BCCC) Group represents the specific interests of UK biscuit, cake, chocolate, and confectionery manufacturing/supplying/marketing companies. The Group is the representative and influential voice of the sector, promoting the industry and representing its viewpoint to the government, to all relevant regulatory, scientific, and enforcement authorities, and other public bodies.

In addition, the Group is the UK representative body on the EU organization representing the product sector, i.e., CAOBISCO, so as to play a full part in influencing EU policy matters which impact on the sector.

Food and Drink Federation Vision and How We Work

The core areas of FDF's work are:

- Food safety
- Promoting the competitiveness of the food and drink industry
- Health and well-being
- Sustainability
- Communications

Food Safety

The overall FDF vision enshrined in its core areas of work is that stakeholders, including government and consumers, trust the UK food and drink industry and have confidence both in the safety of FDF members' products, and from a health and well-being perspective, to see the industry as socially responsible and to know that all products can fit into a balanced lifestyle. The vision from a competitiveness point of view is for stakeholders to understand and value the economic contribution of the UK food and drink sector and for this to be fully taken into account in government policy-making and to realize that the industry is working to improve its sustainability – economically, environmentally, and socially.

The Food Safety and Scientific Steering Group, supported by its committees, is at the core of FDF activities and seeks to ensure that all FDF policies are rigorously science-based, including the scientific underpinning of the work of other divisions in FDF and their activities such as the Health and Well-being, Competitiveness, and Sustainability Steering Groups. The Food Safety and Scientific Steering Group represents, promotes, and safeguards the interests of the UK food industry in relation to scientific issues and regulatory developments. Its role is central to supporting and communicating food safety and science as pillars of FDF's work.

Within the Food Safety and Scientific Steering Group, there are seven issue-led committees to facilitate more detailed discussion of specific issues; these are:

- Food Chain Issues, covering issues relating to the safety of the supply chain in respect of food raw materials and animal feedstuffs, and including the overarching issues of GM, novel foods, and nanotechnology;
- Food Contact Materials, dealing with the safety of packaging materials in immediate contact with food and food ingredients;
- Food Hygiene, including EU food hygiene legislation, hazard analysis critical control points (HACCP), and microbiological food safety;
- Food Ingredients, dealing with developments relating to the use of additives, flavorings, enzymes, and processing aids;
- Food Law and Labeling, covering general food law and food labeling;
- Nutrition, which includes developments in the science in relation to diet and health, obesity strategy, reformulation, portion sizes, nutrition and health claims, and nutrition profiling; and
- Residues and Contaminants, which deals with the safety of raw materials throughout the supply chain in terms of chemical and other contaminations. This includes process contaminants and plant protection products.

Each work area comprises a list of priority topics followed by issues to be monitored, with a view to action if appropriate. A continuing requirement for each committee will be to identify emerging issues and assess the need for action, which may necessitate a revision of established priorities. The work program for each committee is reviewed and updated annually.

All these committees assist FDF members in meeting the requirements of the legislation. In addition, FDF uses the science base to drive development, innovation, and reformulation of products. For example, under nutrition, the science of nutrition and our ability to apply it to drive health and well-being in consumers is seen as a key indicator of whether the food and drink industry is perceived as being one that is responsible and able to move with the times – be these developments in the realms of pure or applied (e.g., social) science.

The roles of the Competitiveness and Sustainability Steering Groups are to provide strategic and proactive leadership on competitiveness issues and lead on sustainability and environmental issues affecting the well-being of the food and drink manufacturing industry, respectively.

From time to time, FDF will also form *ad hoc* technical groups when input is required on particular issues.

FDF's communication programs aim to enhance the industry's reputation as a responsible provider of safe food products and reliable information.

Informing and Consulting Members

FDF ensures that members stay abreast of the advances in science that change our understanding of food safety issues and FDF promotes the need for appropriate risk assessment and reduction strategies by regulators.

FDF enhances the coordination of the industry's approach and response to scientific and regulatory issues, and food safety incidents, by provision of timely information and advice to members, and by undertaking consultation as appropriate.

FDF liaises and develops partnerships, including where appropriate joint positions, with supply chain organizations, including retail, food service, farming, scientific, academic, and other organizations that are part of the food chain including consumer organizations.

Food Safety and Science is Key to Our Work

The safety of food products is the top priority for the food industry and this is reflected in FDF's positions on key issues. As part of their due diligence requirement, manufacturers need to have robust systems in place to ensure that from the time of harvest or slaughter to the time of consumption, product safety is monitored and maintained – whether the potential threat comes from chemical contamination, physical objects, or microbiological infection.

As such FDF ensures that members stay abreast of advances in science that may change FDF policy toward food safety or approach to appropriate risk assessment, risk management, and risk communication by national and European regulators.

It is important to remember that science also brings opportunities for product and process innovation and FDF works with members to develop policy positions to address emerging technologies which explain how these developments can provide real benefits for consumers. FDF's secretariat and committees also work to ensure that regulatory approaches to food information to consumers and nutrition are based on science.

Link with Food Research Organizations

To keep abreast of advances in science, FDF member companies are also members of independent membership-based organizations that carry out research and development for the food and drinks industry worldwide, such as Leatherhead Food Research and Campden BRI. These food research organizations are also affiliate members of FDF.

These food research organizations help the food industry meet their daily and long-term technical, scientific, information, and training needs. They help the industry to innovate and evolve by offering services including market intelligence, food research and analysis, food legislation, business and technical information, and training.

Compliance with Food Legislation

The safety of food and drink products, and their delivery in prime condition along the food chain to consumers, is the key priority of the food and drink manufacturing industry. In this respect, the food and drink industry is highly regulated and carefully monitored to ensure the safety and integrity of the food supply chain.

The Food Safety Act 1990 (as amended) provides the framework for all food legislation in Great Britain – similar legislation applies in Northern Ireland. This is an important act in Great Britain, which updated previous law on food safety and consumer protection. This has been in existence since before the 'General Food Law' Regulation (EC) No 178/2002, which covers EC legislation on general food safety.

According to the General Food Law, in the market, it is prohibited to put food which is unsafe (article 14). The legislation covers hygiene, microbiological contamination, residues, and contaminants. There are also more specific regulations, for example: on food additives, Regulation (EC) No 1333/2008; Enzymes Regulation (EC) No 1332/2008; Flavourings Regulation (EC) No 1334/2008, and Regulation (EC) No 258/97 on novel foods, which is currently under review. These regulations are applicable to the risk assessments of specific ingredients and how they are processed, as appropriate.

Framework Regulation (EC) No 1935/2004 on materials and articles intended to come into contact with food as well as legislation on active and intelligent materials, and articles in contact with food are applicable to packaging material. Compliance with this legislation is necessary to ensure compliance with general food law. There are also commodity specific 'directives' or regulations, for example, on chocolate, jam, etc.

As the body of legislation bearing down on the food industry continues to grow, FDF works closely with its European counterpart, FoodDrinkEurope, and European sector associations, to ensure that the industry's voice is heard in the right places and at the right time within the EU. The industry's input ensures that harmonized legislation is in place in order to ensure the free movement of food and feed in the EU and to comply with international standards where necessary.

The Food Chain

The inherent perishability of food from the time of harvest or slaughter requires food manufacturers to be continually vigilant in maintaining product safety – whether from the threat of chemical, physical contamination, or microbiological infection. FDF's work covers developments and issues that impact on the production and sale of food and drink products, specifically:

- food safety, processing, and composition;
- the labeling and presentation of the final product; and
- nutrition, diet, and health.

This involves a whole food chain approach, constructively influencing the regulatory environment in which the industry operates, working with Government and others to improve confidence in the safety of the food supply, and addressing sustainability and competitive issues in the related regulatory systems.

The food chain encompasses a number of players from primary producers through to consumers. This chain is wide, complex, and global (Figure 2). As such, transparency and compliance with legislation is needed throughout the chain to ensure that food safety is achieved. Hence, responsibility for

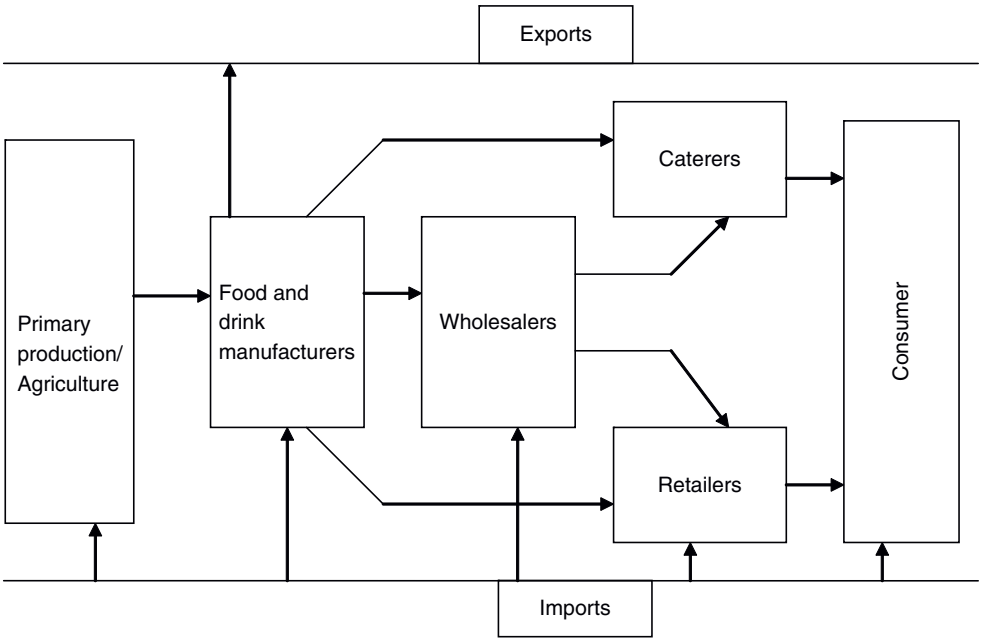

Figure 2 Interaction of the food chain stakeholders. *Source*: Institute of Manufacturing, University of Cambridge, Report to FDF, Value of Food and Drink Manufacturing to the UK, July 2010.

food safety is shared by everyone involved with food, from primary production to consumption, including growers, processors, regulators, distributors, retailers, and consumers.

Responding to Food Incidents

In addition to the objective to seek proportionate and evidence-based legislation that control food, FDF also assists members in the prevention of incidents through the work of the Incident Management Working Group, including identifying the root causes of incidents and horizon scanning for emerging issues. Although the food and drink industry strives to follow and maintain food standards, guides, and codes of practice and legislation along the food chain, incidents do happen now and again. Notable examples of incidents affecting the food industry in recent years are melamine contamination of milk in China, Sudan I contamination of spices originating from India, and dioxins contamination of guar gum from India. With the increasing globalization and complexity of the food chain, trade of food, food ingredients, and agricultural products around the world are continuing to increase; this also increases chances of food incidents happening along the food supply chain. Some of the contaminations are deliberate, i.e., fraud, and some are accidental. As such, FDF promotes and fosters the proportionate handling of food safety incidents at national and EU level.

FDF's Incident Management System assists the food industry in taking prompt action in response to emerging or potential food safety incidents. At national level, the Food Standards Agency (FSA) leads on incidents in liaison with the EU Rapid Alert System for Food and Feed (RASFF), which is managed by the European Commission. FDF helps its members by ensuring that food safety incidents, when they happen, are handled in a proportionate way, at national and EU level, and FDF coordinates an effective industry response to any such incident. FDF assists by cascading information from FSA, FoodDrinkEurope, and the Commission. FDF also consults members for the necessary information that may be required from industry regarding a particular incident.

Partnership

FDF helps its members operate in an appropriately regulated marketplace to maximize their competitiveness. FDF communicates the industry's values and concerns to Government, regulators, consumers, and the media. FDF also works in partnership with key players in the food chain to ensure that our food is safe and that consumers can have trust in it. This involves working with various UK Government departments including FSA, Department for Environment Food and Rural Affairs (Defra), Department of Business, Innovation and Skills (BIS), Local Government Regulation (Enforcement Authority), and others including the European Commission, European Parliament and EFSA, Codex, and FoodDrinkEurope to improve confidence in the safety of the food supply by seeking proportionate and evidence-based legislation.

Annex

The following Associations are members of the FDF:
ABIM Association of Bakery Ingredient Manufacturers
ACFM Association of Cereal Food Manufacturers
BCA British Coffee Association
BOBMA British Oats and Barley Millers Association

BSIA British Starch Industry Association
CIMA Cereal Ingredient Manufacturers' Association
EMMA European Malt Product Manufacturers' Association
FA Food Association
FOB Federation of Bakers
FPA Food Processors' Association
GPA General Products Association
BSNA British Specialist Nutrition Association
MSA Margarine and Spreads Association
SB Sugar Bureau
SIBA Society of Independent Brewers
SMA Salt Manufacturers' Association
SNACMA Snack, Nut, and Crisp Manufacturers' Association
SPA Soya Protein Association
SSA Seasoning and Spice Association
UKAMBY UK Association of Manufacturers of Bakers' Yeast
UKTC UK Tea Council

Within FDF there are the following sectoral organizations:
BCCC Biscuit, Cake, Chocolate, and Confectionery Group
FF Frozen Food Group
MG Meat Group
ORG Organic Food and Drink Manufacturers' Group
SG Seafood Group
VEG Vegetarian and Meat-Free Industry Group
YOG Yoghurt and Chilled Dessert Group

See also: Institutions Involved in Food Safety: FoodDrinkEurope. Public Health Measures: Challenges of Industrialized Countries in Food Safety Management; Modern Approach to Food Safety Management: An Overview

Relevant Websites

http://www.campden.co.uk/content.htm
Campden BRI.
http://www.defra.gov.uk/
Department of Environment Food and Rural Affairs.
http://ec.europa.eu/food/food/rapidalert/index_en.htm
European Commission.
http://www.efsa.europa.eu/
European Food Safety Authority.
http://www.codexalimentarius.org/
FAO/WHO Food Standards.
http://www.fdf.org.uk/
Food and Drink Federation.
http://www.food.gov.uk/foodindustry/links/
Food Standards Agency: A Selection of Websites with Information Related to the Food Industries.
http://www.fooddrinkeurope.eu/
FoodDrinkEurope: Information on European Food and Drink Industry.
http://www.leatherheadfood.com/
Leatherhead Food Research.
http://www.lacors.gov.uk/lacors/home.aspx
Local Government Regulation: Information on UK Enforcement of Food Safety.
http://www.opsi.gov.uk
Office of Public Sector Information: Food Safety Act (1990) (as amended).
http://www.pesticides.gov.uk/
Pesticides Safety Directorate: Information on Pesticides Controls in the UK.
http://www.food.gov.uk/
UK Food Standards Agency (FSA).
http://www.vmd.defra.gov.uk/
Veterinary Medicines Directorate: Information on Veterinary Medicines Controls Accessible on the Veterinary Medicines Directorate (VMD) Website.

INSTITUTIONS INVOLVED IN FOOD SAFETY

International Organization for Standardization (ISO)

M Petró-Turza, formerly Budapest Technical University, Budapest, Hungary

Glossary

Control measure Action or activity that can be used to prevent or eliminate a food safety hazard or reduce it to an acceptable level.

International standard Standard that is adopted by an international standardizing/standards organization and made available to the public.

International standardization Standardization in which involvement is open to relevant bodies from all countries.

Operational prerequisite program (oPRP) PRP identified by the hazard analysis as essential in order to control the likelihood of introducing food safety hazards and the contamination or proliferation of food safety hazards in the product(s) or in the processing environment.

Prerequisite program (PRP) Basic conditions and activities that are necessary to maintain a hygienic environment throughout the food chain suitable for the production, handling, and provision of safe end products and safe food for human consumption. Note: The PRPs needed depend on the segment of the food chain in which the organization operates and the type of organization. Examples of equivalent terms are: good agricultural practice (GAP), good veterinarian practice (GVP), good manufacturing practice (GMP), good hygienic practice (GHP), good production practice (GPP), good distribution practice (GDP), and good trading practice (GTP).

Process An activity using and managing resources in order to enable the transformation of inputs into outputs. Often the output from one process directly forms the input to the next.

Process approach Application of a system of processes within the organization, together with the identification of interactions and the management of these processes.

Standard Document, established by consensus and approved by a recognized body, that provides, for common and repeated use, rules, guidelines, or characteristics for activities or their results, aimed at the achievement of the optimum degree of order in a given context. Note: Standards should be based on the consolidated results of science, technology, and experience, and aimed at the promotion of optimum community benefits.

Standardization Activity of establishing, with regard to actual or potential problems, provisions for common and repeated use, aimed at the achievement of the optimum degree of order in a given context. Note: (1) In particular, the activity consists of the processes of formulating, issuing, and implementing standards; (2) important benefits of standardization are improvement of the suitability of products, processes, and services for their intended purposes, prevention of barriers to trade, and facilitation of technological cooperation.

Standardizing body Body that has recognized activities in standardization.

About International Organization for Standardization in General

The International Organization for Standardization (ISO in each language) is the world's largest developer and publisher of International Standards. ISO was the first established international standardizing organization. Officially, ISO began operating in 1947 with 25 national standards institutes as its members (one per country) and at present, the number of its member bodies is 162. The system of this global scale network of national standards institutes is coordinated by the ISO Central Secretariat, located in Geneva, Switzerland.

ISO has more than 3000 technical bodies, among them approximately 200 active technical committees for developing ISO standards; and more than 50 000 experts for participating in its activity. ISO has more than 17 500 International Standards and other types of normative documents in its current portfolio. The technical work of ISO is regulated by a stringent procedure which was developed and continuously improved since ISO's existence. The overall coordination of this technical work is made by the ISO Technical Management Board. The above mentioned data speak for themselves and show that in the past more than 60 years ISO has kept its leading role in international standardization.

ISO deals with the full spectrum of human activity with the exception of electrical and electronic engineering where International Standards are developed by the International Electrotechnical Commission (IEC) as well as the

telecommunication sector, which is the area of International Telecommunication Union (ITU). ISO work program ranges from standards for traditional activities, such as agriculture and construction, through mechanical engineering, manufacturing, and distribution, to transport, medical devices, information, and communication technologies, and to standards for good management practice and services.

Considering the fact that ISO members are not national governments, but the national bodies most representative of standardization in their respective countries, ISO is a non-governmental organization that forms a bridge between the public and private sectors. Many of its member institutes are part of the governmental structure of their countries, or are mandated by their government. Other ISO members have their roots uniquely in the private sector, having been set up by national partnerships of industry associations. Therefore, ISO enables to reach consensus that meet both the requirements of business and the broader needs of society.

ISO standards are voluntary and it does not enforce their implementation. A certain percentage of ISO standards – mainly those concerned with health, safety, and environment – has been adopted in some countries as part of their regulatory framework, or is referred to in legislation for which it serves as the technical basis. However, such adoptions are sovereign decisions by the regulatory authorities or governments of the countries concerned. ISO itself does not regulate or legislate.

Although ISO standards are voluntary, the fact that they are developed in response to market demand, and are based on consensus among the interested parties, ensures their widespread use.

Food Standardization in International Organization for Standardization

Since the beginning, ISO has dealt with food standardization. The majority (70%) of this type of ISO standards specifies analytical or test method. A part of them is suitable for the determination of physical or sensory properties of foods or for the determination of their chemical compound(s); others are used for the measure the extraneous and/or foreign matter content or microbiological contaminations; again others are suitable for the detection and/or determination of toxic materials or genetically modified organisms in food products; whereas other methods are developed for the detection of food adulteration. The suitability of most of the ISO methods is checked by international ring tests. Therefore, many ISO methods are recognized by the Codex Alimentarius Commission.

The second group of ISO food standards (12%) deals with product specifications, majority of them concerns spices and a few concern cereals. The relatively low proportion of this type of ISO standards can be explained by the fact that most of the product specifications are standardized by the Commodity Codex Committees.

There are ISO food standards dealing with storage conditions and transport (9%), whereas others specify sampling procedures (4%). Among the remainder (5%) the group containing vocabularies or nomenclatures is worth highlighting. These International Standards edited trilingually or bilingually are comprehensively used in the world trade and accepted by other international organizations as well.

Food Safety Standards of International Organization for Standardization

As the food safety is a typical area of the Codex Alimentarius Commission, ISO started to deal with this question rather late, in 2001, because of the huge demand of its member bodies. At that time the hazard analysis and critical control point (HACCP) system was already well known and used around the world. Therefore, it is a fair question why does the need for an ISO standard arise?

The main problem with the HACCP system is its general and brief text, which:

- allows various interpretations resulting in huge differences in the implementation and in the acceptance criteria;
- does not give enough help for the food producers in the practical application of the system; and
- does not contain auditable requirements as this Codex document is only a recommended guideline.

Following the success of the ISO 9000 standard series, certified management systems have increasingly become a general requirement for access to both national and international markets. Therefore, many retailer syndicates have prepared food safety auditing standards e.g., the British Retailer Consortium (BRC), the European Food Safety Inspection Service (EFSIS), or the International Food Standard (IFS) developed by German and French companies on the basis of BRC and EFSIS, and require their suppliers to develop food safety management systems and obtain certification to these standards. Although it is stated that these standards do not differ significantly from each other, certification according to one is accepted only in a few cases by another.

The reasons that led to the development of ISO 22000, the basic food safety standard of ISO, can be summarized as follows:

- increasing number and severity of regulations and controls relating to food safety;
- greater extent of customers' requirements (owing to their increased knowledge of the health effects of nutrition and the risks of food safety);
- HACCP systems in place but with differing requirements and implementation in different countries and for different foodstuffs;
- food producers have to comply with different requirements depending on the country and customer; and
- retailers are certifying and inspecting food producers and have set a variety of specific criteria.

The solution to the above mentioned problems was awaited from ISO, as it was the most recognized international standardization organization, which developed the well-known management system standards (ISO 9001, ISO 14001), new ones (e.g., for information security (ISO/IEC 27001), and for supply-chain security (ISO/PAS 28000)).

International Standard ISO 22000:2005, Food Safety Management Systems – Requirements for Any Organization in the Food Chain

In the development of this new ISO standard, experts from more than 40 countries took part and it was published in September 2005. ISO 22000 constitutes an internationally recognized food safety management system allowing an organization to implement and demonstrate compliance with the relevant documents of Codex, thus simplifying/clarifying the customer/supplier relationship. ISO 22000:

- although arranged differently, includes each principle and application step of the HACCP system, so standardizes its implementation and the basic criteria for its acceptance;
- completes HACCP with more detailed instructions regarding its application;
- contains auditable requirement; and
- Annex B contains cross references between HACCP and ISO 22000 to promote the combination of the two systems.

However, ISO 22000 is much more than the HACCP itself, as it combines the HACCP system with the prerequisite programmes (PRPs) and with the quality management system (QMS).

ISO 22000 categorizes the control measures into the following three groups:

- PRPs,
- operational prerequisite programmes (oPRPs), and
- HACCP plan and its critical control points.

PRPs ensure the basic conditions and activities which each member of the food chain should perform. These help in maintaining a hygienic production, processing, and handling environment. PRPs do not need validation but their effectiveness shall be verified periodically.

Both of the other types of control measures are managing control measures identified by the hazard analysis and both shall be validated and verified.

ISO 22000:2005 introduced the new term, oPRP, to handle those control measures that, for any reason, cannot be included or those that it is not necessary to include in the HACCP plan as critical control points. For example, the critical limit of the given hazard cannot be determined, or can be determined but cannot be validated, or the incidence rate of the given hazard is relatively low. This makes it possible to focus the HACCP plan at the most critical hazards, and keep all others under appropriate control.

Among the management system elements of ISO 22000:2005, the following should be highlighted:

- interactive communication (internal and external);
- structured processes/process approach (help to identify and manage numerous linked activities);
- commitment of top management (management responsibility); and
- keeping the system alive (by verification, updating, review, and continual improvement).

ISO 22000:2005 emphasizes the necessity that personnel whose activities have any impact on food safety shall be aware of the relevance and importance of this fact. Therefore, the organization shall provide training or take other appropriate action to ensure personnel have the necessary competencies and awareness. It is important to mention that within the top management responsibilities, the establishment, implementation and maintenance of procedures to manage potential emergency situations and accidents that can impact food safety are also included.

Documentation forms an integral part of management systems. as it has a key role in the case of certification. ISO 22000:2005 deals with the general rules of the documentation in a separate clause, but every clause which specifies any form of documentation draws the attention to it. The Standard differentiates the following types of documents:

- Documented procedures, methods or statements. This means much more than a simple putting down in writing. This type of document shall be developed, implemented, and updated as a part of the food safety management system. Such document is, for example, the food safety policy, the procedure for handling of potentially unsafe products, or the method of hazard analysis.
- Records. The Standard requires this type of document in many cases as they provide evidence of activities performed. Records can be used to demonstrate the suitable operation and the effectiveness of the food safety management system.
- Other documents, for example product specifications.

The main advantages of ISO 22000:2005 can be summarized as follows:

- gives one set of requirements that can be applied to any organization in the food chain in any country;
- internationally recognized;
- auditable, as it contains requirements;
- flexible, organizations can choose which methods and approaches they use to fulfil the requirements of ISO 22000;
- can be applied independently of other management system standards;
- can easily be integrated with existing other management systems and/or with the HACCP method;
- allows small or less developed organizations to implement an externally developed combination of control measures; and
- supported by other ISO deliverables (ISO/TS 22002-1:2009; ISO/TS 22003:2007; ISO/TS 22004:2005; ISO 22005:2007 and *An easy-to-use checklist for small business*).

According to the data of *The ISO Survey of Certifications – 2008* during 3 years, after the publication of ISO 22000, at least 8206 organizations in 112 countries were independently certified to ISO 22000:2005 and their number continuously increases every year.

International International Technical Specification ISO/TS 22004:2005, Food Safety Management Systems – Guidance on the Application of ISO 22000:2005

This ISO document assists the adoption of ISO 22000:2005. As there was a great demand to publish the guidance close to

the date of publication of ISO 22000, it was decided to prepare it as a Technical Specification (TS). In this case, the ISO procedure is shorter; in addition, the required degree of consensus for publication is lower than in the case of an International Standard. ISO/TS 22004 was published in November 2005.

The guidance deals with those parts of the requirements of ISO 22000:2005 which should be handled with special attention. For example, it provides the small and/or less developed organizations with additional information. ISO/TS 22004 helps to avoid misinterpretation of ISO 22000 and to clarify the meanings of its terms. It provides the users with such explanations which were not possible to include in the standard but promoted its application.

International Technical Specification ISO/TS 22003:2007, Food Safety Management Systems – Requirements for Bodies Providing Audit and Certification of Food Safety Management Systems

Certification of the implemented food safety management system (FSMS) is not a requirement of ISO 22000, but – as mentioned before – it is one means of providing assurance that an organization has implemented a system for the management of food safety in line with its policy. Therefore, the number of organizations in the food chain which have certified FSMS continually increases.

ISO/TS 22003 was developed to promote decreasing quality differences among activities of the certification bodies, and giving certification only to those suppliers whose management commitment to food safety ensures that they will operate an effective FSMS.

ISO/TS 22003 was developed in harmony with ISO/IEC 17021:2006, *Conformity assessment – Requirements for bodies providing audit and certification of management systems*, which contains the general requirements for bodies providing audits and certification of management systems.

ISO/TS 22003 defines specific rules applicable for the audit and certification of a FSMS complying with the requirements given in ISO 22000:2005 (or other sets of specified FSMS requirements), and provides the necessary information and confidence to customers about the way certification of their suppliers has been granted.

International Standard ISO 22005:2007, Traceability in the Feed and Food Chain – General Principles and Basic Requirements for System Design and Implementation

The traceability of food products 'from the farm to the fork' is an important requirement of ISO 22000, therefore ISO developed a separate standard for this purpose. ISO 22005 complements the relevant Codex documents and helps food producers and manufacturers to comply with the relevant statutory requirements by providing an internationally recognized approach for designing a traceability system.

ISO 22005:2007 can be applied by an organization operating at any step in the feed and food chain. It is intended to be flexible enough to allow feed organizations and food organizations to achieve identified objectives.

The traceability system is a technical tool to assist an organization to conform to its defined objectives, and is applicable when necessary to determine the history or location of a product or its relevant components.

ISO 22000, *Food Safety Management Systems – An Easy-To-Use Checklist for Small Business – Are You Ready?*

This handbook is a joint publication of ISO and the International Trade Centre (ITC). It is built up as follows: it quotes a part from ISO 22000, and raises a question relating to the quoted part.

- If the reply is 'Yes', it says 'Go to the next question'.
- If the reply is 'No' it says 'See guidance below.'

This structure makes possible to detect each weak and missing point of an existing FSMS in an effective and rapid way, and at the same time offers suitable solution(s) for their elimination.

International Technical Specification ISO/TS 22002-1:2009, Prerequisite Programs on Food Safety – Part 1: Food Manufacturing

This is the newest member of the ISO 22000 standard family and at the same time the first member of a new series, within this family, planned to develop relevant food sectors. ISO/TS 22002-1 were published on 15 December 2009.

The new technical specification applies to all organizations involved in the manufacturing step of the food chain, regardless of size or complexity. It is not a requirement of ISO 22000 and may be used in parts or in its entirety, depending on the nature of food manufacturing operations.

Availability of International Organization for Standardization Deliverables

The hard copy of each member of ISO 22000 standard family can be ordered from ISO national member institutes, and from the ISO Store at the Central Secretariat. The whole ISO 22000 standard family is also available on a CD format (bilingual, English and French).

Further Reading

Faergemand J (2008) The ISO 22000 series – Global standards for safe food supply chains. *ISO Management Systems* May–June: 4–7.

Frost R (2006) Early adopters underline benefits of new ISO standard for safe food supply chains. *ISO Management Systems* March–April: 21–25.

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Relevant Website

www.iso.org

International Organization for Standardization. Also see the websites of ISO member bodies.

INSTITUTIONS INVOLVED IN FOOD SAFETY

International Life Sciences Institute (ILSI)

N van Belzen, ILSI Europe, Brussels, Belgium
E Hentges, ILSI North America, Washington, DC, USA
J Chen, ILSI Focal Point in China, Beijing, China
YB Yee, ILSI Southeast Asia Region, Singapore
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Glossary

Acceptable daily intake (ADI) Estimate of the amount of a substance in food or drinking water, expressed on a body weight basis, which can be ingested daily of a lifetime by humans without appreciable health risk.

As low as reasonably achievable (ALARA) A risk management approach that aims to keep exposure to a compound at the lowest level that is realistically achievable.

Exposure assessment Assessment of the amount of a particular chemical agent that reaches the target population, organism, organ, tissues, or cells, usually expressed in numerical terms of substance concentration, duration, and frequency.

Genotoxic carcinogen A compound that causes cancer by binding to DNA.

Harmonization Agreement on general principles of food safety standards as well as technical aspects, for example, methodologies, information requirements, or criteria for determining unacceptable risks, by groups representing national and international governmental bodies and others.

Hazard analysis critical control point (HACCP) A system that identifies, evaluates, and controls hazards that are significant for food safety.

Margin of exposure (MOE) The ratio between a reference concentration of a compound (often taken from an animal study and corresponding to the dose that causes a low but measurable response in animals) and the estimated dietary intake in humans.

Probabilistic modeling Use of statistical tools to estimate the likelihood of an event occurring based on historical data.

Risk analysis A discipline comprised of three components: risk assessment, risk management, and risk communication, that is used to provide food safety regulators with the information and evidence they need for effective decision-making to improve public health.

Risk assessment A process intended to calculate or estimate the risk to a given target organism, system, or (sub)population, including the identification of attendant uncertainties, following exposure to a particular agent, taking into account the inherent characteristics of the agent of concern as well as the characteristics of the specific target system.

Purpose and Structure of International Life Sciences Institute

The International Life Sciences Institute (ILSI) is a nonprofit, worldwide organization established in 1978 to advance scientific understanding of nutrition, food safety, toxicology, risk assessment, and the environment. Working through its 15 branches around the world and Research Foundation, ILSI brings together scientists from academia, industry, government, and the public sector for a balanced approach to solving problems of common interest for the health and well-being of the public. Figure 1 provides an organizational chart for ILSI.

The ILSI branches are membership organizations. Food processors, ingredient manufacturers, pharmaceutical manufacturers, agrochemical companies, and others are members of one or more branches. The ILSI Research Foundation does not have members.

All ILSI entities use a tripartite model involving scientists from academia, government, and industry to address scientific questions. ILSI entities address topics of interest through committees, task forces, or working groups made up of experts in the topic. All of ILSI's work becomes public knowledge through publications and meetings.

Each ILSI entity is governed by a group of nonindustry and industry scientists. The ILSI Board of Trustees, a 31-member international body, sets policy for the whole organization. All of the governing bodies are at least 50% nonindustry.

The branches work with their national and regional governmental bodies to ensure that these groups have access to the latest scientific information. For example, ILSI Europe aims to provide scientific information that can support the work of organizations like the European Food Safety Authority (EFSA), the Health and Consumer Protection Directorate-General of the

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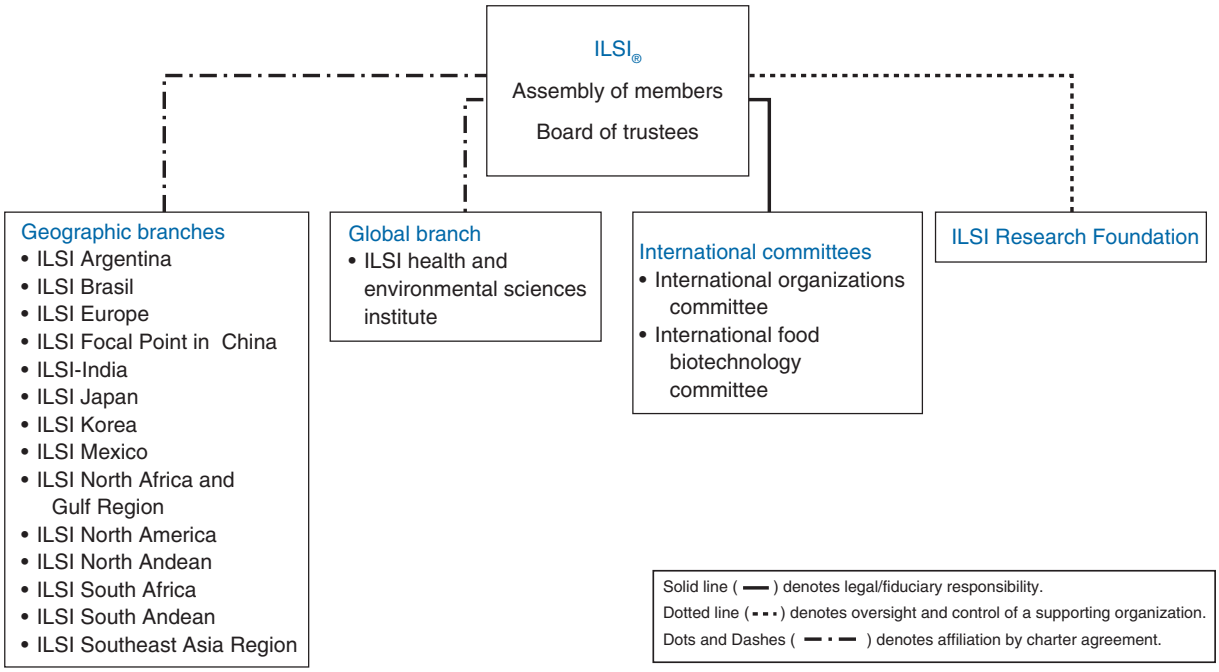

Figure 1 Organizational chart for the International Life Sciences Institute (ILSI). Reproduced from www.ilsi.org, with permission from International Life Sciences Institute.

European Commission (DG SANCO), and the national authorities in Europe. ILSI Focal Point in China provides current scientific information to the Ministry of Health on hot topics in food safety as a reference for their risk-management decisions. ILSI North America has cooperative programs with the US Food and Drug Administration, Centers for Disease Control and Prevention, Department of Agriculture, and other federal food safety agencies. ILSI Southeast Asia (SEA) Region has a long-standing working relationship with the ASEAN Health Authority on capacity building training and harmonization of safety standards. ILSI is also recognized by the World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO) as a nongovernmental organization.

International Life Sciences Institute's Role in Microbial Safety

ILSI Europe has published an extensive series of monographs on different foodborne pathogens, including their occurrence, toxicity, and mitigation options; the latest edition concerns food-related viruses. Other activities include guidelines on food safety objectives and a monograph on hazard analysis critical control point (HACCP), which is currently in its third edition; the first edition was translated into seven languages.

ILSI North America Committee on Food Microbiology is committed to providing sound science to improve understanding and control of microbial food safety hazards to enable scientifically-informed decision-making. For example, 4 years before the 1993 *Escherichia coli* outbreak in Washington state, the committee recognized the potential public health threat of this emerging pathogen and began supporting research that provided critical data on the prevalence of *E. coli* 015:H7 in retail foods and the development of detection methods still being used today. Data from these research projects were critical to the rapid recognition and control of the 1993 outbreak.

In addition, ILSI North America maintains a reference strain collection, including *Listeria monocytogenes* and *Cronobacter* (*Enterobacter*) *sakazakii*, for distribution to researchers worldwide. Current research priorities include *Salmonella* in low-moisture foods, control of sporeforming bacteria, and foodborne viruses.

International Life Sciences Institute's Role in Chemical Food Safety

Increasing sensitivity of analytical equipment and its concomitant decrease in detection levels result in the identification of low amounts of undesired chemicals in food and beverages, which requires assessment of their risks. Using the Paracelsus theorem which defines chemical substance as a health concern based largely on its dose, ILSI has helped develop the threshold of toxicological concern (TTC) concept, that provides a rationale and decision tree to indicate whether a chemical of known structure and exposure is a potential health concern, or not. The TTC concept is being applied for flavorings and its application for other purposes, such as cosmetic products, is being investigated. This is one example of how ILSI has contributed to scientifically based risk assessment methodologies.

Genotoxic carcinogens, which are chemicals that cause cancer by directly binding to the DNA, are exceptions to this approach. Although from a cell and tissue biology point of view it could be argued that thresholds might exist (e.g., due to DNA repair mechanisms and cell turnover), risk assessment has traditionally assumed the absence of a threshold for these compounds. Thus, it is assumed that any exposure above zero to genotoxic carcinogens could be a concern. Therefore, levels of these compounds must be kept as low as reasonably achievable (ALARA) in food. This ALARA approach, however, does not provide guidance to assess levels of concern and priority setting. Together with WHO and EFSA, ILSI has investigated the use of the margin of exposure (MOE) concept, which determines the ratio between experimentally derived toxicity data and exposure data in humans and can be used for prioritization of risk management actions.

As a result of the data from the ILSI North America Committee on saccharin's 13-year mechanistic research program, the International Agency for Research on Cancer (IARC) downclassified it in a precedent-setting process in its monograph evaluation series in 1999, the National Toxicology Program removed saccharin from its Congressionally-Mandated List of Carcinogens (also a precedent setting event) in 2000, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) increased its temporary acceptable daily intake (ADI) level, and the Canadian Government accepted a reasonable no-effect level for saccharin. Even more importantly, these data have been applied by the US Environmental Protection Agency in developing new guidelines for risk assessment that include the concept of mode of action as a key determinant of risk.

Recently, the ILSI Research Foundation has been exploring how the dose–response relationship at very low doses can be an approach in quantitative risk assessment. The concept of a threshold for adverse effects is fundamental in regulatory toxicology, underlying basic values such as ADI and tolerable daily intake. Analyzing the fundamental biological processes underlying human health effects for chemicals, allergens, pathogenic organisms, and nutrients led to a cross-disciplinary mode-of-action based analytical approach – Key Events Dose–Response Framework – that systematically examines important, required biological and chemical events along the pathway between exposure/intake and the ultimate effect of concern. The approach is described in a series of five papers published in the September 2009 issue of *Critical Reviews in Food Science and Nutrition*.

This new work provides an evidence-based approach and powerful new tool for using our growing understanding of fundamental biology and mechanistic data to reduce reliance on default assumptions, to quantify variability, and to better characterize biological thresholds.

Allergen Safety

Like many other allergens, food allergens are harmless to most people but a significant health risk to a minority, inducing a wide range of effects from mild irritation of the oral mucosa and the skin to anaphylactic shock and death. ILSI-funded research has used clinical data on eliciting allergen doses in combination with the population distribution of allergic individuals for probabilistic modeling of allergen risks. ILSI North America has reviewed a unique set of clinical challenge data for peanut allergens. A dose–response curve for risk characterization of the sensitized population was developed to validate the concept of a threshold level.

Food Safety Training and Education Activities

ILSI Europe organized a continuing education course on TTC at the meeting of European toxicological societies (Eurotox) in 2009. Training programs in collaboration with FAO and WHO to encourage the use of risk assessment in food safety control have been and are being organized in China, Southeast Asia, and Latin America. These workshops address topics such as setting of food safety standards, safety, and exposure assessment of food additives, microbial, mycotoxins, and other contaminants in the food chain. ILSI North America organized and sponsored 52 symposia, technical sessions, and workshops (1993–2009) on emerging global issues in food microbiology through its symposium series with the International Association for Food Protection.

Harmonization across Countries/Regions

Recognizing the difficulty that many countries in Central, Northern, and Southern Eastern Europe experience to harmonize their nutrition and food safety approaches with that of Western Europe, ILSI Europe provides travel grants for scientists from these countries to attend selected scientific events and courses in Western Europe.

In 2001, ILSI SEA Region collaborated with FAO–UN and WHO to establish an ASEAN (comprising 10 countries of the Association of Southeast Asian Nations) Working Group on Food Safety Standards Harmonization. Since then, a series of annual workshops have been conducted to promote scientific understanding and facilitate consensus on the application and implementation of risk analysis, exposure assessment, and harmonization of food safety standards in the region in line with Codex general standard for food additives (GSFA). A database was developed for the working group and the countries to tract the harmonization effort.

ILSI branches in China, Japan, and Korea exchange information on food safety regulations and standards, with the goal of facilitating harmonization these regulations and standards for Asian countries.

Summary Statement of What International Life Sciences Institute as an Nongovernmental Organization has been Able to Accomplish

For more than three decades, ILSI has advocated the use of science in public health decision-making. ILSI has established risk assessment and benefit assessment methodologies for evaluating diet, pharmaceuticals, and chemicals in relation to human and environmental health. Its unique mode of operation that brings together scientists from academia,

government, and industry to address food safety issues has proven effective in generating valuable scientific knowledge applicable for improving human health. The ILSI approach will continue to be relevant in the future for food safety decision-making.

For more information about ILSI and its scientific contributions, please visit the ILSI website (www.ilsi.org). The site has an advanced search function that allows the visitor to retrieve scientific publications sponsored by ILSI. The Scientific Issues section of the website has a subject-matter specific interactive risk assessment diagram that provides information on all ongoing activities in this area.

See also: Bacteria: *Cronobacter (Enterobacter) sakazakii* and Other *Cronobacter* spp.; *Listeria monocytogenes*. Disciplines Associated with Food Safety: Food Safety Toxicology. Food Additives: Sweeteners. Food Safety Assurance Systems: Hazard Analysis and Critical Control Point System (HACCP): Principles and Practice. Hazards of Food Contact Material: Nanotechnologies and Nanomaterials. Other Significant Hazards: Food Allergies and Intolerances. Public Health Measures: Assessment of Novel Foods and Ingredients; Safe Use of Wastewater for Agricultural Production. Risk Analysis: Risk Analysis of Hazards in Food: An Overview; Risk Assessment: Chemical Hazards; Risk Assessment: Microbiological Hazards. Safety of Food and Beverages: Safety of Genetically Modified Foods

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Relevant Websites

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Benefit-Risk Analysis of Foods.

www.ilsi.org
International Life Sciences Institute.

INSTITUTIONS INVOLVED IN FOOD SAFETY

FoodDrinkEurope

B Kettlitz, FoodDrinkEurope, Brussels, Belgium

Glossary

Codex Alimentarius (Latin for 'food code' or 'food book') is a collection of internationally recognized standards, codes of practice, guidelines and other recommendations relating to foods, food production, and food safety.

ETP European Technology Platform – A European network bringing together researchers, industry and, other relevant stakeholders in a particular technological field in order to foster European research and development in the concerned area.

European Rapid Alert System (RASFF) European system aiming at providing control authorities with an effective tool for the exchange of information on measures taken to ensure food safety.

FACET Flavors, Additives and Contact materials Exposure Task – European 7th Framework Project.

SMEs Small and medium enterprises – not having more than 250 employees.

Introduction to FoodDrinkEurope (formerly CIAA)

FoodDrinkEurope is the Umbrella Organization of the European Food and Drink Industry. FoodDrinkEurope membership is comprised of 26 national federations, including 3 observers; 26 EU sector associations; 18 major food and drink companies, of which the majority are Small and Medium Sized Enterprises (see Tables 1 and 2).

17 Major Companies

Agrokor, Cargill, Coca-Cola, Danone, Ferrero, General Mills, Heineken, Heinz, Kellogg, Mondelez (formerly Kraft Foods), Mars, Nestlé, PepsiCo, Südzucker, Tate & Lyle, Ülker, and Unilever.

The EU food and drink industry serves almost 500 million consumers with a wide variety of safe and high quality products.

The mission of an industry association is to represent the interests of its members. For FoodDrinkEurope, this means representing their interests at both the European and international institution levels.

FoodDrinkEurope contributes to the development of a legislative and economic framework for the food and drink industry, addressing competitiveness, food quality, and safety, consumer information and respect for the environment.

Access to safe food is an imperative for health, social, and economic reasons.

Although food in Europe is more varied and safer than ever before and the consumer is better informed and more aware of food related issues, consumers still do not appear to have sufficient trust in the supply chain.

FoodDrinkEurope Priorities

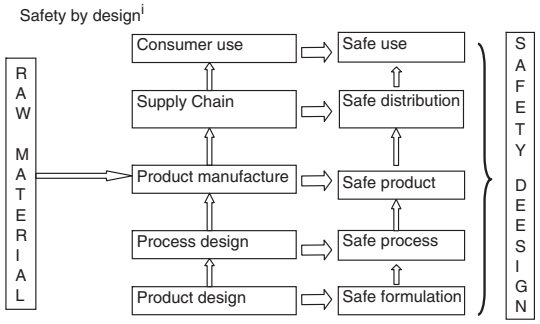
One of the key priorities of FoodDrinkEurope and its membership is therefore to increase consumer confidence in the food and drink industry.

To respond to this priority, FoodDrinkEurope is working together with more than 500 industry experts to address challenges related to food safety in the areas of process contaminants, contaminants, ingredients, food contact materials, in addition to novel foods, emerging technologies and others.

Very recently, a nanotechnology expert group was created by FoodDrinkEurope. This is a group in which both expert knowledge and communication skills are necessary to contribute to the public dialogs which are key to establishing trust in the food and drink industry.

Holistic Approach

The food and drink industry has developed an integrated and holistic approach to food safety. Safety is not guaranteed by 'safe' product manufacture – the total chain must be taken into account (see figure below):



 doi:10.1016/B978-0-12-378612-8.00009-3

Table 1 26 National food industry federations (including 3 observers)

ANIA – Association Nationale des Industries Alimentaires
BLL – Bund für Lebensmittelrecht & Lebensmittelkunde e.v.
DI – Fødevarer
ÉFOSZ – Elelmiszer–Feldolgozok Orszagos Szövetsége
ETL – Eesti Toiduainetööstuse Liit
ETL – Elintarviketeollisuusliitto Ry
FDF – Food & Drink Federation
FDII – Food & Drink Industry Ireland
FEDERALIMENTARE – Federazione Italiana Dell'Industria Alimentare
FEVIA aisbl – Fédération de l'Industrie Alimentaire/Federatie Voedingsindustrie
FIAA – Fachverband der Nahrungs- & Genussmittelindustrie
FIAB – Federación Española de Industrias de Alimentación y Bebidas
FIAL – Fédération des Industries Agro-Alimentaires Luxembourgeoises
FIPA – Federação das Indústrias Portuguesas Agro-Alimentares
FNLI – Federatie Nederlandse Levensmiddelen Industrie
GZS – Gospodarska Zbornica Slovenije, Zbornica Kmetijskih in Živilskih Podjetij
LI – Livsmedelsföretagen
LPUF – Latvijas Partikas Uznëmumu Federäcija
PFPZ – Polska Federacja Producentów Ywno ci zwi zek Pracodawców
PK ČR – Potravinárská Komora Ceské Republiky
PKS – Potravinárska Komora Slovenska
ROMALIMENTA – Federatia Patronala Romana din Industria Alimentara
SEVT – Federation of Hellenic Food Industries
UFPS – Union of Food Producers in Slovakia
HUP – Hrvatska udruga Poslodavaca
NHO – Mat og Drikke
TGDF – Türkiye Gida ve Içecek Sanayii Dernekleri Federasyonu

Table 2 26 European sector associations

- Bakery
- Bottled waters
- Broth & soups
- Dairy products
- Fruit & vegetable preserves
- Ice cream
- Margarine
- Pet food
- Processed potatoes
- Snacks
- Soluble & roasted coffee
- Spirits
- Tea & herbal infusions
- Beer
- Breakfast cereals
- Chocolate, biscuits & confectionery
- Dietetic products
- Fruit & vegetable juices
- Intermediate producers for bakery & confectionery
- Pasta
- Processed meat
- Sauce & condiments
- Soft drinks
- Spices
- Sugar
- Yeast

Work with Food Chain Partners

As not all food chain partners are members of FoodDrinkEurope, a Food Safety Platform, involving consumer representatives, farmers, feed producers, traders, restaurants and retailers was created at the initiative of the FoodDrinkEurope in 2005. The Platform members strive to exchange information related to the safety of the food chain, emerging issues included.

Emerging Issues

FoodDrinkEurope aims to solve emerging issues. These may be raised by individual members, provided that these are relevant for the entire food industry. The Association also analyses the European RASFFs (Rapid Alerts which aim to provide control authorities with an effective tool for the exchange of information on measures taken to ensure food safety.) which are published on the European Commission website in this respect.

FoodDrinkEurope has put in place an 'Incident Management System', which allows the organization to serve its members immediately, or within 48 h at the latest, by means of establishing appropriate:

- Information networks,
- Links with risk managers, and
- Risk assessors

to address the issue as necessary.

The ultimate aim of the above is the protection of consumer health and the prevention of unnecessary 'scare stories', which naturally have the potential to undermine consumer confidence in the food and drink industry.

The establishment of such a system has been proven to be successful and is appreciated by its members, due to its harmonized approach towards addressing issues and seeking solutions.

Example: Dealing with Emerging Issues

FoodDrinkEurope, together with its members, is also developing tools, such as guidelines for the proper interpretation of new legislation. Examples of these are the 'GMO Guidelines' and the 'Acrylamide Toolbox', the latter of which aims to help producers to find tools to mitigate acrylamide. The Toolbox was last updated in 2011.

Given that the majority of FoodDrinkEurope members are small and medium sized enterprises (SMEs), it is important that relevant information is addressed directly to such enterprises in a clear language and in translated versions. FoodDrinkEurope has for example, provided its members with 'acrylamide pamphlets' which offer 'tools to try' for five different sectors, namely:

- Bread products,
- French fries,
- Potato crisps,
- Biscuits, and
- Breakfast cereals.

These pamphlets have been translated into 22 languages and both the Acrylamide Toolbox and the pamphlets have been recognized by risk managers. Links to the papers are available on the European Commission website.

Joint Industry Groups

Where necessary, FoodDrinkEurope also joins coalitions to address particular issues of temporary concern and seeks the expertise of other industry partners in 'joint industry groups' which are particularly relevant in areas related to the packaging and ink industry.

The role of an industry association is also of course to join research projects where these are relevant to its general work. For FoodDrinkEurope, such a research project concerns refinements of exposure assessments for flavorings, additives and food contact materials, (FACET stands for Flavors, Additives and Contact materials Exposure Task) for instance.

Research and Innovation

Project involvement is of course insufficient to address elements to further improve food safety. The European Technology Platform 'Food for Life' was therefore created in 2005 under the auspices of FoodDrinkEurope. This Platform seeks to deliver innovative, novel, and improved food products for and to national, regional, and global markets in line with consumer needs and expectations.

This Platform offers the unique opportunity to merge academic knowledge with industry needs and, by doing so, to improve the integration of research investments and thereby facilitate competitiveness and wealth creation. Innovation and effective technology transfer will stimulate economic growth.

Stakeholders have been involved in the shaping of a Strategic Research Agenda which also addresses:

- The building of consumer trust in the food chain,
- The support of sustainable and ethical production, and
- The improvement of health, wellbeing, and longevity.

Regulation and Self-Regulation

It is the role of an industry association to react to questions which are integrated within regulatory processes and to proactively address questions which do not yet feature on the radar-screen of policymakers. An industry association will offer assistance in both developing self-regulatory measures or, if necessary, intervene in regulatory processes in order to address industry-specific issues.

Involvement in Global Tasks

In addition to acting at European levels, it is necessary to reach out to global players and therefore to actively contribute to developments within Codex Alimentarius.

In this respect, FoodDrinkEurope also addresses trade-related questions and avails of its networks in order to solve eventual issues either internationally, where new legislation has been introduced, or at European level, where the internal market is at risk of being disturbed due to the incoherent implementation of European legislation.

See also: Food Safety Assurance Systems: Essentials of Crisis Management; Food Safety and Quality Management Systems; Hazard Analysis and Critical Control Point System (HACCP): Principles and Practice; Investigation of Incidents in Industry; Management of Allergens in Food Industry; Management of Supplier and Raw Material. Food Technologies: Nanotechnology and Food Safety. Institutions Involved in Food Safety: FAO/WHO Codex Alimentarius Commission (CAC); International Food Information Council (IFIC) and Other Food Information Organizations; International Life Sciences Institute (ILSI). Public Health Measures: Alerts and Early Warning Systems; Challenges of Industrialized Countries in Food Safety Management; Food Inspections and Enforcement Systems; Fundamentals of Food Legislation; Modern Approach to Food Safety Management: An Overview; Monitoring of Contaminants; Risk Governance. Risk Analysis: Risk Communication: Chemical Hazards; Risk Communication: Novel Foods and Novel Technologies; Risk Management: Application to Biological Hazards; Risk Management: Application to Chemical Hazards

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Safety by Design.

INSTITUTIONS INVOLVED IN FOOD SAFETY

International Food Information Council (IFIC) and Other Food Information Organizations

DB Schmidt and AP Benson, International Food Information Council and Foundation, Washington, DC, USA

Glossary

Dietary Guidelines Alliance A private-public partnership among leading food, nutrition and health organizations and societies, food industry organizations and the government, dedicated to providing consumers with science-based, practical advice on how to apply the Dietary Guidelines for Americans to their lives.

Food processor A person, facility, company or machine that transforms raw ingredients into food, or that transforms food into other forms for consumption.

Food safety Assurance that food will not cause harm to the consumer when it is prepared and eaten according to its intended use.

Nutrition The science of how the body uses food.

Risk communication The interactive exchange of information and opinions throughout the risk analysis process concerning risk, risk-related factors and risk perceptions, among risk assessors, risk managers, consumers, industry, the academic community and other interested parties, including the explanation of risk assessment findings and the basis of risk management decisions.

Introduction

Founded in 1985, the International Food Information Council (IFIC) is a nonprofit organization, whose mission is to effectively communicate science-based information about food safety, nutrition and health to health and nutrition professionals, educators, government officials, journalists and others providing information to consumers. Based in Washington, DC, IFIC is supported primarily by the broad-based food, beverage and agricultural industries. The organization does not represent any product or company, nor does it lobby for legislative or regulatory action.

The IFIC Foundation was incorporated as a public education foundation in 1991 and serves as the educational arm of IFIC. The IFIC Foundation is guided by a Board of Trustees, the majority of whom represent public institutions such as universities, governmental bodies, and public foundations.

IFIC participates in, and helps to facilitate cooperation and exchange between the members of an informal network of independent food information organizations in Asia, Europe, New Zealand and South Africa and South America.

Why Do we Need Food Information Organizations?

The food supply chain relies on a complex system involving farmers, growers, ingredient suppliers, food processors, food distributors, food importers, food regulators, and food retailers in many different world regions.

Modern media outlets can transmit news about food safety developments or crises across the world in microseconds. So a crisis in one part of the world has the potential to alarm consumers across the globe.

Technological advances and other new practices in sanitation and detection methods can help reduce potential food safety risks. Yet the task of providing the public with accurate and understandable information to explain these developments remains a daunting one, and it is here that food information organizations play a critically important role.

If the food chain does not function with effective communications among key parties, a potentially disastrous food incident can still result. That is why we need food information organizations, and why their collective reach needs to be local, regional and global.

The Fundamental Underpinnings of Food Safety

Though food safety challenges can be complex, it could be argued that just three key elements constitute the fundamental underpinnings of food safety.

First and foremost is sound science that includes good agricultural and food safety practices, and which has to be a prerequisite. But where science is complex and where the food chain is long, an appropriate level of oversight is clearly necessary.

So the second key element has to be an appropriate level of food safety regulation, together with an adequate measure of regulatory oversight and inspection.

But what happens if key information regarding even basic food hygiene doesn't reach a grower in a developing country, or a food server in a Western quick service outlet? This is the

third key element that underpins food safety, namely effective communications to and from key stakeholders along the length of the food chain.

The IFIC's Food Safety Role

IFIC and its Foundation aim to bridge the gap between science and communications by collecting and disseminating scientific information in a manner consumers can understand, and by working with an extensive roster of experts to help translate research into understandable and useful information and advice for stakeholders, journalists and other opinion leaders and consumers.

In times of a food safety emergency, IFIC plays a key role, briefing the media and informing the public, partner organizations and key stakeholders about important developments that could impact public health and well-being. IFIC serves as a news media resource, and can refer journalists to over 350 independent, credentialed experts on a variety of nutrition and food safety topics.

The Need for International Cooperation

Given the international nature of today's food supply, IFIC recognizes that it is important to disseminate and exchange key information and advice on nutrition and food safety globally.

IFIC participates in and helps to facilitate cooperation and exchange between an informal, global network of independent food information organizations.

Working independently but exchanging knowledge and insight internationally, these organizations all share a common goal of bringing science-based insight and understanding to regional and international food safety and nutrition issues.

Informed decision making by policy makers, stakeholders and consumers helps to maximize levels of food safety, and helps to minimize the consequences of any food safety incident. Informed decision also helps to avoid misinformation and confusion, reducing the risk and the consequences of unfounded public alarm.

Clear and consistent communications – that engage health and nutrition professionals, educators, government officials, opinion leaders and the media – help build broad-based understanding that serves the shared interests of stakeholders and consumers most effectively.

IFIC Collaborations

IFIC has established partnership programs with a wide range of credible professional organizations, government agencies and academic institutions, including a long-standing relationship with the U.S. Department of Agriculture and Food and Drug Administration as part of the Dietary Guidelines Alliance and the *MyPyramid* Food Guidance system, as well as food safety education materials for consumers.

IFIC hosts or participates in major national and international professional conferences and meetings including programs organized by the World Health Organization, the Food and Agriculture Organization of the United Nations, the International Congress of Nutrition and other key international authorities and expert groups.

The IFIC Foundation also participates in a recently formed International Center of Excellence in Food Risk Communication (http://foodriskcommunications.org). The Center is collaborative initiative among global food and health organizations, government agencies, academic institutions, and expert nonprofit communication organizations, founded on the belief that it is important to have a collective international resource of food-specific risk communication materials which are dedicated to enabling informed decision-making to promote global health.

IFIC has helped to develop a number of key training resources for food safety risk communication including *Improving Public Understanding: Guidelines for Communicating Emerging Science on Nutrition, Food Safety and Health* (based on an advisory group convened with Harvard School of Public Health), *A Practical Guide to Risk Communication* (a Continuing Professional Education Module self-study unit developed with the University of Maryland) and *Risk Communicator Training for Food Defense Preparedness, Response and Recovery* (developed through the National Center for Food Protection and Defense).

These and other key resources can be accessed on the IFIC Foundation Web site at www.FoodInsight.org. The IFIC Foundation uses the latest technology, communication tools and interactive formats, including a Blog, a *FoodInsightTV* channel, Web casts, educational videos, a Facebook page and a Twitter account. The popular monthly *Food Insight* newsletter (available online only) helps stakeholders stay on top of the latest research and trends.

IFIC and its Foundation commission primary research on public opinion about food safety and nutrition and shares the results with stakeholders and the public. The *Food & Health Survey* provides information about how Americans connect the food they eat with their health. Recent surveys have explored topics such as food safety, overall diet, physical activity, weight management, dietary fats, functional foods, caffeine, and food allergies.

IFIC (www.ific.us) and the IFIC Foundation (www.foodinsight.org) are headquartered at: 1100 Connecticut Ave, NW, Suite 430; Washington, DC 20036. Tel.: +1 202/296-6540; Fax: +1 202/296-6547.

How Do International Food Information Organizations Cooperate?

The international food information organizations operate autonomously and independently yet they share insight, consumer attitudinal research and best practices in risk communication globally.

The food information organizations realize that, although knowledge and expertise should be shared internationally, communications need to be handled regionally and locally to have the desired impact and effectiveness.

Effective communication organizations need to have the linguistic capabilities to reach stakeholders and consumers in

their regions. But beyond language there are the key questions of credibility and impact.

Who are the trusted authorities in the region? What are the most effective communication channels? What are the regional sensitivities regarding particular food and nutrition issues, and what sort of messaging is needed to communicate accurately, clearly and effectively?

The food information organizations determine locally who have detailed first-hand knowledge of the issues, communications challenges and cultural sensitivities in the region.

So Who are Other Key International Food Information Organizations?

The Asian Food Information Centre (AFIC) was founded in 1998 and registered in Singapore. The AFIC team of scientific, health and communications professionals works in close collaboration with the academic and scientific communities in the region.

AFIC produces and distributes a wide range of science-based, consumer-friendly information on food safety and nutrition through workshops, interviews, articles, information leaflets and multilanguage website. AFIC publications include the *Food Facts Asia* newsletter and an *AFICNews* E-bulletin.

AFIC also conducts research into consumer behavior, perceptions and expectations.

Mailing Address: Shaw Centre #23-14, 1 Scott's Road, 228208 Singapore. Tel.: +(65) 6235 3854; Fax: +(65) 6235 7459. E-mail: info@afic.org. Website: www.afic.org.

The Council for Food Safety and Nutrition Information (CISAN) was newly formed in 2010 and has its operational base in Buenos Aires, Argentina. Its mission is to share science-based information on food safety, nutrition and health with health professionals, communicators and consumers, so as to enhance understanding and enable informed food choices. CISAN is operated as a collaborative initiative between three prestigious institutions, namely the Argentine Nutrition Foundation, the International Life Science Institute of Argentina, and the Argentine Council for Information on Biotechnology.

CISAN's website can be accessed at http://cisan.org.ar. To contact CISAN, please send an E-mail to info@cisan.org.ar.

The European Food Information Council (EUFIC) is a nonprofit organization, which provides science-based information on food safety and quality, and health and nutrition to the media, health and nutrition professionals, educators and opinion leaders, in a way that consumers can understand.

EUFIC's mission is to enhance the public's understanding of such issues and to raise consumers' awareness of the active role they play in safe food handling and choosing a well-balanced and healthy diet.

Information that EUFIC publishes has been subject to a review process by members of its Scientific Advisory Board (SAB). Given the broad range of subjects addressed in EUFIC's popular newsletter, *Food Today*, a dedicated Editorial Board for this publication provides additional insights and feedback.

With its main offices located in Brussels (Belgium), EUFIC counts on and liaises with a European network to enhance the impact and outreach of its communication instruments and programs in other countries.

EUFIC actively participates in European initiatives together with the European Commission Directorate Generals for Research, and for Health and Consumers, where it contributes to a number of projects as dissemination partner.

EUFIC's website is www.eufic.org. To contact EUFIC, please send an E-mail to eufic@eufic.org.

The Food Advisory Consumer Service (FACS) is South Africa's first independent food and nutrition consumer service. First launched in January 1995, its purpose is to provide consumers with information on food and nutrition issues which is both relevant and scientifically correct.

FACS operates a telephone information service, supported by a network of technical experts in food, nutrition and related subjects.

FACS is run by a committee representing SAAFoST, the South African National Consumer Union (SANCU), the Association of Dietetics (ADSA), the Directorate of Food Control of the Department of Health, and professional representatives of other consumer-friendly organizations. FACS has access to information from similar bodies overseas. However, it is committed to offering a comprehensive service in line with South Africa's unique situation. This would include risk groups such as infants, sufferers of malnutrition and others.

FACS has good contact with the media and is a member of the Food Legislation Advisory Group, the body concerned with food legislation in South Africa, and with Codex Alimentarius, the Joint FAO/WHO Food Standards Program.

Website: http://www.foodfacts.org.za/. Postal Address: PO Box 27852, Sunnyside, 0132. Customer Hotline: 012 428 7122 (hotline available from 08:00 to 12:00); Fax: 086 672 8585.

The New Zealand Nutrition Foundation (NZNF) is a professional, nonprofit organization whose members believe all New Zealanders should have access to accurate information to enable them to make informed choices about food and the effect it has on their health. NZNF helps New Zealanders make these choices by providing a balanced viewpoint on important issues around food, nutrition and health.

Founded in 1980, the NZNF was established as a charitable not-for-profit trust, with individual and corporate membership to support its activities. The Foundation's membership includes those working in health, nutrition, education and the food industry, providing the opportunity to co-ordinate and support work between professional groups, food producers and marketers and government agencies.

Contact information: P.O. Box 331 366, Takapuna, Auckland 0740, New Zealand. Tel.: +64 9 489 3417.

See also: Food Safety Assurance Systems: Essentials of Crisis Management. Foodborne Diseases: Overview of Biological Hazards and Foodborne Diseases. Institutions Involved in Food Safety: Food and Agriculture Organization of the United Nations (FAO); International Life Sciences Institute (ILSI); International Union of Food Science and Technology (IUFoST); World Health Organization (WHO). Risk Analysis: Food Safety Training and Health Education: Principles and Methods; Risk Communication: Diet, Nutrition, and

Health; Risk Communication: Novel Foods and Novel Technologies; Risk Communication

Further Reading

Benson A (2010) Communicating risk to consumers in domestic and internationally traded products. *Food Control* 22(9): 1529–1534.

Bostrom A and Lofstedt R (2003) Communicating risk: Wireless and hardwired. *Risk Analysis* 23: 241–248.

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Fineburg H and Rowe S (1998) Improving public understanding: Guidelines for communicating emerging science on nutrition, food safety, and health. *Journal of the National Cancer Institute* 90: 194–199.

Fischoff B, Brewer N, and Downs J (2011) *Communicating Risks and Benefits: An Evidence-Based User's Guide*. U.S. Food and Drug Administration. Available at: http://www.fda.gov/ScienceResearch/SpecialTopics/RiskCommunication/default.htm

Relevant Websites

www.afic.org
Asian Food Information Centre.

http://cisan.org.ar
Council for Food Safety and Nutrition Information (Argentina/South America).

www.eufic.org
European Food Information Council.

http://www.foodfacts.org.za
Food Advisory Consumer Service (South Africa).

http://foodriskcommunications.org
International Center of Excellence in Food Risk Communication.

http://foodinsight.org
International Food Information Council Foundation.

http://www.nutritionfoundation.org.nz
New Zealand Nutrition Foundation.

INSTITUTIONS INVOLVED IN FOOD SAFETY

International Union of Food Science and Technology (IUFoST)

A Mortimer, ABC Blending, Bayswater, VIC, Australia
K Buckle, The University of New South Wales, Sydney, NSW, Australia

Glossary

Adhering body A representative body of the International Union of Food Science and Technology (IUFoST) from each country, or defined geographical area(s), that has an independent science budget.

What is IUFoST?

The International Union of Food Science and Technology (IUFoST) is the sole global, country member organization that represents more than 200 000 food scientists and technologists (Buckle and Meech, 2003). It is a voluntary, nonprofit association that links the world's food scientists and technologists through international cooperation and exchange of scientific and technical information. IUFoST is a full member of the International Council for Science and works closely with several United Nations agencies such as the Food and Agriculture Organization (FAO), World Health Organization (WHO), Codex Alimentarius Commission, and International Atomic Energy Agency as well as other international organizations to transfer technical knowledge and strategies for basic and applied research for the developing world.

IUFoST sponsors international and regional congresses, conferences, symposia, and workshops that are held in member countries, and promotes a safe, secure, and wholesome food supply for the world's increasing population.

Origin

The profession of food science and technology is generally agreed to have emerged in the USA in the early decades of the twentieth century. The subsequent formation of the (US) Institute of Food Technologists (IFT) in 1939 and its successful development and expansion led to the formation of similar associations in many countries during the following 20 years. In 1960, the UK government and several British scientific societies hosted a conference in London to commemorate the centenary of the UK Food and Drugs Act (1860) and the hundred and fiftieth anniversary of the publication in 1810 of Nicolas Appert's book (*L'art de Conserver, Pendant Plusieurs Années, Toutes les Substances Animals et Végétales*) on heat preservation of foods in sealed containers. In the preceding week, the Glasgow Royal College of Science and Technology (later to become the University of Strathclyde) convened a symposium on recent advances in food science, and discussions between several eminent UK and US food scientists raised the concept of an international food society. The formation of an international organization was discussed at the First International Congress of Food Science and Technology held in London in 1962 and organized by the Food Group of the (UK) Society of Chemical Industry.

Following the Congress, an ad hoc International Committee of Food Science and Technology (ICFoST) was formed with eminent scientists from several countries to promote and foster international cooperation in food science and technology. The Second International Congress of Food Science and Technology was held in Warsaw, Poland, in 1966. By the time the Third International Congress of Food Science and Technology (Science of Survival: SOS/70) was held in Washington, DC, USA, in 1970, ICFoST had developed a constitution and working arrangements for an international union (IUFoST), which was inaugurated on 14 August 1970.

Membership

The founding membership consisted of 20 food science and technology organizations from 20 nations; current membership includes organizations from 69 countries (http://www.iufost.org/adhering-bodies). Under the constitution, membership is open to all countries and consists of one representative body, termed as an 'Adhering Body,' from each country. The term 'country' is understood to be a defined geographical area, or areas, that has an independent science budget. Only one Adhering Body can represent each such area. Adhering Bodies can be a national food science and/or technology group, society or institute composed of food scientists, and food technologists or other professionals engaged in food science and technology; an academy of sciences, national research council, or similar organization of scientists; an intersociety committee or similar group representing two or more

Table 1 IUFoST World Congresses of Food Science and Technology

Congress no.	*Year*	*Location*
1	1962	London, UK
2	1966	Warsaw, Poland
3	1970	Washington, DC, USA
4	1974	Madrid, Spain
5	1978	Kyoto, Japan
6	1983	Dublin, Ireland
7	1987	Singapore
8	1991	Toronto, Canada
9	1995	Budapest, Hungary
10	1999	Sydney, Australia
11	2001	Seoul, South Korea
12	2003	Chicago, IL, USA
13	2006	Nantes, France
14	2008	Shanghai, PR China
15	2010	Cape Town, South Africa
16	2012	Iguazu Falls, Brazil

societies composed of food scientists and technologists; or a national committee representing food scientists, engineers, and technologists working in the field.

Mission and Activities

The IUFoST mission is to promote international cooperation and information exchange, to provide education and training to food scientists and technologists around the world, and to promote professionalism and professional organization among food scientists and technologists.

IUFoST conducts a wide range of activities in order to achieve its objectives of fostering international cooperation and exchange of knowledge and ideas. They are conducted in conjunction with the Adhering Bodies and regional groupings, or with other local, national, and international organizations.

Congresses

From 1962 to 1999, World Congresses of Food Science and Technology were conducted about every 4 years, in conjunction with the Adhering Body of that country. From 2001, they have been held biennially, commencing with the 11th World Congress of Food Science and Technology in Seoul, South Korea (Table 1).

Regional Meetings

IUFoST has been associated with more than 200 conferences, symposia, seminars, workshops, training programs, and short courses since its inception in 1970, covering diverse topics in areas such as food science, technology, engineering, and microbiology; human nutrition; national and international food law; food toxicology; product development; food security; and, in more recent years, especially food safety.

IUFoST Involvement in Food Safety Activities

Committees and Panels

The first formal committee with a brief on some aspects of food safety was set up in 1980 as the Committee on Food Composition and Food Safety, initially chaired by Dr Richard Hall (USA), who was later to become the fourth IUFoST President (1983–87). One of the first food safety activities was the First Asian Conference on Food Safety: The challenges of the 1990s, held in Kuala Lumpur, Malaysia on 2–7 September 1990, organized by the Malaysian Institute of Food Technology, International Life Sciences Institute (ILSI), Malaysian Agricultural Research and Development Institute, Malaysian Ministry of Health, and the WHO in conjunction with the Federation of Institutes of Food Science and Technology in Association of South-East Asian Nations (ASEAN) (FIFSTA)…, FAO, ILSI Australia, and IUFoST.

In 2009, IUFoST established a Food Safety Committee in response to the expressed needs of its Adhering Bodies worldwide, with the objective to advise and act within IUFoST, via the Scientific Council, on emerging and current issues in areas of food safety from the national and regional perspective, in conjunction with IUFoST Expert Panels. The Food Safety Committee consists of representatives from Adhering Bodies, including Regional Groups, together with Academy Fellows, and others. The Expert Panel members are drawn from Fellows of the International Academy of Food Science and Technology (IAFoST), from the Union's worldwide members, and from IUFoST's global partners in Europe, FAO, WHO, ILSI, International Commission on Microbiological Specifications for Foods (ICMSF), and others. The Food Safety Expert Panel advises on aspects of chemical and microbiological food safety, allergies, zoonoses, traceability and food safety policy, and regulation and reports to the Scientific Council.

Declarations on Food Security

IUFoST has long recognized the role of food science and technology in improving food security, including food safety, in the developing world, especially in Africa, Southeast Asia, and Eastern Europe. The Victoria Falls Declaration, supporting the plight of consumers in the developing world, especially in sub-Saharan Africa, was approved by delegates at the IUFoST/Eastern, Central and Southern African Association of Food Science and Technology (ECSAAFoST) joint meeting in 1994, the first International food conference held in Zimbabwe in September 1994. The declaration was based on the original declaration from the FAO/WHO International Conference on Nutrition held in Rome, Italy in December 1992, which was endorsed by 159 states and the European Economic Community.

The following year, the 9th World Congress of Food Science and Technology, held in Budapest, Hungary in July–August 1995, the Budapest Declaration, based on the FAO/WHO and Victoria Falls Declarations, was approved by delegates to the IUFoST General Assembly (http://iufost.org/budapest-declaration). Clause 1 states that IUFoST delegates will "… recognise that access to nutritionally adequate and safe food is the right of each individual…", whereas clause 7 states that IUFoST

identifies "… the following areas as being especially significant: promotion of the safety and quality of all foods…".

Delegates to the General Assembly at the 15th World Congress of Food Science and Technology held in Cape Town, in August 2010 approved the Cape Town Declaration (http://iufost.org/cape-town-declaration), which again reaffirmed IUFoST's commitment to food safety as reflected by the appearance of terms such as 'nutritionally adequate and safe food.' 'safe and wholesome foods,' 'promotion of the safety and quality of all foods,' and 'to ensure food safety.'

Publications

Trends in Food Science and Technology

Published by Elsevier, this publication is the official journal of IUFoST and the European Federation of Food Science and Technology (EFFoST), one of IUFoST's regional groupings. It is an international peer-reviewed journal publishing critical reviews and viewpoints of current technology, food science, and human nutrition.

Newsline

The IUFoST newsletter, first published in 1970, became *Newsline* with the 25th edition in March 1993, when it was first published as part of *Lebensmittel-Wissenschaft und Technologie* (*Food Science and Technology*), the official journal of the Swiss Society of Food Science and Technology and published by Elsevier Science. *Newsline* is now published electronically three times per year and is available on the IUFoST website (www.iufost.org/iufost-newsline). *Newsline* has published many articles on food safety issues, especially from IUFoST-cosponsored seminars/symposia conducted in conjunction with national Adhering Bodies.

The World of Food Science

In 2000, IUFoST and IFT, the US Adhering Body, jointly launched the online electronic magazine *The World of Food Science*, directed at food scientists and technologists worldwide. It was relaunched in October 2006, following the 13th World Congress of Food Science and Technology, in Nantes, France. Volume 3 (February 2008) features articles on food safety (http://www.worldfoodscience.org/cms/?pid=1004439). The Editorial Advisory Board includes members drawn from IUFoST's Adhering Bodies.

Scientific Information Bulletins

IUFoST's Scientific Council has produced several Scientific Information Bulletins (SIBs) on topical issues, including the following on aspects of food safety (http://iufost.org/iufost-scientific-information-bulletins-sib):

- Bovine spongiform encephalopathy (February 2004).
- Safety, risk, and the precautionary principle (August 2007).
- Best practices in risk and crisis communication: Advice for food scientists and technologists (October 2007).
- Equivalence in food safety management (December 2007).
- Chemical hazards in food (October 2008).
- Food allergy (March 2009).
- Trans fatty acids (updated February 2010).
- Biotechnology and food (updated March 2010).
- Acrylamide in foods (updated July 2010).
- Nanotechnology and food (updated August 2010).
- Chemical hazards in food (January 2011).
- Radioactive fallout from the 2011 Japan nuclear plant accident and some recommended precautions and countermeasures (May 2011).
- Shiga toxin producing *Escherichia coli*: Germany 2011 *E. coli* 0104:H4 outbreak linked to sprouted seeds.
- Food traceability (March 2012).

SIBs are prepared by members of the Scientific Council or by selected experts from around the globe.

Conferences, Symposia, and Seminars

IUFoST has collaborated with a variety of Adhering Bodies, and both national and international organizations, to provide a forum for discussions on aspects of food safety. Some examples of such activities over the past 5 years include:

- 15th IUFoST World Congress of Food Science and Technology, Cape Town, South Africa, August 2010, featuring sessions on food safety, risk assessment and contaminants, and food allergens.
- International Forum on Food Safety, Beijing, China, 2010 and 2011, with Chinese Institute of Food Science and Technology.
- IUFoST – Japan Food Safety Symposium, September 2009.
- 14th IUFoST World Congress of Food Science and Technology, Shanghai, China, October 2008, featuring technical sessions on food safety of children's foods; modern food safety control systems; food safety forum; food safety monitoring technology; food safety regulations and standards; hazard assessment and foodborne disease; hot topics in safety of food systems; food genetic engineering and food safety; and biotechnology and food safety.
- IUFoST/Food Ingredients Asia/China Conference on Natural and Safe Food Ingredients, Shanghai, China, March 2007.
- 13th IUFoST World Congress of Food Science and Technology, Nantes, France, September 2006, featuring 10 chapters on food safety in the resultant book entitled *Global Issues in Food Science and Technology* (Barbosa-Canovas *et al.*, 2009).

In addition to the above, IUFoST has collaborated with a number of national, regional, and international organizations, including:

- ICMSF,
- WHO,
- International Union of Nutritional Sciences and
- IUFoST Regional Groupings, including:
  - La Asociación Latinoamericana y del Caribe de Ciencia y Tecnologia de Alimentos (Latin American and Caribbean Association of Food Science and Technology),

- EFFoST,
- FIFSTA, and
- West African Association of Food Science and Technology.

Further Reading

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Buckle KA and Meech JS (2003) International union of food science and technology. In: Caballero B, Finglas P, and Trugo L (eds.) *Encyclopedia of Food Sciences and Nutrition (10 vols.)*, pp. 3342–3349. London: Academic Press.

Relevant Website

www.iufost.org
IUFoST Website.

INSTITUTIONS INVOLVED IN FOOD SAFETY

Trust in Animals and Food Safety (TAFS) Forum

U Sperling, TAFS Forum, Bern, Switzerland

The international forum TAFS (Trust in Animals and Food Safety) is a nonprofit making Swiss foundation founded in 2002 in a response to the urgent need to enhance the communication between scientists and stakeholders of the food chain, food industry, and consumers in relation to the bovine spongiform encephalopathy (BSE) and other transmissible spongiform encephalopathy (TSE) diseases. With improvements in the prevention of BSE, TAFS extended its scope to other transmissible animal diseases such as paratuberculosis and influenza, and aspects of livestock rearing that are of relevance for food safety in general.

TAFS aims to contribute toward global food safety by acting as platform for communication on risks of animal diseases and measures for their management, particularly where important aspects of the scientific basis of animal diseases are still unclear. This is done by:

- monitoring scientific developments and provision of stakeholders with early warning and advanced information on the new scientific findings;
- interpretation of the significance of the scientific findings and possible implications on the regulatory development;
- voicing TAFS' opinion on the safety of animal-derived food and food products;
- making recommendations on voluntary risk management measures for the stakeholders;
- advocacy for regulatory measures;
- identification of gaps in science and possible areas for research.

Recommendations for risk management are nonbinding even for members and are meant as a guideline. Implementation may require adaptation to local requirements and possibilities.

The guiding principles of TAFS are: Personal trust and confidentiality, independence and impartiality, a strong commitment to a science- and fact-based approach, acknowledgment of knowledge gaps and their impact on risk management.

Communication of TAFS occurs through various channels, including international conferences, position papers, and a news stream. TAFS has so far published a number of position papers and suggested risk management plans, particularly on BSE and paratuberculosis. They are available at www.tafsforum.org for download.

TAFS has 21 members (status January 2012) from Europe, North America, and Asia, representing the food industry, regulators, and academia. Consumer views and interests are covered by social scientists. TAFS is generally open to admit new members, provided they commit to the guiding principles and actively contribute to the achievement of TAFS' goals.

Relevant Website

http://www.tafsforum.org
The TAFS Forum.

INSTITUTIONS INVOLVED IN FOOD SAFETY

Global Harmonization Initiative (GHI)

H Lelieveld, Global Harmonization Initiative (GHI), Vienna, Austria

Glossary

Food safety Preventing harm from eating food.
Food security Certainty that there is sufficient food.
Global scientific consensus Consensus between scientists (including nutritionists, technologists, and engineers) from all countries of the world.
Harmonized regulations Regulations that are the same and have the same meaning everywhere.
Nutrient security Certainty that there are enough of all essential nutrients.
Sound science Knowledge based on facts that have been accepted by peers and can withstand scrutiny.
Toxic concentration Concentration of a substance in food that upon ingestion causes bodily harm.

Introduction

The Global Harmonization Initiative (GHI) is a global association of individual food scientists (in a wide sense, hence including technologists, nutritionists, and others) with a goal to "Achieving consensus on the science of food regulations and legislation to ensure the global availability of safe and wholesome food products for all consumers." Consensus statements will provide tools for stakeholders to exert pressure on legislators to make them base regulations on sound scientific evidence. Harmonized regulations will reduce the need for destroying safe food for pure differences between regulations. It will also facilitate trade between countries and the acceptance of new products or production methods once proven safe, avoiding testing in many countries with different protocols.

Although the globalization of world trade has created new pathways to economic growth for many nations, the trend toward a 'one-world economy' has also exposed critical differences in international laws and regulations that are designed to protect the world's citizens. Nowhere is this more evident than in the global food supply chain. Gaps in the science used to justify international regulations create confusion for food producers trying to achieve compliance. The result is a world of barriers to achieve food security and effective food safety – and the technological advancements that could ensure both. The need to harmonize global food regulations and laws has never been greater, considering the following challenges.

Food Security and Nutrition

Although the world as a whole produces enough food for everyone, approximately half of the food produced does not reach those who need it. As a consequence, today approximately 'one billion people' suffer from hunger. One of the major reasons is that a significant percentage of the food is destroyed during harvesting, transport, and storage. Much is spoiled before consumption owing to inadequate preservation, but food may also be deemed unfit for consumption – and consequently destroyed unnecessarily – without scientific justification due to laws that have been developed arbitrarily and are not founded on sanitary or other technical (e.g., rights of consumers and fair trade) reasons. In addition, food preserved to prevent microbial spoilage often has a significantly reduced concentration of essential nutrients. This can significantly be remediated by novel technologies, if their applications are not hindered by scientifically unjustified differences in regulations between nations.

Food Safety

Nations enact food safety regulations to protect consumers from foodborne illness. This is needed because some unscrupulous producers and tradesmen are more concerned about making a profit than safeguarding consumer health. Regrettably, significant differences in food safety regulations between nations can, and do, result in situations in which a food that is considered safe in one country is considered unfit for consumption in another country. This, in turn, leads to the destruction of imported foods that are safe but that do not meet regulatory requirements, or prevents countries from exporting food to areas where it is needed. Without globally harmonized, science-based regulations, rules intended to protect consumers from foodborne illness or death merely serve to erect trade barriers that inhibit technologic advancements that ensure public health.

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Technology and Method Development

New technologies and scientific methodologies may have drawbacks, so there must be proof that such technologies offering beneficial solutions for one problem will not jeopardize other safety aspects. Thus, there is a fair and logical requirement to prove that food produced using a new process is still safe. Defining what constitutes a 'safe' process for ensuring food safety is challenging when the laws and regulations of countries differ, because validation must occur in many countries following different protocols, resulting in significant hurdles to new technology application.

For example, to protect crops against insects and other pests, producers use traditional prevention methods. Clearly, traditional methods work but they do have drawbacks, such as potentially toxic residues, destruction of nutrients, and dramatic losses to crushing or foraging animals. All over the world, scientists and engineers are developing methods that minimize, or even eliminate, such drawbacks. Results include more insect-resistant crop varieties and packaging that effectively protects against mechanical damage and pest infestation. Similarly, to prevent microorganisms from adulterating food preservation methods are developed that need neither heat nor chemicals to stop microbes from doing harm and help retain more of the food's essential nutrients. However, without agreement between countries about the validity of these technologic approaches, neither food producers nor consumers will benefit from the increased food safety and nutritional benefits they can provide.

Testing protocols used to establish the safety of the food itself or validate a preservation process' safety also require harmonization. A case in point is the prescribed testing protocols involving the use of animals, which not only poses serious ethical objections but, for processed food to cross borders, meeting legal requirements can be difficult, costly, and time-consuming. There is increasing evidence that the results of animal testing to determine toxicity of foods, cosmetics, and pharmaceuticals have little or no relevance to humans or human health outcomes. In fact, easy-to-apply alternative methods have been developed. Although these animal-free methods have been shown to return a faster, more accurate speed-to-result, expected financial benefits of their use cannot be realized if the protocols for product safety testing are not globally harmonized.

Historical Background

In view of the above, in 2004, members of the International Division of the Institute of Food Technologists (IFT) and representatives of the European Federation of Food Science and Technology (EFFoST), which is the European branch of the International Union of Food Science and Technology (IUFoST), were motivated to contribute to the harmonization of food safety regulations, globally. This resulted in the formation of the GHI in 2007. Shortly after establishing GHI, many scientific organizations joined the effort, which now boasts a growing, truly global membership dedicated to achieving consensus on the science of food regulations and legislation to ensure the global availability of safe and wholesome food products for all consumers.

Modus Operandi and Activities

From its inception, GHI's strategy has been to provide a networking forum in which food scientists could practice science. Its goal is to foster global scientific consensuses on issues for which globally equal regulations will help strengthen global food safety and alleviate global hunger and nutrition problems. By producing independent, authoritative information based on scientific consensus, GHI hopes to inform the world's regulators and lawmakers so that regulations are based on sound science and not reactionary conclusions after food safety failures. Stakeholders may use GHI's objective consensus documents to provide evidence to their governments that harmonization of food regulations is possible and in their interest.

Finances and Membership

As is explicit in the constitution of the organizations, to protect its impartiality, GHI will not accept funding from industries or governments. Funding is accepted only from scientifically independent organizations. GHI's intention is to involve scientists from all countries and, to achieve this, there is no membership fee. Applicants for membership, however, need to qualify and therefore are required to provide details showing that they are food scientists and experts have to provide evidence of their expertise before being invited to judge concepts of consensus documents.

Awareness

To make colleagues aware of the initiative, GHI has held workshops, courses, and meetings in many countries, which led to the formation of working groups (WGs), focusing on a variety of topics, including *Listeria monocytogenes* in ready-to-eat food, nanotechnology application, high-pressure sterilization, and the toxicity of food ingredients. Each WG's task is to develop a concept document for discussion by experts worldwide, followed by global distribution to food scientists, technologists, and engineers, with the goal of reaching a significant global consensus on the science involved in the specific area of interest.

From the start there has been abundant publicity about GHI. Many articles can be downloaded from the GHI website. In 2010, Elsevier published the book 'Ensuring Global Food Safety – Exploring Global Harmonization,' edited and written by GHI Members from all over the world. GHI has a quarterly newsletter, which can be downloaded from the website. The newsletters are announced in many professional magazines and in emails to the membership.

Structure

The Association has an Executive Committee consisting of at least six members, namely, the president, a general secretary, a

treasurer, and members at large. There is a Supervisory Board with the task to watch that the organization remains impartial at all times. The consensus processes take place in WGs and opinions are sought from members from all countries. To maximize the input of scientists from all over the world, GHI has Ambassadors in many countries, so that issues can be discussed in local languages.

See also: Institutions Involved in Food Safety: FAO/WHO Codex Alimentarius Commission (CAC). Public Health Measures: Challenges of Developing Countries in Management of Food Safety; Challenges of Industrialized Countries in Food Safety Management; Fundamentals of Food Legislation; International Standards and Harmonization of Food Safety Legislation

Further Reading

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Relevant Websites

http://www.elsevierdirect.com/9780123748454
Ensuring Global Food Safety – Exploring Global Harmonization.

www.globalharmonization.net
Global Harmonization Initiative (GHI).

Abbreviation of Selected Organizations Involved in Food Safety

This is a nonexhaustive list of some of the frequently mentioned abbreviation in the Encyclopedia of Food Safety.

CAC	Codex Alimentarius Commission.
CDC	Centers for Disease Control and Prevention (USA).
CI	Consumers International.
EFSA	European Food Safety Authority.
EHEDGE	European Hygienic Engineering and Design Group.
EPA	Environmental Protection Agency (USA).
EC	European Commission (European Union).
FAO	Food and Agricultural Organization of the United Nations.
FDA	Food and Drug Administration (USA).
IARC	International Agency for Research on Cancer.
ICMSF	International Commission on Microbiological Specifications for Foods.
IFIC	The International Food Information Council.
ILSI	International Life Sciences Institute.
ISO	International Organization for Standardization.
IUFoST	International Union of Food Science and Technology.
JECFA	Joint FAO/WHO Expert Committee on Food Additives.
JEMRA	Joint FAO/WHO Expert Meetings on Microbiological Risk Assessment.
JMPR	Joint FAO/WHO Meetings on Pesticide Residues.
OIE	World Organization for Animal Health.
PAHO	Pan American Health Organization.
RASFF	Rapid Alert System for Food and Feed.
UNEP	United Nations Environment Programme.
USDA	United States Department of Agriculture.
WHO	World Health Organization.
WTO	World Trade Organization.

Index

Notes
Cross-references terms in *italics* are general cross-references, or refer to subentry terms within the main entry (the main entry is not repeated to save space). Readers are also advised to refer to the end of each article for additional cross-references – not all of these cross-references have been included in the index cross-references.

The index is arranged in set-put style with a maximum of four levels of heading. Major discussion of a subject is indicated by bold page numbers. Page numbers suffixed by *T* and *F* refer to Tables and Figures respectively. *vs.* indicates a comparison.

Where index subentries and sub-subentries pertaining to a subject have the same page number, they have been listed to indicate the comprehensives of the text.

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